



NIRT

National Institute for Research in Tuberculosis



Annual
Report 2014-15

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

**NATIONAL INSTITUTE FOR RESEARCH
IN TUBERCULOSIS**

Research Activities

April 2014 – March 2015

CONTENTS

	Page No.
Committees	
1. Scientific Advisory Committee	i
2. Data & Safety Monitoring Board	ii
3. Institutional Ethics Committee	iii
4. Institutional Committees (Internal)	iv
Guest lectures	viii
Abbreviations	ix
Report of Research Activities	
Clinical Studies	
Department of Clinical Research	3
Department of Socio-behavioural Research	31
Laboratory Studies	
• Bacteriology	43
• Biochemistry & Clinical Pharmacology	57
• HIV	67
• Immunology	83
Department of Statistics	127
Department of Epidemiology	133
Bioinformatics	149
Electronic Data Processing Division	159
International Centre for Excellence in Research	165
Contribution to National Programmes	177
Library & Information Centre	187

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Contents (contd....)

Appendices	(i)
Publications	(ii)
Awards	(xiv)
Special Assignments	(xv)
Participation in Conferences	(xvi)
Capacity building	(xx)
Workshops / Conferences organized	(xxi)
Ph.D. Scholars – Staff and Students	(xxxix)
Staff list	(xxxv)

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S.No.	Date of Lecture	Name of the Speaker	Affiliation of the speaker	Topic of the Lecture
1	Sept. 19, 2014	Dr. Krishna Rajarathnam	Professor, University of Texas Medical Branch, USA.	How do chemokines orchestrate immune surveillance? Insights from structural and animal model studies
2	Oct. 1, 2014	Dr. Manivelan	Medical Officer, Dept. of Public Health & Preventive Medicine, Chennai.	Social determinants of health & hygiene in relation to Millennium development goals: Indian perspective
3	Oct. 20, 2014	Prof. Diana Gibbs	Institute of Clinical Trials & Methodology, University College of London, UK.	Recent trials in HIV and TB in children
4	Nov. 19, 2014	Dr. Salman Keshavjee	Harvard University, USA.	MDR-TB and integrated service delivery for TB care
5	Jan. 7, 2015	Dr. Kelly E. Dooley	Johns Hopkins University, School of Medicine, USA.	Optimizing new and existing drugs in pediatric TB
6	March 16, 2015	Dr. Suvanand Sahu	Deputy Executive Secretary, Stop TB Partnership Secretariat, UNOPS, Geneva.	Fighting TB in the post 2015 era – Vision of Stop TB partnership

ABBREVIATIONS

AFB	Acid fast bacilli
ARR	Acquired rifampicin resistance
ART	Anti-retroviral treatment
ATT	Anti-TB treatment
AUD	Alcohol use dependence
AP	Auramine- O pehnol
CFA	Culture filtrate antigen
CFP	Culture filtrate proteins
CFU	Colony forming units
CIF	Cumulative incidence function
CP	Continuation phase
CRP	C-reactive proteins
C-TRIUMPH	Cohort for TB research by the Indo-US medical partnership
DKO	Double knock-out
DM	Diabetes mellitus
DR-TB	Drug resistant TB
DST	Drug susceptibility testing
ECOFF	Epidemiological cut-off
EMB	Ethambutol
EQA	External quality assurance
FGDs	Focus group discussions
FM	Fluorescence microscopy
FNAC	Fine needle aspiration cytology
Fq	Fluoroquinolone
GIS	Geographical information system
GPS	Global positioning system
HAART	Highly active anti-retroviral therapy
HCS	Healthy control subjects
HHC	Healthy household contacts
ID	Immunodominant
IGRAs	IFN gamma release assays
INH	Isoniazid
IP	Intensive phase
IPT	INH preventive therapy
IPTG	Isopropyl β -D-Thiogalactoside
IRIS	Immune reconstitutiton inflammatory syndrome
LTBI	Latent TB infection
LAM	Lipoarabinomannan
LED	Light-emitting diodes
LPA	Line probe assay
LRP	Luciferase reporter phage
MDP	Model DOTS Project
MDR-TB	Multi-drug resistant TB
MOI	Multiplicity of infection

MFX	Moxifloxacin
MSW	Male sex worker
Nab	Neutralizing antibody
NACO	National AIDS Control Organization
NRL	National reference laboratory
NRTI	Nucleoside reverse transcriptase inhibitor
NSP	New sputum smear positive
NTM	Non-tuberculous mycobacteria
OFX	Ofloxacin
PBP	Penicillin binding protein
PCA	Principal component analysis
PDB	Protein database
PHC	Primary health centre
PI	Protease inhibitor
PMA	Phorbol-myristate-acetate
PP	Private practitioner
PR	Protease
PT	Previously treated
PTB	Pulmonary tuberculosis
PZA	Pyrazinamide
RBRC	Random blinded rechecking
RMP	Rifampicin
RNAP	RNA polymerase
RNI	Reactive nitrogen intermediate
RNS	Reactive nitrogen species
RNTCP	Revised National TB Control Programme
ROC	Receiver operating characteristic
ROI	Reactive oxygen intermediates
ROS	Reactive oxygen species
RPF	Resuscitation promoting factors
RS	Remote sensing
RT	Reverse transcriptase
SCC	Short course chemotherapy
SHG	Self help group
Ss	Strongyloides stercoralis
STPK	Serine / threonine protein kinase
TAT	Turn-around time
TM	Transmembrane
TST	Tuberculin skin test
UTR	Untranslated region
VDR	Vitamin D receptor
ZN	Ziehl Neelsen

CLINICAL STUDIES

**DEPARTMENT OF
CLINICAL RESEARCH**

STUDIES IN PROGRESS:

CL-1: A 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 pulmonary tuberculosis

Principal Investigator : Dr. G. Narendran (email:nareng@nirt.res.in)
Co-PI : Dr. Soumya Swaminathan
Collaborators : Govt. Hospital of Thoracic Medicine,
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Chennai; NJIL and OMD, Agra; Govt. Stanley Hospital,
Chennai; Govt. Otteri Hospital, Chennai; Govt. Vellore
Medical College and Hospital, Vellore; Govt. Rajaji
Hospital, Madurai
Source of funding : USAID (Model DOTS Project)
Study period : 2009-2015
Trial Registry No. : 476/09, NCT No. 933790

Background: HIV-TB is an important dual infection in India demanding attention from programme managers and clinicians with focus on duration of treatment and schedule of administration, which are hot button issues that require generation of scientific evidences, especially from India. We undertook this study to examine intricate aspects of this dual infection including schedule of therapy, sputum conversion, radiological clearance, immune reconstitution inflammatory syndrome (IRIS), treatment emergent adverse drug reactions and drug levels so that concrete answers could be deduced.

Aims:

Primary: (i) To compare daily vs. intermittent therapy of anti-TB treatment (ATT) in reducing failures and emergence of acquired rifampicin resistance (ARR)

Secondary: (i) To correlate sputum conversion, IRIS, emergence of ARR,

radiological improvement, plasma Rifampicin (RMP) and Isoniazid (INH) concentrations 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, with stratification based on CD4 cell counts and sputum smear grading. Blood samples at 2-hr post dosing is being collected at months 2 and 6 of ATT. Toxicity is monitored using modified CTC and DAIDS criteria. Unfavorable responses in each regimen during treatment and follow-up are compared. Both intent to treat analysis and per protocol analysis were performed in the first interim analysis.

Results: 290 patients have been enrolled so far. Table 1 provides the baseline parameters of enrolled subjects.

far. Table 1 provides the baseline parameters

Table 1: Baseline demographics of enrolled patients (Mean \pm SD)

Variables	Regimen		
	Daily (n=96)	Part Daily (n=100)	Intermittent (n=94)
Age (yrs)	38 \pm 8	38 \pm 9	39 \pm 8
Weight (kg)	42.4 \pm 8.2	42.9 \pm 7.6	44.4 \pm 7.3
Hemoglobin (Gms%)	9.7 \pm 2.3	9.5 \pm 2.0	10.0 \pm 2.1
Viral Load (units)	4.9 \pm 1.9	4.9 \pm 1.0	4.8 \pm 1.2
ATT-ART interval* (months)	17 (3-39)	19(5-46)	16(1-37)
CD4 * cell counts (cells/mm)	129 (65-212)	148(86-262)	140 (67-266)

*Median (IQR) Above values are mean \pm SD

Interim findings suggest a trend towards better response with the daily regimen, though not statistically significant. The incidence of IRIS is 38% and the overall toxicity is 7%, being more in the daily regimen when patients are already on anti-

retroviral treatment (ART). Sputum smear and culture conversion seem to be better with the daily regimen. Soon the second interim analysis will be done.

The study is ongoing.

CL-2: Evaluation of different strategies (pharmacologic intervention versus enhanced motivation vs. standard motivation) for smoking cessation in tuberculosis patients under treatment in the Revised National Tuberculosis Programme – A cluster randomized effectiveness trial

Principal Investigator : Dr.S. Ramesh Kumar
(email: ramesh@nirt.res.in)
Collaborators : DTOs of Villupuram and Kanchipuram district
Consultants : Dr.Vidhubala, TCC-Adayar Cancer Institute,
Dr.Karthikeyan, Psychiatrist
Source of funding : WHO through Model DOTS Project
Study period : 2013-2016
Study Registry No. : CTRI/2013/07/003830.Registered
on: 23/07/2013)

A cluster randomized effectiveness trial has been started to compare the feasibility, acceptability and effectiveness of pharmacologic therapy (Bupropion SR) versus enhanced counselling package in smoking cessation among TB patients initiating treatment, under program settings in India.

Thirty six direct microscopy centres (DMCs) from two districts (18 DMCs each from Villupuram and Kanchipuram) are randomly selected to receive any of the three, namely (i) Bupropion SR along with standard counselling (ii) enhanced counseling and (iii) standard routine counseling (control arm). Smoking cessation of the pulmonary TB (PTB) patients

thus enrolled is assessed by self-reporting and confirmed by breath carbon monoxide testing, at 0, 2 and 6 months of ATT. TB outcome is recorded at 6th month / end of ATT. The sample size has been calculated to be 1200 patients, 400 in each arm. The study is ongoing.

45 subjects were recruited to the pilot study. The main study started in Jan. 2014.

A total of 2323 patients were screened and 244 TB patients were recruited for the study. Arm-wise and district-wise break-up is as follows (Table 2). The study is in progress.

Table 2: Recruitment details of study subjects

Treatment arm	Kanchipuram	Villupuram	Total
Drug arm	38	47	85
Enhanced counseling	26	37	63
Standard counselling	31	65	96
Total	95	149	244

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

Principal Investigator	:	Dr.Dina Nair (email: dinanair@nirt.res.in)
Co-Principal Investigator	:	Dr.M. Makesh Kumar
Source of funding	:	Intramural
Study period	:	2007-2014
CTRI Registration No.	:	PROVCTRI/2008/091/000024

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: To compare relapse rates upto 24 months of follow-up after treatment, in newly diagnosed smear and culture positive PTB patients treated with 4-month moxifloxacin (MFX) containing regimens, with the relapse rate in those treated with a 6-month regimen (control regimen).

In this trial, the standard 4-drug TB regimen is supplemented with MFX, a fluoroquinolone with potent bactericidal and sterilizing activities against *M. tuberculosis*.

Methodology: 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. 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 3.

Table 3: 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 1163 patients have been enrolled in the study. 35 patients were excluded from the study as per the protocol criteria. The baseline characteristics of 1128 are shown in Table 4. A majority of the

patients were male, had advanced disease evidenced by strongly positive sputum cultures and radiological involvement of more than 2 lung zones.

Table 4: Baseline characteristics of 1128 patients enrolled in study

Regimen	Test Reg. 1 (n = 112)	Test Reg. 2 (n = 268)	Test Reg. 3 (n = 274)	Test Reg. 4 (n = 262)	Control Reg. (n = 212)	Total (n = 1128)
Sex						
Male	89	210	205	188	162	854
Female	23	58	69	74	50	228
Age						
<35 years	58	124	149	141	116	588
≥35 years	54	144	125	121	96	540
Initial sputum culture						
1+	3	26	30	30	11	100
2+ or 3+	109	242	244	232	201	1028
Extent of initial X-ray involvement (zones)						
≤ 2	25	50	53	56	44	228
> 2	87	209	213	197	163	869

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 (92%) in the MFX arm (consolidated for all four test regimens) compared to the control arm

(74%). This observation which was made earlier is sustained even with a larger population. Fig. 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: Sputum culture conversion with treatment in 1044 patients

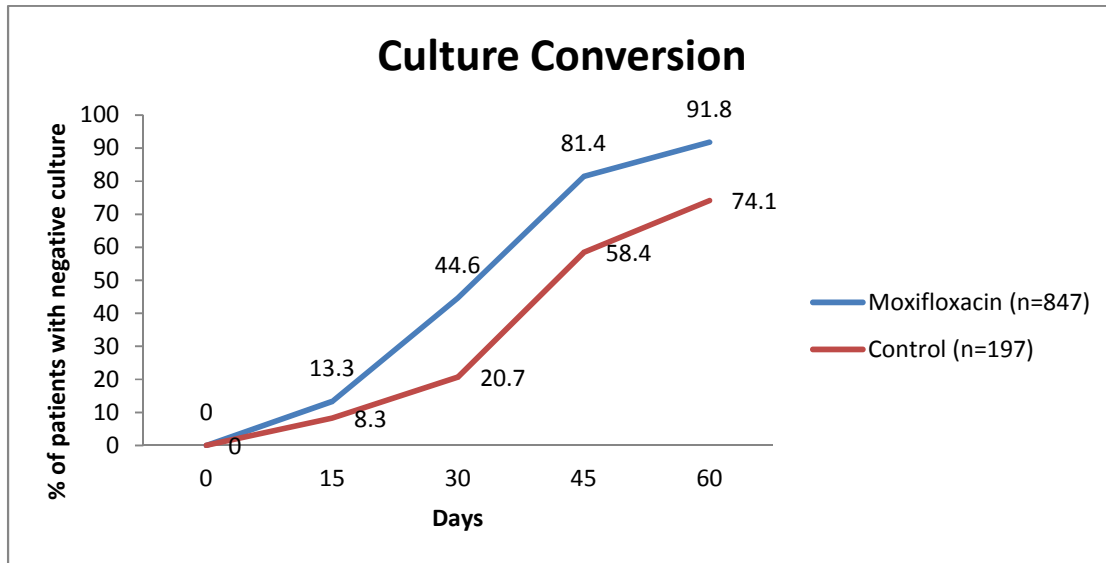


Table 5 describes the results at the end of treatment in 1044 patients, and recurrence of TB among those who had a favourable response at the end of treatment. Of patients treated with MFX regimens, 92% had negative sputum cultures at the end of treatment compared to 82% in the control regimen.

Of the 934 patients with successful outcome at the end of treatment, 56 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. This regimen was discontinued. Intake to the other regimens is continuing.

Table 5: Response at the end of treatment and TB recurrence during follow-up

Regimen	Patients	Favourable response at end of treatment	TB recurrence*
Test regimen 1	112	103 (92%)	20 (19%)
Test regimen 2	246	226 (92%)	9 (4%)
Test regimen 3	247	227 (92%)	18 (8%)
Test regimen 4	241	215 (89%)	15 (7%)
Control regimen	198	163 (82%)	6 (4%)

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

CL-4: 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 lymph node TB

Principal Investigator : Dr. D. Baskaran
(email: baskaran.d@nirt.res.in)
Collaborators : Govt. Stanley Hospital; Govt. Rajaji Gandhi
Medical College Hospital, Chennai
Source of funding : Intramural
Study period : 2013-2016
Trial Registry No. : CTRI/2013/03/003481

Background: TB lymphadenitis is the most common presentation of extra-PTB, accounting for 30–40% 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 RMP, INH, Ethambutol (EMB) and Pyrazinamide (PZA) for the first 2 months followed by 2 drugs

(RMP and INH) for the next 4 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 lymph node TB in Madurai, south India.

In the current study we investigate the 4-drug regimen [RMP, INH, PZA and Ofloxacin (OFX)] daily intensive phase of 2

months, followed by a 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.

Objectives:

Primary objectives:

To compare the efficacy of 2 regimens in terms of:

- a. Response at the end of treatment
- b. Relapse upto 24 months of follow-up after treatment, in newly diagnosed superficial lymph node TB patients

Secondary objectives:

To compare the incidence of:

- a. “Paradoxical reaction” during treatment and follow
- b. Drug adverse reactions

Study design:

Type : Intervention

Design: Prospective, randomized (open-label) parallel arm, controlled clinical trial

Study population:

a) Selection of the study population

Patients attending the surgical, medical clinics of Madurai Rajaji Hospital and Govt.. Hospitals and Corporation RNTCP Centres in Chennai with Fine needle Aspiration Cytology (FNAC) proved superficial TB lymphadenitis or clinical evidence of lymph node enlargement will be considered for the study.

All patients will undergo clinical evaluation every month including an assessment of the lymph node in the clinic. During these visits, patients will be asked about their general well being, will be evaluated for drug toxicity and information about any adverse events will be recorded on a standardized toxicity form. All patients will be followed up for a period of 24 months after completion of treatment, every month upto 12 months and then every 3 months.

Study outcome:

The following outcome measures will be compared between the test and control regimens:

Primary outcome

- a) Favourable, probably favourable but biopsy recommended, Unfavourable at the end of treatment
- b) Relapse during follow-up in those with favourable response at the end of treatment

Secondary outcome measure

- a) Paradoxical reactions

b) Adverse reactions to anti-TB drugs
The study is being conducted in Chennai and Madurai. The estimated sample size for this trial is 320 patients; so far 56 patients

have been enrolled to the trial. The study is ongoing. The interim results are given in Tables 6-8.

Table 6: Patients' details

		2EHRZ3/4RH3 (n=31)	2OHRZ7/2OHR3 (n=31)
Sex	Male	11	11
	Female	20	20
Age (years)	Mean	27.9	31.1
	Std.Deviation	7.5	11.6
BMI (kg/sq m)	Mean	22.2	21.9
BL_0 Smear	Pos	4	7
	Neg	24	22
	NA	3	2
BL_0 Culture	Pos	19	26
	Neg	7	1
	UMB	1	2
	NA	4	2

Table 7: Drug susceptibility pattern of culture positives

Drugs	2EHRZ3/4RH3 (n=31)			2OHRZ7/2OHR3 (n=31)		
	Sensitive	Resistant	NA	Sensitive	Resistant	NA
H	17	-	14	20	3	8
R	17	-	14	21	2	8
E	17	-	14	23	-	8
OF	17	-	14	22	1	8
Sm	17	-	14	20	3	8
K	15	2	14	22	1	8
Eth	7	10	14	5	18	8

Table 8: End of treatment outcomes

Outcome	2EHRZ3/4RH3 (n=22)	2OHRZ7/2OHR3 (n=24)
Favourable	21	16
Probable favourable	0	2
Unfavourable	1	4
NA	0	2

CL-5: Evaluation of newer diagnostic tools and feasibility of consensus case definition in the diagnosis of intrathoracic TB in children

Principal Investigator	:	Dr. Soumya Swaminathan (email: soumyas@nirt.res.in)
Co-Principal Investigator	:	Dr. V.V. Banu Rekha
Collaborators	:	Govt. Stanley Hospital (GSH), Chennai; Institute of Child Health (ICH), Chennai; Christian Medical College (CMC), Vellore; Govt. Vellore Medical College (GVMC), Vellore; Govt. Rajaji Hospital (GRH), Madurai
Source of funding	:	USAID (Model DOTS Project)
Study period	:	2013-2016

Background: The lack of a gold standard for diagnosis of childhood TB is a major obstacle to accurately quantifying the true burden of the disease 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 PCR assay designed for the rapid and simultaneous detection of *M. tuberculosis* (*M.tb*) and RMP resistance. Lipoarabino-mannan (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;

(ii) To compare the yield of *M.tb* from different specimen collection methods (expectorated / induced sputum, gastric lavage) in various age groups and

(iii) To evaluate urine LAM, in the diagnosis of intra-thoracic TB

Methodology: All children aged < 15 yrs attending the pediatric out-patient department with any of the following symptoms are screened for the study - (a) cough (b) weight loss/ failure to thrive (c) persistent unexplained fever (d) persistent, unexplained lethargy or reduced playfulness. Symptom screening, detailed general and clinical evaluations are 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, are also done.

In addition, in infants (i.e. aged < 1 yr), stool samples are collected for 2 consecutive days which are examined by Xpert® MTB/RIF, for AFB smear, culture and DST, if culture positive. Urine for LAM, blood investigations and FNAC of lymphnodes are done, where needed. TB in children will be classified into 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.

Sample size: 2800 children with presumptive TB.

Current status: The study was initiated in August 2013 and is currently enrolling children from Govt. Stanley Hospital (GSH), Chennai; Institute of Child Health (ICH), Chennai; Christian Medical College (CMC), Vellore; Govt. Vellore Medical College (GVMC), Vellore; and Govt. Rajaji Hospital (GRH), Madurai.

As of March 2015, a total of 1191 children have been enrolled in this study and the study is ongoing (Table 9).

Table 9: Site-wise recruitment details of children

	GSH	ICH	GVMC	CMC	GRH	Total

Number of children enrolled in 2013-2014	253	242	27	13	46	581
Number of children enrolled in 2014-2015	310	183	32	26	59	610
Total	563	425	59	39	105	1191

CL-6: Predictors and immunologic characterization of TB-associated IRIS in HIV-TB patients started on antiretroviral therapy

Principal Investigator : Dr. G. Narendran (email: nareng@nirt.res.in)
 Co-Principal Investigator : Dr. Soumya Swaminathan; Dr. Sudha Subramanyam; Mr.S. Anbalagan
 Source of funding : NIH Intramural to India grant 2008 and ICMR adhoc grant from 2012
 Study period : 2009-2015

Background: It is important to successfully predict and further prevent the occurrence of TB-IRIS in HIV & TB co-infected patients. The fine combination of virological and mycobacterial facilities in NIRT proved ideal for a study of this nature. This study is the only one in the world looking at TB-IRIS in a pure cohort of culture confirmed cases of PTB with RMP susceptible isolates.
Methodology: HIV/TB co-infected patients with and without IRIS manifestations were prospectively followed through progressive stages of immune restoration; clinical and lab predictors were serially recorded. The two groups were further classified into IRIS

and Non-IRIS based on INSHI criteria. Both univariate and multivariate analyses were carried out between the groups for evaluation of predictors, using various clinical parameters and immunological markers.

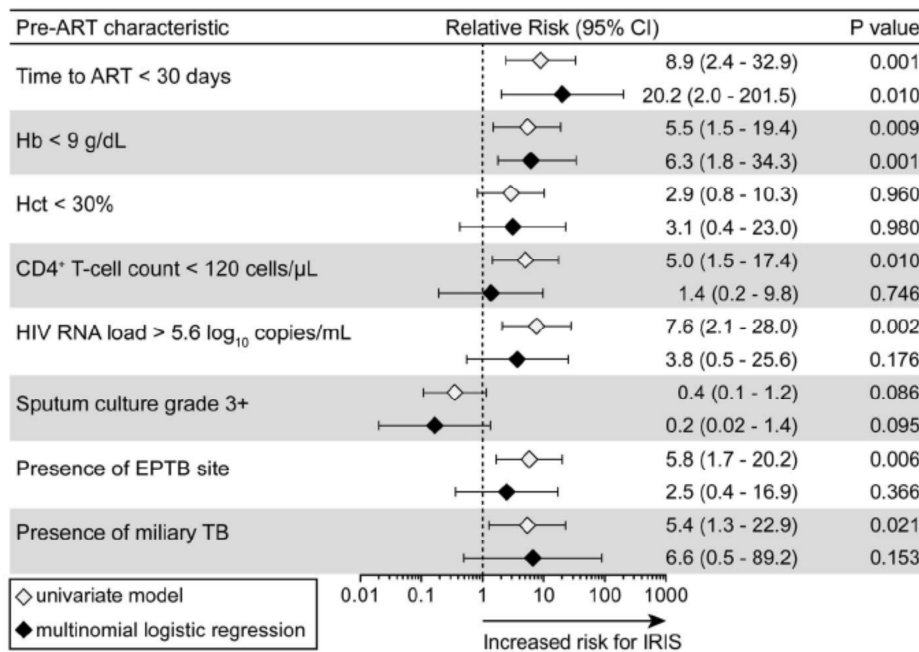
Results: Of the 69 HIV positive patients with newly diagnosed culture confirmed PTB (55 males: 14 females), who were ATT and ART naïve, enrolled in the parent study, 48 had complete evaluation of inflammatory cytokines while for the remaining patients IL-6 and C-reactive proteins (CRP) were evaluated. Univariate analysis showed that low CD4, high viral load, opportunistic

infection, miliary TB, presence of extrapulmonary focus, low hemoglobin and shorter ATT-ART interval were all associated with IRIS. Hemoglobin, CRP and time interval between ATT-ART remained significant in multivariate regression. A particular subset of monocytes

SCD14+CD16- have been found to be involved in TB-IRIS, reflecting the need for better evaluation for predictors than the ones existing (Figs 2 & 3).

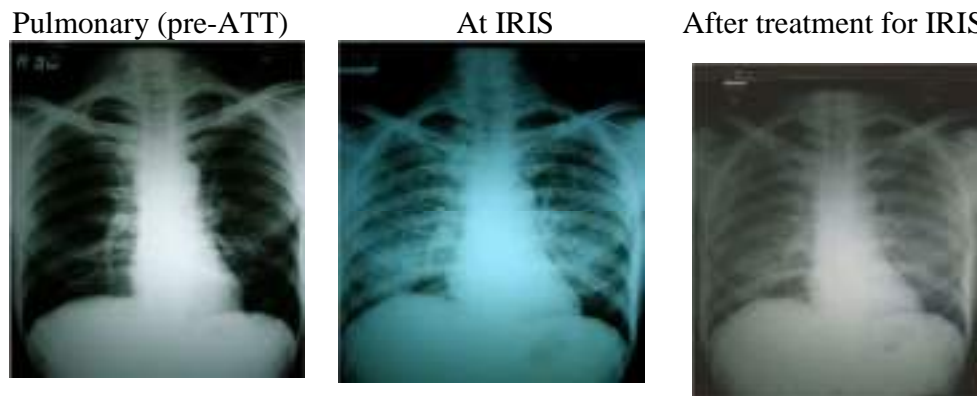
The study is ongoing.

Fig.2: Associations between pre-ART clinical and laboratory characteristics with subsequent TB-IRIS events



Associations between pre-ART clinical and laboratory characteristics with subsequent TB-IRIS events. Baseline (pre-ART) characteristics significantly different between patients that developed TB-IRIS events in the follow up and those who did not were assessed to test associations with risk for IRIS in univariate and multinomial logistic models. Relative risks (RR) are for values below or above the threshold levels are displayed, which were estimated close to median values for the overall study population. Adjustment was performed for all variables presented and also included age and gender. 95% CI, 95% confidence interval. Doi:10.1371/journal.pone.0063541.g001

Fig. 3: Pulmonary IRIS in patients with HIV-TB presenting with miliary opacities



CL-7: A prospective study to determine the incidence of TB among patients with type 2 diabetes mellitus

Principal Investigator	:	Dr.M. Makesh Kumar (email: makeshkumar.m@nirt.res.in)
Co-Principal Investigator	:	Dr. Soumya Swaminathan
Collaborators	:	Dr. Tazibha Hussain (RMRC, Bhubaneswar), Dr. Vijay Viswanathan (MV Hospital for Diabetes, Chennai), Dr.P. Dharmarajan (Institute of Diabetology, Rajiv Gandhi Govt. General Hospital, Chennai), Dr.E. Subbiuah (Dept. of Diabetology, Govt. Rajaji Hospital, Madurai)
Source of funding	:	WHO through Model DOTS Project
Study period	:	2013-2017

Background: The diabetes epidemic has a major impact on the epidemiologic dynamics of TB and poses several challenges to the control of TB in a resource-poor country like India. Diabetes/TB burden can be brought under control by timely diagnosis of TB among diabetics by intensified case finding, by

adequate and effective treatment of detected cases and possibly preventive therapy. Given the serious threat posed by the diabetes epidemic on control of TB, and the current gaps in knowledge related to diagnosis, prevention and treatment of TB among diabetes persons in the Indian population, it is proposed to conduct this

cohort study which is first of its kind to be done in a representative population to establish the incidence of TB in Type 2 diabetes patients.

Aims:

Primary objective:

(i) To determine the incidence of TB among people with Type 2 Diabetes Mellitus (DM)

Secondary objectives:

(i) To identify risk factors for TB among people with Type 2 DM;

(ii) To study the diagnostic accuracy of sputum smear for diagnosis of TB among people with Type 2 DM;

(iii) To correlate clinical and radiographic features of TB with severity of Type 2 diabetes and

(iv) To evaluate the diagnostic accuracy of Gene Xpert MTB/RIF among Type 2 DM patients with suspected TB

Methods: This is a multicentric prospective cohort study among Type 2 diabetic patients to study the incidence of TB. Study participants are recruited from patients who attend Diabetic OPD at Govt. General Hospital, Chennai, Govt. Rajaji Hospital, Madurai, MV Hospital for Diabetes, Royapuram, Chennai, Capital Hospital, Bhubaneswar and District Hospital, Khurdha, Odhisa. The recruitment details are given in Table 10.

Table 10: Details of screened and recruited patients

Sl.No.	Centre	Nos. screened	Nos. recruited	Nos. Refd. back
1	GRH, Madurai	117	73	44
2	RGGGH Chennai	239	187	52
3	MV Diabetes Centre	7	0	7
	Total	363	260	103

CL-8: HIV-associated lipodystrophy syndrome in children: Role of nutrition, ART and genes

Principal Investigator	:	Dr. Soumya Swaminathan (email: soumyas@nirt.res.in)
Collaborators	:	Dr. Christine Wanke; Dr. Alice Tang, Tufts University; Dr. Anita Shet, St. John's National Academy of Health Sciences, Bangalore
Source of funding	:	National Institute of Health (5R01 A1084390)
Study period	:	2011-2015

This is a prospective multi-centric observational study undertaken at NIRT, Chennai and Madurai and at St. John's National Academy of Health Sciences, 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 after initiating ART, as well as to determine the role of genetic factors in the development of fat redistribution, insulin resistance and dyslipidemia.

The study, initiated in June 2011, has completed enrollment. As on 31st March 2015, 377 HIV-infected children between

the age group of 2-12 years are on varying stages of follow-up. Details about their demographics, clinical and dietary history (Food security questionnaire, 24 hr dietary recall), physical and anthropometric details at baseline are shown in Table 11. These children are followed up every 3 months upto 24 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 and every 6 months till 24 months of follow-up. Further follow-up of children is in progress.

Table 11: Baseline demographics of 378 HIV-infected children

Characteristics of children	Values in Mean (SD)
Gender (M:F)	182
Age in years	7.7 (3.0)
Body weight in Kg	18.1 (5.9)
Height in cms	111.9 (17.5)
WAZ score	-2.11
HAZ score	-2.13
BMI (kg/m ²)	14.16 (1.74)
CD4%	16.55 (8.82)
Median CD4 cell count (IQR) cells/cu.mm	388 (269-653)
Median HIV RNA viral load (IQR) / units	141000 (25876-436000)
Blood Total Cholesterol mg/dl	130.5 (34.6)
LDL- cholesterol mg/dl	78.0 (28.8)
HDL- cholesterol mg/dl	29.1 (11.2)
Serum Triglycerides mg/dl	144.5 (73.5)
Blood Glucose mg/dl	85.4 (14.5)
C-reactive protein mg/dl	2.5 (3.4)
Serum Insulin mg/dl	9.3 (12.4)
HOMA-IR	2.1 (3.0)

CL-9: High density lipoprotein cholesterol and gene polymorphisms among HIV- infected south Indians on first line antiretroviral therapy

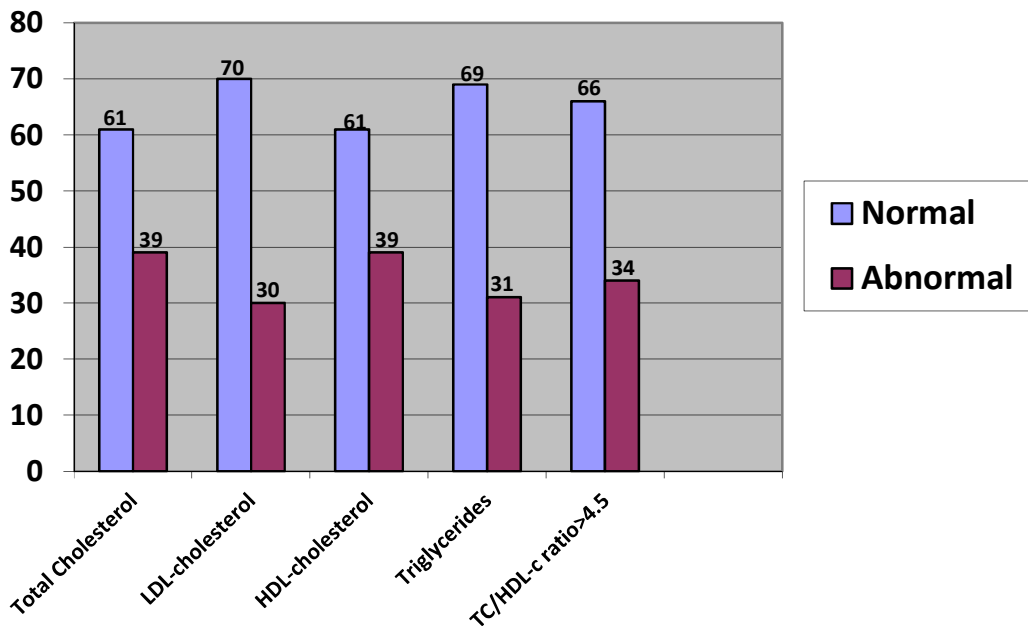
Principal Investigator : Dr.C. Padmapriyadarsini
(email: padmapriyadarsinic@nirt.res.in)
Source of funding : Fogarty International (NIH)
Study period : 2013-2015

This was a cross-sectional study 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 Nevirapine (NVP) based ART regimen.

Aim: To study 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 and as on 31st March 2015, has completed enrolling its target of 300 patients. Abnormal total cholesterol was observed in 116 (39%) of patients. 39% of males and 47% of females had HDL-c levels below reference value after 12 months of ART. Allelic variants of CETP or APOC3-related polymorphisms did not show statistically significant associations with low HDL-c levels in this cohort. The prevalence of dyslipidemia in HIV infected patients on ART is shown in Fig. 4.

Fig. 4: Prevalence of dyslipidemia in HIV-infected patients on ART



CL-10: Study on the effectiveness and feasibility of TB preventive therapy for people living with HIV in India - adults and children

Principal Investigators	:	Dr.C. Padmapriyadarsini; Dr.P.K. Bhavani; Dr. Soumya Swaminathan (email:padmapriyadarsinic@nirt.res.in; soumyas@nirt.res.in; bhavanipk@nirt.res.in)
Collaborators	:	ART MDs of study sites, NACO, CTD
Funding Agency	:	USAID (Model DOTS project)
Study period	:	2013-2015

This is a prospective multicentric study with phased implementation, looking at the feasibility and effectiveness of TB

preventive therapy in HIV-infected adults and children attending ART centres in several states.

Primary aim: (i) To assess the effectiveness of INH preventive therapy (IPT) in people living with HIV (PLHIV) (at different CD4 counts and both pre-ART and on ART)

Secondary aims: (i) To assess the effectiveness of simple algorithms to exclude active TB prior to IPT initiation;
(ii) To assess the feasibility of providing IPT for PLHIV attending ART centers and
(iii) To measure number needed to screen and number needed to treat to prevent one case of TB

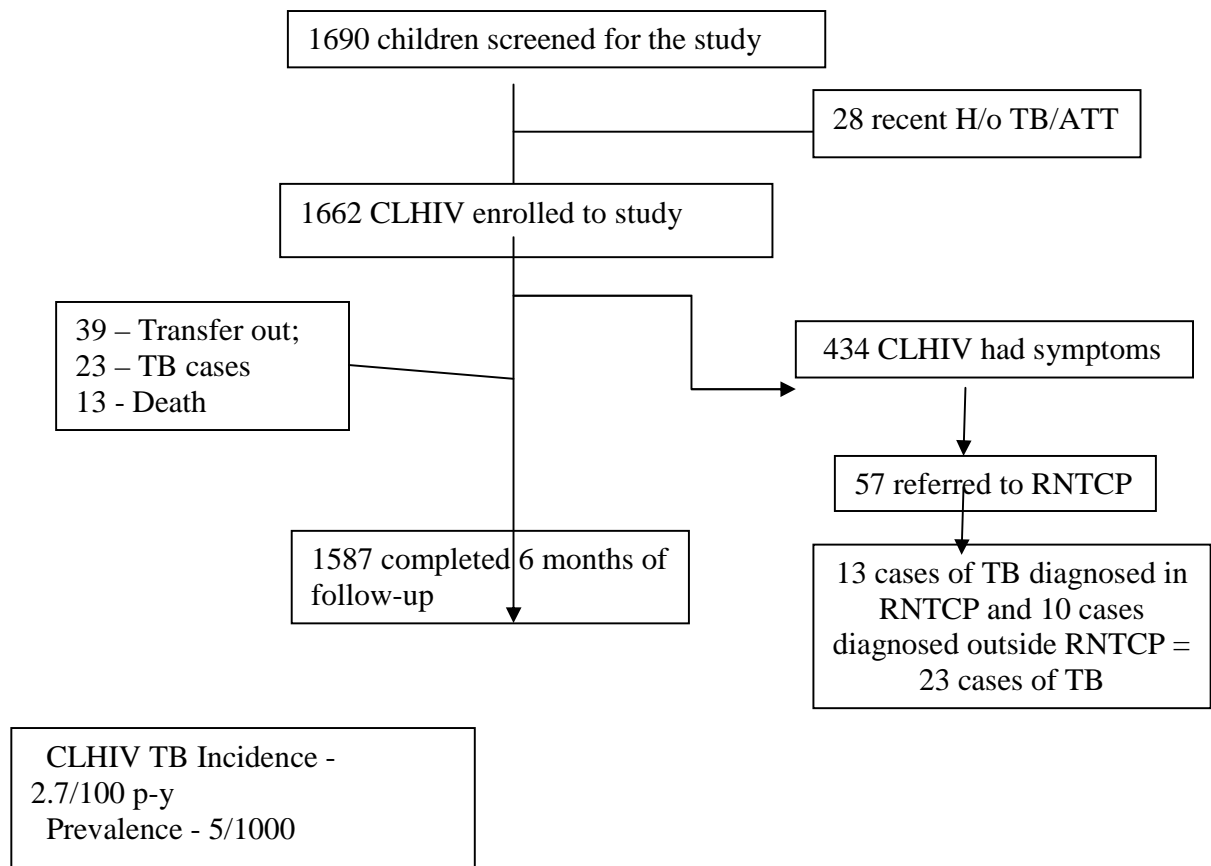
The study consists of two phases – Phase I: Enhanced TB surveillance (all sites, all ART centre attendees) for all HIV-infected adults and children.

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.

The study was initiated in a phased manner at five ART centers in Tamil Nadu, two in Karnataka, one each in Andhra Pradesh and New Delhi. As on 31st March 2015, Phase I of the study was completed. A total of 6099 adults and 1662 children have been recruited. Overall, prevalence of TB among adult PLHIV was 0.8% (52 cases) and TB incidence was 2.4/100 p-y; (95% CI: 1.90, 3.10). Prevalence of TB among children was 0.5% (23 cases), and incidence was 2.7/100 p-y; (95% CI: 1.60, 4.30). Both adults and children are being followed into Phase II of the study and further. Results of the phase I of the pediatric study is shown in Fig. 5.

Fig. 5: Intensified TB screening among children attending ART centres



CL-11: C-TRIUMPH: Cohort for TB research by the Indo-US medical partnership multicentric prospective observational study

Principal Investigators : Dr. Soumya Swaminathan
(email:soumyas@nirt.res.in)
Funding Agency : DBT
Study period : 2013-2018

This is a prospective multi-centric observational cohort study at two sites, Chennai and Pune, which is enrolling TB patients and their household contacts

to study host and microbial risk factors associated with progression from TB infection to disease, TB disease progression, treatment outcomes, and

transmission. A repository of biological specimens will be created, which can be used for future basic science research including biomarker discovery, and be made available to investigators of this Centre / Partnership on request. After receiving all regulatory approvals and

study funding, the study started enrolling patients since August 2014. As of 31st March 2015, 97 TB patients have been enrolled into Cohort A and 218 household contacts to Cohort B. Study is ongoing.

CL-12: Inhibition of host-induced mycobacterial efflux pumps as a novel strategy to counter drug tolerance and virulence of PTB

Principal Investigators	:	Dr. Soumya Swaminathan (email:soumyas@nirt.res.in)
Funding Agency	:	DBT
Study period	:	2013-2015

This is a phase 2, single-centre, open-label dose-finding pharmacokinetic study of verapamil given in conjunction with RMP. The goal of this study is to determine the contribution of the newly discovered efflux pump-mediated tolerance mechanism in delayed or incomplete sterilization in active PTB. We hypothesized that verapamil added to standard TB therapy will accelerate sputum clearance of *M.tb*. The study objectives are:

- (i) To determine the compensatory increase in verapamil dose that can offset the increased metabolism of verapamil when it is co-administered with RMP and
 - (ii) To confirm the safety and tolerability of verapamil in patients with TB without underlying cardiac disease
- The study dossier has been submitted to DCGI for approval.

CL-13: Species identification and response to appropriate treatment of symptomatic pulmonary non-tuberculous mycobacterial disease among patients treated for TB in Tamil Nadu

Principal Investigator : Dr.C. Padmapriyadarsini
(email:padmapriyadarsinic@nirt.res.in)
Funding Agency : ICMR Task Force (Awaited)
Study period : 2013-2015

This is a descriptive study to identify the various species of pathogenic non-tuberculous mycobacteria (NTM) causing symptomatic pulmonary disease and to evaluate their response to treatment, based on ATS guidelines among patients with symptomatic pulmonary NTM disease in Tamil Nadu. The study was initiated in November 2014 and is currently enrolling

pulmonary NTM patients from Chennai and Kanchipuram district. NTM species are identified and appropriate treatment given for the entire duration of 12 months following culture negativity. The study was initiated in November 2014. As of 31st March 2015, 7 patients have been started on appropriate treatment.

CL-14: EDOTS - Effect of diabetes on TB severity

Principal Investigators : Dr. Pradeep Menon
(email:menonpa@nirt.res.in)
Funding Agency : DBT
Study period : 2014-2017

A multicentric study has been started to determine the effect of diabetes on TB severity. In Chennai, the collaborating centres are NIRT and MV Diabetes Centre. The study is being conducted at Pulianthope and Tondiarpet RNTCP centres.

The estimated sample size is 300 TB patients (150 with DM and 150 without DM).

Of the 355 patients screened, 158 were enrolled in to the study. 35 subjects were excluded after enrolment (default-4, death-4, including 2 MDR cases, culture-negative-15, Cat II-6 and

withdrawn from study -6). Smear and culture results are available for 145 patients. Currently 89 patients in the

diabetic-TB arm and 34 in the non-diabetic-TB arm are being followed up. The study is in progress.

CL-15: Multi-centric cohort study of recurrence of TB among newly diagnosed sputum positive PTB patients treated under RNTCP

Principal Investigators	:	Dr. Soumya Swaminathan (email:soumyas@nirt.res.in)
Collaborators	:	National Tuberculosis Institute, Bangalore, NITRD, New Delhi, RMRCT, Jabalpur, Thiruvananthapuram Medical College, Thiruvananthapuram, Mahatma Gandhi Inst. of Medical Sciences, Wardha
Funding Agency	:	Central TB Division
Study period	:	2014-2017

Background: In the RNTCP, newly diagnosed smear - positive PTB patients are treated with a 6-month thrice-weekly regimen, consisting of an initial intensive phase (IP) of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) for two months followed by a continuation phase (CP) of H and R for four months (2H₃R₃Z₃ E₃ / 4 H₃R₃), given under direct observation, fully during the IP and partly during the CP. The country reports around 87% ‘cure’ among smear-positive patients treated with this regimen. A TB recurrence rate of 10-12% has been reported from

localized studies. There is little information on the proportion and predictors of patients who develop recurrent TB among those patients who have had a successful outcome at the end of treatment and on proportion of recurrent TB due to re-infection and endogenous reactivation.

Study Objective:

Primary Objective: (i) To estimate the recurrence of TB among newly diagnosed sputum positive PTB patients who have been successfully treated under RNTCP

Secondary Objective: (i) To distinguish between relapse and re-infection among those who have recurrence of TB and (ii) To identify risk factors for unfavourable treatment outcomes (treatment failed, lost to treatment follow-up and died) and recurrent TB

Methodology: This is a prospective, multi-centric cohort study conducted by six institutes. New smear positive PTB patients treated under RNTCP are enrolled. They will be followed up till treatment completion, and those with successful treatment outcome will be followed up for a period of 12 months after completing treatment. These

patients will be subjected to the following procedures: Structured interview, sputum examination for smear, culture, drug susceptibility testing (DST) and genotyping and blood tests for diabetes mellitus and HIV infection.

Study progress: Enrollment of study participants has been completed in five out of the six study centres. A total of 1535 participants have been enrolled into the study till the end of May 2015. The details are given in Table 12.

Table 12: Site-wise details of total participants screened and enrolled into the study till the end of May 2015

	Screened	Enrolled		
	Total	Total	Rural	Urban
NIRT	298	249	126	123
MGIMS	754	249	80	169
NITRD	505	302	146	156
RMRCT	338	242	121	121
TMCT	355	298	149	149
NTI	258	195	114	81
Total	2508	1535	736	799

Out of the 1535 new smear positive TB patients, 71.6% are males and 89% are in the productive age group of 18-60 years. This cohort has 48% “ever smokers” who have smoked either beedi or cigarette and half of this cohort (51%) have taken alcohol at some point in their life. A total of 68 (4.4%) and 37 (2.4%) are continuing to smoke and take alcohol respectively even while initiating treatment for TB.

Co-infection with HIV is seen in 2.5% of the cohort (38 participants) while 18.3% of the study participants are diabetics at the start of TB treatment.

Bacteriology: 2848 smears were examined prior to start of treatment, out of which 83.2% are smear positive and 78% are culture positive.

The study is ongoing.

Department of Socio Behavioral & Research

COMPLETED STUDIES:**(i) HIV prevention through mobile phone technology among male sex workers in India**

Principal Investigator	:	Dr. Beena E. Thomas (email: beenathomas@nirt.res.in)
Co-Investigator	:	Dr.E. Thiruvalluvan
Collaborators	:	Dr. Mathew Mimiaga, Harvard Medical School / Fenway Institute, USA; Dr. Steve Saffren, MGH (Massachusetts General Hospital)
Source of Funding	:	Indo-US Joint working group on HIV
Study Period	:	2012 -2014

Background: Male sex workers (MSWs) are a significant but invisible population in India with elevated levels of sexual risk behavior. Baseline data from a pilot randomized controlled trial that aimed to decrease HIV risk behaviors among MSWs in Chennai, India are examined to explore sex work-related risk behaviors and identify associations between sexual behaviors and source of income.

Aims: (i) To develop an HIV risk reduction counseling intervention for MSWs 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 and

(iii) To assess if potential mediators of intervention group and if these changes are associated with the primary outcome (reduced sexual risk taking)

Methods: Between December 2013 and May 2014, 100 MSWs completed a baseline assessment. Participants were 18 years and older and reported current sex work.

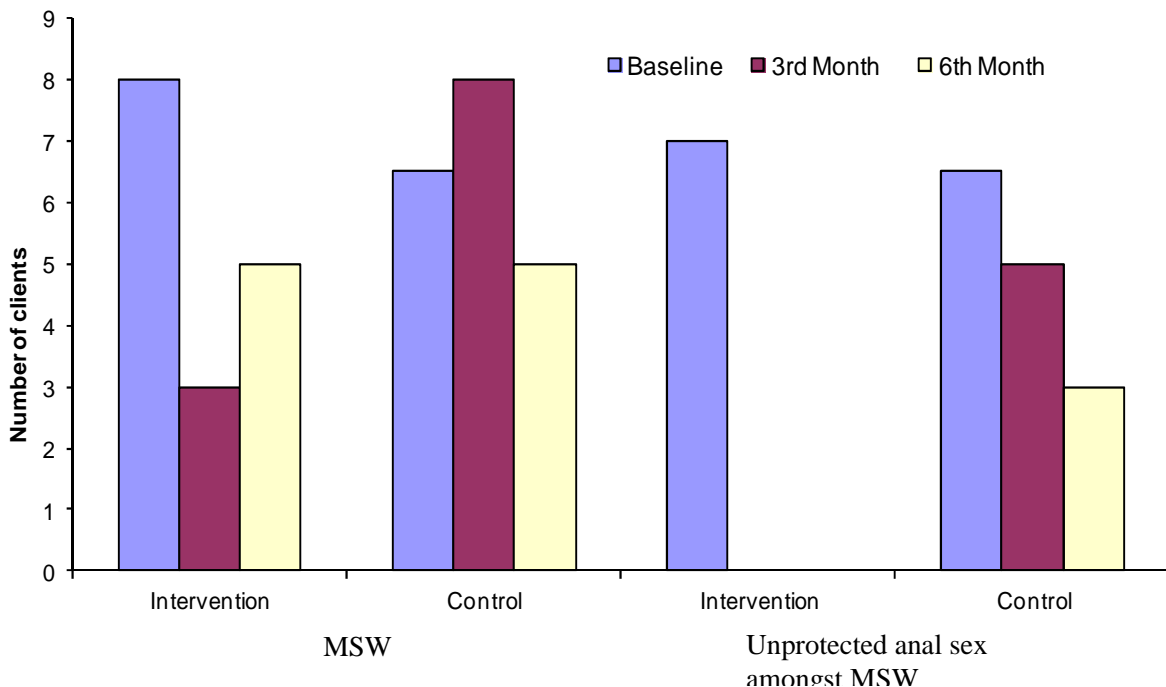
Results: Most (76.8%) participants identified themselves as Kothi, and 50% completed secondary education or less. Participants were engaged in sex work for 5.0 years (IQR=21.0-31.5), and earned Rs.3,000 (IQR=2000-8000) (<50 USD) per month from sex work. Sixty-four percent reported ever testing for HIV and 20.2% for any STI. The most common reasons for starting and continuing sex work were money (83.0% and 93.0%, respectively) and pleasure (56.0% and 50%, respectively). Participants reported 8.0 (IQR=3.0-15.0) male clients and 2.0 (IQR=0.0-6.0) non-paying male partners in the past month. Participants reported 7.0 (IQR=4.0-15.0) condomless anal sex acts with male clients and 3.0 (IQR=1.0-6.0) with non-paying

male partners in the past month. Compared to participants who indicated an additional source of income, participants whose only source of income was sex work reported significantly more male clients in the past week (7 vs. 4.0, $p=0.001$) and in the past month (10.0 vs. 6.0, respectively, $p=0.017$), as well as more condomless anal sex acts with male clients (8. vs. 5.0, respectively, $p=0.007$) and non-paying male partners (5.0 vs. 2.0, respectively, $p=0.024$) in the past month. Nearly 70% were offered more money to not use a condom during a sex work encounter, and two-thirds reported

having difficulty using condoms with clients. In the intervention group unprotected anal sex was reduced significantly as compared to control groups (Fig. 6).

Conclusions: MSWs in India engage in high levels of sexual risk for HIV/STIs. HIV prevention intervention among this group needs to extend its reach to MSWs and address issues of transactional sex, alcohol, drugs and sexual risk and equip them with strategies that encourage safe sexual practices.

Fig. 6: Sexual practices among MSWs



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

Principal Investigator : Dr. Beena E. Thoms (email: beenathomas@nirt.res.in)
Co-Investigators : Mrs. Chandra Suresh; Dr. Chandrasekaran
Source of funding : USAID through MDP
Study Period : 2013-2014

Background: The influence of alcohol with regard to delays in seeking care and non-compliance for treatment has been well documented. There is an urgent need to develop a feasible, acceptable alcohol intervention strategy among TB patients to ensure successful treatment completion and a better quality of life. It is against this background that this study was conducted.

Aims: (i) To enhance treatment adherence of TB patients who consume alcohol by reducing the default rate through intervention strategies and

(ii) To evaluate the impact of intervention strategies on alcohol intake by comparing TB patients who consume alcohol in the experimental group with those TB patients in the control group

Methodology: This is a two-arm randomized controlled study in which 4 zones of the Chennai Corporation were randomly selected and allotted (2 each) to the experimental and control arms. TB patients (>18 years) registered from August 2013 to January 2014, under the RNTCP, were assessed using AUDIT scale (>8 to

<20). The intervention consisted of 4 individual counseling sessions (0, 2, 4 and 6th month) conducted by trained interventionists. An intervention manual using a community participatory approach was prepared to guide the sessions. This involved one to one counseling sessions and visual aids were used to explain facts about TB and the effects of alcohol use on TB treatment and management.

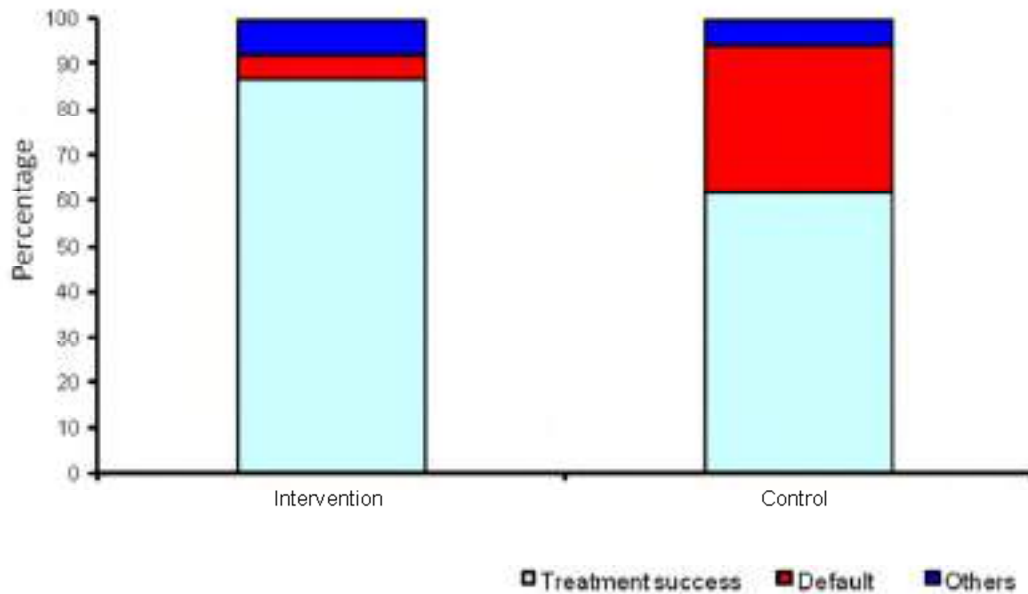
Results: Of the total 872 registered TB patients, 298 (31%) were found to have alcohol use disorders (AUD). The number of TB patients in the experimental and control arms were 113 and 185 respectively. Percentage with favorable treatment outcomes (cured/completed treatment) was significantly higher ($\chi^2 = 20.8$, 1 d.f., p-value<0.001) in intervention (87%) compared to the control group (62%) (Fig. 7). It was also observed that the overall adherence to TB treatment was significantly higher ($\chi^2 = 35.1$, 2 d.f. p-value < 0.001) among the intervention group when compared to the control group. Among the intervention group, 88% completed more

than 2 intervention sessions. Percentage of patients with favorable treatment outcomes increased with the number of interventions attended ($\chi^2 = 43.3$, 4 d.f. p-value<0.001). It was seen that the proportion with regular consumption of doses till the end of treatment increased with the number of interventions attended ($\chi^2 = 44.6$, 4 d.f. p-value < 0.001). Participants expressed interest towards the effectiveness of the interventions especially with regard to the

visual aids that were used and the time taken for the sessions.

Conclusion: Alcohol intervention among TB patients is effective in ensuring favorable treatment outcomes and TB treatment adherence. This intervention was feasible and acceptable to patients. This study suggests the need for trained counselors in TB care settings to ensure effective alcohol intervention strategies to ensure TB treatment compliance among TB patients with AUDs.

Fig.7: Treatment outcomes in the control and intervention groups



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

Principal Investigator : Dr. Beena E. Thomas (email: beenathomas@nirt.res.in)
Co-Investigators : Ms. Niruparani Charles; Dr.P. Paulkumaran; Ms. Basilea Watson
Source of funding : ICMR
Study period : 2012-2014

Background: With TB continuing to be a major public health problem, innovative community based TB prevention and intervention strategies aimed at TB control is crucial. A powerful community task force has gained momentum in many districts in south India by way of Self Help Groups (SHG). This group could be involved in TB control activities considering their reach in communities especially at the grass root level.

Aims: To design a model sensitization programme on TB for SHGs based on participatory action approach which would facilitate their involvement in the RNTCP

Methods: This experimental study had a qualitative phase using Focus Group Discussions (FGD's) and Key informant interview to test the acceptability of SHGs in the community and develop a feasible and acceptable TB sensitization intervention strategy through a community based approach. The quantitative phase was a randomized control trial to test the intervention impact. The sample included

1560 SHG representatives (796 in the intervention and 764 in the control group). Participants were assessed at 0, 3 and 6 months.

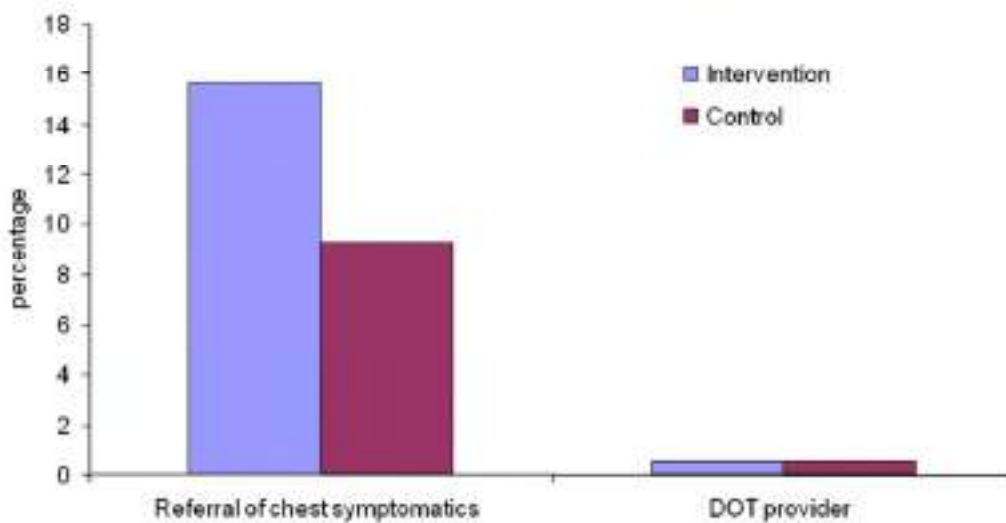
Results: Based on the results of qualitative phase a manual on the TB sensitization programme was prepared. This included messages on TB, visual aids, role plays, folklore (Villupattu), and a CD with a movie on TB, starring famous Tamil artistes. Quantitative findings indicate that 84% in control and 88% in intervention groups have never been involved in any health related activities at baseline. In both the groups, more than 95% of the members were willing to get involved in TB control activities. It was observed that at the end of 3rd month, a significantly higher proportion of members in the intervention group had involved in TB advocacy, identified symptomatics and referred them to the primary health centres (PHCs) when compared to the control group. At the end of 6th month, it was observed that a significant difference was

observed in referral of symptomatics in the experimental group (Fig. 8).

Conclusions: A community driven TB sensitization model based on participatory action approach using SHGs in TB control has proven to be an effective strategy in TB control. SHG's can be considered a

powerful task force in promoting TB awareness, identification and referral of chest symptomatics and also as community DOTS providers.

Fig. 8: Referral of chest symptomatic in different groups



(iv) Perceptions of private practitioners on TB notification

Principal Investigator : Dr.V.V. Banurekha
 (email: banurekha@nirt.res.in)
 Co-Investigator : Dr. Beena E. Thomas
 (email: beenathomas@nirt.res.in)
 Source of funding : ICMR
 Study period : 2012-2014

Objective: To understand the private practitioners (PPs') awareness, perceived barriers and their views to improve notification process among the general and

specialist PPs providing health care services in Chennai, Tamil Nadu.

Methods: The study was conducted from September 2013 to October 2014 in

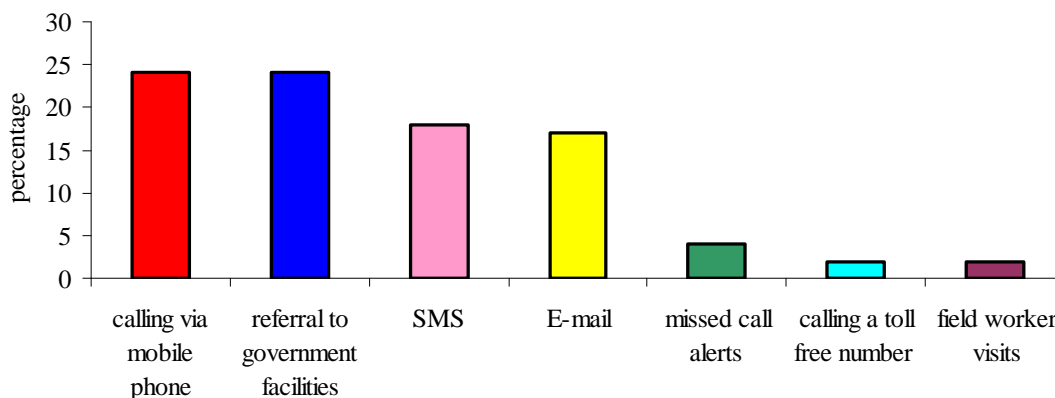
Chennai, south India. A total of 190 PPs were individually approached in their clinic by trained field staff and data collected using questionnaire after getting informed consent. The information included PPs profession, case referrals, awareness about TB notification order, implementation, reasons for not implementing, and their suggestions to improve notification.

Results: Of the 190 PPs from varied specializations, the awareness on TB notification was 137 (72%). Of the 137 who were aware, 66% had not notified. Among the 190 PPs, 56 (30%) had notified TB cases to the government facilities. Patient's personal identities 82 (60%), their demography 7 (5%) and treatment 5 (4%)

were the information which were felt uncomfortable for PPs to provide under notification. Of 190 PPs, 28% of them have expressed the need for specific health personnel from government to facilitate notification process. The preferred means of notification reported by PPs is presented in the Fig. 9.

Conclusion: This study has highlighted that about one-third of PPs do not have awareness about TB notification order and not all those who were aware were notifying. Effective awareness programme, communication and trust building strategies for behavioral change should be established to encourage PPs to notify all TB cases.

Fig. 9: Preferred means of TB notification by PPs



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

Principal Investigator : Dr.E. Thiruvalluvan
(email: thiruvalluvane@nirt.res.in)
Co-Investigator : Dr. Beena E. Thomas
(email: beenathomas@nirt.res.in)
Source of funding : ICMR
Study period : 2012-2014

Background: The treatment for MDR-TB is characterized by rigorous treatment regimens for long duration, higher incidence of adverse side effects, lower cure rate, and high treatment costs. This could lead to number of psychosocial problems that influence treatment adherence.

Objective: To understand the psychosocial issues that impacts the treatment adherence among MDR-TB patients, who are diagnosed and registered for treatment under DOTS Plus programme

Methods: MDR-TB patients registered under DOTS Plus programme during the period of 2013-2014 in Chennai and Madurai districts were included. FGD's were conducted among patients to understand the physical, psychological, social and economical challenges faced during the MDR-TB treatment. Transcribed and translated qualitative data were entered into NVIVO software programme and was analysed.

Results: Analysis revealed that majority of participants were unaware of 'MDR-TB'. Most of the TB patients did not disclose their TB status, even to their family members. Many patients experienced substantial stigma and isolation within the family. Participants were made to feel disgraced and discriminated by the health care workers. Patients and their family feared the loss of their already precarious economic stability because of the disease, and this often generated a great deal of stress.

Conclusion: Study findings highlights significant psychological, social, and financial impact of MDR-TB on patients and their families. There is a need for psychosocial intervention strategies for MDR-TB patients and their caregivers to mitigate the negative effects.

LABORATORY STUDIES

Department of Bacteriology

STUDIES COMPLETED:

(i) Performance of light emitting diode microscope in two different settings for TB diagnosis: A multi-centric study

Principal Investigator : Dr. Gomathi Sekar (email: gomathis@nirt.res.in)
Collaborators : Director, Institute of Thoracic Medicine, Chennai
Source of funding : ICMR - extramural
Study Period : 2013-2014

Background: Early diagnosis and treatment is essential to prevent the transmission of the disease in the community. Recently WHO recommended light emitting diode (LED) microscope for use in TB diagnosis. There is a need to assess the performance of microscope in different settings

Sample size: A total of 1350 samples from consecutive symptomatic patients have been included from two study sites. They are:

(i) National Institute for Research in Tuberculosis, Chennai and

(ii) Regional Medical Research Centre, Bhubaneswar

Screening of patients was initiated at ITM, Chennai.

Methods: Two direct smears and two deposit smears were made for each sputum. The smears were stained by Auramine- O Phenol (AP) and Ziehl Neelsen (ZN) methods and randomized by a statistician. The smears were then read using LED, fluorescence microscopy (FM) and light microscopy. The discrepant slides were resolved by an umpire reader. Cultures were performed for all samples on LJ medium as

per NIRT SOP. Random blinded rechecking (RBRC) for smear microscopy was performed for both methods of smears. Once in 15 days, 10% of slides stained by AP, and, 20 days after ZN staining were selected systematically, coded and examined for external quality assurance.

Results:

Smears: The smear results of direct and deposit smears of LED versus FM were compared. The concordance for direct and deposits between the two microscopies were 96% and 98% (Kappa 0.96 & 0.98) respectively. The sensitivity and specificity of the LED for direct and deposits was 100% & 98.5% and 99.8% & 100% respectively. The observed differences were not statistically significant. The results also revealed that there was no bias in reading of the smears.

Culture: Of the 1347 sputum samples cultured, 28 were contaminants and 47 were identified as NTM. The remaining 1272 samples were included in the analysis. The sensitivity and specificity values between LED direct, deposit smears and culture were

77.4%, 72.1% & 98.6%, 98.5% with no significant differences between them. Of the 28 specimens that showed contaminants, 6 smears were positive by LED (4 from direct and 2 from deposits). Out of the 47 specimens that grew as NTM, 6 direct and 3 deposit smears were positive.

Rate of positivity: The rate of smear positivity of LED direct and deposits were 12.8% and 13.0% respectively. The rate of culture positivity was 14.1% (Table 13).

Drug susceptibility testing: DST was done for first (HR) and second line (KOF) drugs for the culture positives. Out of 136 DST tests, one XDR-TB and 2 MDR-TB were detected.

Random Blinded rechecking: RBRC was carried out after 15 days of first staining for 10% of the smears, stained by AP using LED. RBRC was carried out in 300 sputum smears for ZN after 20 days No statistically significant difference was observed.

Table 13: Details of smear positivity by LED FM and culture

Methods	Smear positive	Percentage positive
LED FM direct	172	12.8
LED FM deposit	175	13.0
Culture	190	14.1

FM- Fluorescence microscopy, LED- Light emitting microscopy

Conclusion: LED is comparable with FM microscopy. In programmatic conditions, LED deposit is more sensitive than ZN.

RBRC can be extended up to 15 days from the first stain because no statistical significance was observed.

STUDIES IN PROGRESS:

B-1: Multi-centric study to evaluate DST by luciferase reporter phage assay

Principal Investigator : Dr. N. S. Gomathi
(email: gomathisharma@nirt.res.in)
Source of funding : ICMR – intramural
Study Period : 2014-15

Background: Techniques involving mycobacteriophages have been developed and evaluated for primary detection and DST. The assays are based on the fact that replication of the phage is dependent on viable mycobacterial cells. DST by Luciferase Reporter Phage (LRP) Assay is done using primary isolates and requires 72 hrs for completion. The assay has been shown a sensitivity of 91% and specificity of 99% for detection of RMP resistance in comparison with the conventional MIC method. A shorter version that can be completed in 24 hrs and can be applied to DST for all drugs has been developed and standardized. The present multi-centric study aims to evaluate both versions of the LRP DST in different settings. The sites include NIRT, Chennai, National Institute for Tuberculosis and Respiratory Diseases - New Delhi, Blue Peters Health Research Centre - Hyderabad and Intermediate Reference Laboratory – Chattisgarh.

Aim: To evaluate LRP assay for DST of clinical isolates of *M. tuberculosis* to first line and second line anti-tuberculous drugs in comparison with reference standard MGIT960

Expected outcome: The study will demonstrate the performance characteristics of LRP DST in comparison with the gold standard MGIT960, reproducibility of the assay in different lab settings and the cost estimation will highlight the usefulness of the assay in program settings.

Progress: Technical and research personnel were trained in LRP assay. Two sets of cultures totaling 114 along with other reagents have been despatched to the sites. Monitoring visits to the sites have been conducted in March and April, 2015. The study is on-going and is likely to be completed by June, 2015.

B-2: Validation of an indigenous test for diagnosis of PTB from sputum sample

Principal Investigator : Dr. N. S. Gomathi
(email: gomathisharma@nirt.res.in)
Source of funding : Department of Biotechnology – Extramural
Study Period : 2014-15

Background: India with its high burden of TB and drug resistant TB (DR-TB) is looking for affordable, high-sensitivity tests with a potential for use at peripheral health facilities with ease of use and minimal infrastructure. The True Nat MTB™ is chip-based nucleic acid amplification test for detection of *M. tuberculosis* from sputum samples. The manufacturers claim that this technology is meant for decentralized, “point-of-care” use, but there are no published data on their feasibility in microscopy centers.

In this validation study protocol, the TrueNAT MTB assay is validated at 4 sites including NIRT Chennai, NITRD New Delhi, AIIMS New Delhi and NJIL & OMD Agra, using the conventional reference standard (cultures) to determine test

accuracy and feasibility. Results of this study will be used to make a decision whether the technology should proceed to a field demonstration study under routine RTNCP conditions, with the test deployed in designated microscopy centers.

Aim: To validate the indigenously developed TruNat MTB test against the laboratory reference standards

Expected outcome of the study: The study will demonstrate the performance characteristics such as sensitivity, specificity, efficiency and predictive values of TruNatMTB in comparison with the lab reference standards MGIT960, solid culture and Xpert across the 4 sites.

Progress: NIRT has recruited 107 patients for the study. The study is ongoing and is likely to be completed by August.

B-3: Validation of indigenous tests for diagnosis of DR-TB from sputum samples

Principal Investigator : Dr. N. S. Gomathi (email: gomathisharma@nirt.res.in)
Source of funding : Department of Biotechnology – Extramural
Study Period : 2014-15

Background: Early diagnosis of drug resistance is crucial for effective patient management and prevention of spread of the severe forms of the disease in the community. Patients with DR-TB require prolonged treatment with more number of drugs. DST on solid media is a standard technology still being practiced in many resource limited settings where TB is endemic. They have a turn-around time (TAT) measured in months. Methods using liquid media have been introduced with a TAT of less than 14 days. But these tests are costly and require expensive bio-safety facilities. India with its high TB and DR-TB burden is looking for affordable, high-sensitivity tests with potential for use at point of care. Recently WHO endorsed nucleic acid amplification tests, such as Line Probe Assay (MTBDRplus from Hains Diagnostics) and Xpert MTB/RIF (Cepheid Inc.), system. The assays can detect *M. tuberculosis* as well as drug resistances simultaneously. With the successes of these technologies, many more fast detectors are being introduced into the arena of rapid detection of DR-TB. Another indigenously

developed, simple cost effective, culture based assay – Indigenous Gold Standard kit for XDR, capable of detecting MDR and XDR in 7-21 days has been developed at AIIMS, Delhi. All these technologies need to be evaluated stringently for use in high burden settings. This multicentric study is carried out at 4 sites including NIRT Chennai, NITRD New Delhi, AIIMS New Delhi and NJIL & OMD Agra.

Aim: To evaluate the emerging indigenous technologies for rapid detection of drug resistance of tubercle bacilli from sputum samples in comparison with the reference standards MGIT960, LPA and Xpert

Expected Outcome: Performance characteristics namely, sensitivity, specificity, efficiency, predictive values, time to detection etc of the new tests - TruNat Rif and IGS kit, will be determined statistically for each site separately and cumulatively for all sites in comparison with MGIT960.

Progress: About 490 patients have been recruited. The study is ongoing and is likely to be completed by June 2015.

B-4: Surveillance of PZA drug resistance among new sputum smear positive patients

Principal Investigator : Dr.N.S. Gomathi
(email: gomathisharma@nirt.res.in)
Source of funding : USAID through MDP
Study Period : 2014-2015

Background: PZA is a drug given to new smear positive patients under category I. Limited information is available on the prevalence of resistance to PZA globally and none from India. Surveillance studies on PZA resistance are crucial for making policy decisions on inclusion of the drug in treatment regimens for management of TB.

Aim: To estimate the prevalence of PZA drug resistance among new sputum smear positive (NSP) cases of *M. tuberculosis* using BACTEC MGIT 960 system

Summary and Progress: Five hundred clinical isolates stored at -80°C from new smear positive patients from the Tamil Nadu Drug Resistance Surveillance were selected based on availability and sub-cultured on to BBL MGIT 7ml tubes for the study.

DST to PZA was set up in BACTEC MGIT960 at a concentration of 100µg/ml as per the manufacturer's protocol. Of the 386 cultures that yielded interpretable results, 97 (25.12%) were found to be resistant to PZA. Retesting using a modified methodology on 60 of the 97 cultures identified 14 cultures as resistant. Sequencing the *pncA* gene is currently being done to resolve discrepancies.

B-5: Determination of cross-resistance among fluoroquinolones in clinical isolates of *M. tuberculosis* from NSP cases

Principal Investigator : Dr.N.S. Gomathi
(email: gomathisharma@nirt.res.in)
Source of funding : USAID through MDP
Study Period : 2014-2015

Background: Fluoroquinolones (Fqs) such as such as OFX, gatifloxacin (Gx), levofloxacin (Lx) and MFX are anti-tubercular drugs included in the treatment of patients with MDR-TB. A recent study on drug resistance surveillance among NSP cases in the state of Tamil Nadu, showed a high level (10.4%) of resistance to OFX. This has been attributed to the common use of OFX for the treatment of common respiratory infections in the community. Conventionally, DST to any Fq in laboratories is done using OFX, though it has been replaced by MFX or Lx in the actual treatment regimens. Earlier reports suggesting cross resistance among Fqs in *M. tuberculosis* are available. Hence, use of OFX alone for DST to Fqs is likely to yield false resistant results. The current study proposes to document any cross-resistance between OFX and MFX in clinical isolates *M. tuberculosis* from Tamil Nadu region.

Aim: To estimate and document cross resistance among Fqs in clinical isolates of *M. tuberculosis* from Tamil Nadu region using BACTEC MGIT 960 system

Summary and Progress: Eighty five clinical isolates identified as OFX resistant using Proportional Susceptibility Testing on solid medium, from NSP patients (56) and previously treated (PT) patients (29) were retested for quinolone cross-resistance using BACTEC MGIT960. DST to OFX and MFX were set up at a concentration of 2µg/ml for OFX and 0.5µg/ml for MFX as per the manufacturer's protocol. Among 56 NSP cultures, 26 were OFX susceptible and all of them were identified as MFX susceptible. Among 30 OFX resistant strains, 7 were found susceptible to MFX (23.3%) (Table 14). Among 29 PT cultures, 19 were Ofx susceptible and all of them were identified as MFX susceptible. Among 10 OFX resistant strains, 5 were found susceptible to MFX (50%) (Table 15). Cumulatively, 12 among 40 OFX resistant strains were found to be MFX susceptible (30%). Sequencing *gyrA/gyrB* genes is currently being done to resolve discrepancies.

Table 14: Cross-resistance between OFX and MFX among NSP cases

	OFX resistant	OFX sensitive
MFX resistant	23	0
MFX sensitive	07	26
	30	26

Table 15: Cross-resistance between OFX and MFX among PT cases

	OFX resistant	OFX sensitive
MFX resistant	05	0
MFX sensitive	05	19
	10	19

B-6: Molecular drug resistance characterization of extensively drug-resistant strains of *M. tuberculosis* from south India

Principal Investigator : Mr. S Siva Kumar (email:shanmugamsiva@nirt.res.in)
 Source of funding : ICMR-Extramural
 Study period : 2014-2017

Background: The ever-increasing burden of drug resistance is a serious concern in developing countries, particularly for patients with *M. tuberculosis* infection. *M tuberculosis* uses various mechanisms to evade killing by therapeutic drugs, including mutations in genes that code for drug target proteins. TB control and prevention programs are based on early diagnosis followed by rapid identification of drug resistance. The development of rapid molecular methods, which can be performed

within 1 or 2 days, is important for the timely detection.

Aim: (i) To determine the molecular drug resistance pattern for 1st and 2nd line anti-mycobacterial drugs and compare it with the phenotypic results and

(ii) To identify mutations in drug target genes of XDR strains of *M. tuberculosis* prevalent in the south Indian population

Methods: Clinical strains of *M.tuberculosis* (XDR, MDR & pan-sensitive) will be collected during the study period. These clinical strains will be subjected to

antimicrobial testing with first line, second line and reserve drugs on solid LJ Medium or Liquid MGIT.

Gene amplification and pyrosequencing: *rpoB*, *katG*, *embB*, *rrs*, *gyrA*, *gyrB*, *rpsL*, *fabG1*, *ahpC*, *thyA*, *embA*, *embC*, *pncA*, *rrl* and the promoter regions of *inhA*, *ahpC*, *embA-B*, *fabG1*, *rpsL*, *thyA* and *pncA* will be amplified and sequencing will be done to determine the hot spots for resistance. Additional SNPs/genes involved in

resistance also will be looked at if the phenotypic and genotypic resistances do not correlate.

Progress: 25 XDR, 25 MDR, 25 pan-sensitive *M.tb* isolates have been collected decoded, grown and DNA has been extracted. Spoligotyping was performed on 19 isolates. Pyrosequencing was performed for the following genes - *katG*, *inhA*, *ahpC*, *rrs*, *gyrA*, *rpoB* on 16 isolates of XDR-TB; 6 isolates of MDR-TB and 7 pan-sensitive *M. tuberculosis* isolates.

B-7: Re-evaluation of the critical inhibitory concentration of INH and RMP among *M. tuberculosis* strains isolated from patients using wild-type MIC distribution

Principal Investigators	:	Dr. V. N. Azger Dusthacker; Dr. K. R. Uma Devi (email: azger@nirt.res.in; email: umadevi.r@ nirt.res.in)
Source of funding	:	ICMR Intramural
Study Period	:	2014-2016

Background: Critical concentration or break point concentration plays a very important role in determining whether a strain is susceptible or resistant to a particular antibiotic. Growing evidence shows that there might be changes in break through points in different populations based on their pharmacokinetic and pharmacodynamic profiles. The validation of the critical concentrations based on the wild type MIC distribution and hence of epidemiological cutoff (ECOFF) becomes

imperative in every population and might not be the same in every geographical setting. Hence in the present work we want to determine the critical concentration level for INH and RMP among the wild type strains from south Indian population. Wild type MIC determination for the existing first line anti-TB drugs would help in re-establishing the critical concentration and thereby help in the treatment of patients.

Aim: To determine the wild type MIC distribution and the ECOFF of INH and

RMP for validating the existing critical concentrations for determining drug susceptibility among the south Indian isolates of *M. tuberculosis*

Summary and progress: Estimated sample size for this study was 284. Until now a total of 130 and 100 strains were tested for RMP and INH. Breakpoint MIC at the range of 0.5 µg/ml was observed in 47 strains for RMP. INH at a concentration of 0.125 µg/ml

was inhibitory for most number of strains tested (n=68) out of 100 using Middlebrook 7H10 agar medium. The lowest concentration of RMP that was found to inhibit 98% of wild-type strains was determined to be 1 µg/ml and for INH it was 0.125 µg/ml among the strains so far tested. The study is in progress.

B-8: Development of a novel method to improve sensitivity of TB diagnosis by culture using Resuscitation Promoting Factor

Principal Investigator : Dr. V. N. Azger Dusthacker (email: azger@nirt.res.in)
Source of funding : ICMR Intramural
Study Period : 2014-2016

Background: *M. tuberculosis* culture-based diagnostic techniques using either solid or liquid media are more sensitive than smear microscopy but they are slow and require at least 100 actively growing bacilli per ml of sputum. In addition, persisting, latent, dormant and semi-dormant bacilli that co-exist with actively replicating bacilli will not grow efficiently, which will contribute to poor sensitivity and underestimation of net bacillary load in patient sputum. Importantly, a sub-population of dormant, persisting *M. tuberculosis* can resume their replication and

phenotypic drug-sensitivity in presence of appropriate growth environment. *In vitro*, this subpopulation of bacteria can resume their growth in the presence of resuscitation promoting factors (RPF), which are a family of secreted proteins produced by *M. tuberculosis* that can stimulate mycobacterial growth, and this underlines the potential of RPF in improving the detection of *M. tuberculosis* in clinical specimens both qualitatively and quantitatively.

Aim: To tap the potential of RPF proteins both from the culture filtrate supernatant of *M.*

tuberculosis H37Rv and their recombinant proteins to enhance the sensitivity/detection limit in the sputum samples of patients with various stages of active PTB

Summary and progress: A total of 131 smear negative culture negative sputum samples from PTB patients were decontaminated by modified Petroff processing method and were frozen in -80°C. These samples were thawed and treated with RPF protein from culture filtrate of wild type H37Rv. They were plated every week for 7 weeks onto Middlebrook 7H11 with OADC

supplement. A total of 13 samples yielded *M. tuberculosis* colonies in the presence of RPF protein alone, 3 yielded *M. tuberculosis* in both RPF treated and RPF untreated. One sample yielded NTM. Optical density was measured in a spectrophotometer at a wavelength of 600nm in all the vials every week for 7 weeks. An increase in the turbidity in 42 of the samples in the presence of RPF protein was observed.

The study is in progress.

**Dept. of Biochemistry &
Clinical Pharmacology**

COMPLETED STUDIES:

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

Principal Investigator	:	Dr.A.K. Hemanth Kumar (email: hemanthkumarak@nirt.res.in)
Collaborators	:	Govt. Hospital of Thoracic Medicine, Chennai; Govt. Rajaji Hospital, Madurai
Study period	:	2009-2013

Background: RMP forms the backbone of first-line ATT, particularly in HIV-infected persons, because of its rapid sterilizing action. However, emergence of resistance to RMP during ATT in patients with HIV infection remains a serious concern. RMP being a concentration-dependent killer of *M.tuberculosis*, intermittent dosing coupled with malabsorption due to HIV infection, increased tissue bacillary load and decreased immunity could cause ARR.

Aim: To compare the pharmacokinetics of RMP during daily and intermittent ATT in HIV-infected TB patients

Methods: HIV-infected patients with newly diagnosed PTB attending the NIRT clinics in Chennai and Madurai were recruited. Patients were randomized to receive either a daily, partly intermittent or totally intermittent ATT regimen for a period of 6 months. The pharmacokinetic study was undertaken during the intensive phase of ATT after patients had received a minimum

of 6 doses of drugs. Serial blood samples (2ml) at pre-dosing and at 1, 2, 4, 6, 8, 12 and 24 hrs after directly observed drug administration were collected. Plasma RMP concentrations were determined by HPLC according to a validated method.

Results: A total of 41 patients (36 were males) have been recruited to the study. Patients treated with the thrice-weekly regimen had significantly lower plasma C_{max} and AUC_{0-24} and higher oral clearance of RMP than those treated with the daily regimen (Table 16). The proportion of patients with RMP C_{max} below the reference range ($< 8.0\mu\text{g/ml}$) was 100% in the thrice weekly and 85% in the daily arm (non-significant).

Conclusions: The study highlights that, although RMP peak concentrations were below the reference range in majority patients, the higher pre-dosing and peak concentrations achieved during daily ATT appear to pose a distinct advantage, as

evidenced by a higher exposure. This could enhance the exposure-dependant killing of

Mycobacteria by RMP, thereby maximising benefits.

Table 16: Patient demographics & RMP pharmacokinetics in daily (n = 26) and intermittent (n = 15) treatment groups

Variables	Daily treatment (n = 26)	Thrice weekly treatment (n = 15)	P value
Age (years)	35 (31.5 – 40.5)	39 (31 – 42)	NS
Body weight (kg)	45 (38 – 48.5)	50 (39 – 55)	NS
Males	22	14	NS
RMP dose mg/kg body wt	9.9 (9.1 – 11.7)	9.8 (8.2 – 11.6)	NS
CD4 cell counts (cells.mm ³)	240 (16 – 877)	177 (45 – 899)	NS
C _{max} (µg/ml)	6.4 (5.6 – 7.6)	3.7 (2.1 – 4.6)	<0.001
T _{max} (h)	4.0 (2.0 – 4.0)	2.0 (2.0 – 4.0)	0.047
AUC ₀₋₂₄ (µg/ml.h)	29.4 (20.6 – 41.0)	20.7 (14.3 – 29.3)	0.03
Cl (ml/min)	15.3 (11.0 – 21.9)	21.7 (15.3 – 31.4)	0.03
t _{1/2} (h)	2.2 (2.1 – 2.3)	2.6 (2.4 – 2.8)	<0.001

The above values are Median (Inter-quartile range).

C_{max} – Peak concentration; T_{max} – Time at which C_{max} was attained; AUC₀₋₂₄ – Exposure; Cl – Clearance; t_{1/2} – Half-life

(ii) Food significantly reduces plasma concentrations of first - line anti-TB drugs

Principal Investigator	:	Dr.Geetha Ramachandran; Dr.A.K. Hemanth Kumar (email: geethar@nirt.res.in; hemanthkumark@nirt.res.in)
Collaboration	:	Chennai Corporation
Source of funding	:	USAID through MDP
Study period	:	2014-2015

Background: Food intake exerts a complex influence on the bioavailability of drugs. Concomitant food intake and ATT drug administration is likely to reduce nausea and enhance compliance to treatment. However, food could lower plasma drug concentrations.

Objectives: To examine the effect of food on two-hour plasma concentrations of RMP, INH and PZA and pharmacokinetics of these drugs

Methods: Newly diagnosed adult TB patients were recruited from the RNTCP treatment centres in Chennai Corporation. An open-label, cross-over study design was adopted; each patient was tested on two occasions with an interval of one week between occasions. On one occasion, the patient took breakfast followed by anti-TB drugs, and on another occasion the patient took the anti-TB drugs after an overnight fast of 12 hrs and took breakfast after two hours of drug administration. Drug administrations were completely supervised. All patients were provided with a uniform breakfast, which was rice –based

(carbohydrate-rich). The study was conducted in two phases. In the first set of patients, a single blood sample was collected two hours after drug administration, on both occasions. During the second phase in a different set of patients, a complete pharmacokinetic study was conducted on both occasions. Blood samples were drawn at pre-dosing and at 1, 2, 3, 4, 6 and 8 hrs after anti-TB drug administration.

Results: Median two-hour concentrations, as well as peak concentrations and exposures of RMP, INH and PZA were significantly lower when the drugs were taken immediately after food, compared to drug administration when fasting (Tables 17 & 18). Time to attain peak concentrations of RMP, INH and PZA was higher when taken with food. The proportion of patients with sub-therapeutic two-hr RMP ($< 7.0\mu\text{g/ml}$), INH ($< 2.0\mu\text{g/ml}$) and PZA ($< 20.0\mu\text{g/ml}$) was significantly higher when drugs were administered after food compared to a fasting state.

Conclusions: Food lowers anti-TB drug concentrations and exposure significantly

and delays absorption. It is advisable to administer anti-TB medications under

fasting conditions in order to attain therapeutic drug concentrations.

Table 17: Two-hr RMP, INH and PZA plasma concentrations during fasting condition and after food

Two-hour drug concentration			
Drug	With food Median (IQR)	Fasting condition Median (IQR)	p-value
RMP	2.9 (1.3-4.4)	6.3 (4.5-7.5)	<0.001
INH	6.1 (3.2-7.9)	11.4 (8.7-13.3)	<0.001
PZA	26.1 (18.3-34.0)	38.3 (31.1-49.1)	<0.001

Table 18: Pharmacokinetics of RMP, INH and PZA during fasting condition and after food

	With food Median (IQR)	Fasting condition Median (IQR)	p-value
RMP			
C_{max}	3.4 (3.3-4.1)	5.8 (5.2-6.2)	0.028
T_{max}	4.0 (3.8-6.0)	3.0 (1.8-3.0)	0.039
AUC_{0-8}	17.3 (13.8-20.3)	22.1 (15.9-28.2)	0.116
INH			
C_{max}	9.7 (4.9-10.2)	13.5 (9.5-15.3)	0.028
T_{max}	3.0 (2.8-4.0)	1.0 (1.0-1.2)	0.026
AUC_{0-8}	43.5 (21.5-52.5)	50.6 (37.7-69.4)	0.028
PZA			
C_{max}	35.9 (31.1-40.8)	48.5 (42.1-60.7)	0.028
T_{max}	4.0 (3.8-5.0)	3.0 (1.8-3.2)	0.039
AUC_{0-8}	220 (189-233)	267 (229-317)	0.028

(iii) *SLCO1B1* gene polymorphisms do not influence plasma RMP concentrations in a south Indian population

Principal Investigator : Dr.Geetha Ramachandran; Dr.A.K. Hemanth Kumar
(email: geethar@nirt.res.in; hemanthkumark@nirt.res.in)
Collaboration : Chennai Corporation
Source of funding : USAID through MDP
Study period : 2014-2015

Background: Several factors can impact plasma RMP concentrations. Hepatocellular uptake of RMP is mediated by an organic anion-transporter polypeptide 1B1 (OAT1B1) coded for by the gene, *SLCO1B1*. It has been reported that polymorphisms (rs11045819 and rs4149032) in this gene influence RMP pharmacokinetics significantly and are implicated in low RMP exposure.

Objective: To determine the role of *SLCO1B1* gene polymorphisms (rs11045819, rs4149032 and rs4149033) on RMP concentrations in adult TB patients from south India

Methods: Adult TB patients were genotyped for three *SLCO1B1* gene polymorphisms, namely, rs11045819, rs4149032 and rs4149033, and compared two-hr post-dosing RMP concentrations between the different genotypes for each of these polymorphisms. Plasma RMP was determined by High Performance Liquid Chromatography. Genotyping was performed by direct sequencing.

Results: A total of 256 TB patients (males 165; females 91) were studied. Their mean (SD) age and body weight were 39.0 (14.1) years and 48.5 (9.8) kg respectively. Of them, 255, 188 and 188 patients were genotyped for rs11045819, rs4149032 and rs4149033 polymorphisms respectively. The distribution of genotypes for the three polymorphisms and RMP concentrations in the different groups are given in Table 19. The genotype distribution for all the polymorphisms followed Hardy-Weinberg equilibrium, the variant allele frequency being 0.01 (A), 0.54 (T) and 0.07 (A) respectively for rs11045819, rs4149032 and rs4149033 polymorphisms. With respect to the rs 11045819 gene polymorphism, there were 4 and 251 patients respectively, belonging to the CA and CC genotypes, but none to the AA genotype. None of the differences in RMP concentrations between the different genotypes of all the three polymorphisms were statistically significant.

Conclusion: This study is the first from India that has examined the influence of 3

SLCO1B1 gene polymorphisms on RMP concentrations in TB patients. This study has shown that three polymorphisms in the *SLCO1B1* gene (rs11045819, rs4149032 and

rs4149033) do not have any influence on plasma concentrations of RMP in the south Indian population.

Table 19: RMP concentrations in the different genotypes

Gene polymorphism*	RMP- 2hr Median (IQR)	p-value
rs11045819		
AA	-	-
CA (4)	2.43 (0.56 - 4.75)	
CC (251)	2.93 (1.01 - 6.24)	
rs4149032		
CC (44)	2.93 (1.02 - 5.90)	0.849
CT (85)	2.74 (0.89 – 5.70)	
TT (59)	2.23 (0.78 - 5.91)	
rs4149033		
AA (1)	0.25 (0.25 - 0.25)	0.595**
AG (25)	3.54 (0.90 - 6.38)	
GG (162)	2.75 (0.91 - 5.58)	

* N given in brackets

** denotes significance value between AG and GG genotypes

STUDIES IN PROGRESS:

BCP-1: Effect of plasma MFX on treatment outcome in PTB patients treated with MFX-containing anti-TB regimens

Principal Investigator : Dr. Geetha Ramachandran
(email: geethar@nirt.res.in)
Source of funding : Intramural
Study period : 2013-2015

Background: Among the newer generation of Fqs, 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. We have undertaken a prospective study to relate MFX blood concentrations with TB treatment outcomes.

Aim: To estimate plasma concentrations of MFX, RMP, INH and PZA and correlate with TB treatment outcome

Methods: This study is 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 end of treatment. Plasma concentrations of MFX, RMP, INH and PZA are estimated by HPLC. 227 patients have been recruited from Chennai and Madurai; of them, 141 have completed treatment. Further recruitment of patients to the study is in progress.

BCP-2: Pharmacokinetics of first-line anti-TB drugs in adult TB patients treated in the RNTCP

Principal Investigators : Dr. Geetha Ramachandran; Dr.A.K. Hemanth Kumar
(email:geethar@nirt.res.in; hemanthkumarak@nirt.res.in)
Collaboration : Chennai Corporation
Source of funding : USAID through MDP
Study period : 2013-2015

Background: Low serum concentrations of anti-TB drugs have been associated with treatment failure, relapse and acquired drug resistance in HIV-infected and non-HIV-infected TB patients. A number of factors have been reported to influence anti-TB drug levels.

Aims: Primary aim:

(i) To study the influence of factors such as diabetes, HIV infection, malnutrition, old age (> 60 years), smoking, alcohol intake, gene polymorphisms, on 2-hr concentrations of RMP, INH and PZA

Secondary aims: (i) To determine 2-hr post dosing concentrations of RMP, INH & PZA in TB patients receiving treatment (Category I/II) under the RNTCP and (a) relate to TB treatment outcome;

(b) To determine cut-off levels for RMP, INH and PZA that would define sub-optimal treatment outcomes and

(ii) To study the pharmacokinetics of RMP, INH & PZA in a sub-group of patients admitted to the study

Methods: A prospective cohort study is ongoing, in which adult TB patients with pulmonary or extrapulmonary TB started on category I or II ATT are recruited. All the patients are receiving ATT in the RNTCP treatment centres in Chennai Corporation. Each patient is investigated on two occasions, during the intensive phase and at end of treatment. During both occasions, blood at two hrs after directly observed drug administration is done. Patients are genotyped for *NAT2* and *SLCO1B1* gene polymorphisms. Random glucose >200mg/dl is considered as diabetes. Sputum at baseline and at end of ATT are collected and processed for smear and culture. Patients are followed up to determine treatment outcome. We have recruited 1975 patients, of which 842 have completed treatment. The complete pharmacokinetic study has been completed in 101 patients. Further recruitment of patients to the study is in progress.

HIV-LAB

COMPLETED STUDIES:

(i) Structure based rational design and synthesis of inhibitors for various enzymes of HIV and *in vitro* testing of activity

Principal Investigator	:	Dr. Luke Elizabeth Hanna; Dr. Soumya Swaminathan, Dr.D. Sriram; Dr. Yogeeswari, BITS-Pilani (email: hanna@nirt.res.in; soumyas@nirt.res.in)
Source of funding	:	DBT/ICMR
Study period	:	2012-2015

Rational: Treatment of HIV patients with combinations of potent antiviral agents targeting the viral enzymes reverse transcriptase (RT) and protease (PR), termed highly active anti-retroviral therapy (HAART), has been more successful than mono-therapeutic regimens. There are, however, problems with drug toxicity and multidrug resistance after prolonged therapy. A new generation of drugs that are more effective, with potency against resistant strains, and higher resilience to new mutations is therefore required.

Aim: To design newer leads based on computer-aided drug design employing structure-based drug design, development of pharmacophoric models, synthesis and *in vitro* testing for enzyme inhibition and anti-HIV activity

Methodology: A set of 12 RT inhibitors, 14 IN inhibitors and 12 PR inhibitors were identified as leads and commercially

synthesized. These molecules were synthesized commercially and tested for *in vitro* testing by enzyme inhibition studies as well as virus inhibition assay. Commercially available RT assay, HIV-1 integrase assay and Protease assay kits were used for enzyme inhibition studies and HIV-1 p24 antigen detection assay was employed for measuring virus inhibition.

Results: Among the different categories of HIV inhibitors tested against circulating virus, only a few compounds showed a target-specific inhibitory effect; however, even in these cases, the percentage inhibition was <50. Two anti-HIV-1 RT molecules showed inhibition of 29.5% and 28% at 100 μ M concentration, and one integrase inhibitor showed an inhibition of 41% at 100 μ M concentration. None of the protease inhibitors demonstrated protease inhibitory activity at the two concentrations tested.

Conclusion: The findings suggest that the compounds which demonstrate minimal *in vitro* activity need to be further modified

chemically to enhance their anti-HIV property.

(ii) Evaluation of chemokine production in HIV-1 infected subjects exhibiting immunovirological discordance

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2014-2015

Background: During HAART, immune recovery is characterized by suppression of HIV-1 replication and increasing CD4+ T-cell counts. Although many patients continue to have CD4+ T-cell recovery for several years after receiving ART, the degree of immune recovery achieved during viral suppression is highly variable. In some individuals, increases in the CD4+ T-cell count appear to plateau after the first few months of ART. This suboptimal CD4+ T-cell response during therapy, otherwise known as “immunologic discordance,” can have detrimental clinical consequences.

Aim: To investigate alterations in the synthesis of host chemokines following ART for HIV infection in order to ascertain the potential role of specific chemokines in the progression of disease

Methodology: We measured different chemokines (CXCL8 / IL8, IP10, RANTES, MIG, MCP-1, MIP-1 α and MIP-1 β) levels in stored plasma of 42 HIV-1 infected children who had suppressed viremia (20 copies/mL) after 12 months of ART. Successive measurements of CD4 and plasma HIV-1 RNA levels were obtained before and during receipt of ART. On the basis of increases in the CD4⁺ T-cell counts, subjects were classified as immunologically concordant [N=28] (demonstrating a very high increase in CD4 count) or discordant [N=14] (demonstrating a stable or low increase in CD4 count) after 12 months of ART. Plasma samples were obtained from 16 HIV negative healthy children at one time point to serve as controls. Levels of above mentioned chemokines in 25 μ l of

stored plasma sample were measured using BD CBA Flex Kit using appropriate standards. Samples were acquired on a flow cytometer and analyzed using the FCAP array software version 1.0.2. The results are expressed in pg/ml.

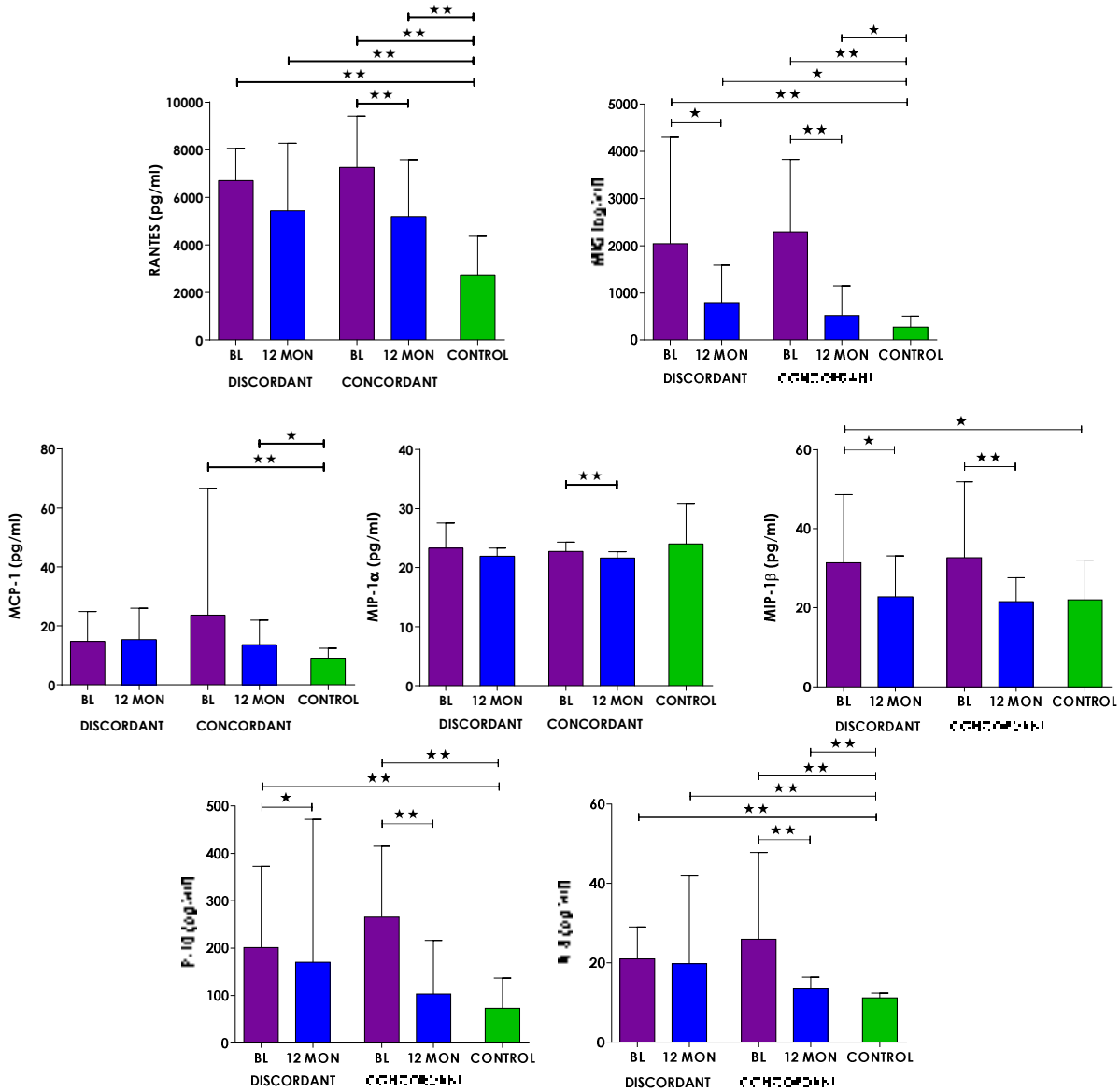
Statistical Analysis: Data were analyzed using Graphpad Prism 6 software (GraphPad Software, Inc, USA) and expressed as mean \pm SD. The following statistical tests were used: (1) Wilcoxon matched pairs signed rank test was used to compare the baseline (BL) vs 12 MON (12th month) sample of the same group; (2) Non parametric Mann-Whitney U test was used to compare the BL and 12 MON samples of different groups and control. A p value of <0.05 was considered to be statistically significant.

Results: Mean plasma chemokines (CXCL8/IL8, IP10, RANTES and MIG) levels were significantly higher at baseline in both the immunologic discordant and concordant HIV+ children when compared to controls. Although there was a slight decrease in chemokine levels after 12 months of HAART, higher chemokines

levels were maintained in both the groups as compared to the controls. There was no significant difference between plasma levels of any particular chemokine between the immunologically discordant and concordant HIV+ children. However, among immunologically concordant HIV+ children, chemokines IP10, IL8, RANTES, MIG, MIP-1 α and MIP-1 β levels were significantly reduced after treatment. On the other hand, in children with immunological discordance, a significant reduction was seen only with three chemokines (IP10, MIG, MIP-1 β) (Fig. 10).

Conclusions: Our data demonstrates that up-regulated chemokine production was significantly associated with higher HIV viral load, suggesting that the observed chemokine storm during infection is induced at least in part, in response to the presence of HIV and viral products. However, no significant difference in chemokine levels was observed between individuals with immunologic concordance and discordance following 12 months of HAART.

Fig. 10: Plasma chemokines (IL8, IP10, RANTES, MIG, MCP-1, MIP-1 α and MIP-1 β) levels in healthy subjects and perinatally HIV-infected children with concordant and discordant immunologic failure



The levels of chemokines were analysed using the Cytometric Bead Array and flow cytometry. The results are represented as mean \pm SD. $P < 0.05$ was considered to be statistically significant. The statistical significance is shown as * when $p < 0.05$, ** when $p < 0.01$

STUDIES IN PROGRESS:

HIVL-1: Identification and characterization of neutralizing antibodies in clade ‘C’ HIV-1 infected individuals in south India

Principal Investigator : Dr. Luke Elizabeth Hanna
(email:hanna@nirt.res.in)
Source of funding : ICMR Intramural
Period of study : 2012-2016

Background: Cross-reactive antibodies capable of neutralizing heterologous primary viral isolates develop during the course of HIV-1 infection, and it is this neutralizing antibody (NAb) response that is of interest to vaccine researchers. An HIV-1 vaccine that can generate preexisting antibodies that can neutralize most circulating virus strains has the potential to protect against infection with HIV-1. Only a handful of broadly cross reactive neutralizing antibodies (bNAbs) have been identified till date; interestingly, the Indian clade C HIV-1 strains are largely resistant to many of these bNAbs. The incidence of NAbs with similar specificities or other as-yet uncharacterized specificities and their relative contributions to broad cross clade neutralization, including the Indian subtype C strains is largely unexplored. Identification of such novel neutralizing antibodies could in a major way contribute to the design of a preventive vaccine for HIV.

Aim: To identify broadly cross-reactive neutralizing antibodies in sera of HIV-1 subtype C infected individuals from India and characterize their binding specificities

Methodology: Whole blood from 100 ART-naive, recently infected HIV-1 infected individuals were collected and their sera were screened for the presence of broadly cross reactive neutralizing antibodies using the standard panel of Tier-1, 2 and 3 pseudo viruses (easy to neutralize, moderately easy to neutralize and difficult to neutralize viruses respectively) obtained from the NIH AIDS Repository.

Results: A total of 58 samples were able to neutralize at least 50% of the tier-1 pseudo viruses. Of the 58 samples, 30 samples were found to neutralize at least 50% of the tier-2 pseudo viruses. As expected most of the plasma samples exhibited very strong neutralization with the Indian pseudo viruses 16936-2.21 and pIndie, indicating a geographical neutralization specificity. Thirty samples were screened with a panel of tier-3 pseudo viruses and it was found that 5 plasma samples (NAB001, NAB016, NAB059, NAB063 and NAB069) neutralized all the tier-3 pseudo viruses tested. An additional seven samples (NAB004, NAB033, NAB046, NAB062, NAB065, NAB0120 and NAB0122) exhibited moderate levels of neutralization of tier-3 pseudo viruses (Table 20).

Table 20: Samples exhibiting neutralization of tier-1, 2 & 3 pseudoviruses

(percentage inhibition is mentioned for each virus)

Samples	Tier-1 pseudo viruses				Tier-2 pseudo viruses						Tier-3 pseudo viruses		
	SF162 LS	GS015 EC12	ZM197M PB.7	CRF0 2AG.2 42	TRO .11	CRF02 AG/A1 .280-5	1693 6.2.2 1	Pindie*	CAP 210	DU 156.12	PVO -4	CRF0 2AG.3 3	CRF 02A G.25 3
NAB06 3	99	100	83	99	92	95	99	99	95	96	91	96	92
NAB01 6	100	100	85	74	86	98	98	100	65	99	87	98	91
NAB00 1	100	100	95	75	92	96	98	100	98	99	86	93	93
NAB06 9	97	100	85	98	86	95	97	97	94	97	82	89	94
NAB05 9	100	100	96	89	95	99	98	98	96	99	86	90	87
NAB12 0	89	100	86	88	90	94	97	92	79	95	92	75	91
NAB06 5	98	99	84	96	85	98	99	98	93	98	84	77	95
NAB06 2	99	100	71	98	91	94	97	96	51	95	96	98	69
NAB04 6	99	99	93	95	91	98	96	94	90	97	93	95	63
NAB03 3	100	100	68	0	66	86	89	99	78	96	91	90	70
NAB12 2	98	100	49	81	76	90	95	98	58	87	79	85	66
NAB00 4	93	100	75	92	39	63	88	100	81	91	61	95	62

Samples which exhibited broad cross clade neutralization were further characterized for the binding specificity of the antibodies by

means of Pep Scan method using gp160 linear overlapping peptides of the envelope protein of the Indian molecular clone pIndie.

Results suggest that majority of the samples that exhibited broad cross clade neutralization had antibodies that bind to the V3 region, CD4 binding site, immunodominant loop (ID) of gp41, and

MPER. Two of the samples also showed binding to the Kennedy epitope present in the transmembrane (TM) region of gp41 (Table 21).

Table 21: Binding specificity of antibodies in the broadly cross neutralizing sera

Binding location/pep no	Avg OD at 450 nm
V3, ID and Kennedy	2.0, 1.2 and 1.0 respectively.
V3, V4, ID and Kennedy epitope	2.0, 0.3, 1.6 and 1.3 respectively.
CD4BS, V3, C4 and ID	0.268, 1.4, 0.4 and 1.8 respectively.
V3, ID and MPER	1.0, 1.6 and 1.4 respectively.

The nature and strength of the antibodies in the broadly cross neutralizing sera was further evaluated using the limiting dilution TCID₅₀ assay. The results of this experiment showed that the V3 peptide

binding antibodies had the greatest strength and could neutralize at least 50% of the pseudoviruses at a dilution of approximately 5000-fold.

The study is ongoing.

HIVL-2: Multicentric Study on the development of a national HIV drug resistance database by generating and utilizing clinical, genotypic data of the prevalent DR strains of Indian HIV-1 Clade-C

Principal Investigators: Dr. Srikanth Tripathy; Dr. Soumya Swaminathan
(email:tripathysp@icmr.org.in;
soumyas@nirt.res.in)
Source of funding : ICMR Task Force Project
Study Period : 2014 -2017

Background: The current practice of HIV drug resistance testing by genotyping requires amplification and sequencing of the genes coding for reverse transcriptase and protease from the HIV-1 virus. The sequences are then compared with the HIV Drug Resistance database from the Stanford University for interpreting the drug resistance in the analyzed samples. The database interprets the genotype with the resistance data mainly from the clade B HIV and lacks information on the clade C HIV-1 viruses which are predominately circulating in India. In order to perform better interpretation of the resistance in Indian clade-C HIV-1 sequences, there is need of drug resistance database which will help in developing country specific ART algorithm.

Aim: To develop a HIV Drug resistance Database based on pol gene sequences and mutations submitted through the network of HIV DR laboratories across India utilizing circulating Indian isolates

Methodology: HIV-1 drug resistance testing by genotyping using an in house method was undertaken for 20 HIV-1 infected subjects treated with a first line ART (anti-retroviral therapy) regimen, but have shown evidence of clinical and virological failure.

Results: Of the 20 samples, 19 samples were amplified and genotyped for the pol gene using in-house method. Mutational analysis of Non-nucleoside reverse transcriptase inhibitors (NNRTIs), Nucleoside reverse transcriptase inhibitors (NRTIs), Protease inhibitors (PIs) was performed. The analyzed sequence data was submitted to NARI for development of the database.

The study is on going. More number of sequences will be generated in the current year.

HIVL-3: Identification of virological factors that contribute to the varying pathogenicity of HIV-1 and HIV-2

Principal Investigator : Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding : Intramural
Study period : 2013-2015

Background: HIV-2 is the second lentivirus that is known to cause AIDS and is genetically and biologically related to HIV-1. Despite the similarity, HIV-2 infection is associated with slower disease progression and transmission, longer latency period and low or undetectable plasmatic viral levels and reduced likelihood of progression to AIDS than HIV-1. Better understanding of the viral determinants and the magnitude of genetic variation among the circulating strains can give an idea of their implications in disease progression in the population.

Aim: To perform genetic and functional analysis of the regulatory elements in the LTR and regulatory genes like Tat and Rev, of the circulating HIV-2 strains in our population with a view to understand the contribution of viral factors responsible for the diminished pathogenesis and delayed disease progression characteristic of HIV-2 infection

Methodology: Blood was obtained from 15 HIV-2 infected individuals with healthy CD4 cell counts (>500 cells per microlitre of

blood). Genomic DNA was extracted from whole blood and the LTR fragment (~ 850 bp) was amplified by nested PCR and subjected to direct sequencing on a Avant 3100 Genetic Analyzer.

Results: The sequences were aligned and the transcription factor* binding sites like *(NF-kB, TCF-1 α), Tar elements and cis acting elements were identified and analyzed. The sequences obtained showed 80-90% similarity with HIV-2 sequences previously reported from India. An interesting observation was the presence of an additional potential NF-kB site, one of the important transcription factor binding sites and potential marker for increased viral expression and pathogenesis in 3 of the 15 samples.

Functional analysis of the significance of the second potential NF-kB site with regard to viral pathogenesis needs to be investigated. Also studies are on going to characterize other regulatory elements such as the tat and rev genes.

HIVL-4: Cohort for TB research by the Indo-US medical partnership (C-TRIUMPh)

study

Principal Investigator : Dr. Soumya Swaminathan (email: soumyas@nirt.res.in)
Source of funding : Intramural
Study period : 2013-2015

Cohort for TB research by the Indo-US Medical Partnership (C-TRIUMPh) is a multicentric study conducted by National Institute for Research in Tuberculosis and Byramjee Jeejeebhoy Medical College CTU (BJMC), Pune. C-TRIUMPh includes both epidemiologic/clinical and basic laboratory research studies that will address three specific aims:

Aim 1: To measure the host and microbial factors associated with TB treatment outcomes in Indian adults and children (Active TB cohort);

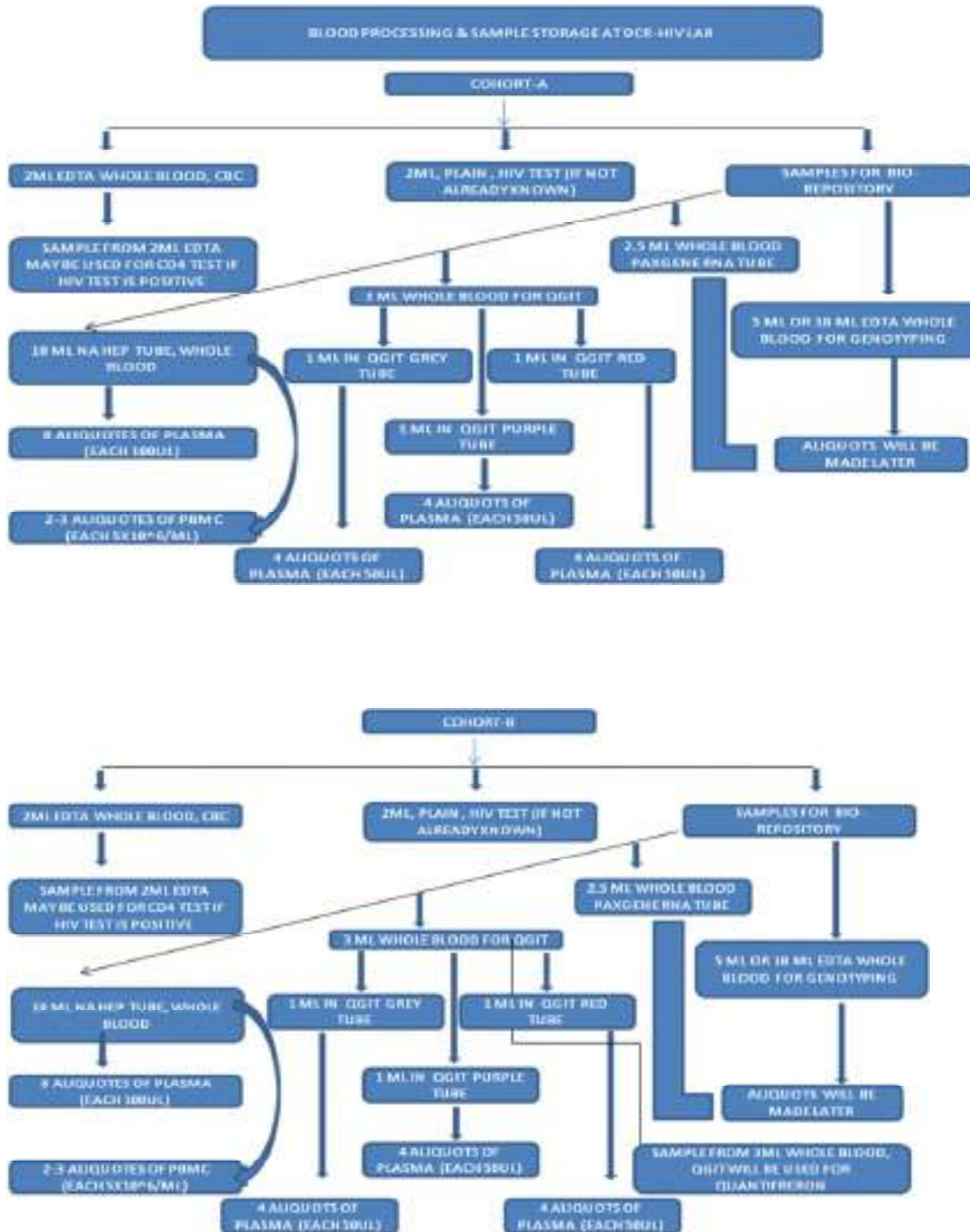
Aim 2: To investigate the host and microbial factors associated with progression from infection to active TB disease in adults and children. (Household Contacts) and

Aim 3: to explore the host and microbial factors associated with TB transmission. (Household Contacts and Control Cohorts)

The HIV Laboratory undertakes processing of blood samples and storage of specimens for this study.

The sample flow is given in Fig. 11. Details of samples received and processed in this study are given in Tables 22-24.

Fig. 11: Flow diagram of HIV Laboratory’s activities undertaken for the C-TRIUMPh study



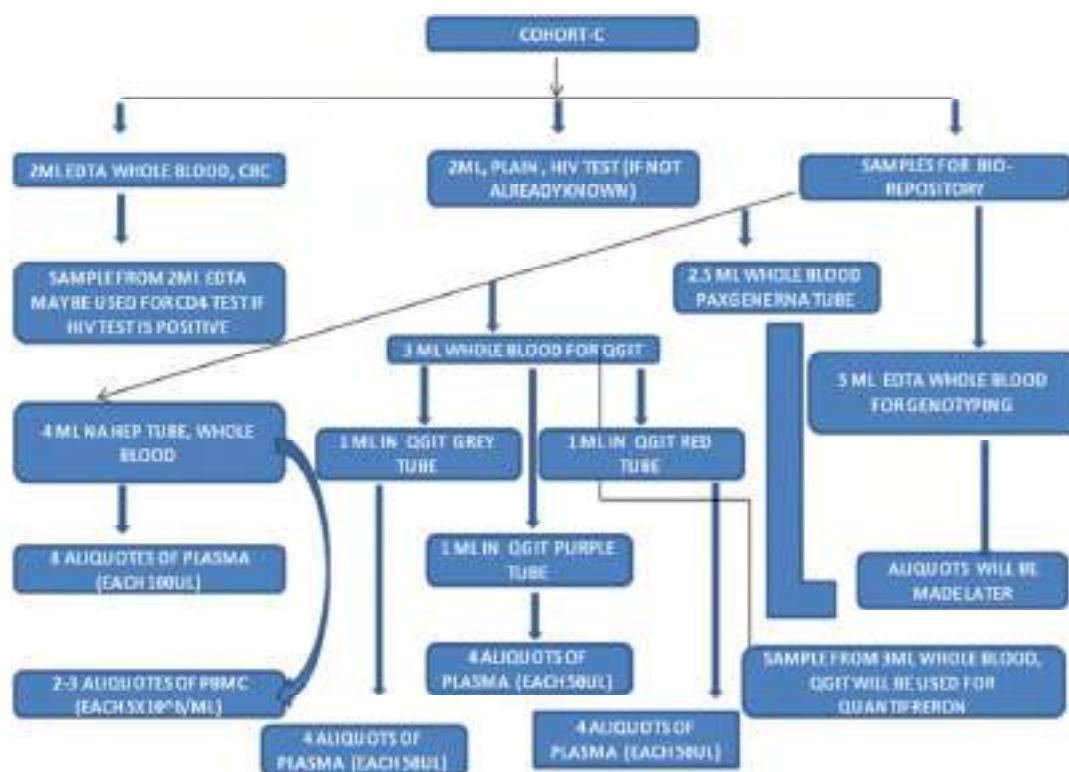


Table 22: No. of study samples received and processed during the period Aug 2014 to Mar 2015

Cohort	A	B	Total
No. of baseline samples received	89	187	276
No. of follow up samples received	165	54	219
Total No. of samples received and processed	254	241	495

Table 23: No. of study samples (aliquots) stored during the period Aug 2014 to Mar 2015

Cohort	PBMC	PLASMA	QGIT (PLASMA)	PAXGENE (WHOLE BLOOD)	DNA (WHOLE BLOOD)
A	641	1872	2616	234	18
B	719	1920	2877	241	186
Total	1360	3792	5493	475	204

Table 24: No. of samples processed for QGIT (Cohort-B) for C-TRIUMPh study during the period Aug 2014 to Mar 2015

Visit	Samples Received	Samples Tested
Entry Level	187	186
4 th month	48	24
Total samples	235	210

Department of Immunology

COMPLETED STUDIES:**(i) Role of PknL, a serine/threonine kinase in the adaptive responses of *M.tuberculosis***

Principal Investigator	:	Dr. Sujatha Narayanan (email: sujathan@nirt.res.in)
Co-Investigator	:	Ms. Ahmed Kabir Refaya
Source of funding	:	ICMR Fellowship / ICMR-Intramural
Study Period	:	2014- 2015

Background: Serine / threonine protein kinases (STPK), the novel signal transduction system identified in *M. tuberculosis* plays a role in physiology and pathogenesis of this organism. This pathogen survives the dynamic environmental cues by adaptive responses modulated by 11 serine/threonine protein kinases (STPKs PknA-PknL). These kinase proteins are mainly localized in cell membrane and cell wall of *M. tuberculosis*, except for *PknG* which is present in the cytoplasm. Interestingly, among the 11 STPKs only 4 of them (PknA, PknB, PknG and PknL) are conserved in *M. leprae*, of which PknA, PknB and PknG have been predicted to be essential in *M. tuberculosis*. As the massive genome decay shown by *M. leprae* suggests that only essential genes (coding for functional proteins) have been left unmutated; therefore, the essentiality of *pknL* in *M. tuberculosis* should be analyzed.

The present work focuses on down-regulating the expression of *pknL* gene by antisense technology and to identify its function in adaptive responses encountered within the phagosome.

Aim: (i) To knock-down the expression of *pknL* in *M.tuberculosis* using antisense approach and

(ii) To identify the function of *pknL* in the adaptive response of *M.tuberculosis*

Methods:

Constructing a conditional knock-down strain of *pknL*: The whole gene *pknL* was PCR amplified and cloned in sense (Pkn-S) and antisense (Pkn-AS) orientation in the IsoPropyl b-D-ThioGalactoside (IPTG) inducible vector pAZ19018b. Both the constructs along the vector were electroporated in H₃₇Rv competent cells and plated in 7H10 agar with 50ug/ml of hygromycin. Selected colonies were grown in 7H9 media supplemented with 0.5 %

glycerol, 0.05 % Tween 80, and 10 % ADS along with 50µg/ml of hygromycin. All the three strains - wildtype, knock-down strain (PknL-AS) and over-expressed strain (PknL-S), were used for further experiments.

Regulation of *M. tuberculosis* PknL gene expression during different phases of growth:

All the *M. tuberculosis* strains wildtype, PknL and PknL-AS were grown in Middlebrook 7H9 broth supplemented with 0.5% glycerol, 0.05% Tween 80, and 10% albumin, dextrose along with 50 µg/ml of hygromycin. IPTG was used in the concentration of 0.1 and 10Mm to induce the expression of sense and antisense *pknL*. The cultures were harvested at regular intervals of 0, 2, 5, 7, 10, 14 & 21 days. Total RNA was purified using an RNeasy purification kit (Qiagen). The first-strand cDNA was synthesized from 1 mg total RNA using high capacity cDNA reverse transcriptase kit (Applied Biosystems). Expression level of *pknL* was estimated in comparison to the house-keeping gene 16sRNA for all the time points. Real-time quantitative RT-PCR (qRT- PCR) was performed in an ABI 7500 system (Applied Biosystems) using TaqMan assays. After baseline correction and determination of threshold settings, relative expression (R) was calculated using the 2^{-2ΔΔCt} method of

Livak & Schmittgen. Results were expressed as log₁₀ (R), which denotes the fold change in expression of the gene at different phases of growth.

***In vitro* growth kinetics of the pknL knock-down strain (PknL-AS):**

Logarithmic phase cultures of the wild type H37Rv, PknL-S and PknL-AS were washed thrice and diluted to 0.1 OD₆₀₀ in enriched Middlebrook 7H0 broth supplemented with 0.5% glycerol, 0.05% Tween 80, and 10% albumin, dextrose along with 50 µg/ml of hygromycin. IPTG was used in different concentration of 0mM, 1mM and 10mM as inducer. Diluted cultures were then grown in the shaker incubator at 200 rpm and at 37°C. Aliqotes of the cultures were withdrawn at regular intervals on day 0, 2, 5, 7, 10, 14 and 21 and the growth monitored by plating serially diluted cultures on the 7H10-ADS plates at specified time points. Colony forming unit (CFU) measurements were made after incubation of the plates at 37°C for 4-5 weeks.

pH, SDS and lysozyme sensitivity: Log phase cultures of all the strains were used to carry out the pH sensitivity experiment at pH5.5 and 7.0 using in 7H9 media. SDS sensitivity was carried out using 7H10 agar plates containing SDS at 0.1%, 0.01% or 0.001% concentrations and CFU were

counted after 14 days. Lysozyme sensitivity was carried out by adding 2.5ug/ml of lysozyme into the logphase cultures and CFU performed at 0, 24 & 48 hrs. Single and viable colonies were counted after 2 weeks.

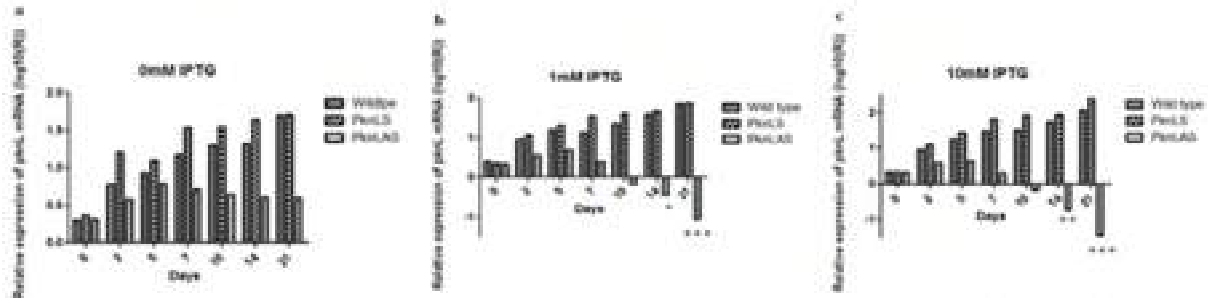
Results: The expression profile of *pknL*-AS showed a considerable decrease compared to *pknL*-S and wildtype (Figs. 12 a-c). Transcription of *pknL* in the antisense construct was less irrespective of the presence or absence of inducer, but showed a significant decrease after day 10 in the presence of 1mM & 10mM IPTG. The rate of growth in *pknL*-AS decreased after day 10 when compared with *PknL*-S and wild type. Whereas *pknL*-AS showed no change in growth in the absence of the inducer IPTG, but reduced considerably in the presence of maximum inducer concentration of 10mM. Similarly the survival rate of *PknL*-AS decreased after day 7 when compared to wildtype and *PknL*-S (Figs. 13 a-c).

The growth and survival kinetics of wild type remained unaltered in both pH 7.0 and pH 5.5, whereas *PknL*-AS survived better in pH 5.5 and *PknL*-S showed a reduced growth in pH 5.5 when compared to its growth in pH 7.0 and also survival capacity of *PknL*-S

was reduced after encountering the acidic stress of pH 5.5 (Fig. 14). Similarly, *PknL*-AS was resistant to both SDS (0.01%) (Fig. 15) and lysozyme stress (24 & 48 hrs) compared to wild type and *PknL*-S (Fig. 16). There was no changes in the colony morphology of all the strains in the presence of SDS (0.01%) but there was a visible change in the colony morphology of all the strains under lysozyme stress exhibiting different phenotype in wild type, *PknL*-S and *PknL*-AS (Fig. 17).

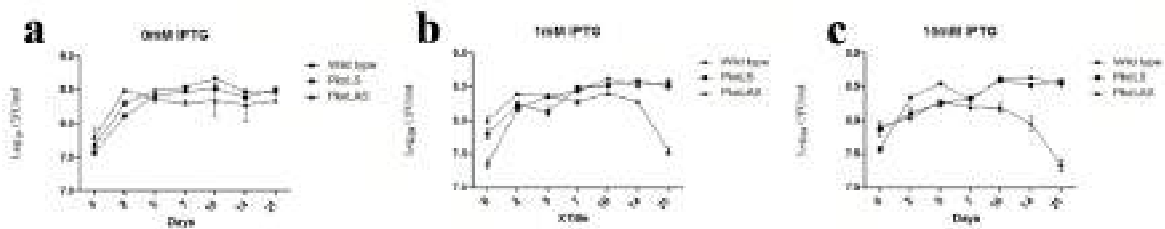
Conclusions: In this study, we have shown that down regulation of *PknL* has a growth advantage under conditions of acidic pH, SDS and lysozyme stress, which probably mimic the macrophage environment as soon as the bacilli are taken into the host. Our studies also indicate that internal signals used to activate *PknL* are most likely the host-associated internal signals and the ability of *PknL* to respond to such stresses is relevant to their survival *in vivo*. In conclusion, it is evident that *PknL* is able to sense the environmental cues and act accordingly, thereby helping the bacteria in adaptation to stress conditions inside the host by slowing down the growth of the bacteria leading to dormant state, thereby persisting within the host.

Figs. 12 (a-c): Relative expression of *pknL* at different concentration of IPTG



Change in expression of *pknL* in Wildtype, PknLS and PknLAS
 a –Relative expression of *pknL* at 0mM IPTG induction..
 b – Relative expression of *pknL* at 1mM IPTG induction.
 c– Relative expression of *pknL* at 10mM IPTG induction.
 There was a significant decrease in the expression of the gene ($p < 0.01$ to $p < 0.001$) in PknLAS compared with PknLS and Wild type in the presence of 1mM and 10mM IPTG.

Figs. 13 (a-c): Survival kinetics of Wild type, PknL-S and PknL-AS at different concentration of IPTG



Log₁₀ CFU/ml of the wild type, PknLS and PknLAS
 a: Log₁₀CFU/ml at 0mM IPTG
 b: Log₁₀CFU/ml at 1mM IPTG
 c: Log₁₀CFU/ml at 10mM IPTG

The data is representative of three independent experiments. There was a significant decrease ($p < 0.01$ to $p < 0.001$) in the growth of PknLAS compared with PknLS and Wildtype after day 10, when induced with 1mM and 10mM IPTG. Error bars represent the standard error of the means.

Fig. 14: Survival kinetics at pH 5.5 and pH 7.0



Log₁₀ CFU/ml of the wild type, PknLS and PknLAS at 10mM of IPTG

a: Log₁₀ CFU/ml at pH 5.5

b: Log₁₀ CFU/ml at pH 7.0

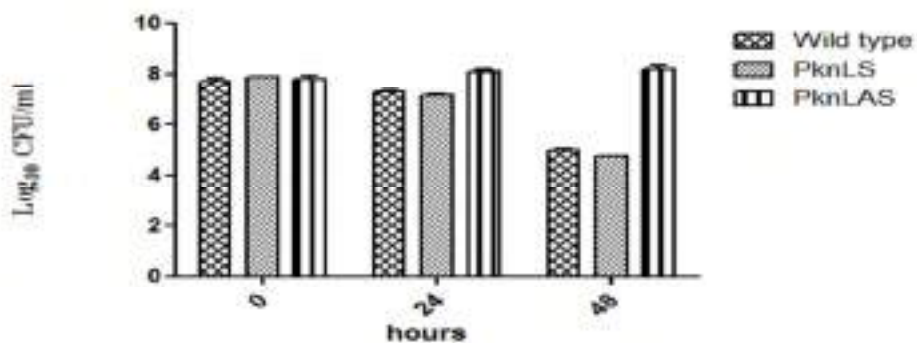
The data is representative of three independent experiments. There was a significant decrease ($p < 0.01$ to $p < 0.001$) in the viability of PknLAS compared with PknLS and Wildtype after day 10. Error bars represent the standard error of the means.

Fig. 15: SDS(0.01%) sensitivity



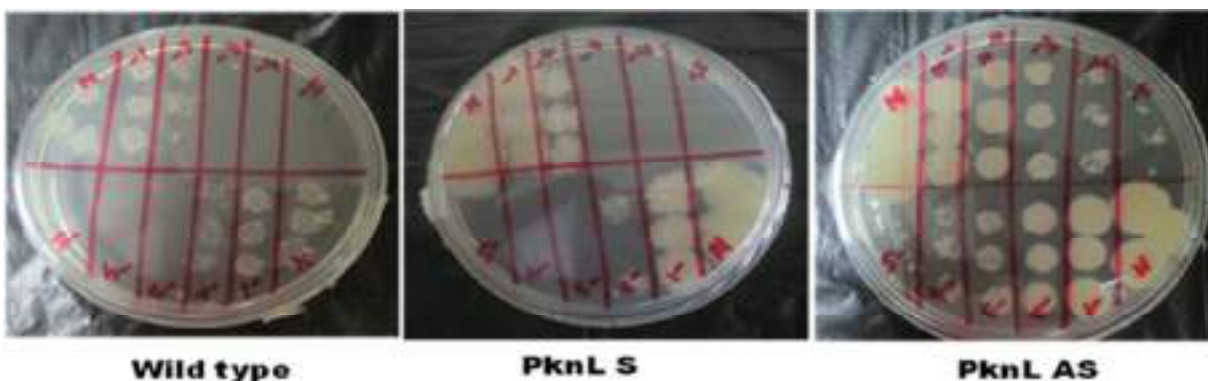
Sensitivity of Wildtype, PknLS and PknLAS to SDS (0.01%)

Fig. 16: Lysozyme sensitivity



PknLAS is resistant to lysozyme. The lysozyme sensitivity of bacteria was monitored in the presence of 7H9 broth with 2.5mg/ml of lysozyme . The data is representative of three independent experiment..There was asignificant increase ($p < 0.01$ to $p < 0.001$)in the growth of PknLAS compared with PknLS and Wildtype. Error bars represent the standard error of the means

Fig. 17: Colony morphology after lysozyme stress



Lysozyme sensitivity of Wild type, PknLS and PknLAS after 48hrs incubation with 2.5mg/ml of lysozyme in 7H9 media

(ii) **Starvation survival response of *pknL* in *M.tuberculosis***

Principal Investigator : Dr. Sujatha Narayanan
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Co-Investigator : Ms. Ahmed Kabir Refaya
Source of funding : ICMR Fellowship / ICMR-Intramural
Study Period : 2014- 2015

Background: *M. tuberculosis* is exposed to low or restricted nutrient concentrations in the host and is able to survive these conditions for long periods of time. Little is known about the condition in which the bacilli survive, although laboratory models have shown that *M.tb* can exist in a non-growing, drug-resistant state that may mimic persistence *in vivo*. Bacterial cells enter into the stationary phase due to limitations in major nutrients such as carbon, nitrogen and phosphorous or in trace elements. There is limited understanding of how bacteria sense environmental changes and thereby produce signals for the genetic machinery to respond appropriately. *In vitro* models that mimic the persistent state are required for the identification and testing of novel agents. Serine/threonine protein kinases play a pivotal role in sensing and transmitting the environmental cues into cellular process. Involvement of *pknL* in the starvation response of *M. smegmatis* has already been analysed. We attempted to investigate the role of *pknL* in the starvation response of *M. tuberculosis*.

Aim: To analyse the survival pattern of *pknL* in completely starved and phosphate starved media

Methods: All the strains wild type, *pknL*-S and *pknL*-AS were grown in supplemented Middlebrook 7H9 broth (7H9 broth, Difco), modified 7H9 broth with 0uM Pi (Pi depletion), and 1XPBS containing 0.05% Tween-80 (nutrient starvation, NS) used to study the survival pattern of the gene. Pi-depleted broth was prepared by reconstituting Middlebrook 7H9 broth except for the phosphate buffering components, which were replaced with 20 mM MOPS pH 6.6. Initially all the strains were allowed to grown in 7H9 medium supplemented with 0.5 % glycerol, 0.05 % Tween 80, and 10 % ADS along with 50 µg/ml of hygromycin until log phase. IPTG was used in the concentration of 10mM as an inducer. Later the cultures were pelleted down, washed thrice with the required medium (phosphate depleted medium and nutrient starved medium) and diluted to an OD₆₀₀ of 0.2. The growth or lysis of the cells were monitored by measuring the OD₆₀₀ and

the viability of the cultures were analysed by performing a colony count in nutrient rich 7H10 agar plates at different time points (Days 0, 2, 5, 7, 10, 14 and 21).

Results: There was a clear lysis observed in all the three strains in nutrient starved media (Fig. 18a). After day 10 there was a slight increase in growth in case of wild type and *pknL-S* whereas *pknL-AS* was not able to grow in the absence of nutrients. The viability of the strains exhibit the *in vivo* phenomenon of reactivation of the mycobacteria when it encounters a favourable environment (nutrient rich). The survival pattern was also similar wherein the strains wild type and *pknL-S* were able to revive its growth in nutrient rich medium but *pknL-AS* was unable to revive and there was no colony growth observed on day 21 (Fig. 18b).

In case of phosphate starvation there was a visible growth until day 5 followed by a steep decline on day 7 and again an increase in growth was noticed for all the three strains except for *pknL-S* which showed a

significant increase in growth after day 10 compared to wild type and *pknL-AS* (Fig. 19a). The viability of all the strains was good upto day 5, but *pknL-AS* showed a significant decrease in its ability to revive its growth after day 5 (Fig. 19b).

Conclusions: There is evidence that *M. tuberculosis in vivo* survives in the nutrient limiting condition and are able to reactivate its growth under favourable conditions. Our data shows the involvement of *pknL* in two of the *in vitro* culture models employed to observe the persistence of the bacilli based on phosphate starvation and complete nutrient starvation. The growth pattern exhibited by *pknL-AS* shows that *pknL* is required for survival under starvation conditions which in turn shows that it is responsible for the persistence inside the host. Finally, the death of the *pknL-AS*, which was unable to revive its growth upon nutrient deprivation, suggests that this could be an essential step in the adaptation process upon the shift down of *M. tuberculosis* to non-replicating state.

Fig. 18 (a&b): Growth and survival kinetics of wild type, PknL-S and pknL-AS in completely starved medium

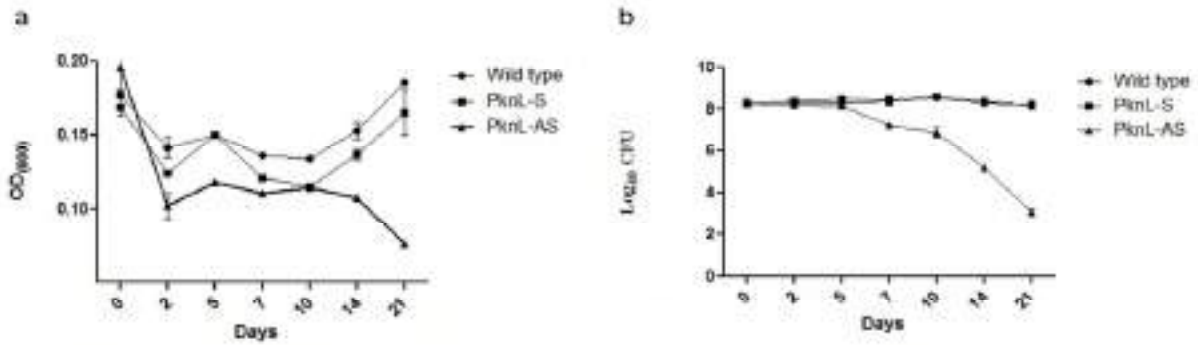


Figure a : Growth rate of Wild type, *PknLS* and *PknLAS* in nutrient starved medium

b : Viability of Wild type, *PknLS* and *PknLAS*

The data is representative of three independent experiments..There was a significant decrease ($p < 0.01$ to $p < 0.001$) in the growth and viability of Cells in *pknL AS* compared with *pknLS* and wild type .when induced with 10mM IPTG. Error bars represent the standard error of the means.

Fig. 19 (a&b): Growth and survival kinetics of wild type, PknL-S and pknL-AS in phosphate starved medium

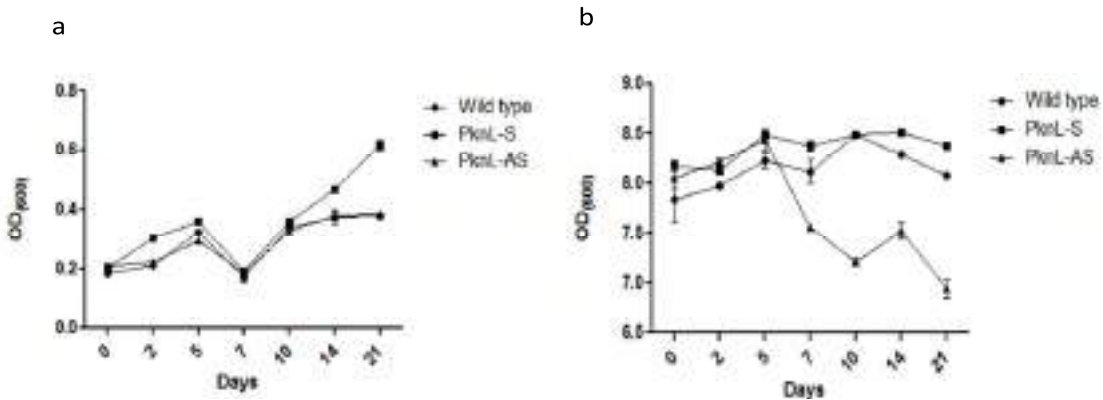


Figure a : Growth rate of Wild type, *PknLS* and *PknLAS* in phosphate starved medium

b : Viability of Wild type, *PknLS* and *PknLAS*

The data is representative of three independent experiments..There was a significant increase ($p < 0.01$ to $p < 0.001$) in the growth of *pknLS* when compared to Wild type and *pknLAS*. The viability of Cells in *pknL S* and Wild type improved except for *pknLAS* which lagged behind significantly ($p < 0.01$ to $p < 0.001$) when induced with 10mM IPTG. Error bars represent the standard error of the means.

iii) The *M. tuberculosis* PknI/DacB2 double knockout strain was attenuated *in vitro* and *in vivo* models

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(email: sujathan@nirt.res.in)
Co- Investigator : Mr.K Srinivasan
Source of funding : ICMR Fellowship / ICMR-Intramural
Study Period : 2010-2014

Background: The physiological roles of several mycobacterial STPKs and Penicillin Binding Proteins (PBPs) are connected to cell division/shape and cell envelope biosynthesis but the exact mechanisms remain unexplored. Earlier, we reported the construction of double knockout (DKO) strain lacking PknI (STPK) and DacB2 (PBP) and its phenotypic characterization.

Aim: To evaluate the virulence of DKO strain *in vitro* and *in vivo* models

Materials and Methods:

1. Infection of human THP-1 macrophages and bacterial enumeration:

Briefly, THP-1 macrophages were seeded at 1×10^6 cells per well in 24-well tissue culture plates and differentiated to macrophages by using 30 nM phorbol 12-myristate 13-acetate (PMA) (Sigma, India) for 16 hrs at 37°C in 5% CO₂. The monolayers were infected in triplicates with wild-type H37Rv, DKO, Δ PknI, Δ DacB2 and DKO comp separately, at a multiplicity of infection (MOI) of 1:10 for 4 h at 37°C. The infected macrophages were then

incubated with fresh RPMI supplemented with 10% FBS at 37°C in the presence of 5% CO₂. At designated time points, day 0, 1, 3, 5 and 7, macrophages were lysed and intracellular bacteria were enumerated by plating appropriate dilutions on MB 7H10 agar.

2. Estimation of viability under *in vitro* stress conditions:

For testing susceptibility to reactive nitrogen intermediates (RNI), bacterial suspensions washed and resuspended in the acidified medium (MB7H9-Tween-ADS broth adjusted to pH 5.4), diluted as necessary and divided into two 10ml aliquots. Sodium nitrite (3mM final concentration) was added to one of the aliquots, whereas the other aliquot served as untreated control. The treated cultures were then incubated at 37°C for 3 days, serially diluted and plated. For testing susceptibility to reactive oxygen intermediates (ROI), mycobacterial strains prepared as above but in non-acidified medium were exposed to hydrogen peroxide (0, 5 and 10 mM). The stress was applied for 24 hrs, and the

cultures were then serially diluted and plated to determine viability.

3. *In vivo* guinea pig experiments:

Pathogen-free out-bred female guinea pigs of the Duncan-Hartley strain in the weight range of 250 to 350 g were housed in stainless steel cages and were maintained in a biosafety level III facility at National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra, India. To study the influence of PknI/DacB2 double deletion on the growth and pathogenesis of *M. tuberculosis*, guinea pigs were infected by the aerosol route (Inhalation Exposure System, Glasscol Inc., IN, USA) with 10 to 30 bacilli of either wild type H37Rv, DKO, Δ PknI, Δ DacB2 and DKO comp strains. Animals (n=5) were euthanized at 5 weeks and 10 weeks post infection by i.p. injection of Thiopentone sodium (100mg/kg body weight) (Neon Laboratories Ltd., India). After dissecting the animals, the bacterial burden was seen in lungs and spleen.

Results:

1. DKO strain exhibits a severe growth defect in human THP-1 macrophages:

The wild-type H37Rv and DKO comp strains grew normally in THP-1 macrophages upto 7 days post-infection. During this time period, Δ DacB2 and Δ PknI showed a better growth inside THP-1 cells

compared to wild type H37Rv strain. Initially, upto 1 day post-infection, no significant difference was observed in the growth of DKO strain when compared with the wild type H37Rv. However, thereafter, the wild-type H37Rv continued to grow normally but the DKO strain exhibited an almost complete attenuation of its growth. At 7 days post-infection, we observed a 6-fold difference in the CFU between DKO and the wild type H37Rv strain (Fig. 20).

2. DKO strain exhibits hypersensitivity to oxidative and nitrosative stress:

After exposure to H₂O₂, the DKO were killed 11-fold more than the H₂O₂ wild-type H37Rv, whereas the Δ PknI and Δ DacB2 mutants did not show any differences in sensitivity to H₂O₂ (Fig. 21). Similarly, after exposure to NaNO₂ the DKO are killed, 7 fold higher than the wild type H37Rv strain, whereas the Δ PknI and Δ DacB2 mutants did not show any differences in sensitivity to NaNO₂ (Fig. 22). These findings suggest that both PknI and DacB2 have a role in oxidative and nitrosative stress.

3. DKO strain was severely attenuated for growth in guinea pig tissues:

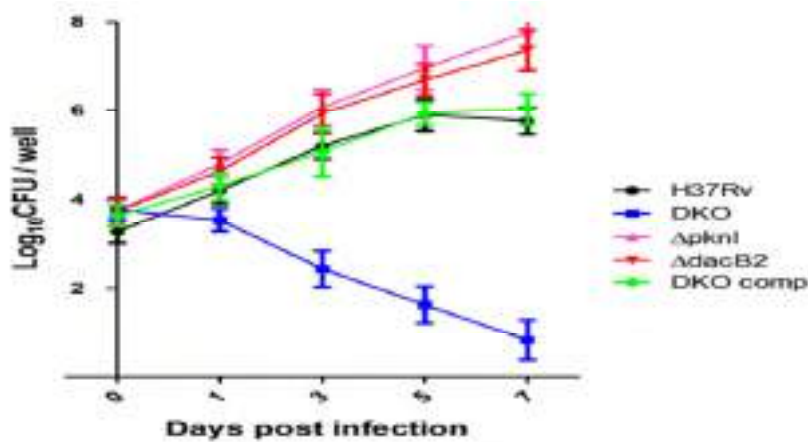
At 5th weeks post infection, the DKO infected groups showed significantly reduced bacterial burden in lungs and spleen. The

DacB2 single knockout also showed less bacterial CFUs when compared to wild type H37Rv but the CFUs was more when compared to DKO strain. The wild type H37Rv, PknI and DKO comp strains showed equivalent bacterial load in the both organs (Fig. 23). In 10th week post infection

also, we observed similar bacterial burden both lings and spleen (data not shown).

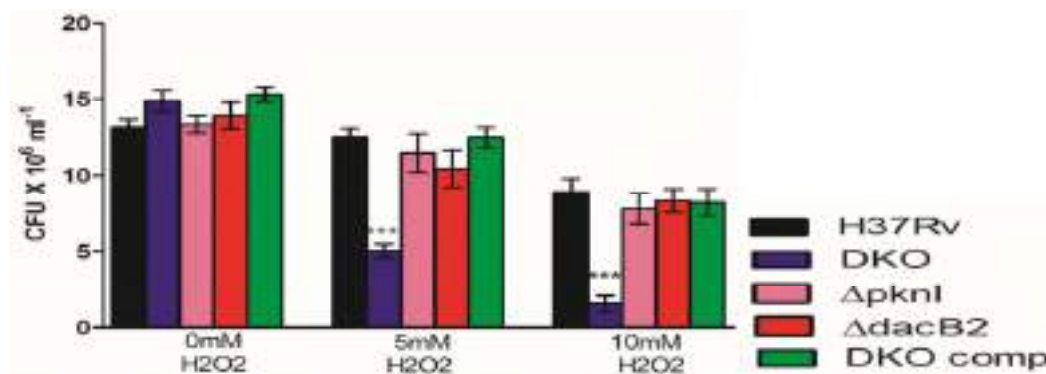
Conclusion: The attenuation of DKO strain *in vitro* and *in vivo* suggests that both PknI and DacB2 together play a role in the pathogenesis of TB.

Fig. 20: Intracellular growth of DKO within THP1 derived macrophages



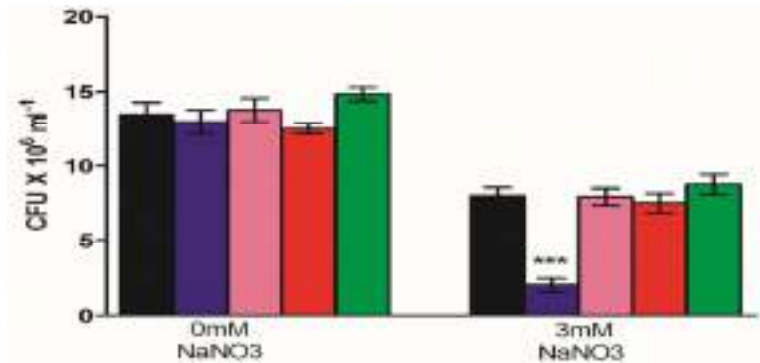
THP-1 derived macrophages were infected with wild type H37Rv, DKO, ΔPknI, ΔDacB2 and DKO comp for 4 h. Infected cells were then lysed with ice-cold 1% trypsin in RPMI and plated for counting CFUs after making serial dilutions at specified time points. All data are representative of three independent experiments carried out using biological replicates. The error bar represents the standard error of the mean.

Fig. 21: Susceptibility to reactive oxygen intermediates



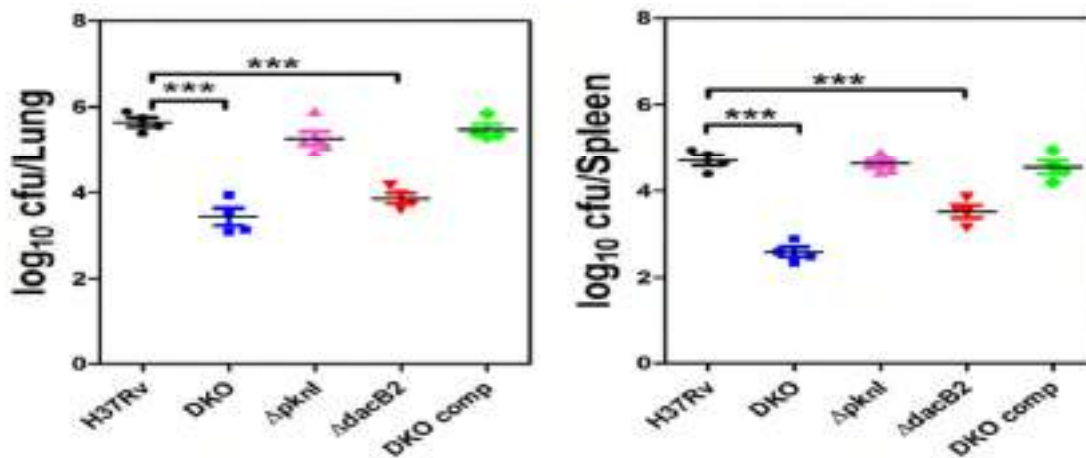
Colony forming units of H37Rv, DKO, ΔPknI, ΔDacB2 and DKO comp strains after 24 hrs exposure to 0 mM, 5 mM and 10 mM H₂O₂. Data are means ± s.d. of triplicate cultures and representative of three independent experiments.

Fig. 22: Susceptibility to reactive nitrogen intermediates



Colony forming units of H37Rv, DKO, Δ PknI, Δ DacB2 and DKO comp strains after three days exposure to 0 mM and 3 mM sodium nitrite at pH 5.5. Data are means \pm S.D. of triplicate cultures and representative of three independent experiments.

Fig. 23: Bacterial burden in lung and spleen at 5th week



(Right) This graph depicts the bacillary load in the lungs
 (Left) spleen of guinea pigs (n=5) infected with H37Rv, DKO, Δ PknI, Δ DacB2 and DKO comp at 5 weeks post-infection. Guinea pigs infected with DKO exhibited a significantly reduced bacillary load in lung and spleen compared to animals infected with wild type H37Rv. Each data point represents the log₁₀ CFU value for an individual animal, and the bars depict means (\pm standard errors) for each group.

STUDIES IN PROGRESS:

I-1: Identification of serologically reactive antigens in CF of *M. tuberculosis*

Principal Investigator	:	Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar	:	Mr.P. Pukazhvandhen
Source of funding	:	ICMR – Intramural; ICMR - Fellowship
Collaborators	:	Superintendent, Otteri Hospital
Study period	:	2010 – 2015

Background: Detection of *M. tuberculosis* (*M. tb*)-specific human antibodies has been an important 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: To identify serologically reactive fractions in CF antigens of *M. tb*

Methods: Culture filtrate antigen (CFA) of *M. tb* was subjected to preparative 2-Dimensional electro-phoresis 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 by indirect ELISA.

In the initial standardization experiments, it was observed that 110 fractions showed ≥ 2

fold difference and 224 fractions exhibited >1.5 fold but <2 fold difference in terms of OD value between TB and healthy controls. Among 224 fractions, 96 fractions were further assessed for their serodiagnostic potential with 110 sera from healthy control subjects (HCS), 110 sera from HHC, 110 sera from healthy household contacts (HHC), 88 sera from PTB patients and 88 sera from HIV-infected TB patients (HIV-TB). Cut off value for each fraction was ascertained by mean $+2SD$. Diagnostic potential was assessed for individual fractions in terms of sensitivity and specificity. Mass spectrometry analysis was carried out to identify antigens in these sero reactive fractions.

Results: Scatter dot blot of representative fractions are depicted in Figs. 24 & 25.

Fig. 24: Scatter dot plot of IEF_WGE fractions

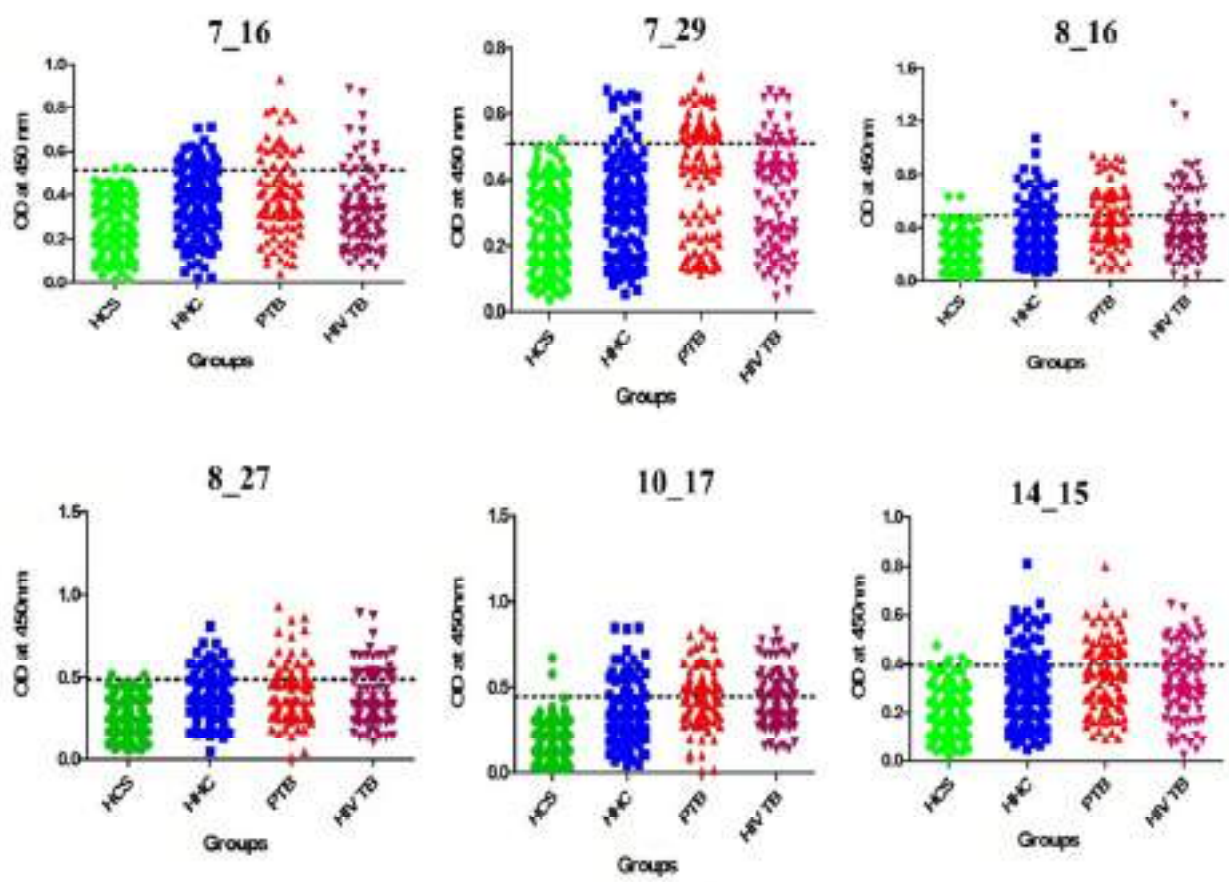
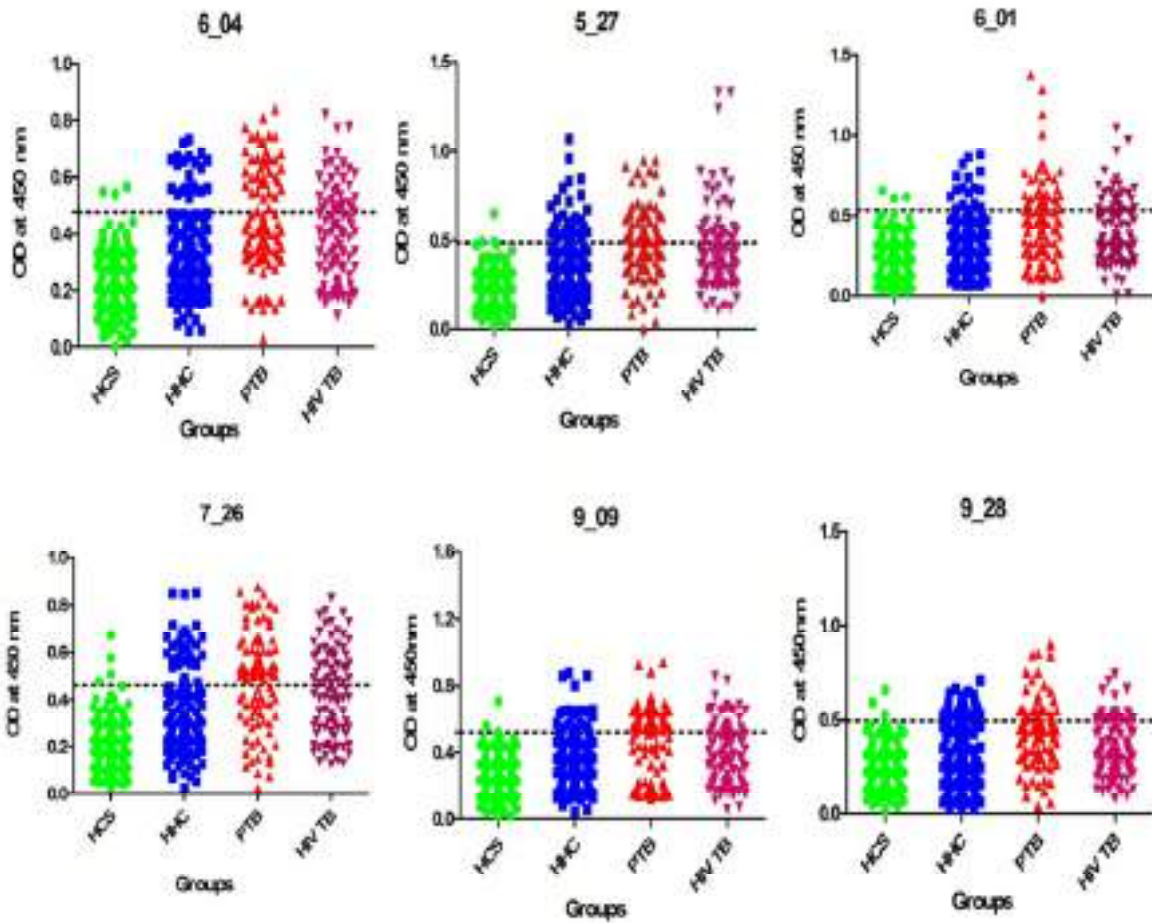


Fig. 25: Scatter dot plot of IEF_WGE fractions



Horizontal dotted line in Y-axis indicates cut off point

On assessing individual fractions, sensitivity + 6_09 + 6_11 + 8_27 + 18_21 + 10_03 and 18_19) increased sensitivity to 78% in PTB, 45% in HHC and 56% in HIV-TB with the specificity ranging from 88% to 96% in HCS. On combination, 7 fractions combination (7_16

Table 25: Sensitivity and specificity of individual fractions and combination of fractions

S.no	IEF_WGE Fractions	% Sensitivity			
		% Specificity	HCS	HHC	PTB
1	7_16	100	11.1	26.5	23.8
2	7_01	100	21.8	19.7	21.5
3	2_06	99	14.5	22.2	17.5
4	2_19	99	17.2	26.5	31
5	6_09	99	15.4	23.1	28.4
6	7_04	99	12	28.6	19.3
7	10_12	99	24.5	20.9	24.0
8	2_30	98	21.8	22.7	15.9
9	14_15	98	22.7	28.6	21.5
10	5_11	98	16.3	10.6	15.9
11	6_18	98	19.0	23.8	17.0
12	8_16	98	14.5	15.4	27.2
13	9_09	98	23.6	20.0	27.2
14	10_09	98	20.0	26.5	27.2
15	11_06	98	25.4	24.3	28.4
16	18_19	98	22.7	25.2	31.8
17	7_29	98	21.8	20.0	22.0
18	6_11	97	15.4	24.0	26.1

S.no	IEF_WGE Fractions	% Sensitivity			
		% Specificity	HCS	HHC	PTB
19	5_27	97	12.7	22.2	27.5
20	10_03	97	25.4	39.6	21.8
21	17_03	97	19.0	22.0	26.1
22	10_17	97	19.0	24.3	12
23	7_10	97	16.3	13.4	16.3
24	5_21	97	27.2	37.7	28.6
25	15_14	97	25.4	24.3	27.5
26	5_27	97	12.7	32.2	17.5
27	8_27	97	35	34.0	26.1
28	6_16	97	24.5	23.1	11.8
29	18_21	97	24.5	10.0	29.5
30	8_06	97	24.5	21.1	29.5
31	16_18	97	27.2	28.8	29.5
32	9_28	97	24.5	36.3	25.0
33	10_05	97	15.4	24.3	28.4
34	18_22	97	20.0	22.0	30.6
35	13_08	97	27.2	29.7	32.9
36	17_25	96	28.1	26.8	25.4
	7_16 + 6_09 + 6_11 + 8_27 + 18_21 + 10_03 + 18_19	90	45	78	56

Exploration of these serologically reactive fractions (7_16 + 6_09 + 6_11 + 8_27 + 18_21 + 10_03 and 18_19) by LC-MS/MS method identified 37 antigens.

Most of these sero reactive fractions contained already reported immunodominant antigens such as phoS1, adk, bfrB,

dnaK, fbpA, Frr, GlcB, glnA1, groEL2, grpE, hspX, icd-2, katG and lppZ. But they also contains 7 novel B cell antigens such as Rv1411c, Rv0815c, Rv1630, Rv2185c, Rv3914, Rv2721c, Rv3036c which are not reported elsewhere (Table 26).

Table 26: Antigens identified in seroreactive fractions

S.No	Protein name	Gene number	Molecular weight
1	acpM	Rv2244	12473.8
2	adk	Rv0733	20075.6
3	bfrB	Rv3841	20423.6
4	cysA2	Rv0815c	30997.2
5	dnaK	Rv0350	66812.9
6	fadA3	Rv1074c	42638.1
7	fbpA	Rv3804c	35668.3
8	fir	Rv2882c	20809.9
9	GlcB	Rv1837c	80402.0
10	glnA1	Rv2220	53522
11	groEL2	Rv0440	56709.3
12	grpE	Rv3418c	10754.1
13	hspX	Rv2031c	16209.6
14	Rv2721c	Rv2721c,	82552.2
15	katG	Rv1908c	80555.9
16	lppZ	Rv3006	38734.2
17	lpqH	Rv3763	15096.2
18	LprA	Rv1270c	24843.7
19	modD	Rv1860	32702.8
20	mpt53	Rv2878c	18364.9
21	mpt64	Rv1980c	24805.3

S.No	Protein name	Gene number	Molecular weight
22	phoS1	Rv0934	38211.0
23	pks13	Rv1815	186428
24	ppa	Rv3628	18278.3
25	ProA	Rv2427c	43774.6
26	rpsA	Rv1630	53183.5
27	sucB	Rv2215	57070
28	wag31	Rv2145c	28259.6
29	hypothetical protein	Rv1926c	16495.8
30	hypothetical protein	Rv3874	10775.6
31	TB16.3	Rv2185c	17200.5
32	TB22.2	Rv3036c	16617
33	hypothetical protein	Rv1926c	16495.8
34	ESAT-6	Rv3875,	6000.2
35	LprG	Rv1411c,	72321.5
36	hypothetical protein	Rv3881c	47575.7
37	trxC	Rv3914,	56862.2

Conclusion: These seven fractions were identified as serologically reactive fractions and the antigens present in these fractions

would be the promising candidates for serodiagnosis of TB.

I-2: Multiple cytokine response to immunoproteomically identified human T-cell antigens of *M. tuberculosis*

Principal Investigator	:	Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar	:	Ms. Maddineni Prabhavathy
Collaborators	:	Superintendent, Otteri Hospital
Source of funding	:	CSIR Fellowship / ICMR Intramural
Study period	:	2010-2015

Background: The enormous reservoir of latent TB infection (LTBI) poses a major hurdle for global TB control. The existing TST and IFN- γ release assays (IGRAs) are found to be suboptimal for LTBI diagnosis. Previously we had taken an immunoproteomic approach and identified 10 protein fractions (contains 16 antigens), which are solely recognized by LTBI. Initially we had estimated the diagnostic ability of IFN- γ response against these 16 CS antigens and listed the most suitable biomarker for LTBI diagnosis (NIRT AR 2013-2014). In line with this study, we have also evaluated other cytokine responses that are associated with LTBI and PTB.

Methods: In a cohort of 35 HHC and 40 PTB, Tumor necrosis factor-alpha (TNF- α), IL-2, IL-6, IL-8, IL-10 and IL-12p40 responses were measured against 16 antigens by using 1:10 diluted whole blood assay. Mann-Whitney statistical test was used to compare the groups. Receiver-operating-characteristic (ROC) curves were used to determine the cut-off points and

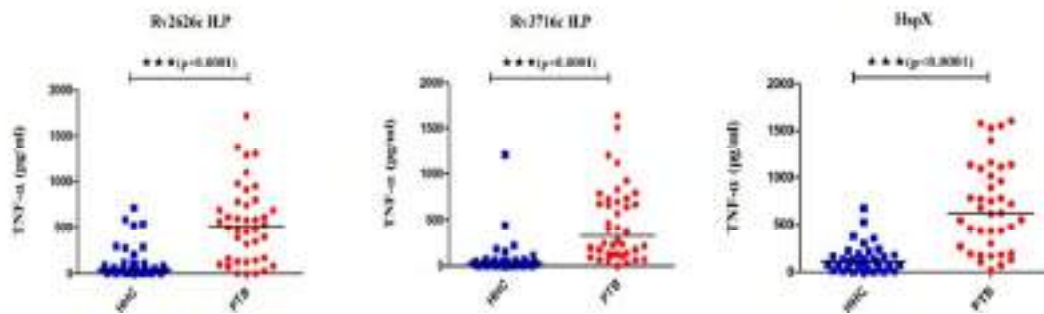
discriminative ability was evaluated by the area under the ROC curve (AUC).

Results: Among the various cytokines, antigen specific TNF- α and IL-10 levels were significantly elevated in PTB compared to HHC ($p < 0.05$). Out of 16 CS antigens, TNF- α response to Rv3716c, HspX and Rv2626c expressed highest positivity in PTB group (Fig. 26). Out of 40 PTB patients, Rv3716c and HspX were positive in 29 (72.5%, 95% CI: 57.03 to 84.02); and Rv2626c was positive in 28 (70%, 95% CI: 54.47 to 82.03) PTB patients respectively. In case of IL-10, only ESAT-6, TB8.4, Rv3716c and AcpM showed significantly elevated levels in PTB compared to HHC ($p < 0.05$), where ESAT-6 showed a maximum of 84.62% positivity in PTB (Fig. 27). Antigen specific IL-2 response did not differ between the study groups ($p > 0.05$). The remaining antigen specific (IL-6, IL-8 and IL-12p40) cytokine levels were higher in HHC compared to PTB; however they showed lesser positivity in HHC compared to IFN- γ .

Conclusion: From this study, we concluded that Rv3716c or HspX antigen specific TNF- α ; and ESAT-6 specific IL-10 response can be used as biomarkers for active TB diagnosis. Antigen specific IL-2

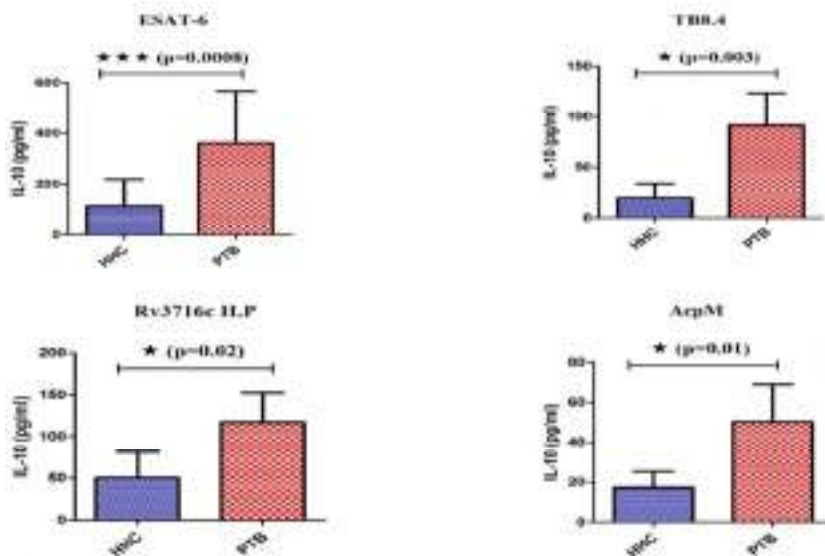
might not be a good biomarker for differential diagnosis of LTBI and PTB. Compared with IFN- γ , the other cytokines like IL-6, IL-8 and IL-12p40 may not be promising biomarkers for LTBI diagnosis.

Fig. 26: TNF- α response to contact specific antigens of *M. tuberculosis*



Each dot represents the study subject and the horizontal line indicates the median value of TNF- α secretion in pg/ml. The levels of antigen specific TNF- α are elevated significantly in PTB compared to HHC ($p < 0.05$).

Fig. 27: IL-10 response to contact specific antigens of *M. tuberculosis*



Antigen specific IL-10 secretion is associated with PTB compared to HHC. Among the 16 CS antigens, only ESAT-6, TB8.4, Rv3716c and AcpM showed elevated secretion of IL-10 in PTB compared to HHC.

I-3: Dormancy associated antigens of *M. tuberculosis*

Principal Investigator	:	Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar	:	Mr.D. Santhi
Collaborators	:	Superintendent, Otteri Hospital
Source of funding	:	ICMR Task Force – Extramural
Study period	:	2011 – 2015

Background: The mycobacterial genes 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 strain was used since literature has not reported dormancy associated antigens from clinical strains.

Aim: To identify differentially regulated genes under hypoxia from laboratory strain H37Rv and 2 clinical strains (S7 and S10) 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). RNA was isolated from pellets collected by standard Trizol reagent method (Sigma Aldrich, USA). 10-200ng of isolated RNA was used for cDNA synthesis by WT primer method and labeled with cy3 (aerobic cultures) and cy5 (anaerobic cultures) by two color labeling

kit of Agilent Technologies, USA. Labeled RNAs were hybridized on a 60mer oligonucleotide based custom array chip from Agilent Technologies in 8x15K format. The image analysis was done using Feature extraction tool version 9.5.3.1 (Agilent Technologies, USA).

The aerobic and anaerobic culture pellets were resuspended in lysis buffer containing 20mM Tris-HCl, 100mM Dithiothreitol (DTT), 1mM PMSF, complete protease inhibitor cocktail (Sigma Aldrich, USA) and 10mg/ml lysozyme and incubated for 15 mins in ice. The cell membrane was then disturbed by ultra sonication (amplitude 40%) and homogenate was collected after high speed centrifugation (18000g for 25 mins). Four hundred (400ug) micrograms of cytosolic fraction proteins from H37Rv, S7 and S10, from both aerobic and anaerobic features were separated by 2DE using 17cm immobilized pH gradient (IPG) strips of pH range 4-7. Spots were then analyzed by mass spectrometry.

Results: Total numbers of differentially regulated genes were discussed in the earlier

annual report. We compared down-regulated genes of H37Rv (502) with the upregulated genes of S7 (454) and S10 (1463) under hypoxia. Total of 10 genes were found in common as represented in H37Rv but expressed in both the clinical isolates under hypoxia (Fig. 28).

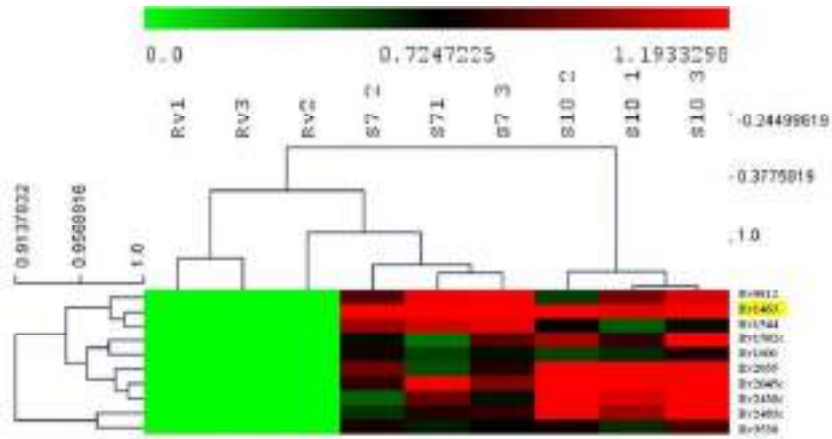
By comparing the protein spots from aerobic and anaerobic cultures of H37Rv, a total of 15 spots that either over-expressed (encircled) or newly appeared (boxed) during hypoxia were identified (Figs. 29 a&b). Eleven protein spots were seen to be increased in intensity and 6 new spots appeared as a result of oxygen depletion from media in S7 (Figs. 29 c&d). Hypoxia induced protein expression in the clinical strain S10 is marked in Figs. 29 e&f.

Conclusion: Ten genes Rv0812, Rv1463, Rv1544, Rv1582c, Rv1601, Rv2035,

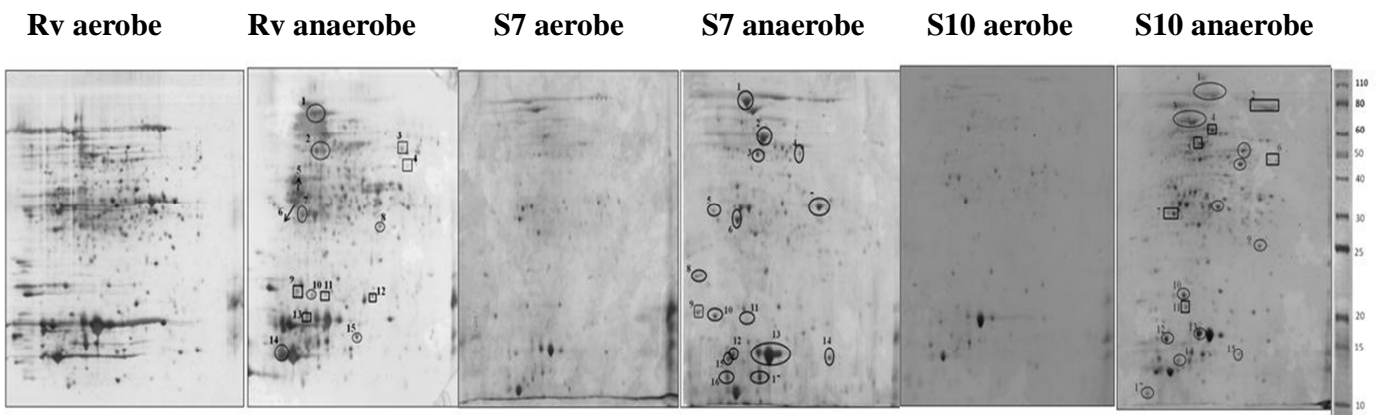
Rv2045c, Rv2430c, Rv2483c and Rv3538 were found to be up-regulated in clinical isolates but they were depressed in the laboratory strain H37Rv. This highlights the need of using most prevalent clinical isolates for *in vitro* studies to minimize strain based variations during vaccine development.

Proteins like Rv0440 and Rv2031c were found as commonly expressed protein in clinical and laboratory strains of *M.tb* and can be targeted for vaccine or drug development. Apart from these, the protein spots Rv0462 (Dihydrolipoamide dehydrogenase) and Rv1240 (Malate dehydrogenase) were found common between H37Rv and S10. This can also serve as vaccine candidates.

Fig. 28: Heat map of down regulated genes of H37Rv but expressed in S7 and S10 under hypoxia



Figs. 29 (a-f): 2DE gels of *M. tuberculosis* strain H37Rv, clinical isolates S7 and S10



I-4: Diagnostic evaluation of novel T- cell antigens of *M. tuberculosis*

Principal Investigator	:	Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar	:	Mr. Pathakamuri Balaji
Collaborators	:	Superintendent, Otteri Hospital
Source of funding	:	ICMR Fellowship / ICMR-Intramural
Study period	:	2010-2015

Background: One third of the world's population is estimated to harbor LTB1. About 10% of them have a life time risk of developing active TB (PTB). Currently used TST and IGRA are inefficient tools for identifying LTB1. Thus diagnosis of LTB1 among the infected is crucial in effective TB control. CFPs of *M. tuberculosis* have been reported as diagnostic markers for TB infection. In our earlier study we were identified Rv2204c (hypothetical protein), Rv0753c (mmsA) and Rv0009 (PpiA) as a novel T-cell antigens, which induced higher IFN- γ response in LTB1 than PTB. Purification of these recombinant proteins and IFN- γ response against these antigens in 35 HHC and 39 PTB has been reported in 2013-14 report. In continuation of this study, we further assessed diagnostic potential of antigen-specific cytokines other than IFN- γ for identifying LTB1.

Aim: To evaluate whether the *M. tb* antigen specific cytokine response can be used as a

diagnostic marker for identifying LTB1 in high endemic setting

Methods: Along with standard antigens (ESAT-6 & CFP-10) tested, Rv2204c, Rv0753c & Rv0009 specific cytokines were measured in the supplement of diluted whole blood, following 6 days stimulation. In this study we measured tumor necrosis factor- α (TNF- α) IL- β , IL-2, IL-6, IL-8, IL-10, IL-12p40, IL-17 and interferon gamma – inducible protein [IP10], monocyte chemotactic protein-1 [MCP-1] and MCP-2 by ELISA (Fig. 30). The differences in various cytokine secretions among the study groups were assessed by Mann-Whitney U test. Cut-off values were determined by ROC.

Results: Although there were altered response of all the cytokines against all antigens, only TNF- α , IL-6, IL-8, IL-12p40, MCP-1 and MCP-2 had shown significant difference between HHC and PTB ($p < 0.05$). In contrast to other cytokines, TNF- α showed significant increase in PTB

compared to HHC against all the antigens. Compared to ESAT-6 and CFP-10, tested antigens showed more discriminatory cytokine response between HHC and PTB. Among the cytokines, IL-12p40 showed higher positivity in HHC than PTB. Rv2204c-specific IL-12p40 showed 89% (31/35) positivity in HHC whereas in PTB it showed 36% (14/39) positivity. Rv0753c

and Rv0009 both showed 86% (30/35) positivity in HHC whereas in PTB, showed 41% (16/39) and 38% (15/39) positivity respectively (Table 27).

Fig. 30: IL-12p40 response to *M. tb* antigens

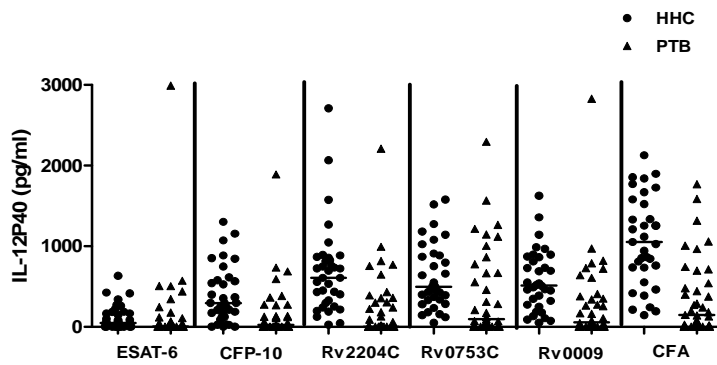


Table 27: Diagnostic performances of cytokines and chemokines in response to ESAT-6, CFP-10, Rv2204c Rv0753c and Rv0009

Protein name	Positivity in HHC (N=35)	Positivity in PTB (N=39)	Cut off value (pg/ml)	Area under the Curve (AUC)
TNF-α				
ESAT-6	14 (5/35)	51 (20/39)	61.2	0.715
CFP-10	14 (5/35)	51 (20/39)	164.8	0.712
Rv2204c	14 (5/35)	59 (23/39)	242.4	0.698
Rv0753c	11 (4/35)	61 (24/39)	535.5	0.798
Rv0009	14 (5/35)	51 (21/39)	381.8	0.664
IL-6				
ESAT-6	71 (25/35)	64 (25/39)	8.0	0.6
CFP-10	86 (30/35)	61 (24/39)	201.1	0.676
Rv2204c	82 (29/35)	72 (28/39)	179.3	0.643
Rv0753c	86 (30/35)	56 (22/39)	1081	0.732
Rv0009	86 (30/35)	41 (16/39)	1046	0.767
IL-8				
ESAT-6	60 (21/35)	28 (11/39)	53.1	0.654
CFP-10	71 (25/35)	41 (16/39)	407.0	0.705
Rv2204c	60 (21/35)	38 (15/39)	82.1	0.597
Rv0753c	60 (21/35)	31 (12/39)	350.8	0.682
Rv0009	71 (25/35)	56 (22/39)	27.00	0.663
IL-12p40				
ESAT-6	69 (24/35)	49 (19/39)	7.1	0.57
CFP-10	89 (31/35)	46 (18/39)	27.0	0.604
Rv2204c	89 (31/35)	36 (14/39)	192.9	0.827
Rv0753c	86 (30/35)	41 (16/39)	221.8	0.703
Rv0009	86 (30/35)	38 (15/39)	171.4	0.788
MCP-1				
ESAT-6	25 (5/20)	15 (3/20)	426.2	0.516
CFP-10	50 (10/20)	45 (9/20)	48.0	0.542
Rv2204c	60 (12/20)	20 (4/20)	179.9	0.69
Rv0753c	60 (12/20)	20 (4/20)	180.9	0.697
Rv0009	-	-	-	-
MCP-2				
ESAT-6	10 (02/20)	55 (11/20)	9.2	0.717
CFP-10	10 (02/20)	50 (10/20)	33.9	0.67
Rv2204c	60 (12/20)	25 (5/20)	718.3	0.683
Rv0753c	60 (12/20)	25 (5/20)	170.7	0.681
Rv0009	80(16/20)	40 (8/20)	11.93	0.777

Conclusion: Measurement of Rv2204c, Rv0753c and Rv0009-specific IL-12p40 response may be a useful marker for

identifying LTBI. Future work will require prospective evaluation of our findings in an independent validation cohort.

I-5: Structural characterization of three essential genes from *M. tuberculosis*

Principal Investigator : Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar : Ms.G. Akilandeswari
Source of funding : DST INSPIRE Fellowship / ICMR-Intramural
Study period : 2011-2015

Background: An important issue to consider in the development of new anti-TB therapeutics is the phenotypic drug resistance of *M. tuberculosis* organisms in the nonreplicative state, which are genetically indistinguishable but distinct from actively multiplying *M. tuberculosis* organisms. Proteomics and structural biology have a clear role to play in this endeavor. In recent years, several papers have reported the identification of potentially interesting new protein drug targets. Understanding the function of these proteins, and indeed also that of existing protein drug targets, often requires detailed knowledge of their structure.

Aim: To clone, overexpress and purify three genes (Rv1294, Rv2515c and Rv2949c) from *M. tuberculosis*, study the structure of their proteins and check whether these proteins can be used as drug targets for TB

Methodology: All the three genes are PCR amplified using H37RV strain of *M. tuberculosis* as template. Cloning of each individual gene is performed using pET 30a vector. The cloned genes are transformed into the expression strain (BL21 star DE3) and the proteins are over-expressed by IPTG induction. The proteins are then purified by Ni-NTA column (affinity chromatography).

Results: Amplification of all the three genes was done by polymerase chain reaction in appropriate conditions of melting temperature (Fig. 31). The amplified and PCR purified genes of interest were cloned into pETa vector and the recombinant vectors were transformed into DH5 α . A colony PCR was performed to confirm the presence of the genes of interest in the colonies (Fig. 32) and the recombinant vectors were transformed into the expression strain (BL21 star DE3).

Fig. 31: PCR amplification of Rv1294 and Rv2515c

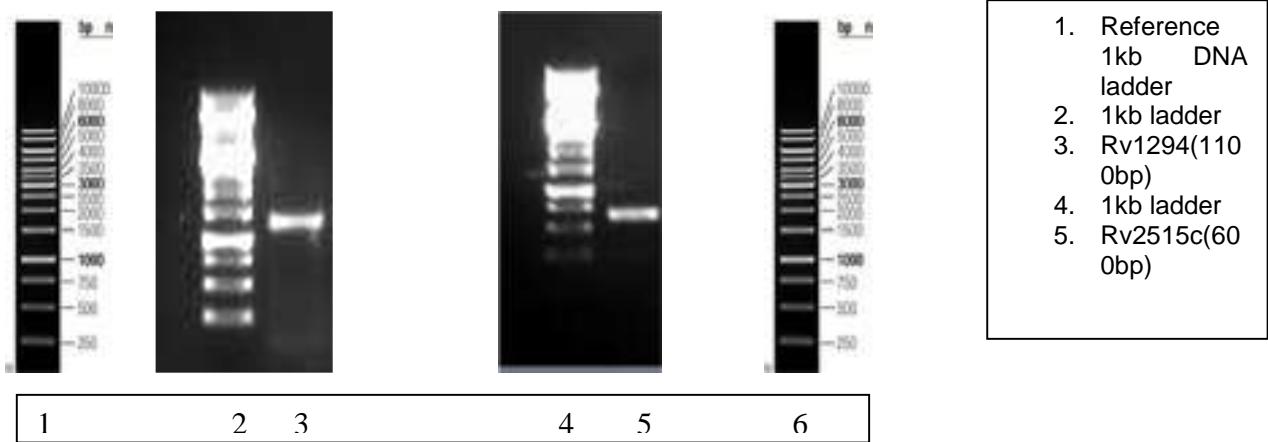
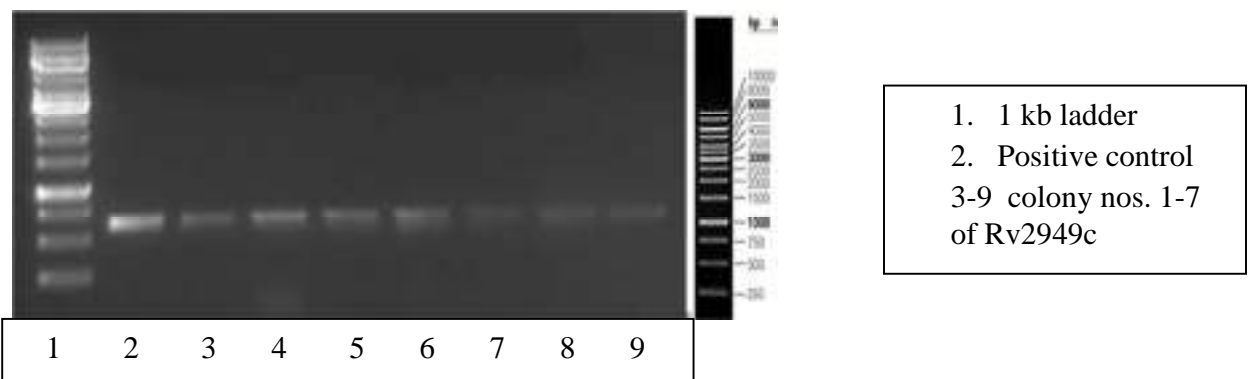


Fig. 32: Colony PCR of the recombinant genes after cloning into pETa vector



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.

I-6: Physiological effects of Rv2159c of *M. tuberculosis* by antisense RNA control

Principal investigator : Dr. Sujatha Narayanan
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Co- Investigator : Mr.V. Arunkumar
Source of funding : ICMR Fellowship / ICMR-ntramural
Study period : 2012-2015

Background: In response to infection, *M. tuberculosis* encounters endogenous reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by the host. As an intracellular bacterium, *M. tuberculosis* uses various defense strategies to overcome the toxicity produced by superoxides and nitric oxides. *M. tuberculosis* strains resistant to INH drug were observed to be devoid of peroxidase activity. Mutations associated with *katG* gene were compensated by increased expression of *ahpC* and thereby controls the oxidative and nitrosative stress. Rv2159c is an AhpD homolog protein, which is predicted to be a member of a large family of proteins, known for its peroxidase activity. Rv2159c as an Alkyl hydroperoxidase, is one of the cysteine based peroxide reducing protein of *M. tuberculosis*. Recent studies from our lab show that PknI, a protein kinase that interacts with Rv2159c might have a functional role in its redox activity. However, the molecular function of Rv2159c is not studied well. In this study, the bacteriostatic and bactericidal effect of

M. tuberculosis Rv2159c to oxidative stress was studied by growth and survival kinetics studies.

Objectives: (i) To develop antisense based gene knockdown technology for Rv2159c of *M. tuberculosis* and
(ii) To phenotypically characterise the Rv2159c knockdown strain

Materials and methods:

Gene cloning: The full length Rv2159c gene from *M. tuberculosis* was amplified using the following primers, FP: 5'CCC GGA TCC CCA GGA GGG AGT CGA ATC ATG AA3' and RP: 5'CCC GAA TTC GGC CAG CGA TGA CAC CCT ACC3'. The amplified gene was inserted into pMV261 vector at *BamHI* and *EcoRI* sites to get Rv2159c in sense orientation (S-Rv2159c). The antisense oriented Rv2159c (As-Rv2159c) was also amplified using the same set of primers and inserted into pCR 2.1 TOPO cloning vector. The resulting plasmid was digested with *HindIII* and *EcoRI* enzymes and further inserted into pMV261 vector to get Rv2159c in antisense orientation. Positive clones were digestion

checked by *Sall* enzyme. The sequence confirmed clones as well as empty pMV261 vector (Rv) were then electroporated into electro competent *M. tuberculosis* H37Rv cells and plated on 7H10 agar media supplemented with OADC containing 20 µg/ml kanamycin.

RNA isolation and qRT-PCR: Total RNA was isolated from S-Rv2159c, As-Rv2159c and control pMV261 *M. tuberculosis* cells using an RNeasy kit (QIAGEN, Inc.). The RNA was subsequently treated with DNaseI at 37°C for 45 min. The DNase was then inactivated by incubation at 75°C for 10 min. RNA was quantified by using a ND-1000 Nanodrop spectrophotometer (Nanodrop Technologies). Purified RNA was stored at -80°C. For the determination of relative mRNA concentrations by quantitative reverse transcription-PCR (qRT-PCR), cDNA was synthesized with 1 µg of RNA using QuantiTect reverse transcription kit (Qiagen), according to the manufacturer's instructions. qRT-PCRs were carried out using Taqman qPCR master mix plus low ROX (Applied Biosystems) according to manufacturer's instruction using the Applied Biosystems 7500 real-time PCR system. To check for DNA contamination, control reactions for each sample was carried out in the absence of

reverse transcriptase. The amplification conditions for all reactions were 1 cycle of 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Analysis of qRT-PCR data was carried out using the comparative C_T method. For each qRT-PCR run, the calculated threshold cycle (C_T) was normalized to the C_T of the internal control 16S rRNA gene amplified from the corresponding sample. Statistical analysis was carried out using GraphPad Prism software. The data presented are averages of three independent experiments and error bars represent standard deviations.

***In vitro* growth determinations:** To determine the *in vitro* growth characteristics of the S-Rv2159c and As-Rv2159c strains, log-phase cultures grown in 7H9-ADS-T medium with 20 µg/ml kanamycin were washed and diluted in fresh 7H9-ADS-T medium with appropriate antibiotics. The bacteria were incubated at 37°C with 100 r.p.m shaking. Aliquots of 100 µl were taken at each time points for OD₆₀₀ nm. Viability was checked at each time points by measuring CFU.

Measurement of sensitivity to Cumene hydroperoxide: The S-Rv2159c, As-Rv2159c and control strains were grown to mid-log phase in 7H9-ADS-T medium and diluted with fresh medium to an OD₆₀₀ of

0.1. Cumene hydroperoxide was added at a concentration of 0, 50 and 100 $\mu\text{M}/\text{ml}$ and grown up to 7 days. The viability of the cultures was measured at 0, 2, 4 and 7 day by OD_{600} nm and CFU was determined using 7H10 agar plates.

Results:

Rv2159c gene knockdown construction:

The sense strand and antisense strands of Rv2159c was successfully cloned in pMV261 vector. The gene was placed under

a constitutive promoter, *hsp*. The sense oriented Rv2159c was inserted in *BamHI* and *EcoRI* site (Figs. 33 a&b) and the antisense oriented Rv2159c was inserted in *HindIII* and *EcoRI* site. Positive clones were digested with *Sall* enzyme. Clones with Rv2159c inserted in the sense orientation gave a fragment of ~ 780 bp and while clones with Rv2159c inserted in antisense orientation gave a fragment of ~ 250 bp confirming their orientation.

Fig. 33 (a & b): Rv2159c cloning in pMV261 vector

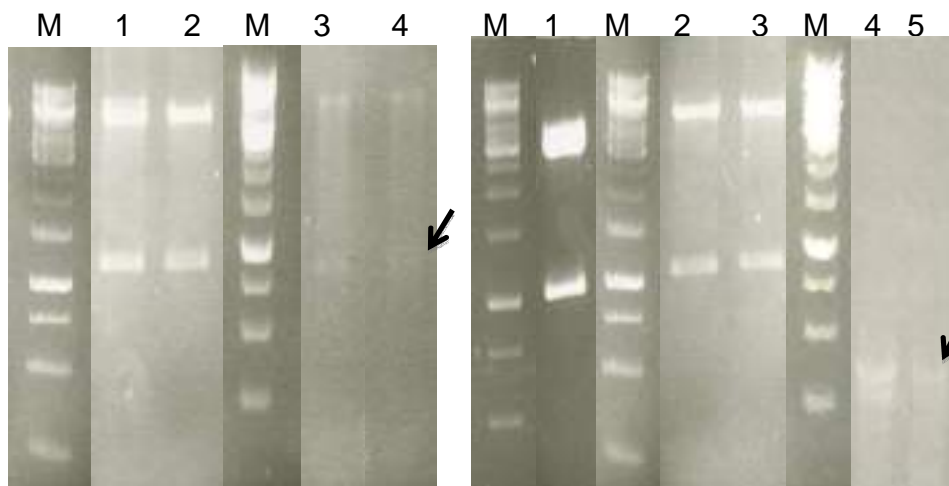


Fig. 33a: Cloning of Rv2159c in sense orientation. Lane 1 and 2, R2159c in pMV261 vector and RED with BamHI and EcoRI; lane 3 and 4, digestion with Sall;

Fig. 33b: Cloning of Rv2159c in antisense orientation. Lane 1, Rv2159c in pCR 2.1 TOPO vector and RED with HindIII and EcoRI; lane 2 and 3, Rv2159c in pMV261 vector and RED with HindIII and EcoRI; lane 4 and 5, digestion with Sall; Lane M, 1 kb DNA ladder.

In vitro growth determination: The growth of S-Rv2159c, As-Rv2159c and Rv was monitored in Middlebrook 7H9-ADS-T medium over 0, 3, 5, 7, 14 and 21 days. OD_{600} shows there was no distinguishable

growth pattern between the Rv, S-Rv2159c and As-Rv2159c strains (Fig. 34a). Survival kinetics also shows that S-Rv2159c and As-Rv2159c was unaltered as compared to Rv (Fig. 34b).

Figs. 34 (a & b): *In vitro* growth kinetics of Rv2159c differentially expressing strains in *M.tuberculosis*

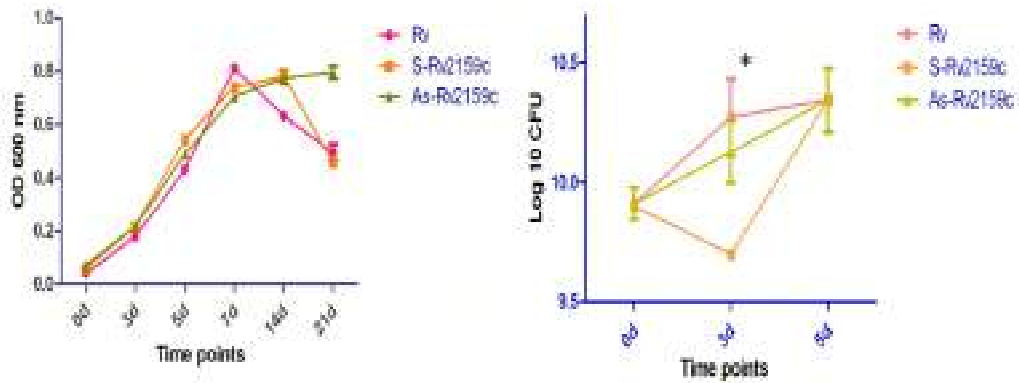


Fig. 34a: S-Rv2159c and As-Rv2159c constructs in *M. tuberculosis* were grown in 7H9 supplemented medium. Graph represents the OD₆₀₀ at different time points and represent by their mean \pm SEM from three different experiments. Comparison of Rv vs S-Rv2159c 7d shows a significance of $P < 0.05$, comparison of Rv vs S-RV2159c 5d, 14d and As-Rv2159c 7d, 14d, 21d shows a significance of $P < 0.001$.

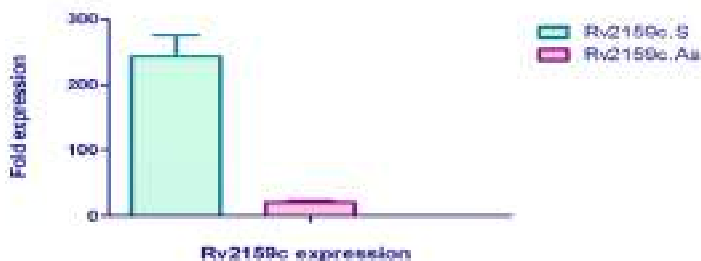
Fig. 34b: Survival kinetics of Rv2159c differentially expressing strains in *M.tuberculosis*. The S-Rv2159c and As-Rv2159c were plated in 7H10 supplemented agar and CFUs were measured at 0, 3 and 5 day and represent by their mean \pm SEM from three different experiments. *denotes degree of statistical significance, $P < 0.05$.

Quantification of Rv2159c RNA

transcript: Since there was no notable difference in growth between the strains, we quantified the Rv2159c RNA transcript in S-Rv2159c and As-Rv2159c over-expressed strains. The total RNA from S-Rv2159c, As-Rv2159c and Rv was isolated from 21 days

culture and quantified using qRT-PCR. The level of Rv2159c transcript in S-Rv2159c was noted to increase 240 fold, whereas in As-Rv2159c the transcript was maintained at 20-fold (Fig. 35). This clearly shows that antisense knockdown system for Rv2159c is well established.

Fig. 35: Quantification of Rv2159c expression



qRT-PCR for Rv2159c. Cell lysate of *M. tuberculosis* over-expressing and under-expressing Rv2159c gene was quantified at 21 days.

Sensitivity to Cumene hydroperoxide: The sensitivity of S-Rv2159c, As-Rv2159c and Rv to peroxide was tested with 50 and 100 μM of Cumene hydroperoxide in 7H9 medium. An OD_{600} show there was no much distinguishable growth pattern between the Rv, S-Rv2159c and As-Rv2159c strains at 0 and 50 μM of Cumene hydroperoxide stress. Both S-Rv2159c and As-Rv2159c strains

with 100 μM Cumene hydroperoxide at 4th day showed a significant increase in growth as comparing to Rv (Fig. 36a). This result was replicated when CFUs of S-Rv2159c and As-Rv2159c were counted at 2 and 4th day. The S-Rv2159c and As-Rv2159c strains showed increased resistance to Cumene hydroperoxide as comparing to Rv (Fig. 36b).

Fig. 36 (a & b): Sensitivity to Cumene hydroperoxide

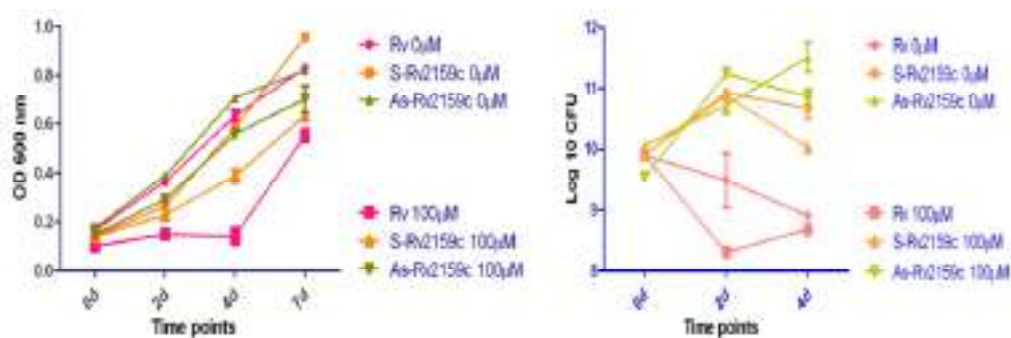


Fig. 36a: The S-Rv2159c and As-Rv2159c constructs in *M. tuberculosis* were diluted to an OD_{600} of 0.1 in complete 7H9 medium with 100 μM of Cumene hydroperoxide. A 100 μl of aliquots were removed at regular interval and OD_{600} was measured at 0, 2, 4 and 7d, represent by their mean \pm SEM from three different experiments. Comparison of Rv 0 μM vs S-Rv2159c 0 μM at 4d shows a significance of $P < 0.05$; Rv 0 μM vs S-Rv2159c 0 μM at 2d, 7d shows a significance of $P < 0.001$. Comparison of Rv 0 μM vs AS-Rv2159c 0 μM at 4d shows a significance of $P < 0.01$. Comparison of Rv 100 μM vs S-Rv2159c 100 μM at 4d and AS-Rv2159c 100 μM at 4d shows a significance of $P < 0.001$; Comparison of Rv 100 μM vs AS-Rv2159c 100 μM at 2d and 7d shows a significance of $P < 0.01$.

Fig. 36b: The S-Rv2159c and As-Rv2159c constructs in *M. tuberculosis* were diluted to an OD_{600} of 0.1 in complete 7H9 medium with 100 μM of Cumene hydroperoxide and CFU was measured at 0, 2 and 4 d, represented by their mean \pm SEM from three different experiments. Comparison of Rv 0 μM vs S-Rv2159c and As-Rv2159c 0 μM at 2d and 4d shows a significance of $P < 0.001$. Comparison of Rv 100 μM vs AS-Rv2159c 100 μM at 2d and 4d shows a significance of $P < 0.001$.

Conclusion: The differentially expressing Rv2159c was constructed and validated successfully in *M. tuberculosis*. Antisense Rv2159c grows more rapidly as compared to

Rv and S-Rv2159c, suggesting that the gene could be involved in slowing down the mycobacterial growth.

I-7: Molecular modeling of Rv2159c, *in silico* analysis and experimental validation of its interaction with PknI from *M. tuberculosis*

Principal investigator : Dr. Sujatha Narayanan
(email: sujathan@nirt.res.in)
Co-investigator : Mr.V. Arunkumar
Source of funding : ICMR Fellowship / ICMR-Intramural
Study period : 2012-2015

Introduction: Proteins control all biological processes inside the cell. They often interact with one another to perform a common function, which is crucial for all the biological processes. The duration of the protein interactions could be either transient or permanent. Studying their physical and functional interactions is critical in understanding the molecular mechanisms beyond the interaction. Protein-protein interaction and reversible phosphorylation are the principle mechanisms in mediating signal transduction. PknI, a serine/threonine protein kinase of *M. tuberculosis* is known to be important for cellular homeostasis. Previous studies from our lab shows, PknI could interact with Rv2159c and Rv0148 of *M. tuberculosis*. In this study, the PknI-Rv2159c interaction pair was further studied [for the critical amino acid residues responsible for the interaction.](#) Rv2159c, a hypothetical protein, is predicted to be an antioxidant with peroxidase activity. We performed homology modeling of Rv2159c protein and molecular docking was

performed using multiple docking servers such as Z-Dock and ClusPro. Further, the most favorable conformation of the protein - protein interaction was obtained using molecular dynamics simulation. We propose that, PknI physically interacts with Rv2159c both *in vitro* and *in silico* studies.

Objectives: [\(i\) To model *M. tuberculosis* Rv2159c protein structure *in silico* and \(ii\) To dock and identify the hot spot residues involved in PknI-Rv2159c interaction](#)

Materials and Methods:

Template selection, homology modelling and structure refinement: To find a suitable template structure for modelling Rv2159c protein, multiple programs like PSI-BLAST, PHYRE2 and I-TASSER were used. Pfam database was further explored for available PDB structures having same domain of Rv2159c. A total of 27 template structures obtained from 4 servers were selected for manual analysis. The final template (PDB Id: 3LVY) was chosen based on sequence identity, domain coverage and

E-value. 3LVY is a crystal structure of Carboxymuconolactone decarboxylase family protein from *Streptococcus mutans*. Homology model of Rv2159c protein was constructed using Modeller 9.14 module in DS. The quality of generated model was checked using PROCHECK and Errat tool. The predicted three dimensional protein structures were visualized using DS 2.1.

Protein-Protein docking: The interactions between PknI and Rv2159c structures were docked using ZDOCK 3.0.2 and ClusPro 2.0 servers.

Z-Dock: The initial docking between PknI and Rv2159c structures were done using Z-dock (3.0.2). The Z-dock is a rigid body based docking protocol, which uses a FFT algorithm to perform a 3D search in all possible binding modes in the translational and rotational space between the two protein structures. The top 10 docked structures were rescored using FiberDock.

ClusPro: ClusPro 2.0 algorithm also uses the FFT correlation approach, and it has been expanded in order to use double logical interaction potentials. ClusPro filters the docked confirmations with near-native structures and ranks them based on their clustering properties. The server outputs the top 10 to 30 docked complexes with highest ranks. By evaluating ten interaction areas

according to thermo dynamical energy calculations, areas where possibility of bonding is high, were determined.

Molecular dynamics simulations of protein complexes:

The best docked WT and mutant PknI-Rv2159c complexes were subjected to MD simulations to refine the protein-protein complexes. Amber ff99SB force field was applied for the complexes. Sodium ions were added to the system using additions module and the complex structures were pre-equilibrated with TIP3PBOX12. All simulations were run with shake on hydrogen atoms, a 2 fs time step and langevin dynamics for temperature control. Further, the production phase of the simulations was performed for a total of 5 ns with same conditions as mentioned above. The backbone RMSD of the C α carbon atoms was calculated using PTRAJ module.

Results:

Modeling and validation of Rv2159c structure:

Rv2159c was found to be a member from Carboxymuconolactone decarboxylase family. Template searching using multiple databases identifies a total of 24 template structures. The 24 template structures were superimposed using PDBefold and 9 structures were shortlisted based on secondary structure alignment and RMSD. The two templates 2GMY and

3LVY were chosen as the best aligned templates based on the threading servers. Finally, 3LVY was selected for homology modelling as it showed maximum identity comparing to 2GMV.

The target (Rv2159c) and the template (3LVY) sequences were aligned and adjusted manually to refine the alignment. Five models were generated and the model with lowest potential density function score was used for further optimization (Fig. 37). PROCHECK results shows that 98% of the residues are in the favorable region and 2% are in the allowed region. The non-bonded interactions in Rv2159c structure were analyzed using ERRAT, which calculates the overall quality factor as 100.000. Taken together, the homology modelled Rv2159c structure was shown to be a reliable model.

Figs. 37: 3D structure of Rv2159c



The 3D structure of Rv2159c was predicted using DS. 3LVY was used as a suitable template and the RMSD was found to be 0.643Å.

Identification of PknI protein binding site:

In order to focus on physiologically relevant interactions, the previously reported kinase structures in complex with proteins / peptides from PDB were analyzed. This resulted in identifying 3 predominant binding sites in kinase proteins with PDB IDs such as 1ZYS, 1WBP and 1UKH. The identified 3 binding sites were then mapped on to the 3D structure of PknI for docking Rv2159c.

***In silico* protein-protein docking:**

The energy minimized Rv2159c protein structure was docked against the PknI protein structure using Z-dock and ClusPro. Docking studies were performed with both blind and knowledge based method. The top 10 docked complexes from blind and flexible receptors were analyzed for residues binding between the two proteins. The global free binding energy of the WT complexes was calculated as -52.00 kcal/mol using FiberDock.

ClusPro, also a FFT based algorithm, it also clusters and filters the docked complexes. The lowest energy values for the docked Rv2159c and PknI complex structures for the four methods such as balanced, electrostatic favored, hydrophobic favored and VdW+Elec are -953.0, -984.4, -1432.2 and -169.2 respectively. ClusPro results

suggest that hydrophobic interaction plays an important role in PknI-Rv2159c complex. The docked poses of Rv2159c structure were observed to be majority in the mapped binding site of 1ZYS when compared to the binding sites of 1WBP and 1UKH. PDBsum generate was used to analyze the protein-protein interfaces of the complexes. The top 10 docked complexes from Z-Dock and ClusPro were subjected to PDBsum to

identify the Rv2159c's interacting residues with PknI protein. Comparative analysis of the top 10 structures identifies a list of amino acids that were shown to be responsible for the interaction such as, Ala1, Gly2, Trp3, Ala4, Gln14a and Val15 (Figs 38 a&b). Based on the binding scores and PDB sum analysis of two docking programs, the most preferred binding pose was selected for further studies.

Figs. 38 (a & b): PknI-Rv2159c protein docking

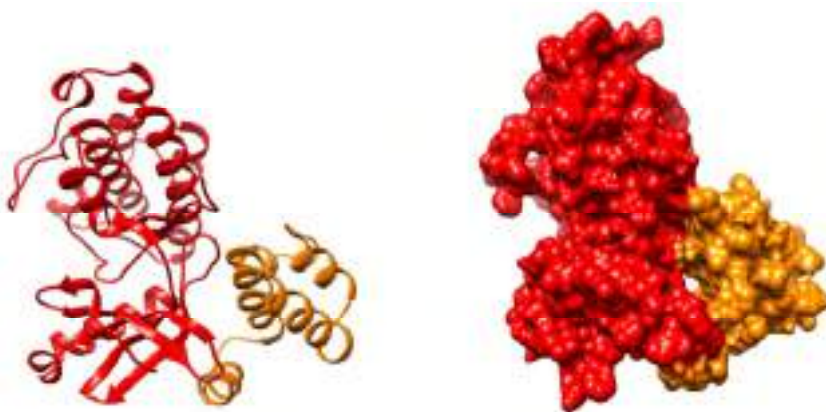


Fig. 38a: PknI-Rv2159c protein interaction using Z-Dock server in line ribbon form and Fig. 38b: surface representation form; Model representing the best among all the docking poses. Chain A colored red is PknI and Chain B colored orange is Rv2159c.

Molecular dynamics simulations of the complexes: The apo form of the protein complex was subjected to MD to study their complex stability. The stability of the complexes was assessed with their RMSD values obtained throughout the MD trajectories. The apo form of the WT PknI-Rv2159c complex shows greater stability

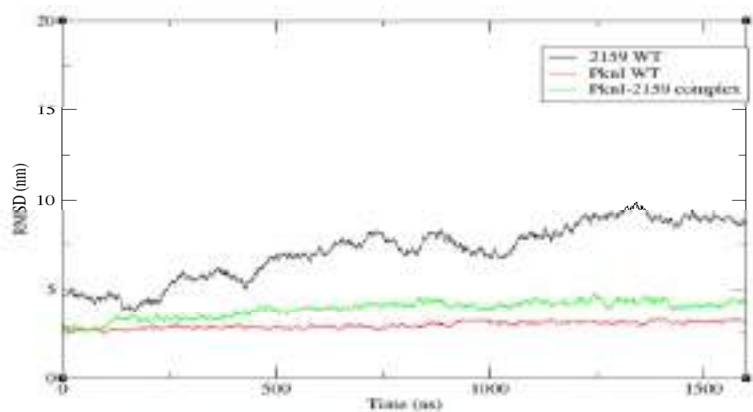
throughout the simulation period. It is noted that Rv2159c in complex with PknI was stabilized after few nanoseconds of simulation where as Rv2159c as a protein alone remained unstable (Fig. 39).

Conclusion: The 3D structure for Rv2159c was predicted using homology modeling and successfully docked against the PknI

protein. The PknI-Rv2159c complex structure was stabilized well as compared to its free form. Collective results from

ClusPro and Z-Dock suggest hydrophobic residues favor the PknI-Rv2159c interaction.

Fig. 39: Structural stability of protein complexes



RMSD analysis of apo (PknI, Rv2159c) proteins in free form and the WT protein complex (PknI-Rv2159c) were shown against time scale in picoseconds.

I-8: Vitamin D receptor gene polymorphisms and sputum conversion during ATT

Principal Investigator : Dr.P. Selvaraj
(email: selvarajp@nirt.res.in)
Co-Investigators : Dr.P. Paulkumaran; Mr.M. Harishankar; Dr.C.
Ponnuraja; Dr.K. Chandrasekar
Source of funding : Intramural
Study Period : July 2013 – June 2016

Background: Vitamin D₃, a potential immunomodulator, is known to influence innate and adaptive immunity. Vitamin D₃ exerts its activity through Vitamin D receptor (VDR), a nuclear hormone receptor. VDR gene variants have been associated with altered VDR expression as well as with susceptibility or resistance to TB. Polymorphic variants of VDR gene have been shown to be associated with faster sputum conversion during ATT.

The present study is planned to understand the role of various VDR gene polymorphisms on sputum mycobacterial culture conversions during ATT in south Indian PTB patients.

Aim: To find out whether VDR gene polymorphisms are associated with sputum mycobacterial smear / culture conversion during ATT and treatment outcome

Experimental Design: Two milliliter blood sample in anti-coagulant is collected from sputum positive PTB patients who received standard ATT (2EHRZ₃/ 4HR₃). DNA will

be extracted from the white cells and used for assessing VDR gene polymorphisms. VDR gene polymorphisms in the 5' regulatory region (Cdx2 and A-1012G), coding region (FokI), and 3' untranslated region (UTR) BsmI, ApaI, and TaqI) will be studied. Data available on sputum mycobacterial culture conversion during ATT will be correlated with the data on allele and genotype frequencies of VDR gene polymorphisms to find out the role on sputum conversion during ATT.

Study subjects: Two hundred patients who received ATT with the standard TB treatment regimen (2EHRZ₃ / 4HR₃) from among the NIRT studies XXII and XXIV (Chennai and Madurai) whose sputum status (smear and culture status) for TB are available for the time points - pretreatment, 15 days, 30, 45, 60, 90, 120, 150, and 180 days of treatment will be included for this study.

So far, 110 patients have been included.

L9: Cytokine gene polymorphisms in HIV and HIV-TB

Principal Investigator:	Dr.P.Selvaraj (email: selvarajp@nirt.res.in)
Co-Investigators :	Mr. M. Harishankar, Dr. Soumya Swaminathan
Source of Funding :	Intramural, NIRT, (Rs. 20 Lakhs)
Study Period :	2014-2017

Background: HIV-1 infection has increased the burden of TB, especially in populations where the prevalence of TB is high among young adults. Host genetic factors have been suggested to serve as genetic markers to find out the susceptible or resistant host to various infectious and non-infectious diseases. Numerous studies have emphasized the role of host genetic factors (HLA and non-HLA genes) on susceptibility or resistance to HIV and HIV-TB.

Studies on cytokine gene polymorphisms in HIV-infected individuals with tuberculosis are meager in Indian population. In the present study, various cytokine gene polymorphisms will be carried out in HIV and HIV-TB co-infection in south Indian population. This study will be carried out in the DNA

samples collected earlier for the ICMR Task Force project.

Aim: To find out whether cytokine gene polymorphic variants are associated with susceptibility or resistance to HIV and HIV-TB in south Indian population

Methodology:

Study Subjects:

The study population consisted of:

1. HIV-1 seropositive patients without tuberculosis (HIV+TB-), (n= 150).
2. HIV-1 seropositive patients with tuberculosis (HIV+TB+), (n=115).
3. HIV-1 seronegative patients with pulmonary tuberculosis (HIV-PTB+) (n=150)
4. Healthy controls (n=150).

During this period following cytokine gene polymorphisms were studied by allele specific PCR method using stored DNA samples extracted from the patients and controls (Table 28).

Table 28: Cytokine gene polymorphisms studied in patients and healthy controls

Cytokine Gene Polymorphisms	HIV+TB- (n=150)	HIV+TB+ (n=115)	HIV-TB+ (n=150)	Healthy Controls (HCs) (n=150)
1) TNF- α -308 (G/A)	148 [#]	106 [@]	150	150
2) IL-10 -819 (C/T)	148	106	150	150
3) IL-10 -1082 (A/G)	148	106	150	150
4) IL-12 +1188 (A/C)	148	106	150	150

n = number of samples included in the study; # - HIV+TB- group, 2 samples not available;
@ - HIV+TB+ group, 9 samples not available

The following cytokine gene polymorphisms will be studied:

- 1) Tumour Necrosis Factor (TNF)- β (TNF- β intron2/exon3).
- 2) Interleukin (IL)-10 (-592 C/A).
- 3) IL-12, (IL-12A 3'UTR G/A and 5'UTR T/G).
- 4) IL-2, (IL-2 -330 T/G and +166G/T).

Study is in progress.

5) IL-4, (IL-4 5'UTR -33C/T, promoter region of IL-4 -589 C/T).

- 6) Interferon (IFN)- γ (IFN- γ +874A/T and 3' UTR 5644A/G).
- 7) IFN- γ inducible protein-10 (IP-10), (IP-10 -1596 C/T, -1447 A/G and -135 G/A).
- 8) CD14 (-159C) and P2X7 (-1513).

I-10: Prevalence of TB (*M. tuberculosis* and *M. bovis*) in cattle and animal handlers in Chennai region

Principal investigator	:	Dr. P. Kannan (email: kannanp@nirt.res.in)
Co- Investigators	:	Dr.C.K.Dolla; Dr.D.Baskaran; Dr.S.Balaji; A.Srividya; Dr Sujatha Narayanan (chandrakumar.d@nirt.res.in; baskar.d@nirt.res.in; sbalaji@nirt.res.in; sujatha.sujatha36@gmail.com)
Collaborators	:	Dr. Dhinakar Raj; Dr.Maroudham. Tamilnadu Veterinary and Animal Sciences University, Chennai
Source of funding	:	Intramural
Study period	:	2015-2018

Background: In most of the developing countries including India the burden of zoonotic TB is not estimated or under estimated. India ranks first in buffalo and second in cattle population in the world, and where bovine TB is not controlled at all. It has been postulated that zoonotic TB represents significant risk in areas where humans and animals share common environment. Agricultural workers may acquire the disease by inhaling cough spray from infected cattle and develop typical PTB and such patients may in turn transmit the infection to cattle and humans. Like animal-human transmission, human-animal transmission occurs at locations of active animal-human interaction. This is posing a formidable challenge in controlling and eradicating mycobacterial diseases.

Objective: To estimate the prevalence of zoonotic and reverse zoonotic transmission of *M.bovis* and *M.tuberculosis* in animal handlers and cattle

Methods: We have screened two government farms and one private farm for TB in animal handlers and animals.

Screening of animal handlers: All the animal handlers in the three cattle farms were interviewed for symptoms of TB (cough for 2 weeks, fever, night sweat, weight loss) and also screened by chest X-ray. Two sputum (spot and early morning) samples were collected from those with symptoms of TB and abnormal chest X-ray for bacteriological examination.

Screening of animals: All the cattle housed in the three farms were screened by using comparative intradermal tuberculin test. The comparative intradermal tuberculin test is

used to differentiate between animals infected with *M. bovis* and those responding to bovine tuberculin as a result of exposure to other mycobacteria. This sensitization can be attributed to the antigenic cross reactivity among mycobacterial species and related genera. The test involves the intradermal injection of bovine tuberculin and avian tuberculin into different sites, usually on the same side of the neck, and measuring the response 3 days later. In the interpretation of the intradermal comparative test, a reaction

is usually considered to be positive if the increase in skin thickness at the bovine tuberculin site of injection is more than 4mm.

Results: A total of 271 animal handlers were screened and 6 of them were positive for TB. A total of 207 cattle were screened by comparative tuberculin skin testing and 23 animals were positive for TB. The study is ongoing.

DEPARTMENT OF STATISTICS

STUDIES IN PROGRESS:

S-1: Kaplan-Meier estimation and cumulative incidence estimation in the presence of competing risks: A simulation approach

Principal Investigator : Dr. C. Ponnuraja
(email: cponnuraja@nirt.res.in)
Study period : 2012-2016

Introduction: In all statistical software packages except very few, the complement of Kaplan–Meier (1-KM) estimates are often used unsuitably instead of cumulative incidence function (CIF). When competing risks are present, the appropriate estimate of the failure probabilities is the cumulative incidence rather than the complement of Kaplan–Meier estimate. This work compares these two methods of estimating cumulative probability of cause-specific events in the presence of other competing events. The simulated data with three competing events is used to demonstrate the different estimates given by 1-KM and CIF. Also this effort evaluates the advantages and suitability of statistical methods using the CIF over 1-KM method in clinical trial time to event competing data.

Methods: Data Simulation was done using STATA command *stcompet*. Two

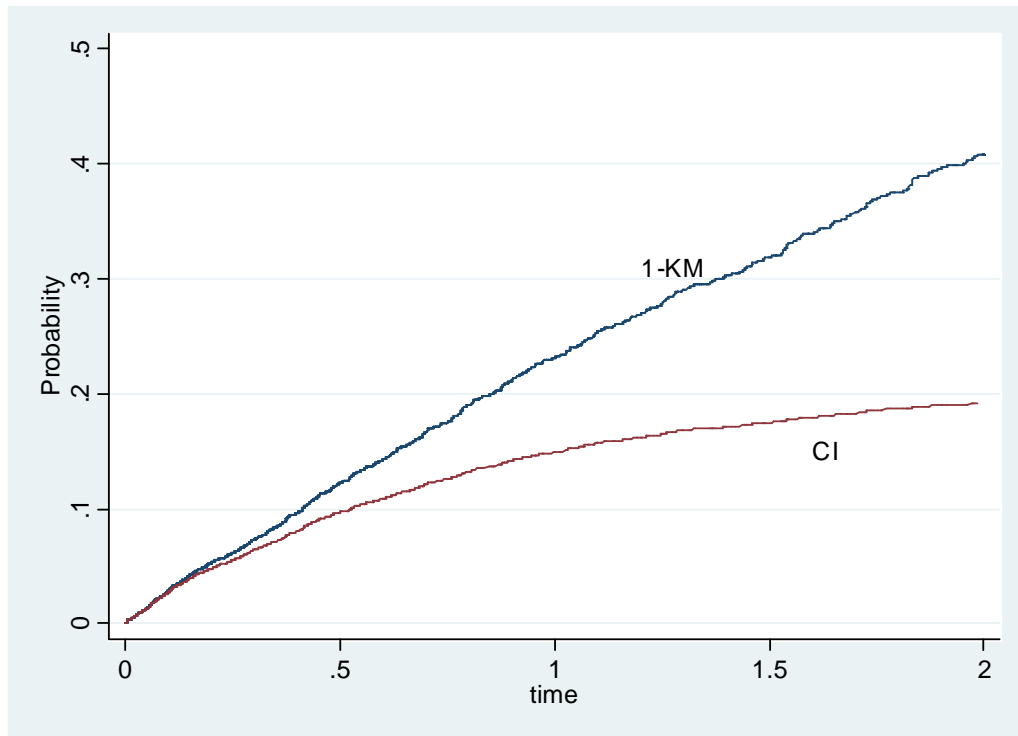
types of failures are assumed, and a time for each type of failure is generated for 10,000 subjects with a constant hazard being 0.25 for the first type of failure and 0.99 for the second type of failure (Table 29). A subject is assumed to fail from the event that occurs early if it occurs before time equals to two units.

Results: The main event of interest for failure is the occurrence of the first type of failure and the competing risk is specified as the occurrence of the second type of failure. The cumulative incidence is created, where the estimate of the cumulative incidence is recorded for both types of failures at each time when a corresponding failure occurs. To attain a plot for comparing 1-KM with the cumulative incidence of the first type of failure, a new variable “Complement” have been created to contain only the approximate pertaining to it.

Table 29: Simulated data using STATA command for 10000 observations with cumulative incidence and the complement of Kaplan-Meier (1-KM)

t25	t99	time	Fail	KM	1-KM	CumInc(CIF)
2.592848	1.703039	1.703039	2	0.65543232	0.3445677	0.7016
1.605753	0.4594849	0.4594849	2	0.8883607	0.1116393	0.3416
1.689981	0.37906	0.37906	2	0.90774236	0.0922576	0.2921
1.771015	0.9418474	0.9418474	2	0.79277793	0.2072221	0.5514
11.43155	0.2922089	0.2922089	2	0.92798877	0.0720112	0.2316
7.85666	3.760532	2	0	0.59522048	0.4047795	
2.803153	0.0987121	0.0987121	2	0.97311244	0.0268876	0.0885
0.3814599	0.4152758	0.3814599	1	0.90730919	0.0926908	0.0785
1.209467	0.6798491	0.6798491	2	0.84342303	0.156577	0.4517
0.5543839	0.0456071	0.0456071	2	0.9878636	0.0121364	0.0419
0.4237287	1.334055	0.4237287	1	0.89755741	0.1024426	0.0851
5.217102	3.439965	2	0	0.59522048	0.4047795	
9.209641	0.9679183	0.9679183	2	0.78811693	0.2118831	0.5597
0.3671193	1.584009	0.3671193	1	0.91161063	0.0883894	0.0755
1.527169	0.6594082	0.6594082	2	0.84923266	0.1507673	0.4424
1.536165	2.728147	1.536165	1	0.6858788	0.3141212	0.1717
4.064649	0.1344252	0.1344252	2	0.96216435	0.0378357	0.118
4.649208	1.724921	1.724921	2	0.65047198	0.349528	0.7051
0.3147765	0.5804275	0.3147765	1	0.92306492	0.0769351	0.0672
1.899437	0.2999618	0.2999618	2	0.92653758	0.0734624	0.2368
4.245422	1.062849	1.062849	2	0.76891417	0.2310858	0.5845
4.357155	0.621662	0.621662	2	0.85503038	0.1449696	0.4255
6.750236	0.4837573	0.4837573	2	0.88359533	0.1164047	0.3558
2.706083	0.3819796	0.3819796	2	0.90730919	0.0926908	0.2941
0.9547318	1.580237	0.9547318	1	0.78968294	0.2103171	0.141

Fig. 40: Complement of the Kaplan-Meier (1-KM) estimate versus cumulative incidence function



The Fig.40 outlines the comparison between the complement of 1-KM with the CIF. In a competing risk setting, the complement of the I-KM overestimates the true failure probability, whereas the CIF is the appropriate method to use in the presence of competing risks.

Conclusion: The complement of I-KM estimates is not reciprocal of the CIF method particularly when in the presence of competing risks. These two methods appear to yield equivalent results when there are no competing risks. The analysis is in progress.

**DEPARTMENT OF
EPIDEMIOLOGY**

COMPLETED STUDIES:

(i) Survival rate of TB disease: a study among TB patients treated under RNTCP in Tiruvallur district of Tamil Nadu

Principal Investigator : Dr. V. Chandrasekaran
(email: chandrasekarav@nirt.res.in)
Source of fundnig : USAID (MDP)
Study Period : March 2014 - September 2015

Background: TB remains an important cause of death from an infectious agent, second only to HIV. TB control is high on the international public health agenda, not only because of the enormous burden of disease, but also because short-course chemotherapy (SCC) is recognized as one of the most cost-effective health interventions. TB mortality has decreased from over 42/100,000 population in 1990 to 24/100,000 population in 2008 as per the WHO Global TB Control-updated 2009 Report. However, there is a need to understand the longevity of these patients after their TB is cured.

Objective: To estimate the survival rate of treated TB patients during 2000 – 2004 under DOTS programme

Study design: Population based matched cohort study

Study setting: TB cases are routinely detected at health facilities through the RNTCP and DOTS which has been implemented since May 1999 in Tiruvallur

district. Through the WHO-USAID funded Model DOTS Project (MDP), Velliyur TB Unit was chosen for close monitoring and follow-up of the DOTS based RNTCP implementation. In addition to TB case detection at health facilities, a community prevalence survey for active TB detection was undertaken in the study area as part of a larger epidemiological investigation.

TB patient cohort: Around 4000 TB patients aged 15 years to 64 years who were registered for TB treatment under Govt. health facilities during 2000-2004 in Velliyur TU of Tiruvallur district was our study cohort.

Control: Controls were defined as a person who was not affected by TB during the same period of 2000-2004. Age (+ or -5 years) and sex matched controls who were: (1) smear and culture negative for AFB, (2) had normal chest X-ray and (3) were asymptomatic at the time of TB prevalence survey 1 and 2 were selected in the ratio of 1:3 to find the 9 year survival rates of TB

treated patients in comparison to patients who had no TB.

Data collection: Semi-structured and a pre-coded interview schedule was used for data collection after getting informed consent from the TB treated patients as well as the controls. The interview schedule included demographic information (age, sex) whether they were alive or dead or migrated. (1) If TB treated patient or control was alive questions were asked regarding the general health status, current symptoms, treatment history including TB, smoking, alcohol consumption and quality of life assessment. (2) If dead the information on the time, date and cause of death was collected. The data collectors were trained to look for any evidence of death from the medical records or death certificates. (3) If migrated we collected information from the time the individual had left the place. In case of TB treated patients or their matched controls who were not alive at the time of interview, details such as year of death and cause of death were collected from any of the patient's close relative. Interviews were conducted by trained field investigators at the respondents' residence in local language.

Results: We entered and cleaned the data using Epi-Info. Data was double entered and cross-checked for consistency. We analyzed

the data using SDPSS software version 17. The male participants were 70% (Table 30). After excluding 23% of TB patients and 28% of controls due to migration at the time of survey, the crude mortality rate among TB patients was 43% against 13% mortality in control group (Table 31). The mortality rate in person years for TB treated cohort was 4 times higher than the matched control (Table 32). Further, (1) the mortality rates for PTB was higher than for extra pulmonary TB (2) mortality rates in defaulters were highest followed by treatment failures (3) the mortality rates of those who had cured of TB were also higher than the controls. We calculated the age standardized mortality rate in the TB treated cohort by comparing with the mortality rate in their matched controls. The excess mortality in this cohort was 2.3 times more than that in the matched controls and young age group had 3 to 4 times excess mortality compared to the matched controls who represented the general population (Table 33). The mortality rate was significantly high among the TB patients during the first 4 years and thereafter there was no significant difference between the TB patients and controls (Fig. 41).

Table 30: Sex distribution among healthy controls and TB patients (2000-2004)

Sex	Control N (%)	TB patients N (%)	Total N (%)
Female	3592 (29.3)	1187 (29.5)	4779 (29.4)
Male	8651 (70.7)	2835 (70.5)	11486 (70.6)
Total	12243	4022	16265

Table 31: Status of the participants at the time of follow-up survey in 2014-2015

Status during the Survey	Control N (%)	TB patients N (%)	Total N (%)
Alive	7768(63)	1733(43)	9501
Died	1634 (13)	1162(29)	2796
Migrated	2841(23)	1127(28)	3968
Total	12243	4022	16265

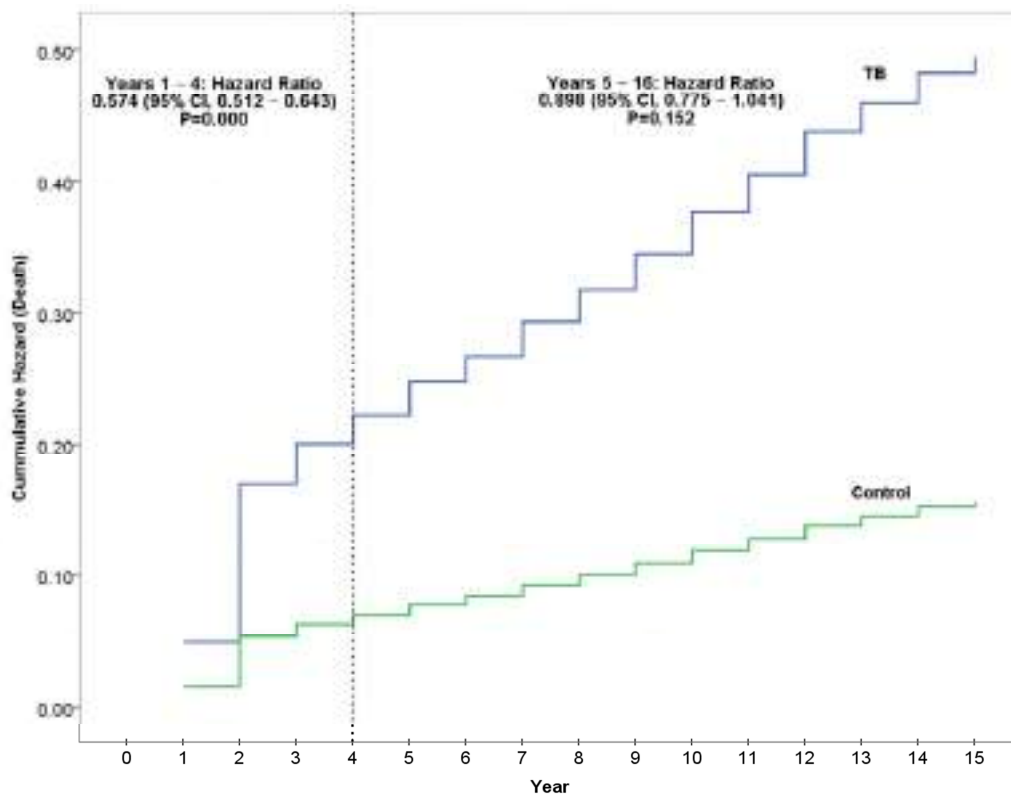
Table 32: Mortality rate of TB patients (stratified by type and treatment outcomes) compared against mortality rate in control group in 2014-2015

Characteristics	Number enrolled	Death (n)	Person years of follow up	Mortality rate per 100 person years
TB patient Cohort	2894	1078	220305	5.87
Control	9399	1146	947366	1.45
Type of TB				
Pulmonary TB	2639	1034	196859	6.30
Extra Pulmonary TB	255	44	23446	2.25
Treatment Outcomes				
Defaulted	320	206	16273	15.19
Failure	96	57	5184	13.19
Cured	1213	361	101332	4.28
Treatment Completed	1134	327	95938	4.09

Table 33: Excess mortality among TB patients compared to the controls

Age group	Mortality in TB Patients			Mortality in Controls			Standardized Mortality Rate (SMR) (%Mortality in TB patients/ % Mortality in Control)
	Died (n)	Total (N)	Died (%)	Died (n)	Total (N)	Died (%)	
15-19	14	168	8.3	22	412	5.3	1.6
20-24	30	231	13.0	31	703	4.4	2.9
25-29	52	301	17.3	41	901	4.6	3.8
30-34	72	269	26.8	52	877	5.9	4.5
35-39	96	317	30.3	86	1072	8.0	3.8
40-44	127	308	41.2	129	1141	11.3	3.6
45-49	170	354	48.0	182	1211	15.0	3.2
50-54	197	333	59.2	284	1137	25.0	2.4
55-59	193	321	60.1	346	1043	33.2	1.8
60-64	211	293	72.0	461	905	50.9	1.4
Total	1162	2895	40.1	1634	9402	17.4	2.3

Fig. 41: Cumulative incidence of death (hazard ratio) among TB patients cohort and controls over the follow-up period



Conclusion: TB patients have significantly higher mortality compared to the matched controls, even after they had completed TB treatment. The mortality was higher in the first 4 years

after treatment. The mortality was high especially among TB patients (1) who were young, (2) who had PTB, (3) who were defaulters and (4) those with TB treatment failure.

STUDIES IN PROGRESS:

E-1: Community volunteers, solidarity and case management of TB study

Principal Investigator	:	Dr. Soumya Swaminathan (email: soumyas@nirt.res.in)
Collaborators	:	Dr. Nancy Luke, Dr.K. Munshi, Pennsylvania State University, USA & Dr. Shanthidani Minz, CMC, Vellore
Source of funding	:	Pennsylvania State University, USA
Study period	:	2012 – 2018

Objective: The first objective of this collaborative project involving NIRT, CMC and Brown University is to assess the feasibility of using DOT providers from the patient's community for the case management of TB. The second objective is to establish where and why these community volunteers will be effective.

Three groups of DOT providers were included:

- Methodology:** (i) Community DOT provider within the patient's kin-group;
(ii) Community DOT provider outside the patient's kin-group;
(iii) Govt. DOT provider (control arm)

DOT provider performance will be based on objective measures of treatment success collected from all TB patients as well as assessment of the patient's (and DOT provider's) experience.

Our first objective is to assess whether community DOT providers (arms 1 and 2) are more effective on average than Govt. DOT providers (arm 3).

The results of this exercise are directly relevant for policy and have the potential to substantially improve the performance of the TB control program, without increasing costs, in the future. To meet our second objective and provide direct support for the role of solidarity in generating differences in volunteer performance across treatment arms, we match TB patients to the kin-groups and villages from which they originate and measure solidarity directly at the community level. We conducted a survey of 12,000 households that are representative of kin-groups and villages in the study area. Measures of solidarity are collected using multiple techniques (survey responses and a behavioral experiment) to assess whether differences in solidarity within kin-groups and in the wider community (general population) match differences in DOT provider performance between arms (1) and (2).

Results: Household surveys (details are listed in Table 34).

Table 34: Household survey details

Name of the TU	No. of HHs completed
Punnai	97,988
Puthupadi	1,10,057
Natrampalli	93,882
Total	3,01,596

E-2: TB treatment outcome among TB-HIV co-infected patients under programme conditions in south India – a retrospective study

Principal Investigator : Mrs. Basilea Watson (email: basilea@nirt.res.in)
Source of fundnig : Intramural
Study Period : 2013 - 2016

Background: The global impact of the converging dual epidemics of TB and HIV is one of the major public health challenges of our time. Worldwide, TB accounts for nearly one in four deaths among people with HIV, according to WHO estimates. People with HIV infection are 20 to 30 times more likely to develop active TB disease than people without HIV.

The periodic HIV survey in TB patients in 2006-07 demonstrated that the prevalence of HIV among TB patients varied substantially in 15 surveyed districts between 1% and 13.8%. According to the RNTCP Annual Report, 2011, it is estimated that nearly 8% are known to be HIV infected among all TB patients tested.

With regard to detecting HIV among individuals with active TB, provider initiated HIV testing is recommended for all TB patients, as standard of care. This policy was rapidly scaled up with over 60% of TB patients being aware of their HIV status in 2011.

In the treatment of HIV-TB co-infected patients, treatment of TB always takes precedence over the treatment of HIV infection. For those patients who are already on ART, some modifications are made to both HAART and ATT.

Though the treatment outcomes among HIV-TB co-infected patients have been studied in controlled situations, the same in programmatic conditions in India have not

been explored in detail. This study was proposed to study the TB treatment outcomes among the TB/HIV co-infected (with both pulmonary/extra PTB) and the possible factors associated with such outcomes.

Study Objectives:

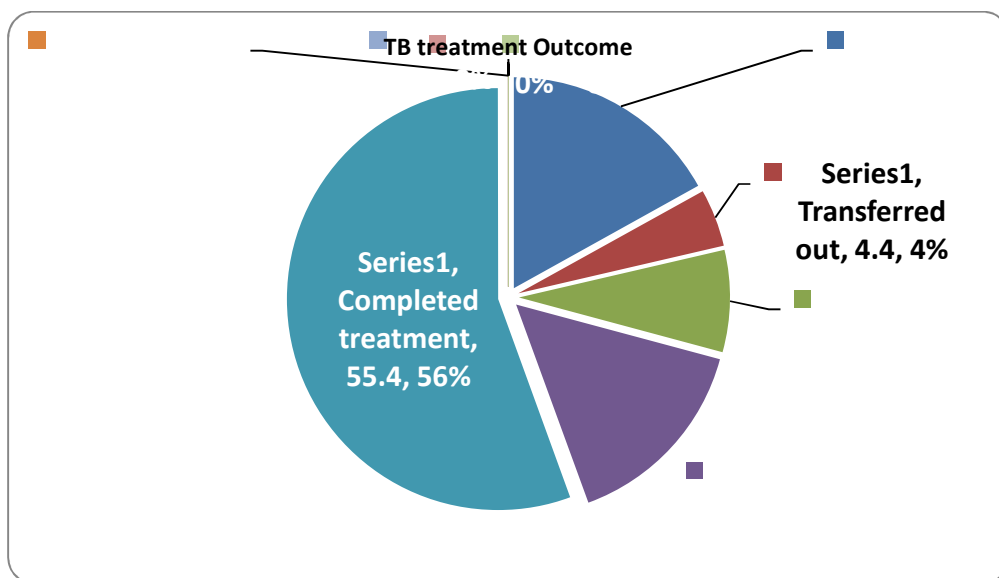
Primary Objective: (i) To determine the TB treatment outcomes among patients with TB/HIV (with pulmonary /extra PTB) at the end of TB treatment

Secondary Objective: (i) To determine risk factors for poor TB treatment outcomes among TB/HIV patients

Study design: The study was a retrospective record review of the HIV-TB co-infected patients registered for TB treatment between January – December 2012. A data capture format was sent to ART centres of Tamilnadu to get the appropriate data for the study.

Progress till date: Data on 2231 HIV-TB co-infected patients from 49 of 52 ART centres of Tamil Nadu have been received so far. Preliminary analysis showed that 56% had completed TB treatment while 15% had died (Fig. 42).

Fig. 42: TB treatment outcome



ART initiation in relation to TB treatment: The time interval between initiation of ART and initiation of TB treatment was categorized as follows: TB diagnosed while on ART, ART initiated ≤

90 days after initiation of TB treatment ('early ART'), ART initiated > 90 days after initiation of TB treatment ('delayed ART'), and ART not started (Table 35).

Table 35: Details of ART initiation

ART initiation in relation to TB treatment	No.	%
TB diagnosed while on ART	685	30.7
Early ART	850	38.1
Delayed ART	316	14.2
ART not started	371	

Factors associated with TB treatment outcome of TB-HIV co-infected:

Unfavourable TB treatment outcome was defined as those who had either died, failed or defaulted during TB treatment. Unfavourable TB treatment outcome was 32% among those TB/HIV patients \geq 45 years which was significantly higher than those in the younger age group. Also, male patients had a higher unfavourable TB

treatment outcome as compared to the females. Unfavourable outcome was significantly higher among those who had a low BMI at baseline. A similar effect was seen among those who had a low CD4 count (<100 cells/mm³) at the beginning of ATT. Not being on ART at the time of initiation of ATT had a significant association with unfavourable TB treatment outcome. Further analysis is in progress.

E-3: Factors associated with early mortality among HIV-infected patients initiating on ART

Principal Investigator : Ms. Srividya A. (email: vidyaadi@gmail.com)
Source of fundnig : Intramural
Study Period : 2014 - 2015

Background: New HIV infections have reduced by more than 50% over the past decade, thanks to ART. This observed trend of reduction in infection and AIDS related deaths are the impact of the nationwide free ART which has converted AIDS from being a deadly disease to a manageable one. While some studies have shown that the mortality among the HIV has drastically reduced since the introduction of free ART since 2004, some have reported more early deaths (say within 6 months) even after taking ART. Of the earlier studies on this issue, one was based on a subgroup of HIV positive individuals and the other study had data from only three urban ART centres, especially during the initial years after start of ART i.e., between November 2004 to January 2005. It is almost a decade since the initiation of free ART in our country. It was proposed to study the survival pattern among HIV-infected individuals initiating ART and the factors associated with mortality.

Aim: To study the survival patterns of the HIV-infected individuals initiating ART and

the factors associated with mortality within the first six months of ART

Data collection: All the details in the ART cards of the HIV-infected individuals who initiated ART between January – December 2012 are available in the information management system with the State Aids Control Society. A pre-defined template in Microsoft Excel for the data to be collected (based on the details available in the ART card), for this study was created and sent to the coordinator of State Aids Control Society.

Progress: As the study proposes to use the secondary data from ART centres, permission from National AIDS Control Organization (NACO) was sought and was granted. Though, initially it was proposed to carry out the study only in Tamil Nadu, NACO wanted to include Maharashtra, Uttar Pradesh and West Bengal in the study.

Data from nine ART centres of Tamil Nadu were only received till date. Records of the 1120 HIV positive patients (age >18 years) who initiated first line ART between January to December 2012 were received. Of this, 31.6% were females, 73% were

heterosexual, 2.4% were MSM. Sixty one percent of them were initiated on ART based on CD4 counts. Out of 91 (8.1%) who died, 46 (50.5%) died within six months of initiation of ART. Of these 46, 28 (60%) had a baseline CD4 cell counts of less than 200 cells/cu.mm. with mean CD4 cell counts of (143.6 ± 14.0 cells/cu.mm). It was also seen that the CD4 cell counts were significantly lower for those who died within 6 months

(p value <0.05) when compared to those who died after 6 months (231.9 ± 30.0 cells/cu.mm).

Progress: The data is now being analysed to compare the survival patterns among various risk groups. Cox regression models are being used to find out about the risk factors associated with mortality at 6 months and 1 year.

E-4: Health impact of quarry works in suburban areas of Chennai

Principal Investigator	:	A. Ilangoan (email: elangoan@nie.gov.in)
Co-PI	:	Dr.C.K. Dolla (chandrakumar.d@nirt.res.in)
Collaborator	:	Sathyabama University
Source of funding	:	Intramural
Study Period	:	2014 – 2015

Background: We propose to determine the effects of the quarry works in the population living around the quarry in sub-urban areas of Chennai by using the latest 3S spatial technology of geographical information system (GIS), Global positioning system (GPS) and remote sensing (RS).

In this study, it is proposed to develop a Spatial Decision Support System by employing a suitable algorithm (fuzzy logic, artificial neural network etc.,) and GIS to identify the spread and magnitude

of the disease caused by the quarries. This model will be used to show the existence and the strength of correlation between causes and effects, which helps in decision-making capabilities of the policy makers in the areas where quarries are situated.

Objectives: (i) To measure the following environmental parameters within 5 km radius of quarry:

- suspended particles in air, and,
- weather

(ii) To measure the health impact of quarry works among the residents within 5 km of radius from the quarries with respect to TB, Silicosis & chronic respiratory diseases

(iii) To develop a spatial risk model using the above environmental and health parameters with the help of GIS

Methodology:

• **Study area:** Thiruneermalai / Kundarathur regions near to Chennai

• **Study design:** One year longitudinal data on weather and air pollution is collected. One time cross-sectional data on health related information with respect to silicosis and TB is collected based on the clinical examinations.

• **Study population:** Nearby residents in and around quarries within a radial distance of 5 km. The sample size is calculated as 541 households.

• **Sampling design:** 541 households are selected based on GIS grid sampling method within a radius of 5 km around the quarries.

• **Inclusion criteria:** Quarry workers & permanent residents (at least 2 years in the study area).

• **Exclusion criteria:** People who do not satisfy the inclusion criteria and children less than 2 years old.

Expected outcomes: This study attempts to develop a database on meteorological, air pollutants and related health hazards (silicosis & TB) in the study area. All these data sets will be integrated to a spatial decision support system in ArcGIS environment to develop a Spatial Risk Model. This GIS based model will be useful for decision maker in framing health care needs and framing guidelines for Quarry related works. This study might be able to provide evidence to State Govt. to initiate appropriate intervention strategies.

The study was initiated during February 2014 and now the data / sample collections are completed. X-ray was done for all the participants whereas the sputa were collected only for the symptomatic and X-ray positive cases. Spirometry test was done only for selected persons identified by random selection method. Table 36 shows the number of samples collected from the quarry workers and the community.

Table 36: Sample collection details

Type of sample	No. Samples collected	No. of positive	%
Quarry workers	236	Symptomatic – 54	16.6
		X-ray positive -- 24	7.4
Community	1957	Symptomatic – 60	3.0
		X-ray positive -- 32	1.6

The environmental parameters are being collected by Sathyabama University as they are one of the collaborators of this study.

After completion of collection of environmental parameters, the desired spatial risk model will be developed.

BIOMEDICAL INFORMATICS

STUDIES COMPLETED:

(i) Substrate identification of lysin A (gp11) of mycobacteriophage Che12 using docking and simulation studies

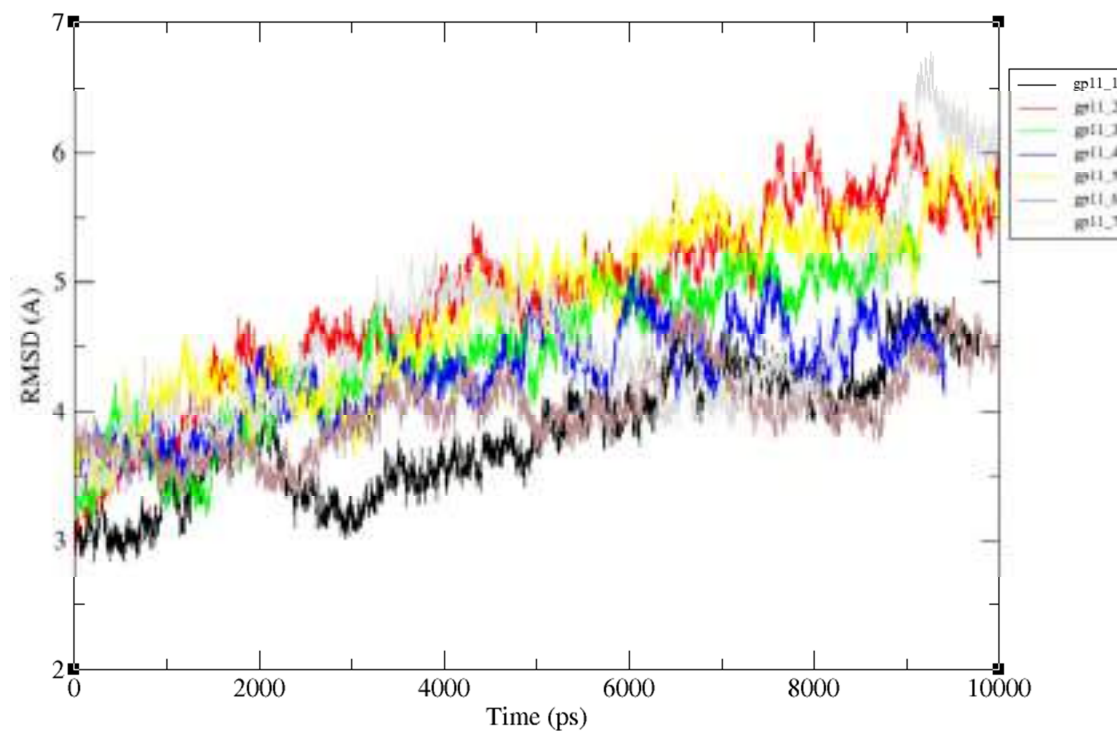
Principal Investigator : Dr. Vanaja Kumar
(email: vanaja_kumar51@yahoo.co.in)
Source of funding : Intramural
Study period : 2014-2015

Mycobacteriophages produce lysins that break down the host cell wall at the end of lytic cycle to release their progenies. The ability to lyse mycobacterial cells make them significant. Mycobacteriophage Che12 is the only reported temperate phage capable of infecting and lysogenising *M. tuberculosis*. Gp11 of Che12 was found to have Chitinase domain that serves as endolysin (lysinA) for Che12. Structure of gp11 was modeled and evaluated using Ramachandran plot in which 98% of the residues are in the favored and allowed regions. Che12 lysin A was predicted to act on the substrate NAG-NAM-NAG in the peptidoglycan of cell wall. The tautomers of NAG-NAM-NAG molecule were generated and docked with lysin A. The stability and binding affinity of lysin A – NAG-NAM-NAG tautomers were studied using molecular dynamics simulations (Fig.43). It is observed from the RMSD plot that there

was increase in RMSD in gp11 - NAG_2, gp11 – NAG_5 and gp11 - NAG_7 indicating a large structural rearrangement within these complexes. When comparing the RMSD plot for the bound NAG-NAM-NAG, it was observed that the NAG-NAM-NAG in NAG_1 gp11 complex has little conformational changes when comparing to NAG-NAM-NAG bound in other complexes. MM-PBSA binding energy value calculated for all complexes and NAG_1 having $\Delta G_{\text{bind}} = -57.86$ kcal/mole is identified as the probable NAG-NAM-NAG tautomer and the right conformation within the binding site of gp11.

Conclusion: Based on the RMSD plot and the MMPBSA energy analysis, we conclude that NAG_1 molecule is the most appropriate tautomer for Che12 Phage lysine A (gp11) protein.

Fig. 43: RMSD plot of C alpha atoms of gp11_NAG complexes during 10 ns of molecular simulation



(ii) A user-friendly web portal for analyzing conformational changes in structures of *M. tuberculosis* proteins

Principal Investigator : Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding : ICMR Biomedical Informatics Project
Study period : 2014-2015

Background: With the initiation of the TB structural consortium, the protein structural space for *M. tuberculosis* (MTB) has been steadily increasing. This has led to furtherance of our understanding of *M. tuberculosis*, and has provided a base for structure-based drug designing. There are

multiple experimental structures available for several MTB proteins that have been solved under different functional conditions such as bound forms with different ligands, mutant proteins, etc.

Aim: (i) To develop a user friendly web portal for analyzing conformations changes in structures of MTB proteins

Methods: All available structures for a given MTB protein were compiled from protein data base (PDB). Detailed systematic analysis of the available multiple structures of each MTB protein was carried out to determine the amount of conformational changes that the given protein structure could accommodate. Torsion angles were used to perform principal component analysis (PCA).

Results: The data base presently contains 970 three dimensional structures for 361 mycobacterial proteins.

Conclusion: This database will serve as a valuable tool for selecting appropriate protein structures, molecular modeling, docking and structure-based drug designing studies. The data base can be accessed online at <http://bmi.icmr.org.in/mtbsd/MtbSD.php>.

(iii) Updating of *M. tuberculosis* structural database

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2014-2015

Background: We developed a database of all experimentally solved protein structures of *M. tuberculosis* and published it in Tuberculosis 2011;91(6):556-562. The database contains systematically analyzed and categorized structural data for each protein and serves as a very useful resource for structure based drug designing studies.

Aim: To update the database with the newly solved MTB protein structures

Results: The database has been updated with 130 newly solved MTB protein structures and their structural homologues. A functional annotation page has been provided for hypothetical proteins and a search option has been added for searching for structures based on PDB id, country, authors and journals. For example, the user can now search for MTB structures solved by different countries. For the selected country, the different institutes and

departments will be listed. Further selecting the institute, the different MTB structures deposited by the institute will be listed in a tabular form. The database is now integrated with BLAST, ClustalW tools. BLAST tool can be used for searching *M. tuberculosis* homologues in MtbSD database. Further, the user can select any number of Blast hits that

are similar to the submitted sequence and submit for multiple sequence alignment using ClustalW. Option for saving both BLAST as well ClustalW results are provided.

Conclusion: The database will be updated on a yearly basis.

STUDIES IN PROGRESS:

BI-1: Database for drug resistant TB – DDR-TB

Principal Investigator : Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding : ICMR Biomedical Informatics Project
Study period : 2013-2018

Background: Emergence of resistance to anti-TB drugs is one of the major challenges for the control of TB globally. MDR-TB has now been reported in almost all parts of the world, and XDR-TB cases have been confirmed in 58 countries. Close follow-up and monitoring of the disease will help us understand and prevent the emergence and spread of TB drug resistance.

Aim: To develop an online resource called 'Database for DR-TB' which can serve as a dynamic source for large scale data analysis

Method: Data on 170 unique variables about DR-TB patients, including demography, contact history, medical history (past illnesses and treatment history), present clinical profile (general and systemic examination reports, presenting complaints, co-morbidity status), treatment profile, (regimen details, adverse drug reactions, treatment monitoring Index), laboratory reports (clinical, microbiology and biochemistry), etc. is captured from the patients' case files and entered electronically. MySQL and HTML has been used to develop the database. In the initial

phase, the database will be local and capture available data on all MDR-TB patients registered at NIRT since February 2008, and all XDR-TB patients registered at NIRT since February 2013. Rights to access, edit and modify data in the database are restricted to persons authorized by the Director of NIRT.

Results: Currently the database contains clinical and laboratory data on 34 XDR-TB and 26 MDR-TB patients. Genotypic NIRT mutation data is available on some of the XDR-TB isolates. The database is being continuously updated on a monthly basis.

Conclusion: Systematically captured clinical data of this nature will help clinical researchers to easily access and review data for evaluation and management of DR-TB cases. Further, large scale analysis of the data will help us to understand the changing pattern of the disease from drug sensitive to drug resistant form, and thus help in timely decision making. The database will also serve as an educational tool for medical students and researchers.

BI-2: *In silico* analysis of the variable regions (v1-v5) of gp120 protein in R5, X4 and R5X4 tropic viruses

Principal Investigator : Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding : ICMR Biomedical Informatics Project
Study period : 2014-2016

Background: The HIV-1 genome is known to mutate rapidly. This heterogeneity enables the virus to infect many cell types, including those expressing different chemokine receptors on the cell surface. HIV-1 infects the host cell by interacting with the primary receptor CD4 and a coreceptor CCR5 or CXCR4. Generally, R5 viruses opt CCR5 as coreceptor for viral entry, whereas X4 viruses use the CXCR4 coreceptor. Some viral strains are capable of using both CCR5 as well as CXCR4 coreceptors and are known as dual-tropic viruses (R5X4). While in general transmitted forms of the virus are preferentially CCR5 tropic, CXCR4 tropic viruses evolve with disease progression, as a result of molecular changes occurring in the variable loop 3 (V3) of the HIV-1 envelope (gp120) protein. The significance of co receptor usage, has led to the development of several *in silico* tools to predict CCR5

and CXCR4 usage based on the V3 sequence.

Aim: To identify molecular signatures and motifs in the variable regions of gp120 besides the V3 region that enables dual tropic viruses to use more than one co-receptor

Methodology: The envelope sequences for the current study were obtained from existing literature. Consensus patterns were generated for each of the variable loops of the three types of viruses (R5, X4 and R5X4) and employed to identify distinct features that characterize each of the virus types.

Conclusion: The study will help in the identification of key signatures in the HIV-1 envelope outside the V3 loop responsible for expanded co-receptor usage in dual tropic viruses and develop a concise algorithm that can distinguish between single tropic and dual tropic viruses. The study is on going.

BI-3: *In silico* analysis of rifampicin resistance in *M. tuberculosis*

Principal Investigator : Dr. Sujatha Narayanan
(email: Sujatha.sujatha36@gmail.com)
Source of funding : ICMR Biomedical Informatics Project
Study period : 2014-2016

Background: Rifampicin (RIF) is an important drug in anti-TB therapy. Although rare, resistance to RIF is increasing because of its widespread usage and this results in selection of mutants resistant to other components of SCC. RIF binds to the β subunit of DNA-dependent RNA polymerase (RNAP), close to the RNA/DNA channel. RIF is bactericidal because it inhibits β -subunit of RNAP of bacterial but not of mammalian origin, and acts early in transcription and physically blocks the elongation of the growing RNA chain after 2 - 3 nucleotides have been added. The RNAP core enzyme of 400-kDa consists of five different subunits, such as α -dimer (α_2), β subunit, β' subunit and ω subunit. These subunits are converted to a holoenzyme following the binding of one σ subunit, which initiates transcription from promoters. The genes encoding α , β , β' , and σ have been designated as *rpoA*, *rpoB*, *rpoC* and *rpoD*, respectively. The majority of mutations responsible for RIF resistance have been mapped to three distinct loci near the centre of the *rpoB* gene. The three regions are RIF-cluster I (512-534), RIF-

cluster II (563-574), and RIF-cluster III (687). In MTB, resistance to RIF develops in a "single step". The genetic basis for RIF resistance in approximately 95% of the cases is due to mutations in an 81-bp RIF resistance-determining region (RRDR) of the *rpoB* gene, corresponding to codons 507 to 533 which encodes for 27 amino acids. Most of the mutations are single amino acid substitutions, however inframe deletions and insertions also occur, but at lower frequencies. Mutations at positions 521, 526, 531 and 533 have been reported to be the most commonly involved codons. These mutants are generally associated with high-level RIF resistance (MIC<32 μ g/ml) and are absent in susceptible organisms. Amino acid changes at position 514 or 533 usually result in low-level RIF resistance. Although minor discrepancies have been reported, in general there is a strong correlation of specific amino acid substitutions and MIC. Therefore, it is of interest to study the interactions between the clinical mutants (MTs) of RpoB and RIF which are responsible for mediating RIF resistance from MTB, using *in silico* approaches.

Aim: To characterize molecular interactions between the wild type and mutant RpoB protein of MTB with rifampicin and investigate the basis of RIF resistance

Methodology: A homology model was generated using crystal structure of 2A68 (3 domains structure), which is considered as template and WT and for MTs (D516V, L521M, H526D, H526Y, H526R, S531L and L533P) substitutions were done at the respective positions with the help of Modeller 9.11 software. Following this, docking of WT and MTs of RpoB proteins with RIF was carried out by software-GOLD 4.0.1.

Results: The docking of RIF with WT and MTs showed higher values with WT as compared to MTs such as D516V, H526D, S531L and L533P, except for L521M, whose score was higher than that of WT. The high score obtained for WT indicates lack of unfavorable substitutions in the MTs. MD simulation for WT RpoB protein with RIF gave a binding energy of -22.88 kcal/mol.

Conclusion: More data on mutants is required to gain further insights into the interactions and to fully understand the clinical relevance of mutations.

**ELECTRONIC DATA
PROCESSING DIVISION**

Electronic Data Processing

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

Overview: Research today needs to have Electronic Data Processing (EDP), and EDP Department is an essential part of any Research Institute. The main objective of the department is to computerize the documents of all the research studies undertaken in our institute and maintain the Local Area Network system. The data entry for all the current studies is being done using *EpiData* software. The department was given an additional responsibility of creation of data entry questionnaire using *EpiData* software for all the clinical and laboratory studies. This helps the data to be available for researchers in the Institute. The data collected during the epidemiological surveys forms the basis of subsequent data analysis which in turn is helpful for writing reports for publications. The EDP staff is being engaged in developing several in-house computer programs for data management and data outputs.

Handling IT equipments maintenance service:

Several computers and its peripherals which needs periodical maintenance services are handled by a service engineer working under comprehensive annual maintenance contract.

Handling Video conferencing system:

The video conferencing system is being handled by the service engineers who are working under ICMR-HCL project.

Handling Network system:

To setup a network of computers, we need to have a proper infrastructure in place. The EDP department plays an important role here. The department ensures that everything is being done with the requirement of the Institute. Maintenance of hardware and software part of computer network system is being handled by the NIRT-ICMR/ICER project staff. Apart from this, configuring for network connection, creation of user accounts, maintenance of internet, intranet facility and Wi-fi connectivity and periodical backup of all the files from the servers are all handled by them.

Highlights:

- Created login facility on the network system for 70 new users and added with existing user account database.
- A new remote Desktop Service was created to provide access to the WS-2012 Server.

- A new Federated Service was created to provide NIH Library access to the Research Scientists.
 - A network monitoring dashboard was installed to monitor the network, video conference and basic services at NIRT, Chennai Campus.
 - The existing network was structured with numbering the network points at NIRT, Chennai Campus.
 - The network system at NIRT, Madurai unit, was upgraded with installation of a new BSNL leased line with 2MBPS (1:2).
 - A new router, switch and access points were provided and a network monitoring dash board was installed to monitor the network system of NIRT Madurai unit.
 - A new server with the domain replication placed at Madurai unit
- and a network tunneling was created between Madurai unit and Chennai for sharing the data resources.
- A new 10 KVA x 2 UPS with 5.00 hours power backup has been installed in the EDP department, NIRT Chennai.
 - A new video conferencing facility was established in the ICER Facility building for the NIRT Scientist to have regular meeting with other institutes for doing presentation and Skype Calls.

Computerization of documents:

The quantum of documents of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2014 to March, 2015 is shown below (Table 37).

Table 37: Study-wise data entry details

Documents computerised			
Sl.No.	Name of the study	Entered	Verified
1	Animal husbandary	271	271
2	Mitun	389	389
3	Children tuberculosis	814	814
4	NIRT Clinic	18682	18303
5	Survival16345	16345	16345
6	Pharmacokinetic	1690	1690
	Total	38191	37812

Research work for publication:

The research reports of one-time independent prevalence survey, Tiruvallur, and mortality surveys of Andhra Pradesh

and Orissa states were finalized for publication. The data outputs of sub-population with tuberculin positive skin test ($\geq 12\text{mm}$) from BCG trial were obtained and made available for application.

**INTERNATIONAL CENTRE
FOR EXCELLENCE IN
RESEARCH**

COMPLETED STUDIES:

(i) Host immune responses in strongyloidiasis: IL-10 - dependent regulation of parasite antigen - specific Th1, Th2 and Th17 responses in *Strongyloides stercoralis* infection

Principal Investigators	:	Dr. Subash Babu; Dr. P. Paul Kumaran (email:sbabu@nirt.res.in / ppkumaran@nirt.res.in)
Co-Investigators	:	Dr C.K. Dolla; Dr. M. Satiswaran
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH); Dr. R. Nandini (GGH); Dr. V. Lakshmi (CDH)
Study Period	:	2014-2015

Background: Chronic helminth infections are known to be associated with modulation of antigen - specific CD4⁺ T responses. However, the role of CD4⁺ T cell responses in human infection with *Strongyloides stercoralis* (Ss) is not well-defined.

Aims / Methodology: To examine the role of CD4⁺ T cells expressing Th1, Th2 and Th17 cytokines in strongyloidiasis, we compared the frequency of these subsets in infected individuals (INF) to frequencies (F_o) in Ss-uninfected (UN) individuals

Results: INF individuals exhibited a significant decrease in the spontaneous and antigen specific F_o of both mono - and dual functional Th1 cells compared to UN. Similarly, INF individuals also exhibited significantly decreased F_o of mono - and dual - functional Th17 cells upon antigen - stimulation compared to UN. In contrast, both the spontaneous and antigen - induced

F_o of mono- and dual - functional Th2 cells was significantly increased in INF compared to UN individuals. This differential T-cell response was predominantly antigen - specific since it was abrogated upon control antigen or mitogen stimulation. The regulation of Th1, Th2 and Th17 cells was pre-dominantly dependent on IL-10, while the regulation of Th2 but not Th1 or Th17 cells was also dependent on TGFβ. In addition, treatment of Ss infection significantly increased the antigen - specific F_o of Th1 and Th17 cells and decreased the F_o of Th2 cells in INF individuals.

Conclusion: Ss infection is characterized by an IL-10 dependent regulation of mono- and dual - functional Th1, Th2 and Th17 cells, a regulation reversible by anti-helminthic treatment.

(ii) Immunology of helminth-TB co-infections: A. Modulation of pro- and anti-inflammatory cytokines in active and latent TB by coexistent Ss infection

Principal Investigators	:	Dr. Subash Babu; Dr. P. Paul Kumaran (email: sbabu@nirt.res.in / ppkumaran@nirt.res.in)
Co-Investigators	:	Dr C.K. Dolla; Dr. V. Banurekha; Dr. Dina Nair; Dr. M. Satiswaran
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2013-2015

Background: Helminth infections are known to induce modulation of both innate and adaptive immune responses in active and latent TB. However, the role of helminth infections in modulating systemic cytokine responses in active and LTB is not known.

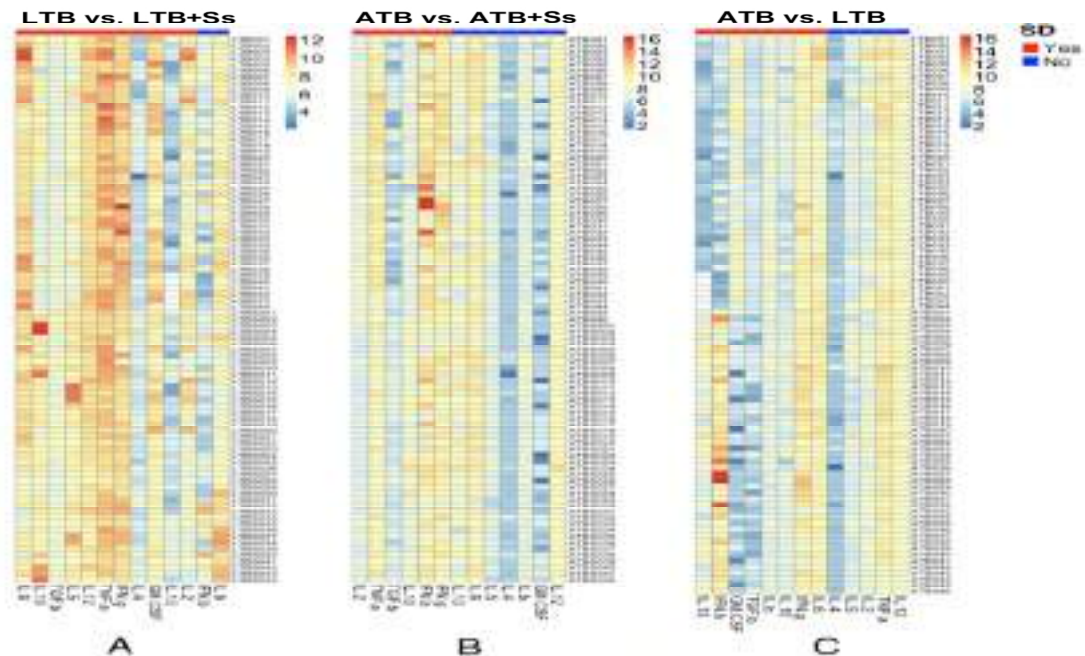
Aims / Methodology: To define the systemic cytokine levels in helminth-TB coinfection, we measured the circulating plasma levels of Type 1, Type 2, Type 17, other pro-inflammatory and regulatory cytokines in individuals with active TB (ATB) with or without coexistent Ss infection by multiplex ELISA. Similarly, we also measured the same cytokine levels in individuals with LTB with or without concomitant Ss infection in a cross-sectional study

Results: Our data reveal that individuals with ATB or LTB and coexistent Ss

infection have significantly lower levels of Type 1 (IFN γ , TNF α and IL-2) and Type 17 (IL-17A and IL-17F) cytokines compared to those without Ss infection. Also, those with ATB and LTB with Ss infection have significantly higher levels of the regulatory cytokines (IL-10 and TGF β), and those with LTB and Ss infection also have significantly higher levels of Type 2 cytokines (IL-4, IL-5 and IL-13) as well. Finally, those with LTB (but not ATB) exhibit significantly lower levels of other pro-inflammatory cytokines (IFN α , IFN β , IL-6, IL-12 and GM-CSF).

Conclusions: Our data therefore reveal a profound effect of Ss infection on the systemic cytokine responses in ATB and LTB and indicate that coincident helminth infections might influence pathogenesis of TB infection and disease (Fig. 44).

Fig. 44: Heatmaps depicting the trends in the modulation of cytokines in Ss-TB coinfection and in LTB versus ATB



Heatmaps of log₂ transformed plasma levels for LTB vs. LTB+Ss, ATB vs. ATB+Ss, and ATB vs. LTB were shown in panel A, B, C respectively, in which each row stands for a sample and each column stands for a cytokine. The annotation bar indicates if there is a significant difference (SD) between two groups or not for each cytokine.

(ii) Immunology of helminth-TB co-infections: B. Coincident helminth infection modulates systemic inflammation and immune activation in active PTB

Principal Investigators : Dr. Subash Babu; Dr. P. Paul Kumaran
 (email: sbabu@nirt.res.in / ppkumaran@nirt.res.in)
 Co-Investigators : Dr C.K. Dolla; Dr. V. Banurekha; Dr.
 Dina Nair; Dr. M. Satiswaran
 Source of funding : ICER
 Collaborators : Dr. Thomas Nutman (NIH)
 Study Period : 2013-2015

Background: Helminth infections are known to modulate innate and adaptive immune responses in active and LTB. However, the role of helminth infections in modulating responses associated with

inflammation and immune activation (reflecting disease activity and/or severity) in TB is not known.

Aims/ Methodology: To measure the markers of inflammation and immune

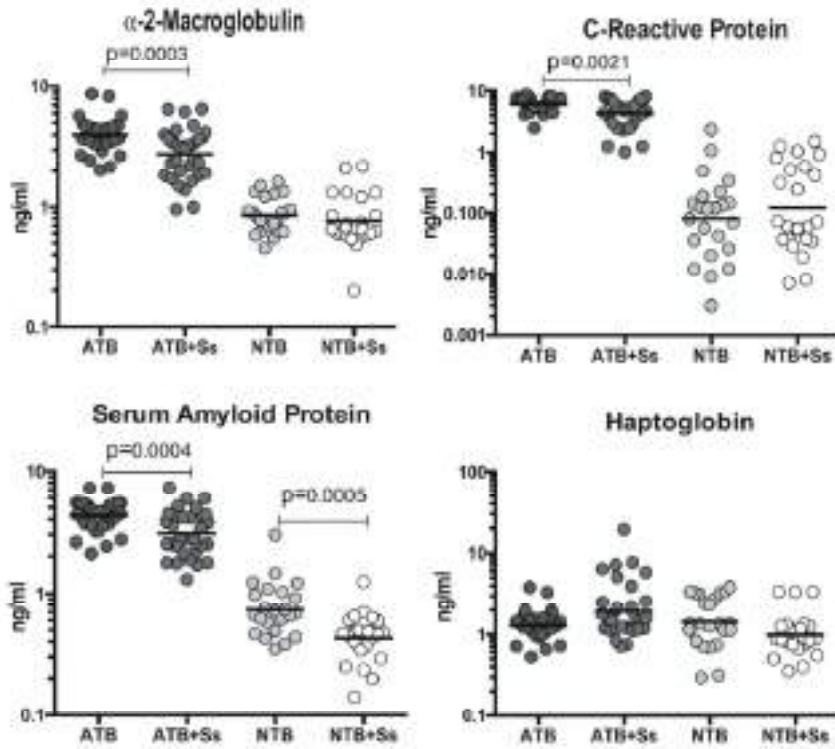
activation in active PTB individuals (ATB) with co-incidental Ss infection. These included systemic levels of acute phase proteins, matrix metalloproteinases and their endogenous inhibitors and immune activation markers. As a control, we measured the systemic levels of the same molecules in TB-uninfected individuals (NTB) with or without Ss infection

Principal Findings: Our data confirm that ATB is associated with elevated levels of the various measured molecules when compared to those seen in NTB. Our data also reveal that co-incident Ss infection in ATB individuals is associated with

significantly decreased circulating levels of acute phase proteins, matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases as well as the systemic immune activation markers, sCD14 and sCD163. These changes are specific to ATB since they are absent in NTB individuals with Ss infection (Fig. 45).

Conclusions: Our data therefore reveal a profound effect of Ss infection on the markers associated with TB disease activity and severity and indicate that co-incidental helminth infections might dampen the severity of TB disease.

Fig. 45: Helminth infections are associated with diminished plasma levels of acute phase proteins in active TB



The plasma levels of acute phase proteins - α -2M, CRP, SAA and Haptoglobin - were measured by multiplex ELISA in active pulmonary TB individuals with (ATB+Ss, n=36) or without Strongyloides coinfection (ATB, n=33) and in non-TB infected individuals with (NTB+Ss, n=23) or without Strongyloides coinfection (NTB, n=23). The results are shown as scatterplots with each circle representing a single individual and the bar representing the geometric mean.

(iii) Immunology of TB and its co-morbidities: A. IL-27 and TGF β mediated expansion of Th1 cells and aTregs expressing IL-10 correlates with bacterial burden and extent of disease in PTB

Principal Investigators	:	Dr. Subash Babu; Dr. V.V. Banurekha; Dr. Dina Nair (email: sbabu@nirt.res.in; banurekha@nirt.res.in; dinanair@nirt.res.in)
Co-Investigators	:	Dr. R. Sridhar (Stanley Hospital)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2012-2014

Background: CD4⁺ T-cell expression of IL-10 is an important mechanism controlling immunity to TB.

Aims/ Methodology: To identify the CD4⁺ T-cell subsets producing IL-10 in human TB, we enumerated the frequencies of IL-10 expressing CD4⁺ T-cell subsets following TB - antigen stimulation of cells from individuals with PTB and LTB

Results: We first demonstrate that TB antigens induce an expansion of IL-10 expressing Th1 (IFN γ ⁺, T-bet⁺), Th2 (IL-10⁺, IL-4⁺, GATA-3⁺), Th9 (IL-10⁺, IL-9⁺, IL-4), Th17 (IL-10⁺, IL-17⁺, IFN γ) and natural and adaptive regulatory T-cells [nTregs; IL-10⁺, CD4⁺, CD25⁺, Foxp3⁺ and

aTregs; IL-10 single⁺, CD4⁺, CD25⁻, Foxp3⁻] in PTB and LTB individuals, with frequencies being significant in the former. However, only Th1 cells and adaptive Tregs expressing IL-10 exhibit a positive relationship with bacterial burdens and extent of disease in PTB. Finally, we show that IL-27 and TGF β play an important role in the regulation of IL-10⁺ Th cell subsets.

Conclusions: Active PTB is characterized by an IL-27 and TGF β mediated expansion of IL-10 expressing CD4⁺ T-cell subsets, with IL-10⁺ Th1 and IL-10⁺ aTreg cells playing a potentially pivotal role in the pathogenesis of active disease.

(iii) Immunology of TB and its co-morbidities: B. Diminished systemic and antigen-specific Type 1, Type 17 and other pro-inflammatory cytokines in diabetic and pre-diabetic individuals with LTBI

Principal Investigators	:	Dr. Subash Babu; Dr. Paul Kumaran (email: sbabu@nirt.res.in; ppkumaran@nirt.res.in)
Co-Investigators	:	Dr. C.K. Dolla; Dr. Satiswaran
Source of funding	:	ICER
Study Period	:	2012-2014

Background: Type 2 DM is known to be a major risk factor for the development of active TB, although its influence LTBI remains poorly characterized.

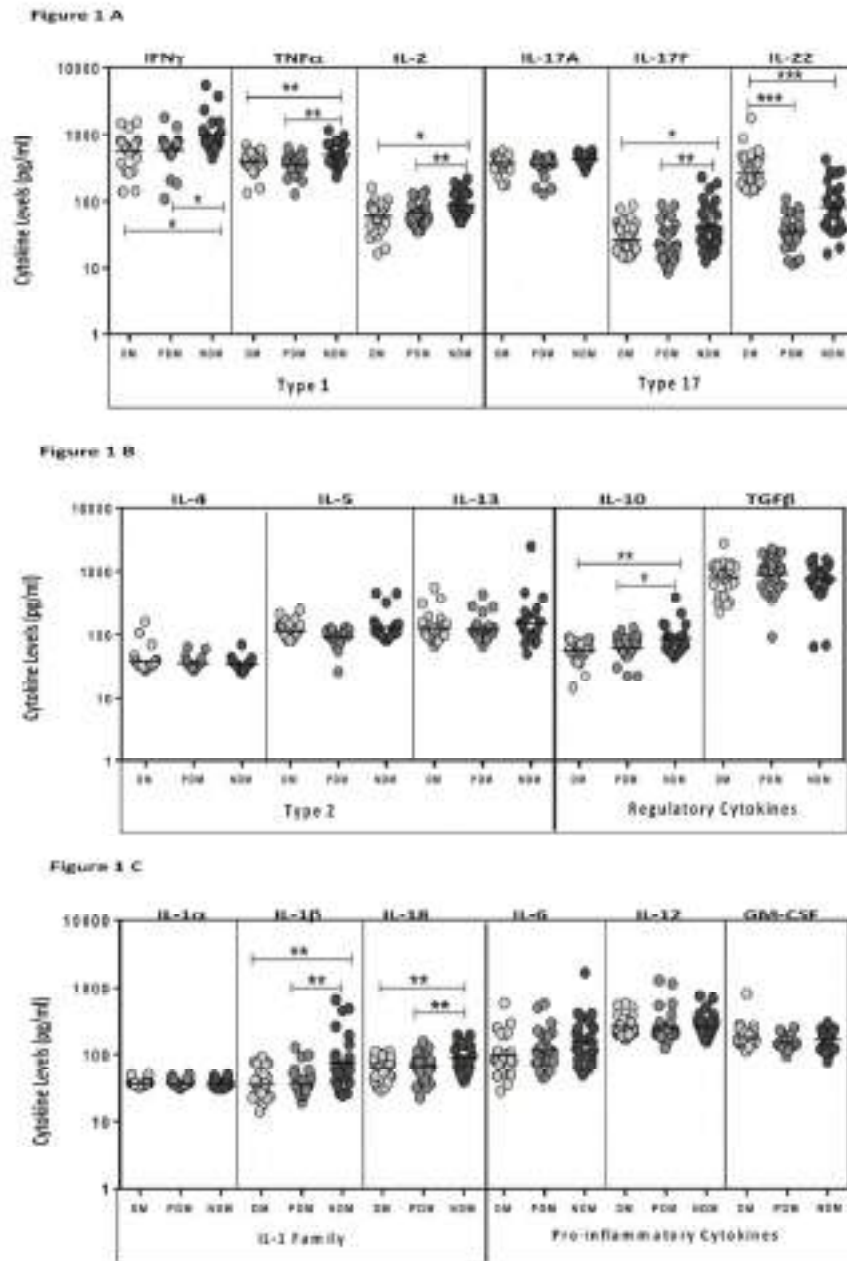
Aims/Methodology: To examine circulating plasma cytokine levels in individuals with LTBI with diabetes or pre-diabetes and compared them to those with LTBI with normal glycemic control

Results: LTBI with DM or pre-DM is characterized by diminished circulating levels of Type 1 (IFN γ , IL-2 and TNF α) and Type 17 (IL-17F) cytokines. This was associated with decreased systemic levels of other pro-inflammatory cytokines (IL-1 β and IL-18) and the anti-inflammatory cytokine (IL-10) but not Type 2 cytokines.

Moreover, LTBI-DM individuals had diminished levels of spontaneous and TB - antigen specific levels of Type 1 and Type 17 cytokines when antigen stimulated whole blood was examined. Finally, there was no significant correlation between any of the cytokines measured (with the exception of IL-22) with hemoglobin A1C (HbA1c) levels (Fig.46).

Conclusion: Our data reveal LTBI in the presence of diabetes or pre-diabetes is characterized by diminished production of cytokines implicated in control of TB activation allowing for a potential immunological mechanism that could account for the increased risk of active TB in DM.

Fig. 46: Diminished systemic levels of Type 1, Type 17, IL-1 family of cytokines and IL-10 in LTBI-DM and LTBI-PDM individuals



The plasma levels of Type 1 (IFN γ , TNF α , IL-2) and Type 17 (IL-17A, IL-17F and IL-22) (A); Type 2 (IL-4, IL-5, IL-13) and regulatory (IL-10 and TGF β) (B); IL-1 family (IL-1 α , IL-1 β , IL-18) and other pro – inflammatory (IL-6, IL-12 and GM-CSF) (C) cytokines were measured by ELISA in LTBI-DM (n=30), LTBI-PDM (n=30) and LTBI-NDM (n=30) individuals. The data are represented as scatter plots with each circle representing a single individual and the bar representing the geometric mean.

STUDIES IN PROGRESS:

ICER-1: Characterization of immune responses in helminth-TB co-infection

Principal Investigators	:	Dr. Subash Babu; Dr.P. Paul Kumaran (email: sbabu@nirt.res.in; ppkumaran@nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2012-2017

We are studying the influence of helminth infection on the immunological responses to TB antigens in LTB infected individuals. This study is being conducted as a prospective case-control study in Kanchipuram district, Tamil Nadu. We will be comparing immune responses to

mycobacterial antigens between individuals with LTB and helminth co-infection and individuals with LTB alone. The expected sample size is 100 per group. We have recruited 30 individuals in each group thus far.

ICER-2: Characterization of immune responses in treatment induced latency in PTB

Principal Investigators	:	Dr. Subash Babu; Dr.V.V. Banurekha; Dr. Dina Nair (email: sbabu@nirt.res.in; banurekha@nirt.res.in; dinanair@nirt/res.in)
Co-Investigator	:	Dr. R. Sridhar (Stanley Hospital)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2010-2015

The immune responses in LTB 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. We are comparing the immune response in PTB individuals before and after treatment. In addition, we are also

trying to determine immunological differences between PTB, extrapulmonary TB, LTB and uninfected individuals. We have performed *ex vivo* phenotyping on a variety of leucocyte subsets in our patients. Recruitment is over and follow-up is in progress.

ICER-3: Host immune responses in lymphatic filariasis and Strongyloidiasis

Principal Investigators	:	Dr. Subash Babu; Dr.P. Paul Kumaran (email: sbabu@nirt.res.in; ppkumaran@nirt.res.in)
Co-Investigator	:	Dr.C.K. Dolla; Dr.M. Satiswaran
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH); Dr.R. Nandhini (GGH); Dr.V. Lakshmi (CDH)
Study Period	:	2012-2017

This study is designed to determine the presence of and the immune response to filarial and Strongyloides infections in an area endemic for lymphatic filariasis and Strongyloidiasis in south India. This study will aim to examine the presence of filarial infection at a community level as well as in hospital settings. We will compare immune responses between filarial

infected, filarial diseased and control patients. Similarly, we will compare immune responses between Strongyloides infected and uninfected individuals. In addition, we will examine the immune responses following anti-helminthic therapy. Patient recruitment is ongoing.

ICER-4: Host immune responses TB lymphadenitis

Principal Investigators	:	Dr. Subash Babu; Dr.D. Baskaran (email: sbabu@nirt.res.in; baskar.d@nirt.res.in)
Co-Inestigator	:	Dr. Alena Srinivasan
Source of funding	:	ICER
Collaborators	:	Dr.R. Sridhar (Stanley Hospital); Dr.N. Meenakshi (GGH)
Study Period	:	2012-2017

Tuberculous (TB) lymphadenitis is the most common presentation of extra-pulmonary TB, accounting for 30–40% of cases. It constitutes a significant disease burden and differs from other forms of TB in that patients have large tuberculin reactions and there is a strong female preponderance. The immune responses in TB lymphadenitis are poorly understood. In addition, no study till date has evaluated the

immune responses pre- and post treatment in TB lymphadenitis. We are comparing immune responses in lymphnode versus peripheral blood in these individuals. We will also compare immune responses between pre- and post-treatment time points in the same individuals in same individuals in peripheral blood. Patient recruitment is ongoing.

CONTRIBUTION TO THE NATIONAL PROGRAMMES

(I) **HIV Laboratory services:**

1. Early Infant Diagnosis Program

(Contact person: Dr. Luke Elizabeth Hanna: email: hanna@nirt.res.in)

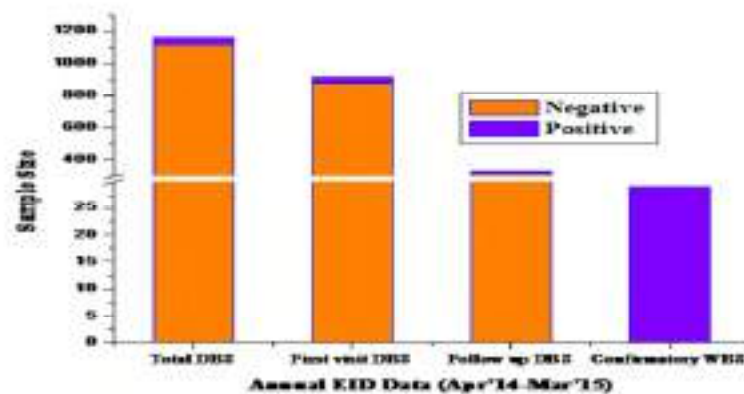
Source of Funding: National AIDS Control Organization (NACO)

Since 2010, NACO has been implementing the early infant diagnosis testing protocol across India and the department of HIV Lab services at NIRT continues to extend its HIV-1 DNA PCR assay testing services in Tamil Nadu. Under this programme, samples from HIV exposed infants/children aged between 6 weeks to 18 months across different districts in Tamil Nadu were referred to this centre for testing.

In the reporting year, 1237 samples were referred for HIV-1 DNA PCR testing

and 38 samples were found positive for HIV-1; out of 1237 total samples tested, 917 were first visit samples (6 weeks to 18 months old) while 320 were follow up visit samples (6 months to 18 months old). Out of the 917 first visit samples, 34 samples were positive while 4 samples of the follow visits were positive for HIV-1. During the period, 29 wbs samples were received and tested for confirmation, all of which yielded positive results (Fig. 47).

Fig. 47: Annual EID Data for the period April 2014 – March 2015 tested at the HIV Lab, NIRT



2. HIV viral load testing services provided to NACO Programme

(Contact Person: Dr. Luke Elizabeth Hanna: email:hanna@nirt.res.in)

Source of Funding: National AIDS Control Organization (NACO)

In the reporting year, 702 samples for the virological monitoring of patients with HIV-1 on II line ART in the NACO programme were received and tested.

Monthwise workload and results summary of the HIV-1 viral load testing are depicted in the table (Table 38).

Table 38: Summary of the HIV-1 viral load testing laboratory month-wise workload and results for the period April 2014- March 2015

Month/ Year	No. of cases			Total Samples tested
	<400 copies/ml	400-10,000 copies/ml	>10,000 copies/ml	
April/2014	34	12	43	89
May/2014	3	1	14	18
June/2014	34	15	46	95
July/2014	35	20	61	116
Aug/2014	21	11	47	79
Sep/2014	29	9	38	76
Oct/2014	17	7	30	54
Nov/2014	11	8	19	38
Dec/2014	17	6	20	43
Jan/2015	10	6	17	33
Feb/2015	9	7	17	33
Mar/2015	4	6	18	28
Total	224	108	370	702

3. Quality Assurance activities

(Contact Person: Dr. Luke Elizabeth Hanna : email: hanna@nirt.res.in)

For improving the quality of testing services, the laboratory has been participating in various External Quality Assurance (EQA) programs. The laboratory received good scores and approved/certified status in the reporting year.

A. EQA Program for HIV Drug resistance: The WHO accredited laboratory HIV-1 drug resistance genotyping laboratory participated successfully in the NIH-VQA Genotyping Proficiency panel testing. In the reporting year, data for two panel of samples were submitted for the HIV Drug resistance testing in the RT and PR genes. The laboratory is currently in the process of validating an in-house assay through VQA Prequalification and Proficiency panel testing.

B. EQA Program for HIV-1 viral load testing: In the reporting year, the HIV-1 viral load testing laboratory participated successfully in two EQAS Programmes - NIH-VQA and RCPA. Data for four panels were submitted to NIH-VQA and one round of panel

testing was completed and results submitted to RCPA.

C. EQA Program for HIV-1 DNA PCR testing: For HIV-1 DNA PCR testing, the laboratory has participated successfully in two rounds of panel of testing in the NIH-VQA Program and CDC.

D. EQA Program for CD4/CD8 Testing and HIV Serology: The routine laboratory that undertakes CD4/CD8 testing and HIV diagnosis (Rapid) has been enrolled in the in-country EQAS Program provided by NARI, Pune. During the reporting year, one round of Proficiency panel testing was completed for the above two assays.

II. Bacteriology Lab services:

The following activities are being conducted by Bacteriology Department as support service to the Central TB Division:

1. Accelerating access to quality TB diagnosis for pediatric cases in 4 major cities in India

Site PI	:	Dr. K. R. Uma Devi (email: umadevi.r@nirt.res.in)
PI & collaborators	:	Dr. Neeraj Raizada, FIND, Delhi, Hyderabad, Kolkata
Source of Funding	:	USAID/FIND
Study period	:	April 2014 – 2015

Pediatric TB diagnosis is still complicated due to the fact that it mimics other common childhood diseases as well as due to the inability of the children to expectorate sputum. Therefore it poses a lot of limitations in the diagnosis of childhood TB. To address this gap, recently in the global guidance document released by WHO, it has been recommended that Xpert MTB/RIF may be used rather than microscopy and culture as the initial diagnostic test in all children presumed to have TB. Xpert MTB/RIF is a cartridge based automated nucleic acid amplification test for TB and RMP resistant-TB case detection. It can be used for detection in pediatric specimen types such as gastric lavage, BAL, induced sputum, lymph node aspirates, etc. for use in Xpert

At NIRT, the project was launched on 17th April, 2014, by Dr. Jagdish Prasad, Director General Health Services, Ministry of Health and Family Welfare, Govt. of

India at NIRT, Chennai, with support from USAID. This project represents concerted efforts of RNTCP, USAID, NIRT and FIND, for a possible solution to the pediatric diagnostic gap under RNTCP.

The aim is to increase the notification of pediatric TB to RNTCP from public and private sector institutions by improving the quality of TB diagnostic services in children. The target populations in this project are pediatric TB suspects (pulmonary and extra pulmonary) in 4 major cities, namely, Delhi, Hyderabad, Chennai and Kolkata. These labs would provide accurate evidence based same day diagnosis in line with internationally accepted standards of TB care. This project would offer this diagnostic solution with no cost to patient or provider both in private and public sector. Any pediatrician both in public and private sector in these 4 cities can send specimens for testing to these laboratories. The tests would be done on the same day

and the results communicated to referring provider electronically and at the same time notified to RNTCP under Nikshay.

At NIRT, so far 2857 samples have been processed within these 12 months (April 2014 to March 2015). The details are listed in the table given below (Table 39):

Table 39: Details of the pulmonary and extrapulmonary specimens processed by Gene Xpert

Type of Specimen	Total no.of samples	Sensitive	Resistant
Pulmonary	1813	35	0
Extra Pulmonary	1044	83	2
Total	2857	118	2

At NIRT, so far 2857 samples have been processed within the 12 month period (April 2014 to March 2015). By Gene Xpert as listed in the table above. Out of the 1813 pulmonary samples processed, 35 were positive for TB and RMP sensitive and 83

out of 1044 extrapulmonary specimen were positive for TB and RMP sensitive while 2 were found to RMP resistant. As of now the project has been extended upto December 2016.

2. Update on diagnosis by Line Probe Assay for programmatic management of DR-TB (Period: 1st April, 2014 and 31st March 2015)

Diagnosis of patients with multi drug resistant (MDR) TB by line probe assay (LPA) was done for 6864 patients during the period between 1st April, 2014 and 31st March 2015 as part of service to the Tamil Nadu PMDT activities of RNTCP. The districts covered were three districts of

Chennai and Kanchipuram. Of them, 237 were true MDR, 116 were resistant to RMP only, 421 were resistant to H only and 3779 were sensitive to both H and R. In addition, 802 follow-up samples were processed by MGIT960 system (Table 40).

Table 40: Number and DR pattern of the specimens processed by LPA

	H Resistant	R Resistant	HR Resistant	HR Sensitive	Neg for MTB	Cult. Neg (of smear negatives)	Follow up
2nd Q/2014	125	27	55	999	60	470	156
3rd Q/2014	108	33	64	926	57	471	211
4th Q/2014	108	26	71	868	35	556	202
1st Q/2015	80	30	47	986	28	550	233
Total	421	116	237	3779	180	2047	802

RNTCP ACTIVITIES IN NATIONAL REFERENCE LABORATORY, NIRT, CHENNAI

(2014-15)

The Department of Bacteriology at NIRT has been designated as one of the National Reference Laboratories (NRL) for monitoring of five states (Andhra Pradesh, Gujarat, Kerala, Tamil Nadu and Telangana) and five Union territories (Andaman & Nicobar island, Lakshadweep, Diu & Daman, Dadar & Nager Haveli and Puducherry) under Revised National TB programme in India. The main responsibilities of NRLs are to monitor EQA of smear microscopy as well as culture and DST for both first line DST (FLD) and second line DST (SLD) by both phenotypic and genotypic methods. The NIRT also provides training of laboratory personnel and is responsible for accrediting state level laboratories, e.g., the IRLs, Medical Colleges and private laboratories for Culture

and DST and for diagnosis of DR-TB. Second line DST has been performed to diagnose XDR cases from presumptive XDR cases under Cat-IV treatment from different states of India. XDR diagnosis at base line has also been implemented for three DR-TB centers in Tamil Nadu Andaman and Nicobar Islands for PMTD services.

A total of 29 laboratory personnel from 10 different institutions have been trained for both first and second line DST using solid and (or) liquid media. Totally 15 laboratories has been certified for either solid, liquid and molecular diagnosis for DR-TB till date. Accreditation process is in progress for 5 Medical colleges/ Private Laboratories for FLD and for 3 laboratories for SLD including IRLs of Andhra Pradesh,

Puducherry and Telangana. Pre-accreditation assessment visit has been conducted for three medical Colleges in Tamil Nadu and Puducherry. Previously accredited 13 laboratories have been renewed based on sixth round proficiency testing. Totally six rounds of proficiency a test has been completed for participating laboratories till date.

A total of 530 follow up cultures and 124 patient samples has been received for XDR diagnosis from presumptive XDR under Cat-IV treatment

and XDR diagnosis at base line respectively; XDR diagnosis details are presented in Table 41. Four states (Andhra Pradesh, Kerala, Tamil Nadu and Telangana) and two UTs (Andaman & Nicobar Island, Puducherry) have been visited for On-site evaluation of smear microscopy. A total of 77 manufactured panel slides were used to check the proficiency of laboratory personnel at IRL and district level as a part of EQA for smear microscopy.

Table 41: Details of XDR diagnosis for PMDT services in RNTCP at NIRT

No of cultures received from states	DST results not available*	No. of results reported as MTB	No. of results reported as XDR	No. of OFX-mono resistance	No. of Kanamycin-mono resistance
XDR diagnosis of presumptive XDR under Cat-IV treatment					
530	60	470	68	178	37
XDR Diagnosis at Baseline (Three DR-TB centres of Tamil Nadu)					
124	12	112	0	18	4

*Due to Contamination, MTB not detected and culture negatives

TRAINING:

As part of Supra National Reference Laboratory activity of NIRT, Department of Bacteriology is committed to provide training facility for students of various education and/or research institutions in the country. Between April 2014 and March 2015, a total of 67 Post Graduate Microbiology students, 25 Medical Lab Technology students, 12 MD Microbiology students, 2 Ph. D scholars and 5 Physicians were trained in the Department of Bacteriology for

mycobacterial procedures. In addition, 12 students belong to Anna University (B. Tech., Biotechnology) and Madras University (M. Sc., Microbiology) successfully completed their final year dissertation work under the guidance of faculties present in the department.

**LIBRARY AND
INFORMATION CENTRE**

Library and Information Centre

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

The Library is constantly evolving to meet the changing needs of the users. During the last fifteen years the Library made significant investments in acquiring e-resources. For providing access to the e-resources, a customized **Digital Library** portal has been established. It provides access to the e-resources being subscribed by the library. It also provides gateway to ICMR-Consortium journals; ICMR Resource sharing portal viz., 'J-Gate@ICMR'; ERMED (Electronic Resource MEDicine) Consortium viz., 'J-Gate@ERMED', Specialized health science databases, open access resources and link to Cochrane Library, which has National Provision License supported by ICMR. It enhances a simplified integrated 24 hrs access facility (intranet) to our users; all it needs are a few mouse clicks.

Collection Building

E-Resources

- Individual Titles

Cumulative Collection

- American Society for Microbiology

E-Bundle

- Annual Reviews Biomedical suite/Life Science

Subject Collection

- Immunology & Microbiology (SciDir)

Package

- NPG Life Science

E-Books

- Books@OVID

Databases

- OVIDSP
- Cochrane Library
- ERMED
 - Infotrac
- Open J-Gate

Archives

- AIDS
- American Society for Microbiology
- Annual Reviews Biomedical suite/Life Science
- JAIDS
- Nature 1950+
- Science Classic (1880-1996)
- Scientific American (1993+)

Consortia

- Lancet
- Nature
- New England Journal of Medicine

- Science
- J-Gate@ERMED

Resource Sharing

- J-Gate@ICMR

Services

The library offers a range of value added services including:

- Access to electronic resources through **digital library portal (24hrs)**
- Electronic Check- in and Check-out services
- Current Awareness Service
- Document Delivery Service (Print & Electronic)
- e-Mail co-ordination
- Face book maintenance
- Internet Lab
- Press Clippings

Publication (TB Alert)

- Reference assistance
- Resource Sharing
- Facilitating digital medial resources and web based services.

Institutional Repository

NIRT Library steps-in to its fourth mile stone in its history followed by the Internet Browsing Lab, Library Automation and Digital Library. Library established its repository viz., the “**NIRT Institutional Repository (NIRTIR)**” and opened to the public (Fig. 48) in order to promote the research on tuberculosis. This is a two-in-one repository i.e., institute cum specific subject repository. This will enhance the visibility and status of our institute further. This repository will facilitate long term preservation of our research output and provide easy access to our publications.

Fig. 48: NIRT Institutional Repository (NIRTIR)



NIRT Web site

The NIRT Web Site (<http://nirt.res.in>) is designed, developed and being maintained by the library.

Publication

As part of our Value Added Services, a monthly publication **TB Alert** is being published and circulated to all ICMR institutes and all the major national and international TB institutes.

APPENDICES

SUMMARY SHEET OF PUBLICATIONS (2014-15)

Parameters

Papers	72
Publishing authors	452 + Collaborative authors
Publishing journals	51

Papers covered in SCI/JCR journals

Sl.No.	Publishing Journals	Impact Factor**	No. of papers	Total Impact Factor
1	Acta Trop	2.270	1	2.270
2	AIDS	5.554	1	5.554
3	AIDS Behav	3.728	1	3.728
4	AIDS Res Hum Retro	2.325	1	2.325
5	American J Med Sci	5.302	1	5.302
6	American J Microbial Res	-	1	-
7	Antimicrob Agents Che	4.476	1	4.476
8	Asian J Biomed Phar Sci	-	3	-
9	Asian J Med Sci	-	1	-
10	Biomed Res Int	1.579	1	1.579
11	Chest	7.483	1	7.483
12	Clin Epid Global Health	-	1	-
13	Clin Infect Dis	8.886	2	17.772
14	Clin Vaccine Immunol	2.37	2	4.74
15	Current Science	0.833	2	1.666
16	Cytokine	2.664	1	2.664
17	Drug Safety	2.824	1	2.824
18	Expert Rev Clin Immunol	2.484	1	2.484
19	Hum Immunol	2.138	1	2.138
20	IOSR J Mathematics	1.759	1	1.759
21	Indian J Appl Res	-	2	-
22	Indian J Pract Pediatr	-	1	-
23	Indian Pediatr	1.040	1	1.040
24	Infect Immun	3.731	1	3.731
25	Int J Bacteriol	-	1	-
26	Int J Computer Sci & Technol	5.09	1	5.09
27	Int J Clin Exp Pathol	1.891	1	1.891
28	Int J Immunogenet	1.247	1	1.247
29	Int Immunopharmacol	2.472	1	2.472
30	Int J Mycobacteriol	-	1	-
31	Int J Scientific Res	-	2	-
32	Int J Tuberc Lung Dis	2.315	1	2.315
33	J Acquir Immune Def Synd	4.556	1	4.556

34	J Coastal Life Med	-	1	-
35	J Chromatograph Separat	-	1	-
36	J Infect Dis	5.997	1	5.997
37	Journal of Microbiology, Biotechnology and Food Sciences	0.9801	1	0.9801
38	J Mole Graph Model	1.722	1	1
39	J Pathol	7.429	1	1
40	J Public Health Policy	1.775	1	1
41	Lancet	45.217	3	135.651
42	Life Sci	2.702	1	1
43	Microbial Res	2.561	2	5.122
44	Nature	41.456	1	1
45	Natl Med J India	0.782	2	1.564
46	Parasite Immunol	2.143	1	1
47	Plant Cell, Tissue Organ	2.125	1	1
48	PLOS Pathogens	7.562	2	15.124
49	PLOS One	3.234	5	16.170
50	PLOS Neg Trop Dis	4.446	3	13.338
51	Tuberculosis	2.711	4	10.844
	Total		72	303.896

DEPARTMENT-WISE PUBLICATIONS:

ICER	:	18
IMMUNOLOGY	:	14
CLINIC	:	13
CLINICAL PHARMACOL.	:	7
BACT.	:	6
STATISTICS	:	6
HIV DVN.	:	6
PATHOLOGY	:	1
DSBR	:	1

LIST OF PUBLICATIONS

Publications in Journals	:	72
Published		
i) International	:	59
ii) National	:	13
Books	:	1
Accepted		
i) International	:	11
ii) National	:	2

International:

1. Afsal K, Harishankar M, Banurekha VV, Meenakshi N, Parthasarathy RT, Selvaraj P. Effect of 1, 25 dihydroxy vitamin D₃ on cathelicidin expression in patients with and without cavitary tuberculosis. *Tuberculosis*.2014;94:599-605.
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1. Ramachandran G. Swaminathan S. Book chapter, ‘Drugs’ in section 30 – TB in postgraduate textbook of pediatrics. Publisher – Jaypee brothers.

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International:

1. Baskaran D, Mohan Kumar P, Srividya A, Chandrasekaran V, Vijayarj S, Sivakumar S, Gomathy S, Menon PA, Wares F, Swaminathan S. Prevalence and risk factors for adult pulmonary tuberculosis in a metropolitan city of south India. *PLoS One.*
2. Thomas BE, Charles N, Watson B, Chandrasekeran V, Senthil Kumar R, Dhanalakshmi A, Wares F, Swaminathan S. Prevalence of chest symptoms among brick kiln migrant workers and care seeking behaviour - A study from South India. *J Public Health.*

3. Hemanth AK, Narendran G, Kumar RS, Ramachandran G, Sekar L, Raja K, Swaminathan S. Rifampicin exposure is lower in HIV-infected TB patients receiving intermittent than daily anti-tuberculosis treatment. *Int J Tuberc Lung Dis*.
4. Kumar NP, Sridhar R, Nair D, Banurekha VV, Nutman TB, Babu S. Type 2 diabetes mellitus is associated with altered CD8⁺ T and natural killer cell function in pulmonary tuberculosis. *Immunology*.
5. Madhumitha H, Mohan V, Kumar NP, Pradeepa R, Babu S, Aravindhan V. Impaired toll-like receptor signalling in peripheral B cells from newly diagnosed type-2 diabetic subjects. *Cytokine*.
6. Mimiaga MJ, Thomas B, et al. Garnering an in-depth understanding of MSM in Chennai India: A qualitative analysis of sexual minority status and psychological distress. *Arch of Sexual Behavior*.
7. Narendran G, Swaminathan S. TB-HIV co-infection: a catastrophic comradeship. *Oral Diseases*.
8. Ramana Rao PV, Anbalagan S, Sikhamani R, Raja A. Interleukins 15 and 12 in combination expand the selective loss of natural killer T cells in HIV infection *in vitro*. *Clin Exp Med*.
9. Selvaraj P, Harishankar M, Afsal K. Vitamin D: Immunomodulation and tuberculosis treatment. *Can J Physiol Pharmacol*.
10. Swaminathan S, Ramachandran G. Challenges in childhood tuberculosis. *Clin Pharmacol Ther*.

National:

1. Kandhakumari G, Stephen S, Sivakumar S, Narayanan S. Spoligotype patterns of *Mycobacterium tuberculosis* isolated from extra pulmonary tuberculosis patients in Puducherry, India. *Indian J Med Microbiol*.
2. Thomas BE, Srividya A, Manogaran C, Swaminathan S. Tuberculosis among tribals in India: A systematic review and Meta-Analysis. *Indian J Med Res*.

Awards/Honours

1. The Institutional Ethics Committee received accreditation by FERCAP.

2. Dr. Soumya Swaminathan - awarded "DRC Gold Medal Oration award" by Prof. M. Viswanathan Diabetes Research Centre, Chennai.

3. Dr. Soumya Swaminathan - awarded "Dr.P.K. Sen TAI Gold Medal Oration award" by 69th National Conference n Tuberculosis and Chest Diseases (NATCON).

4. Dr.G. Narendran – awarded NASI SCOPUS Young Scientist Award in Medicine 2014 by the Minister of Science and Technology organized by the National Academy of Medical Sciences and ELSEVIER.

5. Mr. Jovvian George awarded Bill and Melinda Gates Foundation "Global Health Travel Award" for attending Keystone Symposia, "Co-infection A global challenge fro disease control (C6), MG Brazil in March 2015.

6. Dr.N. Pavan Kumar awarded Bill and Melinda Gates Foundation "Global Health Travel Award" for attending Keystone Symposia, "Novel therapeutic approaches to tuberculosis" Keystone, Colarodo, USA.



Dr. Soumya Swaminathan, Director, NIRT receiving a plaque in connection with the accreditation of the Institutional Ethics Committee by FERCAP.



Dr.G. Narendran awarded NASI SCOPUS Young Scientist Award in Medicine 2014 by the Minister of Science and Technology organized by the National Academy of Medical Sciences and ELSEVIER.

Special Assignments

Membership in Committees

Dr.G. Narendran

Expert Member – CDSCO team for inspection of hospitals – 17-18 2015.

Dr. Beena E Thomas:

- Editorial Board Member for Journal of Health Sciences
- Member of National Advisory Committee on Advocacy Communication Social Mobilization (ACSM)
- Member of DR TB counseling Committee
- Member of International Association of Schools of Social Work International Federation for

social workers

Participation in Conferences / Seminars / Workshops

Mr. Senthil Sellappan:

- Life Member of Professional Social Worker's Association

Dr.M. Makesh Kumar:

- Member of Tamilnadu Medical Council, Chennai.
- Member of Indian Medical Association – Madurai Branch.
- Member of the National Foundation for Infectious Diseases, Bethesda, Maryland, USA.

Dr.K.R. Uma Devi:

- Nominated as a member of IBSC committee for KMS Health Care P Ltd.

Dr.N.S. Gomathi:

- Nominated as a member of the ICMR Expert group on TB diagnostics
- Co-authored white paper on TB diagnostics that has been submitted to ICMR in July 2014.
- Represented NIRT as SNRL member for lab upgradation at Madurai Medical College and BMHR, Bhopal.

Dr.V.N. Azger Dusthacker:

- Was invited to peer review process by PLOS One.
- Was invited to review the manuscripts by Asian Pacific Journal of Tropical Diseases.

Dr.S. Balaji:

- Nominated as a representative of NIRT for “Regional review meeting on Programmatic management of drug resistant TB south states and Delhi” during April, 2014.

1. Guest lecture given - TB_HIV, a catastrophic comradeship – CME program at Meenakshi Medical College, Kanchipuram during March, 2015 - G Narendran.

2. Guest lecture given - 3rd Annual ASIAN experts TB meeting held at Macao, China on Active case finding –strategy for accelerated TB elimination in ASIA- “Feasibility for treatment of LTBI using Quantiferon Gold for resource limited set up” – extempore presentation during August 2014 – G Narendran.

3. Guest lecture given - HIV-TB update at SRM Medical College, Department of Pulmonary Medicine during August, 2014 – G Narendran.

4. Plenary lecture on “Tuberculosis in HIV and “HAART attacks” - Research evidences for maximizing response & recovery at the 7th world Workshop on Oral Health and diseases in AIDS organized by the NIH and ragas at Hyderabad during Nov. 2014 – G Narendran.

5. Participated in “WHO consultation on Research for TB elimination meeting” in Stockholm, Sweden during Nov. 2014 – Beena E Thomas.

6. Poster presentation on “A community based approach in designing a model TB sensitization programme for Self Help Groups (SHGs) - A study from South India” at The 45th Union World Conference on Lung Health in Barcelona, Spain during Oct. – Nov. 2014 – Beena E Thomas.

7. Oral presentation “Disclosure of parental HIV status: Are parents telling their children and what are some factors influencing disclosure – A study from south India” in a session “Addressing health inequalities: International HIV perspectives” at Joint world conference on Social work, education and social development at Melbourne, Australia during July 2014 - Beena E Thomas.
8. “International conference on community empowerment, Coping, resilience and hope” at Centre for Action Research and People's Development, Hyderabad during Dec. 2014 – Priscilla Rebecca.
9. Presented a session on Alcohol & TB on Regional Prospective Observational Research in Tuberculosis (RePORT)-India, 3rd Joint Leadership Meeting Agenda at BJMC Conference, Pune during March, 2015 – Beena E Thomas.
10. Participated and presented “Gender Justice perspectives in Social Work” on the National Conference in Social Workers Association for Staff and Students (SWASS) at Loyola college during Feb. 2015 – Beena E Thomas.
11. Participated in “Strategic Review of the food Security and Nutrition situation in India” at the MSSRF during Jan. 2015 – Beena E Thomas.
12. Participated in “Expert Consultation on Implementation Science Research-Early diagnosis and immediate anti-retroviral treatment” at WHO/SEARO, New Delhi during Dec. 2014 – Beena E Thomas.
13. Presented in “MDR TB Counseling training” organized by CTD, at NTI, Bangalore during Oct. 2014 – Beena E Thomas.
14. Presented a topic on “Socio Economic Impact of TB” at REACH, Chennai during Oct. 2014 – Beena E Thomas.
15. Presented in “ICMR Tribal Health Research Forum Meeting”, RMRCT, Jabalpur during June, 2014 – Beena E Thomas.
16. Participated in “National Advisory Committee on Advocacy Communication and Social Mobilization (ACSM)” organized by Central TB Division, Nirman Bhavan at New Delhi during May, 2014 – Beena E Thomas.
17. Participated in “MDR counseling modules for facilitator and participants” training programme organized by Project Axshya, The Global Fund Round 9 Project, The Union South-East Asia Office, International Union Against Tuberculosis and Lung Disease (The Union), at New Delhi during April, 2014 – Beena E Thomas.
18. Presented in “ICMR Tribal Health Research Forum Meeting”, at DMRC, Belgaum during April, 2014 – Beena E Thomas.
19. Invited speaker for Inauguration of M.Phil Social Work and Psychology at Madras Social of Social Work, Chennai during August, 2014 - Beena E Thomas.
20. Presented a topic on “Trends in Environmental resource management” at Nirmala college for women, Coimbatore during August, 2014 - Beena E Thomas.
21. Delivered a talk on “Role of Social Work in different Settings” at Madras School of Social Work during June 2014 - Beena E Thomas.
22. Participated and Presented in “2nd MDR TB Committee meeting” organized by CTD, at

- Bangalore during April - May 2014 - Beena E Thomas.
23. Participated in “Ethics and Responsible conduct of Research :Basics and Beyond” Organised by Sri Ramachandra University and CITI ,University of Miami, USA held at Sri Ramachandra University, Chennai, India (which offers Ten CME Credit hours) during Nov. 2014 – M. Makesh Kumar. 29.
24. Participated in the training First Joint CReATE Centers Workshop on Ethics, Pharmacovigilance, Biostatistics and Data Management in Clinical Research, Organised by CReATE (Clinical Research Advancement towards Excellence) and Goa Medical College at Goa Medical College, Goa, India during Nov. 2014 – M. Makesh Kumar. 30.
25. Participated in the training workshop on Capacity Building of Ethics Committees for Clinical Research in India, organized by CReATE /PATH and PIMS, at Pondicherry Institute of Medical Sciences, Puducherry during April, 2015 ((it has been awarded for 6 credit hours of Category 2 CME credits by Tamil Nadu Medical Council, Chennai) – M Makesh Kumar. 31.
26. Talk at the National Symposium on "Genomics & Human Health" at IBMS, Taramani, during March, 2015 - K. R. Uma Devi. 32.
27. Presentation on ‘Conventional diagnosis of tuberculosis’ Sree Sastha Institute of Engineering and Technology (Affiliated to Anna University, Chennai, Tamilnadu) during Dec. 2014 - K. R. Uma Devi. 33.
28. Participated in a CME in Madras Medical College, Chennai and presented on new microbiological and molecular techniques for diagnosis of tuberculosis and drug resistant tuberculosis during Jan. 2015 - K. R. Uma Devi. 34.
29. Chaired a session on identifying allergen – Prick test or Immuno CAP, Pediatric Pulmonology at GRT Convention Centre, Chennai, during Dec. 2014 - K. R. Uma Devi. 35.
30. Presented a guest lecture titled “Herbs in Therapeutics” in a National Conference on Natural products as therapeutics, Medical Microbiology, Nanobiology, system biology: current status and emerging trends organized by Mohd. Sathak College and TamilNadu State Council for Science and Technology at Chennai, during Sep. 2014 - N. S. Gomathi.
31. Presented a guest lecture on “Newer TB Diagnostic modalities of Drug Resistant Tuberculosis” in a CME organized by IMA South Chennai, at Kauvery Hospital, Chennai during March, 2015 - N. S. Gomathi.
32. Attended CPTR meeting in Washington DC, USA during Sep. 2014 – K.R. Uma Devi.
33. Attended a workshop, to train the trainers titled “Culture of Responsibility” conducted by American Society of Microbiology at NIMS, Hyderabad during April, 2014 – V.N. Azger Dusthacker.
34. Poster presentation at Keystone symposia, “Novel therapeutic approaches to tuberculosis” held at Keystone, Colorado, USA during April, 2014 - N. Pavan Kumar.
35. Oral presentation at Keystone symposia, “Host responses to tuberculosis” held at Santa Fe, New Mexico, USA during Jan. 2015 – Jovvian George.

36. Poster presentation at Keystone symposia, “Co-infection: A global challenge for disease control (C6) held at Brazil, during March, 2015 – Jovvian George.
37. Poster presentation on “Regulatory role of vitamin D receptor gene variants on vitamin D3 modulated MIG and IP-10 production in pulmonary tuberculosis” at International symposium on genomics in health and disease held at Mumbai during Jan. 2015 – Harishankar M.
38. Poster presentation on “Influence of vitamin D receptor gene variants on vitamin D3 modulated granulysin and perforin positive cells in pulmonary tuberculosis” at International symposium on genomics in health and disease held at Mumbai during Jan. 2015 – Afsal K.
39. Oral presentation on “Promiscuous epitopes specific to HIV-1C strains circulating in India: Potential candidate epitopes for vaccine design” and abstract was published in Conference proceedings in “Indian Genetics Congress” 2015 held at SRM University, Kattankulathur during March 2015 – Jagdish Chandra Bose, *et al.*
40. Presentation titled, “Evolving pattern of mutations in an efflux pump gene Rv1258c in 223 *M. tuberculosis* strains from south India” was published in the Conference proceedings of “Indo-US conference-cum-workshop on “Big data analysis and translation in disease biology (Big data and disease)” held at Jawaharlal Nehru University, New Delhi during Jan. 2015 – Jagdish Chandra Bose, *et al.*
41. Oral presentation at The 45th Union World Conference on Lung Health in Barcelona, Spain during Oct. – Nov. 2014 – A. K. Hemanth Kumar.

CAPACITY BUILDING

Dept. of Social & Behavioral Research:

1. Workshop on 'Developing a Protocol for a Cochrane Systematic Review', organized by CMC Vellore from 17th – 21st November 2014 attended by Ms. Priscilla Rebecca, Ms. Senthandro Ovung and Dr. S. Poonguzhali
2. Workshop on 'Qualitative Research Methods & Data Analysis' organized by SAMARTH foundation at SCARF, Chennai from 6th to 8th November 2014 attended by Mrs. A. Deepalakshmi, Mr. S. Senthil and Dr. E. Thiruvalluvan.
3. Workshop on 'Qualitative research methods and data analysis' organized by Indian Institute of Public Health – Delhi, Gurgaon from 8th – 12th December 2014 attended by Ms. Senthandro Ovung.
4. Workshop on 'Psycho-drama & active group techniques' organized by East West Centre for Counselling & Training, Chennai from 9th – 12th August 2014 attended by Mrs. M. Jamuna & Mr. E. Senthil Kumar
5. Training on 'Sociometry & group psychotherapy' by Magdalene Jeyarathnam at NIRT, Chennai on 17th & 18th June 2014
6. Soft skills training on 'Behavioral Styles' by Ms. Bhanumathi Cousik at NIRT, Chennai on 12th June 2014.
7. Training on MDR-TB Counseling at NTI, Bangalore from 28th April to 2nd May, 2014 attended by Ms. Priscilla Rebecca and Mrs. M. Jamuna

**Conference(s) / Workshop(s)
/Symposium(s) organized:**

1. World TB Day Symposium 2015

Date: 23rd March 2015

Venue: National Institute for Research in Tuberculosis, Chennai

To commemorate World TB Day 2015, National Institute for Research in Tuberculosis (NIRT) conducted a symposium titled “Impact of Tuberculosis on Women and Children” on 23rd March 2015.

The Chief Guest for the Symposium was Mrs. Lalitha Kumaramangalam, Chairperson, National Commission for Women. The Symposium was presided by Dr. C. N. Mahesvaran IAS, Mission Director, National Rural Health Mission, Tamil Nadu State.

The symposium also provided a platform for a cured patient with multi-drug resistant (MDR) TB to share her experiences from a patient's perspective. The chief given lauded the efforts of the NIRT staff in the strong support she received by way of motivation, counselling and urged that more awareness on TB be created among the public. A panel discussion was held which was followed by talks by experts on TB in relation to pregnancy, nutrition (etc.).



Venue: National Institute for Research in Tuberculosis, Chennai

Date: 15th December 2014



On December 15, 2014, a plaque to commemorate the contributions of Dr. Wallace Fox, founder Director of the Tuberculosis Chemotherapy Centre (1956-1961) was unveiled by Dr.S.P. Tripathy, Former Director General, Indian Council of Medical Research at NIRT, Chennai on behalf of Dr.V.M. Katoch, Secretary DHR and DG, ICMR. Mrs. Gaye Fox & five members of the Fox family attended the function which was also graced by

Dr.J. Radhakrishnan I.A.S., Principal Health Secretary, Government of Tamil Nadu, Dr.S. Radhakrishna, former Director, IRMS (now NIE), Dr. Andrew Nunn, Medical Research Council, Clinical Trials Unit, London, representatives from several ICMR institutes, State Government collaborators and retired TRC staff. A symposium on 'Recent Advances in TB Diagnosis and Treatment' was also held. Speakers provided both historical perspectives on TB clinical trials and focused on future needs and priorities. This symposium was funded by ICMR, DHR & DBT.



2. Dr. Wallace Fox plaque installation function & Symposium on 'Recent Advances in TB Diagnosis and Treatment'



diagnostic study designs, incremental value of new tests, and impact of new tests on clinical decision making and impact on patient-important outcomes.

Five protocols were developed by the groups and discussed in detail on the last day of the workshop. These would be carried forward for

doing multicentric studies to evaluate and validate the upcoming new TB diagnostic tests in Indian laboratories.

3. Evaluation of new TB Diagnostics: A capacity building workshop for public sector institutions in India.

Venue: National Institute for Research in
Tuberculosis, Chennai

Date: 8-12th December, 2014

“Evaluation of new TB Diagnostics” – a capacity building workshop for public sector institutions in India was held in collaboration with FIND and McGill global health programs and McGill International TB centre.

This 5-day intensive workshop was designed for investigators who are primarily involved in TB research. The main focus of this workshop was on the methodology for evaluation of new TB diagnostics including diagnostic algorithms, basic principles,



Venue: National Institute for Research in Tuberculosis, Chennai

Date: 2 – 3 September 2014, NIRT, Chennai.

A national workshop was held to discuss and disseminate the findings of operational research studies carried out by NIRT under the Model DOTS Project (MDP). This project which was supported by World Health Organization (WHO) and United States Agency for International Development (USAID) between 1999 and 2014, is a model of cooperation and collaboration between Tamil Nadu State Government, ICMR, Central TB Division, WHO and USAID. Many of the findings of these studies have been translated into policy and implemented in the TB control program. In the words of Dr. Thomas R. Frieden, Director, U.S. Centers for Disease Control and Prevention, Atlanta “This project is an important example of how effective use of research and science can have a real impact on improving health and saving lives. TB is persistent, and this must be matched by persistence with basic tuberculosis control principles: prompt, accurate diagnosis effective treatment begun

4. Research Dissemination workshop on “Operational research findings from the Model DOTS project (1999-2014)”

immediately upon diagnosis and monitored until completion and interruption of transmission”.

The workshop was attended by over 70 scientists, researchers, program managers and NGO representatives. Strengthening TB surveillance system to understand the burden and epidemiology, engaging the private practitioners and NGOs, testing new models of intervention for TB control & translation of research findings to policy in a timely manner were the important recommendations that emerged from this research dissemination workshop.

A monograph that had compiled all the research activities undertaken by NIRT under the Model DOTS Project was released.



5. The ICMR Tribal Forum Annual Meeting 2014

Venue: National Institute for Research in Tuberculosis, Chennai

Date: 9th August 2014

The ICMR 11th Annual Tribal Forum meeting was held alongside the commemoration of the “International Day of the World’s Indigenous People” with the theme “Bridging the gap: Implementing the rights of Indigenous people.”

Around 60 members from 23 institutes across the country participated in the 11th Tribal Forum Annual Meeting. The two day meeting included a series of sessions with presentations, discussions and a visit to tribal areas in Chengalpeta district. The main objective of this meeting was to bring all the ICMR as well the non-ICMR institutes together to address and discuss issues and

challenges facing the tribal population with a motive to 'reach the unreached'. Dr. NK Menon from the Cudalur Adivasi hospital was the key note speaker and a panel discussion was held with Dr. Amit Mitra, Anthropologist, Dr. Chandrika, MSSRF, Dr. Sudhakar and Dr. KR John sharing their experiences of working in tribal areas.



6. Operational Research Workshop in Tuberculosis for faculty of Medical Colleges of South Tamil Nadu

Date: 13-14th November 2014

Venue: National Institute for Research in Tuberculosis, Madurai Unit

A two day workshop on 'Operational Research Workshop in Tuberculosis' at Government Rajaji Hospital, Madurai was organized by NIRT/ICMR in collaboration with Madurai Medical College, RNTCP and The UNION for Medical Officers of Medical Colleges.

The objective of the workshop was to sensitize the Medical Officers in Operational Research (OR) and capacity

building in Medical Colleges and to train the Medical Officers in writing Study protocols on OR in Tuberculosis, and also to aid and guide in conducting the research as per the prepared study protocol and publishing the same.

The Workshop had talks on general topics on TB research, challenges in RNTCP, research priorities and funding options in OR on TB, and then specific topics on Operational research. Practical group sessions were held to develop research questions and protocols. The participants then presented their protocol outline to get inputs and comments from the experts who were in the panel. The participants were informed to communicate with the facilitators for further development of protocol and conducting the research. About 19 study proposals were developed during the workshop, with topics including TB & diabetic management strategies, strategies to improve TB treatment outcomes and Gene Xpert in extra pulmonary TB in adult and pediatric populations.



7. Dissemination meeting on the study titled **“An experimental study to enhance treatment adherence in tuberculosis patients who**

consume alcohol’

Date: 29th September 2014

Venue: National Institute for Research in Tuberculosis, Chennai

A dissemination meeting was held to share the findings of the Alcohol Intervention study undertaken by the Department of Social and Behavioral Science Research (DSBR). The NIRT believes that these meetings will pave the way towards making policy level changes to improve the existing TB control program and strengthen health systems.

This study was an outcome of two pilot studies on “Prevalence of alcohol use disorders (AUDs) among TB patients” and “Feasibility of alcohol use intervention programmes for patients with AUD” . These published studies reported a high prevalence of AUD among TB patients, the need to address AUDs in TB care and that an alcohol intervention programme was both acceptable and feasible.

Around 65 members participated in the dissemination workshop which included representatives of the Chennai Corporation, NGOs, representatives from the RNTCP corporation clinics, DTOs from Chennai and Thiruvallur, former WHO representative and representatives from NIRT. A copy of the intervention manual which was prepared as an outcome of this study was presented to all the participants who attended.

Venue: National Institute for Research in Tuberculosis, Chennai

The NIRT and Immunology Quality Assessment (IQA) team, Duke Human Vaccine Institute, US conducted a PBMC Training Workshop as part of the Regional Prospective Observational Research in Tuberculosis (RePORT) India study, at NIRT during Oct. 2014.

This 4-day workshop imparted knowledge on several topics including the principle and methodology of PMBC separation and cryopreservation as well as relevant documentation. Hands on training was provided to the participants on plasma isolation, blood dilution, overlay process, PBMC isolation, subsequent washes, counting of PBMCs and determination of volume of cryopreservation media, aliquoting and cryopreservation of PBMC, thawing of PBMC, determination of percentage viability and viable recovery. The participants including laboratory personnel from the LEPR (Hyderabad), BJMC (Pune), JIPMER (Pondicherry), MV Diabetics (Chennai) and NIRT.

8. PBMC Training workshop:

Date: 7-10 October, 2014

Ph.D. Scholars

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

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	Sl.No.	Name of the candidate	Title of the Ph.D. thesis
			1.	Ms.R. Lakshmi	Molecular studies on mycobacteria
1.	Ms. N. Amudha	Screening of selected medicinal plants and characterization of <i>Andrographis paniculata</i> against clinical isolates of <i>M. tuberculosis</i>	2.	Mr. Brijendra Singh	Chemokine gene polymorphism and chemokine expression in PTB
			3.	Mr.K.Srinivasan	Comparative genomics and pathogenesis of TB
2.	Mr.L. Sekar	Semi-parametric and competing risk models in analysis of censored survival data	4.	Mr. Jovvian George	Helminth Immunology
3.	Mr.N. Arunkumar	Causal modeling of treatment effects using propensity scores			
4.	Mr.N. Pavan Kumar	Characterization of T-cell responses in pulmonary and extra-pulmonary TB with other co-morbidities			
5.	Ms.V. Malini	Characterization of components of signal recognition particle pathway from <i>M. tuberculosis</i> H37Rv			
6.	Ms.M. Mullai	A study on self-organizing map neural networks in disease diagnosis and classification			
7.	Ms.R. Anuradha	Characterization of immune responses in lymphatic filarial infection and disease			
8.	Ms.S. Suba	Characterization of lipoproteins of <i>M. tuberculosis</i>			
9.	Mr. Jagdish Chandra Bose	Immunodominant epitopes against HIV subtype C			

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

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. thesis	Supervisor/Guide
1.	Mr.P. Pugazhvendhan	ICMR	Immunoproteomic identification of B-cell antigens of <i>M. tuberculosis</i>	Dr. Alamelu Raja
2.	Ms.D. Santhi	ICMR-TASK FORCE	Novel subunit vaccine targets from <i>M. tuberculosis</i>	Dr. Alamelu Raja
3.	Ms.Maddineni Prabhavathi	CSIR	Comparative genomics and pathogenesis of TB	Dr. Alamelu Raja
4.	Mr.S. Balaji	ICMR	Diagnostic evaluation of novel T-cell (Rv2204c, Rv2394) antigens of <i>M. tb</i>	Dr. Alamelu Raja
5.	Ms.G. Akilandeswari	INSPIRE FELLOW	Structural characterization of 3 essential genes from <i>M. tb</i>	Dr. Alamelu Raja
6.	Ms. Ahmed Kabir Refaya	ICMR	Mycobacterial transcriptional regulators in pathogenesis	Dr. Sujatha Narayanan
7.	Mr.V. Arunkumar	ICMR	Gene regulation of mycobacteria	Dr. Sujatha Narayanan
8.	Mr.K. Afsal	ICMR	Effect of vitamin D3 on innate and adaptive immunity in pulmonary TB	Dr.P. Selvaraj
9.	Ms.J. Nancy Hilda	ICMR	Neutrophil mediated innate immune response in TB	Dr.D. Sulochana
10.	Mr. Narayanaiah Cheedarla	UGC	Comparative studies between HIV-1 and HIV-2 cases in India	Dr. Luke E. Hanna
11.	Ms.A.S. Shainaba	Lady Tata Fellowship	Phage based drug target identification and anti-mycobacterial drug discovery	Dr. Vanaja Kumar

**Staff (Part-time) registered 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. Sivakumar S.	Molecular epidemiology of TB	Dr. Sujatha Narayanan
4.	Mr. Sukumar B.*	Statistical methods for micro array data analysis	Dr.P. Venkatesan
5.	Ms. Vasantha M.	Structural equation modeling	Dr.P. Venkatesan

* Ex-staff

NIRT - STAFF LIST

(As on 1 April, 2015)

SCIENTIST 'G' & Director

1. Dr. Soumya Swaminathan, M.D., DNB, FIAP, FASc., FNASc.

SCIENTIST 'G'

1. Dr. Alamelu Raja, M.Sc., Ph.D.

SCIENTIST 'F'

1. Dr. P. Selvaraj, M.Sc., Ph.D.
2. Dr. Mohan Natrajan, MBBS, Ph.D., Dip. in Der.

SCIENTIST 'E'

1. Dr. P. Paul Kumaran, MBBS, MPH
2. Dr. Pradeep Aravindan Menon, MBBS, DPM
3. Dr. C. Padmapriyadarsini, MBBS, DNB, M.S.
4. Dr. D. Baskaran, MBBS
5. Dr. Sudha Subramanyam, M.Sc. Ph.D.

SCIENTIST 'D'

1. Dr. Geetha Ramachandran, M.Sc., Ph.D.
2. Dr. KR Uma Devi, M.Sc., Ph.D.
3. Dr. S. Ramesh Kumar, MBBS
4. Dr. G. Narendran, MBBS, DTRD, DNB
5. Dr. C. Ponnuraja, M.Sc., Ph.D.
6. Dr. Luke Elizebath Hanna, M.Sc., Ph.D.
7. Dr. C.K. Dolla, MBBS, MPH
8. Dr. Beena E Thomas, M.A., Ph.D.

SCIENTIST 'C'

1. Dr. A. Sheikh Iliayas, MBBS
2. Dr. P. Kannan, M.V.Sc., Ph.D.
3. Dr. V.V. Banurekha, MBBS, PGDPH
4. Dr. P.K. Bhavani, MBBS, PGDPH
5. Dr. S. Syed Hissar, MD, MPH
6. Dr. Dina Nair, MBBS, PGDPH
7. Dr. N. Poorana Ganga Devi, MBBS, PGDPH
8. Dr. A.K. Hemanth Kumar, M.Sc., Ph.D.
9. Dr. M. Makesh Kumar, MBBS
10. Dr. Bella Devaleenal, MBBS, PGDOL, MPH
11. Dr. S. Sriram, MBBS, MPH
12. Dr. Angeline Grace G, MBBS, APGDCRMW, MPH

SCIENTIST 'B'

1. Mr. S. Sivakumar, M.Sc.
2. Dr. N.S. Gomathi, M.Sc., Ph.D.
3. Ms. A. Srividya, M.Sc.
4. Mr. Sukhendu B Barman

Sr. Library & Information Officer

1. Dr. R. Rathinasabapati, MLIS

Nursing Superintendent

1. Ms. Jayalakshmi Vadivel, M.Sc.

Asst. Nursing Superintendent

1. Ms. A. Gunasundari, M.Sc.
2. Ms. G. Mangalambal, M.Sc.

Nursing Sister

1. Ms. Valarmathi Nagarajan, M.Sc.
2. Ms. C. Kavitha, B.Sc.
3. Ms. S. Chellam
4. Ms. K. Sureswari
5. Ms. Mary Eunice George
6. Ms. A. Gomathy
7. Ms. Shyamala Gopu

Staff Nurse

1. Ms. A. Komathi, B.Sc.
2. Ms. V. Revathy
3. Ms. R. Valarmathy
4. Ms. R. Manimegalai
5. Ms. V. Farthimunnisa
6. Ms. Shakila Shankar
7. Ms. V. Indirani
8. Ms. K. Porselvi, B.Sc.
9. Ms. A. Selvi
10. Ms. S. Stella Mary
11. Ms. A. Stella Mary
12. Ms. R. Saraladevi
13. Ms. P. Pandeewari
14. Ms. M. Rathinam

15. Ms. S. Theensuwai, B.A.
16. Ms. P. Kowsalya
17. Ms. M. Mohana
18. Ms. R. Vetrichselvi, M.A.

Junior Staff Nurse

1. Ms.OR Vijayalakshmi
2. Ms.A.Vijayalakshmi
3. Ms.A.Poongodi
4. Ms.S.Vaishnavi, B.Sc.
5. Ms.R.Suganthi
6. Ms.K.Maheswari, B.Sc.
7. Ms.V.Senthamizhselvi
8. Ms.V.Shunmugajyothi, M.Sc.
9. Ms.J.Jemima, B.Sc.
10. Mr.N.Lokeswaran, B.Sc.
11. Ms.R.Selvi
12. Ms.J.Vanitha

Female/Male Nursing Orderly

1. Mrs. Rosily Edwin
2. Mrs. Padmavathi Asaithambi
3. Mr. K. Jayavel Anandan
4. Mrs. D. Sundari

Technical Officer – B

1. Dr. K. Jayasankar, M.Sc., Ph.D.
2. Mr. K. Sankaran, M.Sc.
3. Mr. M. Ponnambalam, B.Sc.
4. Dr. Subhas Chandra Bose, M.Sc., Ph.D.
5. Dr. E. Thiruvalluvan, M.A., Ph.D
6. Mrs. Chandra Suresh, M.A.
7. Mrs. D.Kalaiselvi, M.A.
8. Mr. M. Rajasakthivel, M.A.

Technical Officer – A

1. Mr. J. Samuel Vasanthan Goodwill, B.Sc.
2. Mr. S. Manoharan, B.Sc.
3. Mr. R.K. Rajendran
4. Ms. K. Silambu Chelvi, M.Sc., M.Phil.
5. Dr. L. Sekar, M.Sc., Ph.D.
6. Dr. K. Chandrasekaran, Ph.D.

7. Mr. E. Kirubakaran
8. Mr. T. Gowri Shankar
9. Dr. R. Srinivasan, M.Sc., Ph.D.
10. Ms. M. Vasantha, M.Sc., M.Phil.
11. Ms. S. Sivagama Sundari
12. Mr. M. Subramani
13. Mr. K. Ramesh, M.Sc.
14. Mr. M Harishankar, M.Sc.
15. Mr. S. Anbalagan, M.Sc.
16. Ms. Lucia Precilla, M.Sc., M.Phil.
17. Mr. S. Senthil, M.A., M.Phil
18. Ms. V.M. Girijalakshmi, B.Sc.
19. Ms. A. Deepalakshmi, M.A.
20. Dr. K. Ramakrishnan, M.Sc., Ph.D.
21. Ms. D. Saraswathi, M.Sc., M.Phil.
22. Dr.. S. Balaji, M.Sc., Ph.D.
23. Dr. D. Anbarasu, M.Sc., M.Phil., Ph.D.
24. Mr. S. Murugesan, M.Sc.
25. Mr. M. Anandan

Technical Officer-A (Eng Support)

1. Mr. B. Kanagasabapathy, M.A.

Technical Assistant

1. Mr. C. Thirukumar, B.A.
2. Mr. M. Asokan
3. Ms. R Mahalakshmi, M.Sc.
4. Mr. M Tamizhselvan, M.Sc.
5. Mr. D Thangaraj
6. Ms. K. Devika, M.Sc.
7. Mr. M. Baskaran, B.Sc.
8. Mr. S. Rajakumar, M.Sc.
9. Ms. B. Angayarkanni, M.Sc., M.Phil.
10. Ms. J. Chitra, M.Sc.
11. Mr. V. Thiyagarajan, M.Sc., M.Phil.
12. Mr. D. Ravikumar, M.Sc.
13. Ms. K. Sumathi, B.Sc.
14. Mr. S. Govindarajan, M.Sc.
15. Mr. K. Krishnan
16. Mr. K. Ramakrishnan
17. Mr. M. Michel Prem Kumar, M.Sc.
18. Ms. B. Pricilla Rebecca, M.A.
19. Ms. S. Rani, B.S.W.
20. Ms. A. Dhanalakshmi, M.A.
21. Ms. Senthenro Ovung, M.S.W.
22. Ms. V. Mythily, M.Sc.
23. Ms. M. Muthu Vijayalakshmi, B.Ed., M.Sc., M.Phil.
24. Mr. P. Palaniyandi, M.Sc., M.Phil.
25. Mr. T. Balamurugan, M.Sc., M.Phil.

26. Dr. C. Manogaran, M.A., M.Phil., Ph.D.
27. Ms. G. Radhika, B.Sc.
28. Ms. H. Hemalatha, M.Sc.
29. Ms. Devi Sangamithrai, M.Sc.
30. Ms. K. Jeyasree, B.Sc.
31. Mr. A. Madheswaran, B.Sc.

Technician – C

1. Mr. S. Venugopalan
2. Mr. D.Madhavan
3. Mr. RK.Syed Nisar
4. Ms. B.Brindha
5. Mr. P.Nagarajan
6. Mr. P.Sivaraman
7. Ms. V.Sudha
8. Ms. R.Nithya
9. Ms. Rohini Punaneshwari
10. Mr. R.Rajkumar
11. Mr. D.Srinivasa Raju

Technician – C (Engg. Support)

1. Mr. K.Parthiban
2. Mr. S.Iyyappan
3. Mr. K.S.Venkatesan

Technician – B

1. Mr. M. Thanigachalam
2. Mr. E.A. John Washington
3. Mr. N. Ramakrishnan

Technician – B (Engg. Support)

1. Mr. R. Anbulingam

Technician – A

1. Mr. A. Manoharan
2. Mr. P. Chandran
3. Mr. M. Kawaskar
4. Mr. Santhana Mahalingam
5. Mr. T. Bharathiraja
6. Mr. S. Mangaiyarkarasi
7. Mr. M. Pandidurai
8. Mrs. R. Sathya
9. Mr. T.M. Loganathan
10. Mr. A. Vijayakumar

Technician – A (Engg. Support)

1. Mr. P.Johnson Kennedy
2. Mr. Harihara Ganapathi Subramanian

Sr. Administrative Officer

1. Mr. Jagdish Rajesh, B.Com.

Administrative Officer (Stores)

1. Mr. M. Mani, B.A.

Accounts Officer

1. Ms. Santhi Velu, M.A.

Section Officer

1. Ms. M. J. Nirmala, B.A.

Private Secretary

1. Dr. P. Karthigayan, M.A., Ph.D.

Assistant

1. Mr. C. Gopala Krishnan, B.Sc.
2. Ms. M. Rasheetha Begum, M.A.
3. Ms. N. Thamilselvi, B.Sc.
4. Ms. Chithra Sivakumar, B.Sc.
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6. Mr. A. Lakshmanan
7. Ms. L. Vijayakumari
8. Mr. B. Durai Raj
9. Ms. P Kavitha

Personal Assistant

1. Ms. PS Shanthi
2. Ms. A.L. Rajalakshmi, B.Sc., MLIS, MBA

Senior Tel.Opr.-cum-Receipt.

1. Mrs. V.Shailaja Devi

Upper Division Clerk

1. Mr. S. Anandaraj
2. Ms. S.Nirmala
3. Ms. J.Suguna
4. Ms. T.Sheela
5. Mr. A.Gopinathan
6. Mr. V.Velmurugan
7. Mr. R.Hariharan

Stenographer

1. Ms. M Revathy, M.Com.

Lower Division Clerk

1. Mrs. K.Sumathi, B.A
2. Mr. M.Mohan Shankar

3. Ms. D Tamilselvi
4. Ms. S Sundari
5. Mr. D. Sukumar

Senior Record Sorter

1. Ms.Molly Joseph
2. Mr.K.Ganesan
3. Mr.J.Loganathan

Staff Car Driver (Special Grade)

1. Mr. Arthur Sundar Singh

Staff Car Driver (Grade-I)

1. Mr. K.Vadivel
2. Mr. K. Jayaraman
3. Mr. P.Anbu
4. Mr. S.Sri Rama Chandran
5. Mr. A.Ravi

Staff Car Driver (Grade-II)

1. Mr. A. Elangovan
2. Mr. I.Seenivasan
3. Mr. P.Sivakumar
4. Mr. N.Rajan Babu

Staff Car Driver (Ordinary Grade)

1. Mr. M.Thiyagarajan
2. Mr.M.S.Mani
3. Mr. M.Sekar
4. Mr. P.Yuvaraj
5. Mr. E.Selvaraj
6. Mr. M. Sathish Kumar
7. Mr. C. Sivaraman

Multi Tasking Staff

1. Mr. V. Mohan
2. Mr. D. Bose
3. Mr. N.Murali
4. Mr. C.K.Chittarasu
5. Mr. M.Jayaraj
6. Mr. A.Rajavarman
7. Mr. G.Moshe
8. Mr. C.Nagaraju
9. Mr. P.Vijayakumar
10. Mr. A.Annamalai
11. Mr. R.Damodharan
12. Mrs. K.V.Rajamma

13. Mr. J.Venkatesan
14. Mrs. R.Ankamma
15. Mr. R. Ankaiah
16. Mr. R.Ravichandran
17. Mr. V.Adikesavan
18. Mr. V.Sundarajan
19. Mr. K.Kuttappan
20. Mr. J.Selvam
21. Mr. G.Easwaran
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23. Mrs. Kasiammal
24. Mrs. P.Arul Mani
25. Mr. C.Uthara Bahadur
26. Mrs. S.Lakshmi
27. Mr. Keshbraj Paudel
28. Mrs. D.Sharadha
29. Mrs. B.Nageswari
30. Mr. G.Durai
31. Mr. B. Venkateswaralu
32. Mrs. J.Neelavathy
33. Mrs. H.Ponrose
34. Mrs. T.Thilakavathy
35. Mr. G.Nithyanandam
36. Mr. S.Venkatesan
37. Mr. R.Mohanraj
38. Mr. J.Santhakumar
39. Mr. S.Anjaiah
40. Mr. P.Senthilvelan
41. Mr. P.Kosalaraman
42. Mr. S. Nagarajan
43. Ms. P. Hemalatha
44. Mrs. R.Sakila
45. Mr. M. Manikandan
46. Mr. KN Thirumalai
47. Ms. K. Kamatchi
48. Mr. D Rajasekaran
49. Mrs. P Pandiselvi
50. Mrs. V. Amudhavalli
51. Mr. JV Mohanraj

NIRT - Epidemiology Unit

Scientist 'F'

1. Mr.R. Subramani, M.Sc.,

Scientist 'B'

1. Ms.R. Mahalakshmi, M.Sc.,
2. Dr.V.N. Azger Dusthacker, M.Sc., Ph.D.

Technical Officer – B

1. Mr.N. Ravi, DEE.
2. Mr.T. Krishnamoorthy, M.Sc.,

Technical Officer – A

1. Mr.L. Ranganathan, M.Sc (Stats), M.Sc.,
(Maths), M.Tech (IT)
2. Mr.S.Stanley Jones Rajasingh, B.Sc.,
3. Mr.D. Sargunan, M.A.,
4. Mr.M. Kalyanaraghavan, M.Sc.,
5. Mr.S. Egambaram, M.A.,
6. Mr.A.S. Tholkappian, M.Com.,
7. Dr. Gomathi Sekar, M.Sc., Ph.D.,
8. Mr.S. Vijayaraj, M.Sc.,
9. Mr.T. Nataraj, M.Sc.,
10. Mr.J. Devan, M.Sc.,
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12. Mr.Mohd. Ghouse, PUC
13. Mr.K. Balakaliyan, B.Sc.,
14. Mr.S. Nambirajan, M.Sc, M.L.T.,
15. Mr.Senthil Kumar K, M.Sc, M.Phil.,
16. Mr. Radhakrishnan A, M.Sc.,
17. Mr. Ranganathan K, B.Sc.,
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21. Mr.P. Munivarathan, B.Sc.,
22. Mr.D. Nithyakumar, M.Sc.,
23. Ms. Thangam (a) Meenakshi, B.Sc.,
24. Mr.V. Ramesh Babu , C R A
25. Mr.A.M. Ramesh, M.A.,
26. Mr.P.K. Venkataramana, B.Com.,
27. Mr.N. Premkumar, B.Sc.,

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2. Mr.S.V. Joseph Rajkumar
3. Mr.T. Thangaraj M.A, B.Ed.,
4. Ms. Malathi M, M.Sc, C.L.T.,
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6. Mr. Lakshmikanthan N, M.A.,
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8. Mr. John Arokiya Doss Y, M.Sc, DMLT
9. Ms. Mahizhaveni B, M.Sc, D.M.L.T.,
10. Mr. Basilea Watson, M.Sc.,
11. Mr.M. Karthikesan, M.Sc.,
12. Mr.A. Vasudevan, M.Sc.,(Bio-Chem.)
M.Sc., (Clinical Microbiol.)
13. Mr.M. Mahesh Kumar, M.Sc, D.M.L.T.,
14. Mr.P. Chandrasekaran, M.Sc, M.B.A,
15. Mr.K. Rajaraman, M.Sc, C.L.T.,
16. Mr.B. Ananda kumar, M.Sc, M.B.A.,
17. Mr.P. Kumaravel, M.A, D.M.L.T.,
18. Mr.C. Saravanan, BLIS, M.A.,
19. Mr.S.S. Jeganathan, B.Sc.,
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21. Ms.P. Devi Bhagavathy, BCA, MBA.,
22. Mr.R. Vijayakumar, B.Sc.,
23. Mr.T. Kannan, M.Sc., M.Phil.,
24. Ms.R. Vijayalakshmi, M.Sc.,
25. Mr.A. Devanathan, M.Com., MSW.,
26. Mr.S. Govindaraj, DMLT, M.Sc.,
27. Mr. Manohar Nesa Kumar, M.Sc.,PGMLT
28. Mr.M. Kannan, B.Sc.,
29. Ms.V. Rani, M.Sc.,
30. Dr. Kiran Kumar Angadi, M.Sc., Ph.D.
31. Mr.P. Balaji, M.A., B.Ed.
32. Mr.P. Sathyamurthi, M.Sc.,
33. Mr.T.K. Bharath, M.Sc.,

Technician - C

1. Mr. Srikanth Dhawani
2. Mr.K. Munuswamy
3. Mr.P.C. Nagaraja
4. Ms.N. Lakshmi
5. Mr. Levelin David Rajkumar
6. Mr.P. Srinivasulu
7. Mr. Ishwori Dhakal
8. Mr.M. Mohan
9. Mr.C. Saravanan
10. Mr.J. Udayakumar
11. Mr.V. Raja

Technician – C (Eng. Support)

1. Mr.B. Vijayakumar
2. Mr.G. Vasu
3. Mr.R. Balu

Technician - B

1. Mr.L. Venkatesan
2. Mr.W. Wilkingson Mathew
3. Mr.R. Gangadhar Sharma
4. Mr.R. Krishna Bahadur

Technician – B (Eng. Support)

1. Mr.K. Poongavanam

Technician – A (Eng. Support)

1. Mr.J. Ravi

Technician - A

1. Mr.N. Srinivasan
2. Mr.N. Srinivasan

Administration**Administrative Officer**

1. Mr.K. Sampath Kumar, B.Sc.,

Section Officer

1. Ms.D. Devaki, B.Sc.,
2. Ms.M. Meenal, M.Com.,

Private Secretary

1. Ms.S. Rangamma

Personal Assistant

1. Mr.B. Duraisamy, B.A.,
2. Mr.R. Senthil Murugan, B.Sc.,

Assistant

1. Mr.T.N. Surendranath, B.Sc.,
2. Ms. Visalakshi R, M.A.,
3. Ms. D. Vijayakumari B.Sc.,
4. Ms. R. Geetha B.Com.,
5. Mr. S. Rajendran M.A.,
6. Ms. R. Latha B.E, M.B.A.
7. Ms.M.J. Nagalakshmi, M.A.,

Upper Division Clerk

1. Mr.R. Senthilnathan, Dip. In Comp. & Sci & Eng., BCS
2. Mr.S.N. Babu, B.A., B.L,
3. Ms.K. Kanaga, M.A.,

Lower Division Clerk

1. Mr.A.S. Sivaraj, M.A., D.C.A.,
2. Mr. Solomon Priyakumar, M.A.,
3. Ms.A. Uma, B.Com.,

4. Mr.P. Madhan Kumar
5. Mr.V. Navalan, D.C.A.,
6. Ms.P. Kowsalya, B.A.,
7. Mr.M. Senthilkumar
8. Ms. Supriya Srinivasan
9. Ms.B. Manjula, M.Com., MCA.,
10. Mr.S. Santhosh Kumar, B.Sc., (Vis.Com.)

Staff Car Driver (Mech.)

1. Mr.R. Balu

Staff Car Driver (Grade-I)

1. Mr.V. Thanigaivel
2. Mr.J. Prakash
3. Mr.P. Soundararajan
4. Mr.M. Manokaran
5. Mr.V.S. Senthilkumar

Staff Car Driver (Grade-II)

1. Mr.K. Thulasingham
2. Mr.K. Saravanan
3. Mr.P. Subbaiah
4. Mr.B. Sureshkumar

Staff Car Driver (Ordinary Grade)

1. Mr.A.S. Dayalan
2. Mr.S. Doss
3. Mr.G. Vasu
4. Mr.L. Gunalan
5. Mr.V. Babu
6. Mr.J. Loganathan
7. Mr.K. Jagadeesan
8. Mr.M. Anbalagan
9. Mr.M. Pushparaj
10. Mr.U. Murugan
11. Mr.K. Govindan
12. Mr.V. Udayachandran
13. Mr.M.N. Balaji
14. Mr.J. Jayabarath Veeran

Multi Tasking Staff

1. Mr. Gangadhar Sharma
2. Mr.M.S. Devakumar
3. Mr.M.B Mohanan
4. Mr. Yam Bahadur
5. Mr. Hariprasad Sharma
6. Ms.G. Devaki
7. Mr. Tilbahadur
8. Mr.E. Duraiavel
9. Ms.J. Rajathi

10. Mr.T.D. Ponnuswami
11. Mr.S. Prakasam
12. Mr.E. Pongavanam
13. Ms.N. Vasantha
14. Mr.R. Krishna Bahadur (T)
15. Mr.J. Jeeva
16. Mr.S. Karunakaran
17. Mr.D. Sundaramurthy
18. Mr.R. Karunanidhi
19. Mr.C. Anandan
20. Mr. Innamuthan
21. Mr.R. Yuvarajan
22. Mr.F. Albert
23. Mr.A.M. Sivakumar
24. Mr.N. Srinivasan
25. Mr.K. Vasudevan
26. Mr.B. Ammavasai
27. Mr.R. Narasimman
28. Mr.K. Damodharan
29. Mr.K. Selvakumar
30. Mr.S. Kathiravan



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