Annual Report 2015-16

NIRT

National Institute for Research in Tuberculosis

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

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS

Research Activities

April 2015– March 2016

CONTENTS

Cont'd…

Contents (cont'd….)

PREFACE

The year under review at NIRT saw continued activity in all aspects of TB research: clinical, socio-behavioural, bacteriological, epidemiological and basic science.

In the ongoing daily versus intermittent anti-TB treatment study conducted at NIRT, the daily anti-TB regimen was found to have better efficacy that the intermittent arm. Efforts are ongoing to develop a four month regimen for the treatment of newly diagnosed pulmonary TB patients using moxifloxacin along with INH, rifampicin, ethambutol and pyrazinamide. The study participants who received a moxifloxacin containing regimen became less infectious earlier compared with those treated with the standard RNTCP regimen.

A study was carried out to identify clinical predictors of TB IRIS after initiation of ART in HIV/TB patients. Fever was found to be the most common symptom with lymphadenopathy being the major sign associated with IRIS. Higher mycobacterial burden was associated with IRIS. The frequency of IRIS associated with TB was more closely associated with initiation of ART than with the initiation of anti-TB treatment. The levels of high density lipoprotein and gene polymorphisms were studied in HIV positive individuals on a NVP based ART regimen.

A study is ongoing to evaluate to evaluate enhanced motivation versus standard motivation for the cessation of smoking in tuberculosis patients. Studies are ongoing for evaluation of newer diagnostic tools (Xpert MTB/Rif, Cepheid, Sunnyvale USA) and Urine LAM for the development of consensus case definition in the diagnosis of intrathoracic TB in children. A study is ongoing for determining the incidence of TB among patients with Type-2 diabetes mellitus. The role of nutrition, ART and genes in HIV associated lipodystrophy in children is also being evaluated. A repository of biological specimens from TB patients and their contacts is being developed as a part of the C-TRIUMPh study. The study for the effectiveness of IPT in HIV infected study participants has been completed and the data is being analysed. A study for determining the recurrence rate of TB among newly diagnosed pulmonary TB patients is ongoing. Another study to determine the optimal regimen in the treatment of INH resistant PTB is ongoing. Prevalence of TB infection and disease among pediatric household contracts of MDR TB patients is being studied.

Burden of TB among tribal population and development of a system to strengthen TB control in tribal areas is ongoing. Intervention to reduce STIs and HIV among MSMs is being studied. Studies related to determining the reasons for pre-treatment Loss to Follow Up in smear positive TB patients are ongoing.

Studies on Transitmycin-A for evaluation of its anti-TB activity are ongoing. Evaluation of indigenous tests for the diagnosis of PTB as well as DR-TB is ongoing. Cross resistance among fluoroquinolones is being evaluated. Molecular characterization of X-DR strains of MTB is ongoing.

Quality TB diagnosis in pediatric cases is being studied. Pharmacokinetic studies are ongoing for INH, RMP and PZA in adult TB patients in India. Anti-TB drug concentration in TB patients with and without diabetes mellitus is another ongoing study. Pharmacokinetic of rifabutin during concomitant administration of ritonavir is ongoing in HIV-TB patients.

Pharmacokinetics of second line Anti-TB drugs are being studied in MDR-TB patients. Neutralizing antibodies are being studied both in HIV-1 infected and HIV-2 infected individuals. Full length genome analysis of HIV-1 from recently infected pediatric samples is ongoing. Studies related to HIV drug resistance in HIV-1 infected children are being carried out.

Other studies ongoing are related to (1) Biomarkers in TB (2) Development of better anti-TB vaccines (3) Vitamin-D receptor polymorphism, (4) Neutrophil mediated innate immune responses in TB (5) Cytokine gene polymorphisms in HIV and HIV-TB (6) Prevalence of TB in cattle and cattle handlers (7) TB prevalence in Tiruvallur area (8) Host immune response to filariasis and strongyloidiasis.

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GUEST LECTURES

S.No.	Date of Lecture	Name of the Speaker	Affiliation of the speaker	Topic of the Lecture
1	May 5, 2015	Dr. Varadharajan Sundaramurthy	National Center for Biological Sciences, Tata Institute of Fundamental Research, Bangalore.	Smile and kill: Targeting host pathways to combat TB
2	July 9, 2015	Dr.U.D. Gupta	Director, National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra	Biosafety practice for infectious diseases
3	Oct. 29, 2015	Dr. Philip Bergin	IAVI, Human Immunology Laboratory, Imperial College, London, UK.	An IAVI approach to novel HIV-1 preventive vaccines
4	Jan. 12, 2016	Prof. Sir. Tom Blundell	Dept. of Biochemistry, University of Cambridge, UK	Genomes, structural biology and understanding antimicrobial resistance
5	Jan. 20, 2016	Prof. Dr. Roy Santosham	Santhosam Chest Hospital, Chetput, Chennai.	CT guided biopsies in the chest
6	Feb. 18, 2016	Mr. Jaskiran Singh	Clinical Data Architect, NIAID, USA	Clinical research data management tools

ABBREVIATIONS

ARR	Acquired rifampicin resistance
ART	Anti-retroviral treatment
ATT	Anti-TB treatment
BCN	Broad cross-clade neutralization
CTRIUMPh	Cohort for TB research by the Indo-US medical partnership
CRP	C-reactive proteins
DR-TB	Drug resistant-TB
DRMs	Drug resistance mutations
DSMB	Data and safety monitoring board
DST	Drug susceptibility testing
EID	Early infant diagnosis
ELISA	Enzyme linked immunosorbent assay
EMB	Ethambutol
FGDs	Focus group discussions
Fqs	Fluoroquinolones
HHC	Healthy household contacts
INH	Isoniazid
IPT	Isoniazid preventive therapy
LPA	Line probe assay
IRIS	Immune reconstitutiton inflammatory syndrome
LTBI	Latent TB infection
LRP	Luciferase reporter phage assay
Lx	Levofloxacin
MDR-TB	Multi-drug resistant TB
MOX	Moxifloxacin
MSM	Men having sex with men
NACO	National AIDS Control Organization
NC	Number of codons
NNRTI	Non-nucleotide reverse transcriptase
NNS	Number needed to screen
NNT	Number needed to treat
NSP	New sputum smear positive
NTM	Non-tuberculous mycobacteria
NVP	Nevirapine
OFX	Ofloxacin
OD	Optical density
PCR-RFLP	Polymerase chain reaction based restriction fragment length polymorphism
PI	Protease inhibitors
PLHIV	People living with HIV
PLTFU	Pretreatment loss to follow-up
PMDT	Programmatic management of drug-resistant TB
PNGs	Potential N-linked glycosylation sites

PTB	Pulmonary tuberculoiss
PTCT	Parent-to child transmission
RBT	Rifabutin
RC	Resuscitated cells
RLU	Relative light units
RMP	Rifampicin
RNTCP	Revised national TB control programme
ROC	Receiver operating characteristic
RT-PCR	Reverse transcription polymerase chain reaction
RTV	Ritonavir
STI	Sexually transmitted infection
SRP	Signal recognition particle
VDR	Vitamin D receptor
XDR-TB	Extensively drug resistant TB

CLINICAL STUDIES

DEPARTMENT OF CLINICAL RESEARCH

STUDIES COMPLETED:

(i) (CL-6): Predictors and immunologic characterization of tuberculosis - associated immune reconstitution inflammatory syndrome 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
Collaborators	:	GHTM, Tambaram
Source of funding	:	NIH Intramural to India grant 2008 and ICMR adhoc grant from 2012
Study period	:	2009-2015

A. Primary objectives: (i) To identify clinical predictors (age, pulmonary versus disseminated tuberculosis (TB), duration of TB therapy prior to anti-retroviral treatment (ART) initiation) of IRIS-TB patients who develop immune reconstitution inflammatory syndrome (IRIS) based on baseline clinical characteristics (age, < or >4 weeks on TB therapy, pulmonary versus disseminated TB, X-ray < or > than 3 zones, sputum smear and culture grading) for prediction

(ii) To identify laboratory predictors (hemoglobin, CD4 and CD8 T-cell counts) of IRIS-TB in patients co-infected with HIV and TB

(iii) To estimate serum cytokines prospectively at three subsequent time points [baseline, at IRIS event or 1-2 months after ART in controls and at the end of anti-TB treatment (ATT)] to further elucidate the type of inflammatory responses that constitute IRIS

B. Secondary objectives:

(i) description of radiological IRIS (cryptic IRIS)

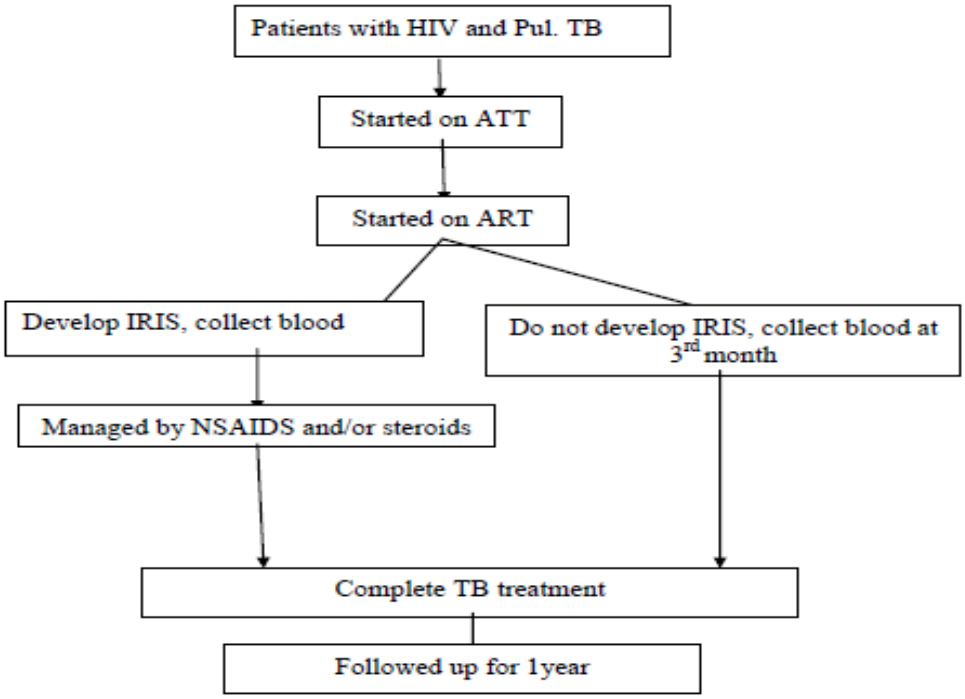
(ii) incidence of IRIS in daily and intermittent therapy

(iii) outcome of IRIS

Methodology: Patients with proven culture confirmed pulmonary TB (PTB) with HIV, who were ATT and ART – naïve at baseline, were started on ATT

first with either daily ($2EHRZ_7/4HR_7$) or intermittent regimen ($2\ EHRZ_3/4HR_3$), all doses being supervised. Subsequently, ART (Zidovudine /Stavudine along with Lamivudine and Efavirenz) was initiated, based on the prevailing National AIDS Control Organization (NACO) ART guidelines. Some of them developed symptoms and signs suggestive of IRIS. Typical signs depend on the location of IRIS occurrence. General symptoms included fever, breathlessness, superficial and abdominal lymphadenopathy with or without radiological deterioration. Febrile episodes were thoroughly investigated to rule out local/ endemic causes of fever at the time of IRIS event. The patient needed to have sputum culture negativity / down regulation of sputum culture grade, to be classified as IRIS and to differentiate it from progression of TB disease. Initial culture should have been sensitive to rifampicin (RMP) to rule out multi-drug resistant TB (MDR-TB) which is the closest mimic. The flow of patients is projected as a flow chart below (Fig. 1):

Fig.1: Flowchart shows the patients details



Patients with IRIS had venipuncture done and immunological parameters and markers estimated. International Network for the Study of HIV

associated IRIS (INSHI) criteria was used in confirming IRIS. At IRIS event, the viral load is expected to reduce by at least 0.5 log_{10} to confirm therapeutic response to HIV. Those who experienced IRIS were treated with NSAIDs for 3-5 days followed by steroids, if symptoms persisted after diagnostic confirmation. When IRIS occurred at extra pulmonary sites, appropriate investigations were carried out in addition to sputum examination and blood tests. Most of the patients were admitted for safe initiation of ART and monitored vigorously for IRIS occurrence which helped to pick up IRIS cases early and start anti-inflammatory medication promptly.

Results: Of the 63 HIV positive patients with newly diagnosed culture confirmed PTB (49 males: 14 females), who were ATT and ART naïve, 52 were included in the TB-IRIS sub-study. The pre-ART baseline characteristics of IRIS vs. non-IRIS, both clinical and laboratory parameters were taken into consideration for the multi-logistic regression analysis. The two groups were comparable with respect to gender, age, body weight, sputum culture grade and levels of ALT and AST at baseline (Table 1).

Table-1: Baseline demographics of cases (IRIS) and controls (Non-IRIS)-total group

Baseline demographics (Mean ± SD)	IRIS (n=28)	Non-IRIS (n=24)
Age (years)	**38 ± 10.0**	**36 ± 7.0**
Weight (kg)	**44.4 ± 8.6**	**43.2 ± 9.4**
Hematocrit (%)	**24.5 ± 6.0**	**30.0 ± 5.4**
Viral load(Log10) copies/ml	**5.9 ± 0.3**	**5.2 ± 0.9**
CD4 cells/mm^3 [Median (IQR)]	**87 (32-135)**	**146 (88-287)**
CD8 cells/mm^3 [(Median (IQR)]	**756 (320-1086)**	**461 (287-738)**

CD4/CD8 ratio	**0.10 (0.04-0.18)**	**0.31 (0.23-0.48)**
Time to ART (Median (IQR) days)	**18 (14-32)**	**46 (23-65)**

This was probably the only study globally looking into TB-IRIS in a group of ATT-ART naïve population with RMP susceptible isolates at baseline, after ruling out drug resistance and failure. We found that individuals who experienced IRIS during follow up had a significantly lower $CD4^+$ T-cell counts at baseline compared to those who did not (median 93 vs. 156 cells/mm^3, P=0.005). The median time to ART initiation was the most significant modifiable factor and the interval from ATT to ART initiation was half the period in the IRIS group (20 days {IQR: 14–30}) compared to patients who had not developed IRIS (Non-IRIS group) which was 43 days (IQR: 23–68) (P=0.002).

Fever was the most consistent symptom (96%) with lymphadenopathy being the major sign (76% - which was either superficial lymphadenopathy or intra-thoracic or intra-abdominal). Radiological deterioration was found in 88% of patients. Miliary TB and extrapulmonary involvement pre-disposed to IRIS occurrence (Figs. 2 & 3). Higher mycobacterial burden as evidenced by a higher sputum culture grade was also significantly associated with IRIS. The schedule of ATT, whether administered daily or intermittently did not influence IRIS occurrence. We found no difference in IRIS incidence with respect to schedule of ATT regimen which hypothesizes that all regimens brought down the antigenic burden equally. (P=0.197). We also inferred hence that IRIS was more closely associated with ART initiation than start or schedule of TB therapy.

Fig.2: Pontine abscess as a manifestation of TB-IRIS in MRI

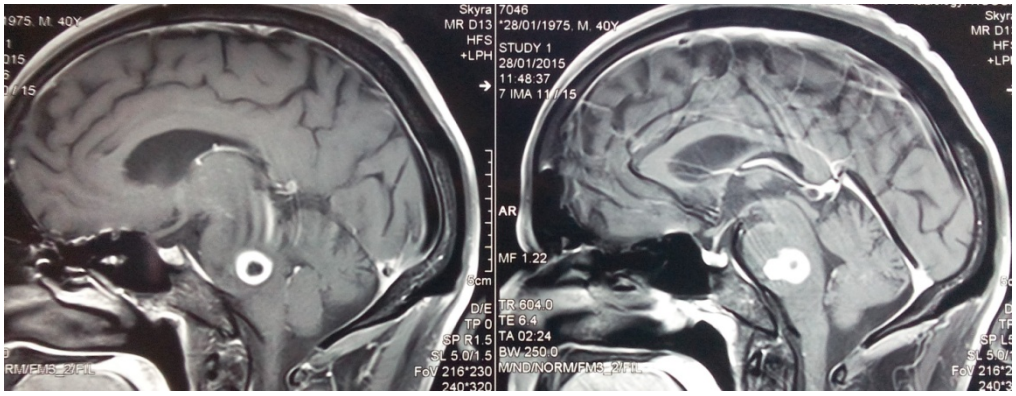
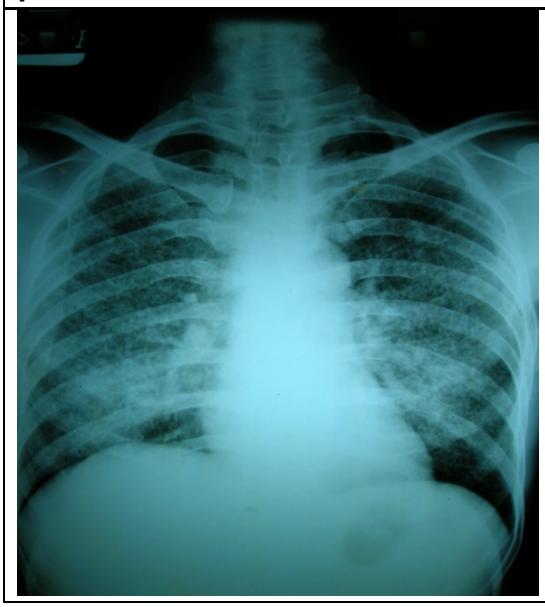
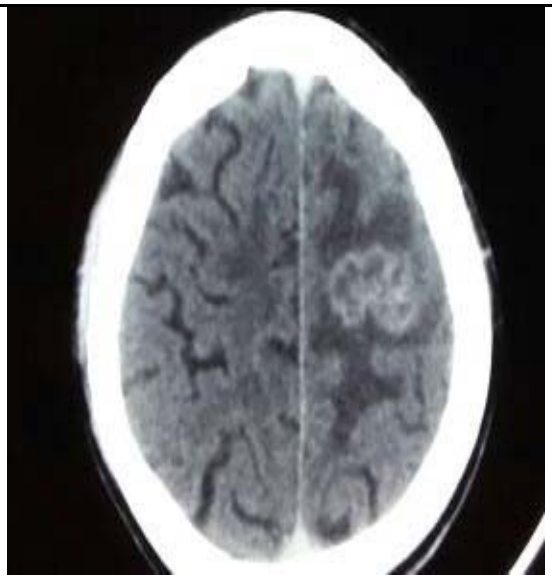


Fig.3:

Miliary TB in a HIV-TB co-infected patient	**Tuberculoma Brain with perilesional oedema**
	

In the univariate analysis, the baseline characteristics that proved to be significantly associated with the emergence of IRIS were low CD4, high viral load, presence of opportunistic infection, miliary TB, presence of extra pulmonary focus, low hemoglobin (Hb) and shorter ATT-ART interval in the unadjusted analysis. Hb and time interval between ATT-ART remained

significant in multivariate regression analysis along with IL-6 and C-reactive proteins (CRP). Extent of radiological involvement did not influence IRIS. This is probably because of the bimodal presentation of TB in advanced cases of HIV where the type of lesion may vary

from extensive involvement as in miliary TB to near normal Chest X-ray due to paucity of CD4 cell alveolitis.

Among various inflammatory cytokines, it was IL-6 and CRP which was grossly different between the groups which emerged as promising predictors and was helpful in differentiating IRIS and non-IRIS cases based on their levels at baseline. This difference was exaggerated at the time of IRIS making our observations more reliable. At pre-ART, the number of persons having levels of IL-6 and CRP simultaneously above the median values of the study population was higher in the group of patients that developed IRIS (14/26, 53.8%) than in those who did not (3/22, 13.6%) (Fisher's exact test P=0.023). IL-6 and CRP remained a promising predictor even after adjustment for age, time to ART, CD4, CD4/CD8 ratio and HIV RNA levels. The sensitivity and specificity increased when both the factors (IL-6 and CRP) were combined.

(ii) (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)
Collaborator	:	Rajiv Gandhi General Hospital, Chennai
Source of funding	:	Fogarty International (NIH)
Study period	:	2013-2015

This was a cross-sectional study to determine whether HDL-cholesterol gene polymorphisms are associated with unfavourable blood HDL-cholesterol levels, in HIV-infected adults in south India, after 12 – 15 months of Nevirapine (NVP) based ART regimen. The study enrolled 300 patients on (NVP)-based ART. In this cohort, high risk lipid profiles for atherosclerosis and cardiovascular disease were observed among HIV- infected individuals, even after 12-months of NVP-based ART. 39% of males and 47% of females had HDL-c levels below normal. Body mass index (aOR=1.70, 95%CI 1.01-2.84, p=0.04) and viral load (aOR=3.39, 95%CI: 1.52-7.52, p=0.003) were negatively associated with serum HDL-c levels. The 10-year risk score of developing CVD was 11-20% in 3% of

our patients. Allelic variants of APOC3 showed a trend towards low HDL-c.

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 PTB – Study XXV

Principal Investigator	:	Dr. G. Narendran (email:nareng@nirt.res.in)
Co-PI	:	Dr. Soumya Swaminathan
Collaborators	:	Govt. Hospital of Thoracic Medicine, Tambaram; Govt. Rajiv Gandhi General Hospital, 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 (Thro' WHO 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 issues that requires generation of scientific evidences, especially from India. We undertook this study to look into the intricate aspects of dual infection including schedule of therapy, sputum conversion, radiological clearance, IRIS, treatment emergent adverse drug reactions and drug levels so that concrete answers could be deduced.

Aims:

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

Secondary: To ascertain sputum conversion, IRIS, emergence of ARR, radiological improvement, pharmaco-kinetics of ATT drugs and toxicity profile with respect to dosing schedule

Methods: HIV-TB patients with culture positive TB are randomized to three regimens viz.: (1) Daily regimen ($2EHRZ_7/4HR_7$), (2) part daily ($2EHRZ_7/4HR_3$) and (3) a fully intermittent regimen ($2EHRZ_3/4HR_3$), given for 6 months duration and followed up for a further period of one year, 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.

Progress: 331 patients have been enrolled so far, 224 from Chennai and 107 from Madurai. Table 2 provides the baseline parameters of enrolled subjects. Sputum smear and culture conversion seem to be better with the daily regimen (98% vs 87%, p=0.002). Incidence of IRIS was 36%. The second interim analysis showed daily regimen to be better in efficacy than the intermittent arm (90% vs 77%, p<0.05) but with more frequency of hepatotoxicity that requires to be carefully monitored and treated.

The patients are being followed up.

Table 2: Baseline demographics of enrolled patients (Mean ± SD)

	Regimens		
Variables	**Daily**	**Part daily**	**Intermittent**
Mean (±SD)	(n=111)	(n=111)	(n=109)
Age (yrs)	38 ± 8.0	38 ± 9.0	39 ± 8.0
Weight (kg)	42.6 ± 8.1	42.9 ± 7.5	44.4 ± 7.6
ATT-ART days	16 (3-36)	17 (5-45)	15 (1-34)
Hb	9.8 ± 2.1	9.4 ± 2.1	9.8 ± 2.0
Log Viral Load	4.9 ± 1.2	4.9 ± 1.1	4.9 ± 1.2
CD4 cell counts	130 (65-226)	144 (71-259)	138 (68-259)
CD8 cell counts	600 (3345-957)	591 (342-1038)	702 (353-1028)
CD4/CD8 ratio*	.23 (.11-.40)	.23 (.15-.40)	.21 (.11-.38)

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

Principal Investigator	:	Dr.S. Ramesh Kumar (email: ramesh@nirt.res.in)
Collaborators	:	DTOs of Villupuram and Kancheepuram Dt; TCC – Adyar Cancer Institute.
Source of funding	:	USAID through MDP
Study period	:	2013-2016
Study Registry No.	:	CTRI/2013/07/003830

Background: Smoking prevalence among males (both rural and urban) has increased in India (NFHS III Vs NFHS II). Evidence for smoking as causal association for TB has been established, and it has been found that higher relapse, increased morbidity , mortality (4 times) occur in smokers with TB. The pilot study at NIRT (Madurai) showed at month 1that 35-45% of TB patients quit smoking when counseling/advice given by Dr / SW. We proposed to evaluate the different smoking cessation strategies at TB programme level.

Primary Objective: 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.

Methods:

Study Design:

Cluster randomized effectiveness trial

Study Procedure:

DMCs from two districts - Villupuram and Kanchipuram randomly selected (cluster randomisation) to receive T1 – Bupropion SR along with Standard counseling, or T2 –Enhanced Counseling arm including provisions of Educative materials on smoking cessation, Flip charts presentation, posters display, Video presentation or C – Standard routine counseling/Control arm, so that each TB patient who are current smokers enrolled to the study receives the intervention allotted to that study centre. Smoking cessation will be assessed by self-reporting and confirmed by Carbon monoxide monitors, done at 0, 2 and 6th month/end of ATT. TB outcome is

recorded at 6th month/end of ATT for these patients. Total sample size has been calculated to be 600 patients.

Progress: After recruiting of 45 subjects to the pilot study, recruitment for the main study started in January 2014. As of March 2016, 4290 patients were screened, of whom 512 patients have been allocated to the study.

The study is ongoing.

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 - Study XXIV

Principal Investigator	:	Dr.V.V. Banu Rekha (email: banurekha@nirt.res.in)
Co-Principal Investigator	:	Dr.M. Makesh Kumar
Collaborators	:	RNTCP Clinics, Chennai Corporation; Govt. Rajaji Hospital Madurai, GVMCH, Vellore
Source of funding	:	Intramural
Study period	:	2007-2016
CTRI Registration No.	:	PROVCTRI/2008/091/000024

Background: Shortening the duration of TB treatment from the currently recommended 6-month regimen for newly diagnosed PTB patients is a global research priority. A randomised clinical trial is being conducted by the NIRT in Chennai and Madurai to compare relapse rates up to 24 months of follow-up after treatment in newly diagnosed smear and culture positive PTB patients treated with 4-month and 3 month moxifloxacin (MFX) containing regimens with a 6-month regimen (control regimen). In this trial, the standard 4-drug TB regimen is supplemented with MFX, a fluoroquiniolone with potent bactericidal and sterilising activities against *M. tuberculosis*.

Methodology: HIV sero-negative, non-diabetic, newly diagnosed sputum positive PTB patients were randomly allocated to 3-month or 4-month MFX regimens, or a 6-month control regimen (Table 3). Treatment was directly observed and response to treatment

was assessed by monthly clinical evaluations and sputum examinations. The patients were closely monitored for adverse drug reactions. Patients with successful treatment outcome were followed up for 24 months after completion of treatment with monthly sputum and clinical 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 1327 patients have been enrolled in the study (May 2007 to March 2016). Forty patients were excluded from the study as per the protocol criteria. The baseline characteristics of 1286 patients are shown in Table 4. Majority of the patients were male (75%), had advanced disease with 2+/3+ sputum cultures (95%), radiological involvement of more than two lung zones (79%). There were 1114 (87%) patients who harboured bacilli susceptible to H, R, E and O.

Table 4: Baseline characteristics of new sputum positive PTB patients enrolled in the study (N=1286)

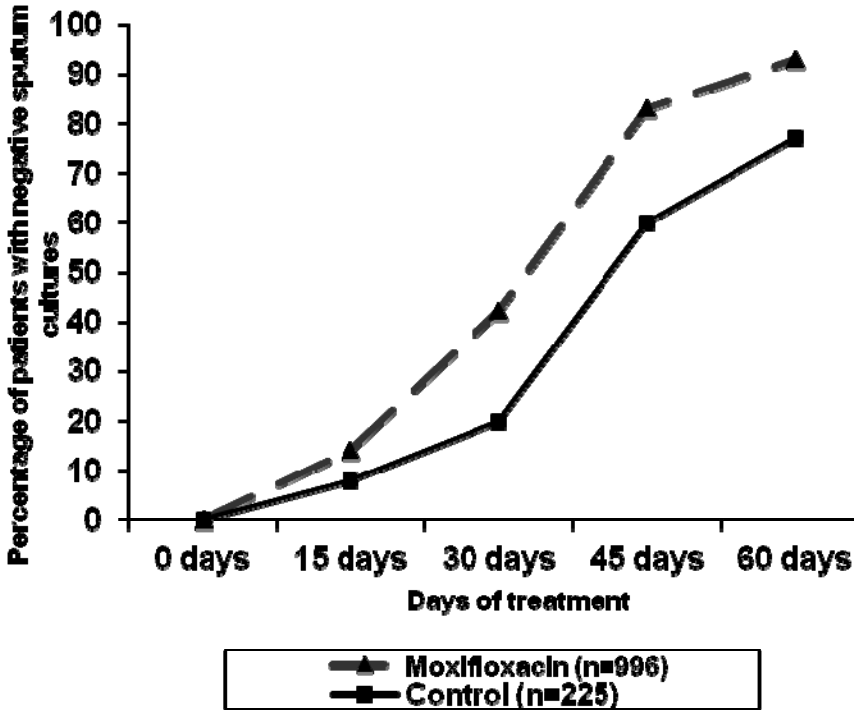
Patient characteristics		Regimen					Total N = 1286 n (%)
		Test Reg. 1 (N= 112) n (%)	Test Reg. 2 (N = 308) n (%)	Test Reg. 3 (N = 314) n (%)	Test Reg. 4 (N = 318) n (%)	Control Reg. (N = 234) n (%)	
Age (years)	< 35	56 (50)	131 (42)	156 (50)	153 (48)	123 (53)	619 (48)
	≥ 35	56 (50)	177 (58)	158 (50)	165 (52)	111 (47)	667 (52)
Gender	Male	89 (79)	238 (77)	236 (75)	225 (71)	177 (76)	965 (75)
	Female	23 (21)	70 (23)	78 (25)	93 (29)	57 (24)	321 (25)
Pre-treatment sputum culture grading	≤ 1 +	3 (3)	13 (4)	19 (6)	22 (7)	6 (2)	63 (5)
	2 +	11 (10)	47 (15)	53 (17)	55 (17)	53 (23)	219 (17)
	3 +	98 (87)	248 (81)	242 (77)	241 (76)	175 (75)	1004 (78)
Extent of initial chest x-ray involvement (zones)	≤ 2	25 (22)	60 (20)	62 (20)	68 (21)	49 (21)	264 (21)
	>2	87 (78)	248 (80)	252 (80)	250 (79)	185 (79)	1022(79)
Drug susceptibility profile							
Susceptible to H, R, E, O		98 (87)	277 (90)	268 (85)	279 (88)	202 (86)	1124 (87)
Resistant to any drug		14 (13)	31 (10)	46 (15)	39 (12)	32 (14)	162 (13)

R – Rifampicin; H – Isoniazid; E – Ethambutol; O- Ofloxacin

Fig. 4 illustrates the proportion of patients with negative sputum cultures at 15, 30, 45 and 60 days of treatment in 1221 patients (May 2007 to September 2015). Sputum culture conversion at the end of 2 months of intensive phase of treatment was significantly higher in the MFX regimen (93%) [Consolidated for all four test regimens] compared to the control regimen (77%) [$p < 0.001$]. This observation which was reported earlier (Annual Reports 2009-2010 to 2014-

2015) is sustained even with the larger population. This indicates that significantly higher proportion of patients treated with MFX containing regimens become less infectious earlier compared to those treated with the control regimen.

<u>Fig. 4:</u> Sputum culture conversion in MFX and control regimen in new sputum positive PTB patients



At the end of treatment, 89% to 92% of patients treated with MFX regimens had a favourable response compared to 83% in the control regimen. [$p<0.05$ for Test regimen 1, 2, 3 compared to control regimen] (Table 5). Of the 1093 patients with favourable response at the end of treatment, 74 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 control regimen. Based on this information the Data and Safety Monitoring Board (DSMB) had earlier recommended suspension of intake to this regimen. Enrollment of patients to the remaining regimens is ongoing.

Table 5: Response at the end of treatment and TB recurrence during follow-up (Intent-to-treat analysis) (N=1220)

Regimen	Patients n	Favourable response at end of treatment n (%)	TB recurrence*	
			n	Per 100 person years
Test regimen 1	112	103 (92%) #	20	11.22$
Test regimen 2	291	268 (92%) #	9	1.90
Test regimen 3	296	271 (92%) #	21	4.56$
Test regimen 4	296	264 (89%) #	17	3.61
Control regimen	225	187 (83%)	7	2.11

* in those with favourable response at the end of treatment (column 3)
# p<0.05 compared with the control regimen
$ p<0.05 compared with the control regimen

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)
Co-Principal Investigator	:	Dr.V.V. Banu Rekha
Collaborators	:	Govt. Stanley Hospital; Govt. Rajiv Gandhi Medical College Hospital, Kilpauk Medical College, Chennai; Govt. Vellore Medical College, Govt. Rajaji Hospital, Madurai and Corporation RNTCP centere in 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, Isoniazid (INH), Ethambutol (EMB) and Pyrazinamide (PZA) for the first 2 months followed by 2 drugs (RMP and INH) for the next 4 months. The delivery of TB chemotherapy in the field would be much easier if the duration of therapy could be shortened without sacrificing efficacy.

In the current study we propose to 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 in continuation phase. The control regimen for comparison of outcome measures with the test regimens proposed for this study will be the standard 6-month regimen.

OBJECTIVES:

Primary objectives:

To compare the efficacy of the two 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 in newly diagnosed superficial lymph node TB patients treated with the 2 regimens

Study regimens

Test regimen:

RMP, INH, PZA and OFX daily for 2 months followed by RMP, INH and OFX thrice weekly for 2 months (2 RHZO daily / 2RHO thrice weekly) - Duration 4 months

Control regimen:

RMP, INH, EMB and PZA thrice weekly for 2 months followed by RMP and INH thrice weekly for 4 months (2 RHEZ thrice weekly / 4 RH thrice weekly) – Duration 6 months

Methodology: Patients attending the surgical, medical out-patient clinics of Govt. Stanley Hospital, Govt. Rajiv Gandhi Medcial College Hospital, Kilpauk Medical College, Chennai, Govt. Vellore Medical College, Govt. Rajaji Hospital, Madurai and Corporation RNTCP Centre in Chennai, in whom a diagnosis of TB lymphadenitis is made in FNAC with clinical evidence of lymph node enlargement, are considered for the study.

They are given counseling and informed consent are obtained for invesigatons for TB and HIV infecton. Those diagnosed as HIV positive will be sent back to the referring unit (with the result) for further management. After providing adequate information about the trial, consent is obtained for enrolment in the study. The patient is then referred to the surgeon for open biopsy of the lymph node (under general anesthesia). The open biopsy specimen of the lymph node, divided into 2 parts, is collected in 2 separate bottles for histopathology and bacteriological examinations. The specimen meant for histopathology is collected in formalin. Serial sections are made and the slides are read by an independent pathologist and graded. The biopsy specimens are also examined by smear and culture methods for tubercle bacilli at NIRT. If the diagnosis of TB of the lymph node is confirmed either by histopathology or bacteriology the patient is considered eligible for enrolment to study if he/she satisfies the other inclusion criteria. Those with lymph node histology not suggestive of TB are referred back for further management. Among this group of paitents, if the lymph node culture yields *M. tuberculosis*, they are re-assessed and enrolled in the study if they have not been startd on ATT elsewhere. They are re-evaluated again for PTB before allocation to the study. The patients found ineligible for the study are referred back to the referring centre for appropriate management.

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

Study outcome:

The following outcome measures are compared beween 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

Results: The study is being conducted in Govt. Stanley Hospital, Govt. Rajiv Gandhi Medcial College Hospital, Kilpauk Medical College, Chennai, Govt. Vellore Medical College, Govt. Rajaji Hospital, Madurai and Corporation RNTCP Centre in Chennai. The estimated sample size for this trial is 330 patients; so far 104 patients have been enrolled to the trial. The interim findings are shown in tables 6 - 8.

The study is ongoing.

Table 6: Patient's details

		2EHRZ3/4RH3 (n = 34)	2OHRZ7/2OHR3 (n = 35)
Sex	Male	11	12
	Female	23	23
Age	Mean	28.1	31.1
	SD	7.5	11.2
BMI	Mean	22.5 (4.4)	22.0 (4.2)
Baseline Smear	Positive	6	9
	Negative	28	26
Baseline Culture	Positive	22	32
	Negative	12	3

Table 7: **Drug susceptibility pattern of culture positives**

	2EHRZ3/4RH3 (n = 34)			2OHRZ7/2OHR3 (n = 35)		
Drugs	Sensitive	Resistant	NA	Sensitive	Resistant	NA
H	20	-	14	29	3	3
R	19	1	14	31	1	3
E	20	-	14	31	1	3
OF	20	-	14	30	2	3
Sm	20	-	14	27	5	3
K	18	2	14	31	1	3
Eth	8	12	14	12	20	3

Table 8: **End of treatment outcomes**

Outcome	2EHRZ3/4RH3 (n=34)	2OHRZ7/2OHR3 (n=35)
Favourable	32	32
Prob.Favour	1	1
Unfavourable	1	2
NA	1	-

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

Principal Investigator	:	Dr. Syed Hissar (email: syed.hissar@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 is a major obstacle to accurately quantifying the true burden of childhood TB which is probably both over and under-diagnosed among children in different settings. The need for improved TB diagnostics in children is consistently acknowledged. Promising novel techniques (Xpert® MTB/RIF, urine LAM) that have been developed for the diagnosis of TB need to be tested and validated in children. Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) is an automated, user-friendly real-time PCR assay designed for the rapid and simultaneous detection of *M. tuberculosis (M.tb)* and RMP resistance. Lipoarabinomannan (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 intrathoracic 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

(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 are screened for the study - (a) cough (b) weight loss/ failure to thrive (c) persistent unexaplained fever (d) persistent, unexplained lethargy or reduced playfulness. Symptom screening, detailed general and clinical evaluation are done. Chest X-ray, tuberculin skin test (TST), collection of gastric lavage / induced / expectorated sputum for Xpert® MTB/RIF, AFB smear, culture and DST if culture positive, are done.

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

Results: The study was initiated in August 2013. Required sample size is 2761 children with respiratory symptoms. The study is currently enrolling children from five centres, namely 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, we have screened 2999 children and out of which 1703 children were successfully enrolled in the study (Table 9). Baseline characteristics of the enrolled children are detailed in the Table 10. Overall % of children were bacteriolgoically positive for smear and/or Xpert and/or MGIT/LJ culture. Children positive only for AFB smear were 1.8% and children positive only for Xpert Mtb were 3.5% (Table 11). The study is ongoing.

Table 9: Site-wise recruitment details of children

	GSH	ICH	GVMC	CMC	GRH	Total
Number of children screened	973	805	132	239	850	2999
Number of children enrolled	708	622	97	60	216	1703
Number of children on ATT and follow-up	107	98	22	14	11	252

Table 10: Baseline characteristics of children

		No. of children
		N = 1142 (%)
Age	0-1 yr	36 (3.2)
	2-5 yrs	448 (39.2)
	6-10 yrs	482 (42.2)
	11-14 yrs	176 (15.4)
Sex	Male	500 (43.8)
	Female	642 (56.2)
TST	**Positive**	**180 (15.8)**
	Negative	962 (84.2)
Previously treated with ATT	**Yes**	**80 (7)**
	No	1062 (93)
Contact with TB case	**Yes**	**495 (43.3)**
	No	647 (56.7)
Chest X-ray Abnormality	**Yes**	**273 (23.9)**
	No	869 (76.1)
HIV	**Positive**	**12 (1.1)**
	Negative	1130 (98.9)

Table 11: Bacteriology results

	Total N = 1527 (%)
No. of Children positive for smear and/or LJ and/or MGIT and/or Xpert	**92 (6%)**
No. of Children Negative for available microbiological investigations	1435 (94%)
No. of children with available smear results	1527
No. of children with smears positive	**27 (1.8%)**
No. of children with available LJ culture results	1468
No. of children with LJ culture positive	**49 (3.3%)**
No. of children with available MGIT culture results	1393
No. of children with MGIT culture positive	**64 (4.6%)**
No. of children with available Xpert results	1217
No. of children with Xpert positive	**43 (3.5%)**
No. of children with RIF resistance	2 (4.7%)

CL-6: A prospective study to determine the incidence of TB among patients with type 2 diabetes mellitus

Principal Investigator	:	Dr.M. Makeshkumar (email: makeshkumar.m@nirt.res.in)
Co-Principal Investigator	:	Dr. Soumya Swaminathan
Collaborators	:	Govt. General Hosiptal, Chennai), Govt. Rajaji Hospital, Madurai; MV Hospital for Diabetes, Royapuram, Chennai, RMRC, Bhubaneswar and Capital Hosiptal, Bhubaneshwar and Dt. Hospital Khurdha Odhisa.
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 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.

Objectives:

Primary objective:

(i) To determine the incidence of TB among people with Type 2 diabetes mellitus

Secondary objectives:

(i) To identify risk factors for TB among people with Type 2 diabetes mellitus

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

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

(iv) To evaluate the diagnostic accuracy of Gene Xpert MTB/RIF among Type 2 diabetes mellitus 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, and MV Hospital for Diabetes, Royapuram, Chennai and Capital Hospital, Bhubaneshwar and District Hospital, Khurdha, Odhisa. The recruitment details are given in Table 12.

Table 12: Recruitment details

Sl.No.	Centre	No. of screened	No. of recruited	Refer back
1	GRH, Madurai	257	165	92
2	RGGGH Chennai	336	271	65
3	MV diabetes	7	0	7
	Total	590	436	164

Two cases were smear positive during screening and they were referred to RNTCP for treatment. Four cases were smear positive during follow-up. All these patients' cultures were negative and these patients were evaluated for further smears cultures and X-ray which were found to be normal. One patient has isolated one culture positive and this was further evaluated with smears, cultures and X-ray, which were found to be negative / normal.

The study is ongoing.

CL-7: HIV-associated lipodystrophy syndrome in children: Role of nutrition, ART and genes

Principal Investigator	:	Dr. Padmapriyadarsini C (email: padmapriyadarsinic@nirt.res.in)
Collaborators	:	Tufts University; St. John's National Academy of Health Sciences, Bangalore
Source of funding	:	National Institute of Health (5RO1 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 at 12 & 24 months after initiating ART.

The study, initiated in June 2011, has completed enroment. Of the 393 HIV-infected children enrolled to the study, 316 children have completed 12 months of follow-up. After ART initiation, these children had periodic estimation of anthropometric parameters, blood lipid profile, serum insulin and CRP, haematology, CD4 cell counts and viral load measurements (Table 13). The follow-up of all enrolled children will be completed in next 4 months. Manuscript preparation is ongoing.

Table 13: Blood lipids of HIV-infected children on ART over 12 months

S. No.	Parameters (n=316)	Baseline [mean ± SD]	6 months [mean ± SD]	12 months [mean ± SD]
1.	Weight	18.1 ± 5.9	20.2 ± 6.3	21.8 ± 8.9*
2.	Height	111.4 ± 17.7	116.3 ± 16.9	118.9 ± 17.3*
3.	Total Cholesterol	132.3 ± 34.6	161.6 ± 35.8	162.8 ± 35.5*
4.	LDL-cholesterol	78.4 ± 28.1	93.8 ± 28.9	92.6 ± 28.8*
5.	HDL-cholesterol	29.8 ± 11.1	46.2 ± 14.7	48.8 ± 15.2*
6.	Triglycerides	139.9 ± 71.4	126.3 ± 72.2	117.7 ± 69.0*
7.	Blood glucose	84.3 ± 13.3	86.4 ± 14.1	84.9 ± 13.6
8.	CD4	522.4 ± 416.9	851.4 ± 477.8	95.5 ± 57.9*
9.	Serum. Insulin	8.6 ± 12.4	105 ± 14.4	10.5 ± 20.2
10.	Serum. CRP	2.5 ± 3.4	2.9 ± 3.6	2.8 ± 3.5

CL-8: C-TRIUMPh: Cohort for TB research by the Indo-US medical partnership multicentric prospective observational study

Principal Investigator : Dr.C. Padmapriyadarsini
(email: padmapriyardarsinic@nirt.res.in)
Collaborators : Johns Hopkins University, USA; B J Medical College, Pune
Funding Agency : DBT
Study period : 2013-2018

This is a prospective multi-centric observational cohort study at two sites, Chennai and Pune, that is enrolling TB patients and their household contacts, to study host and microbial risk factors associated with progression of TB disease, response to treatment, progression from TB infection to disease as well as transmission. A repository of biological specimens is being created, that can be used for future basic science research including biomarker discovery, and can be

made available to investigators of this Centre / Partnership on request. The study is currently enrolling and as of 31st March 2016, 182 TB patients (Cohort A) and 386 household contacts (Cohort B) have been enrolled to the study.

CL-9: **An open-label, non-randomized, two stage, dose-finding study of Verpamil [IR] tablet formulation in adult TB patients in continuation phase of anti-TB treatment**

(Previous title was: "Inhibition of host-induced mycobacterial efflux pumps as a novel strategy to counter drug tolerance and virulence of PTB", now renamed as per suggestion of DCGI)

Principal Investigator	:	Dr.C. Padmapriyadarsini (email: padmapriyardarsinic@nirt.res.in)
Collaborators	:	NITRD, New Delhi
Funding Agency	:	DBT
Study period	:	2013-2018

This is a phase 2, single-centre, open-label, verapamil dose-finding pharmacokinetic study of verapamil given in conjunction with RMP. The goal of this study is to determine the contribution of the efflux pump-mediated tolerance mechanism in delayed or incomplete sterilization in active PTB i.e. whether verapamil when added to standard TB therapy can accelerate sputum clearance of M. tb. DCGI of India approved the study in August 2015. The study obtained NITRD IEC approval in October 2015 and NIRT IEC approval in February 2016. Negotiation with insurance companies for insurance policy for sutyd participants is ongoing. Once the insurance policy is in place, the study will start enrollment.

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:padmapriyardarsinic@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 was a prospective multicentric study with phased implementation, looking at the feasibility and effectiveness of Isoniazid preventive therapy (IPT) for HIV infected adults and children attending ART centres in several states.

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

(ii) To include the assessment of the effectiveness of simple algorithms to exclude active TB prior to IPT initiation

(iii) To measure the number needed to screen (NNS) and number needed to treat (NNT) to prevent one case of TB

Methods: The study was conducted in three phases – Phase I: Enhanced TB surveillance (all sites, all ART centre attendees) for all HIV infected adults and children. Phase II: Provision of IPT: 6 months (following the completion of Phase I) and Phase III: Follow-up of these patients for a period of 6 months.

Results: IPT was initiated in 4528 PLHIV {mean age: 37+8 years; 86% were on ART}. 4015 (89%) successfully completed IPT, while in 121 adults, IPT was terminated by the medical officer for adverse events, 326 patients were lost to follow-up and 17 died. 25 PLHIV developed TB while on IPT {13 PTB and 12 extra PTB). The incidence of TB, while on IPT was 1.17 /100 p-y (95% CI 0.8-1.73) as compared to TB incidence of 2.42 /100p-y (95% CI 1.90-3.10) during the pre-IPT period at these centres, the difference being statistically significant (p=0.017). Follow up of all these patients post-IPT period is completed now, and data analysis is ongoing.

CL-11: 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:padmapriyardarsinic@nirt.res.in)
Collaborators	:	Chennai Corporation, GHTM
Funding Agency	:	ICMR Task Force (Awaited)
Study period	:	2013-2016

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 – 18 months of culture negativity. The study was initiated in November 2014. As of February 2016, 17 confirmed cases of NTM have been enrolled to the study. Of the 17 patients enrolled, there are 10 men and 7 women with mean age of 50.3 years. Nine patients had *M. kansasii*, 2 had *M. abscessus*, 4 had *M. intracellulare*, 1 had *M. avium*, and 1 had *M. fortuitum.* All of them have been started on appropriate treatment.

CL-12: EDOTS - Effect of diabetes on TB severity

Principal Investigator	:	Dr. Pradeep Menon (email:docpradeep@yahoo.com)
Collaborator	:	MV Diabetics Research Centre; NIRT, Chennai
Funding Agency	:	DBT
Study period	:	2014-2017

This is a multicentric study to assess the effect of diabetes on TB severity. The collaborating centres in Chennai are NIRT and MV Diabetes Centre. The study is being conducted at Pulianthope and Tondiarpet RNTCP centres. The estimated sample size is 300 (150 DM-TB and 150 TB cases without DM).

Of the 1100 patients screened, 209 were eligible for the study. 113 were classified as DM-TB and 96 TB without DM. Diabetes was newly diagnosed in 37 (32.7%) patients. TB outcomes are to be analysed. The study is in progress.

CL-13: Multi-centric cohort study of recurrence of TB among newly diagnosed sputum positive PTB patients treated under RNTCP

Principal Investigator	:	Dr. Mohan Natrajan (email:mohan.n@nirt.res.in)
Collaborators	:	National Tuberculosis Institute, Bangalore, NITRD, New Delhi, RMRCT, Jabalpur, Thiruvananthapuram Medical College, Thiruvananthapuram, Mahatma Gandhi Inst. of Medcial 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 ($2H_3R_3Z_3$ E_3 / $4H_3R_3$), 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: 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

(ii) To identify risk factors for unfavourable treatment outcomes (treatment failed, lost to treatment follow-up and died) and recurrent TB

M

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 TB smear, culture, drug susceptibility testing (DST) and genotyping and blood tests for diabetes mellitus and HIV infection.

Study progress: A total of 2473 participants were screened out of which 1578 participants (64 percent) were enrolled into the study. Of the 1269 analysed, 925 (73%) were

males and 672 (53%) were from urban site. The mean age of the population was 40.7 years (range 18-90 years). The mean BMI was 17.5 (10.2-38.5) kg per meter square. The treatment outcomes and TB recurrence rates are being analysed.

The patients are on follow-up and the study is ongoing.

CL-14: Randomized trial to study the optimal regimen in the treatment of patients with INH resistant PTB – Study XXVII

Principal Investigator	:	Dr.P. Paul Kumaran; Dr V V Banu Rekha, (email: ppaulkumaran@nirt.res.in; banurekha@nirt.res.in)
Co-Principal Investigators	:	Dr Mohan Natrajan; Dr. Syed Hissar; Dr.K.R. Uma Devi; Dr.N.S. Gomathi
Funding Agency	:	Intramural
Study period	:	2015-2019

Background: The TB Control Programme in India is dependant on molecular diagnosis for screeing of DR-PTB suspects (for INH and RMP sensitivity) using Line Probe Assay (LPA) technique. RNTCP manages RMP resistance under programmatic management of drug-resistant TB (PMDT), but does not address the management of INH resistant PTB patients; they are currently left to continue the existing retreatment regimen. This study is undertaken to assess the effectiveness of an optimal retreatment regimen, either fully oral or an injectable containing regimen, in the treatment of INH resistant genotypically PTB.

Objectives: (i) To compare the effectiveness of ATT regimens (one fully oral, another an injectable containing regimen) in the treatment of patients with INH resistant PTB

(ii) To compare the relapse rates upto 12 months of follow-up after successful treatment completion

Methodology: Patients whose LPA test results are suggestive of INH

resistance (resistance by presence of *katG* gene and/or *inhA* gene mutation) and are eligible for the study (based on inclusion and exclusion criteria) are randomized (1:1:1) to any one of the treatmet regimens after stratification based on sputum smear grade and diabetes status. The treatment regimens and the drug doses are as shown in Table 14.

Table 14: Treatment regimens with drug dosages

Regimen	Intensive Phase	Continuation Phase
Test regimen – fully oral	3 $LHREZ_7$	5 REZ_7
Test regimen – Injectable	3 S_5 $HREZ_7$	
Control regimen	2 $SHREZ_3$ / 1 $HREZ_3$	5 HRE_3

[S = Streptomycin, H = Isoniazid, R = Rifampicin, E = Ethambutol, Z = Pyrazinamide, L = Levofloxacin]

Inj. Streptomycin (S) – 750mg per day (500mg for patients aged more than 50 yrs)

Tab. Levofloxacin (L) - 750mg per day (1000mg for patients weighing 50 kg or more)

Tab. INH (H) – 600mg per day (along with Tab Pyridoxine 10 mg for those on daily H therapy)

Cap. RMP (R) – 450mg per day (600mg for patients weighing 50 kg or more)

Tab. EMB (E) – 800 mg per day (daily); 1200mg per day (thrice-weekly)

Tab. PZA (Z) – 1500mg per day

As of 31st March 2016, 70 PTB patients with *M. tuberculosis* resistant to INH based on gene mutation were enrolled, out of whom 43 (61%) were allocated to the study (Table 15).

The study is ongoing.

Table 15: Recruitment details in Chennai & Madurai

Recruitment status (upto 31 March 2016)	Chennai (three districts)	Madurai (one district)	Total
Suspects pre-screened	1230	555	1785
INH resistant cases identified	**78**	**47**	**125**
Pre-screening Ineligibles	36	19	55
Study enrolled	42	28	70
√ Inelgiibles	18	9	27
√ Allocated (Non-diabetic: Diabetic)	**24** (14 : 10)	**19** (14 : 5)	**43** (28 : 15)
➢ Test regimen – fully oral	7 (5 : 2)	8 (6 : 2)	**15** (11 : 4)
➢ Test regimen – Injectable	9 (4 : 5)	5 (4 : 1)	**14** (8 : 6)
➢ Control regimen	8 (5 : 3)	6 (4 : 2)	**14** (9 : 5)

CL-15: Prevalence of TB infection and disease among pediatric household contacts of MDR-TB patients – A multicentric propsectice cohort study

Principal Investigator	:	Dr. Dina Nair (email: dinanair@nirt.res.in)
Collaborators	:	NJIL, Agra, Govt. Hospital for Thoracic Medicine; Tambaram; Govt. Medical College & Hospital, Vellore; Govt. Rajaji Hospital. Madurai; Sarojini Naidu Medical College, Agra
Funding Agency	:	ICMR-Task Force
Study period	:	2015-2018

Background: In countries worldwide, the reported percentage of all TB cases occurring in children exposed to TB in the household varies from 24.2% to 69.2%. These children become the reservoir of future disease in adult-hood thus perpetuating the epidemic. The transmission dynamics of drug-resistant bacilli among the household contacts of MDR-TB patients is uncertain. Contact

investigations though recommended to aid early diagnosis and prevention of further transmission thereby reducing the disease burden, morbidity and mortality in children is not prioritized in TB control programmes. The data on the rate of TB infection and subsequent risk of active disease among MDR-TB contacts have not been consistent. Studies done in Brazil have reported that the prevalence of TB infection and progression to active TB was comparable in close contacts of MDR-TB and drug-susceptible TB patients, where as in Romania a study on pediatric population observed that TB infection rates were significantly lower in contacts of MDR-TB patients. A study done in South Africa concluded that MDR-TB is readily transmissible and is associated with a high risk of infection and active disease in children less than 5 years. Tuberculous infection with or without disease was present in 78% of children by 30 months, 95% of whom were already infected by 12 months. In India the data on screening and prevalence of latent TB (LTB) Infection among child contacts of MDR-TB is sparse. The value of contact tracing and the prevalence of LTBI requires more exploration in India.

Objectives:

Primary objective: To measure the prevalence of LTB infection among pediatric household contacts (less than 15 yrs of age) of MDR-TB patients

Secondary objectives: i) To estimate the prevalence of active TB and DR-TB disease among the pediatric household contacts of MDR-TB adults

ii) To describe and compare the drug resistance pattern and *M. tuberculosis* genotype in adult-child contact pairs

iii) To identify the risk factors for disease progression in the pediatric house hold contacts

Progress: This is a prospective cohort study and the sample size required is 600. As of June 2016 we have enrolled 304 index cases with MDR-TB and 75 pediatric household contacts in total from all sites. The study is ongoing.

Department of Socio Behavioral Research

STUDIES IN PROGRESS:

SB-1: Estimatlon of the burden of TB among tribal population and development of an innovative health system model to strengthen TB control in the tribal areas

Principal Investigator	:	Dr. Beena E. Thomas
Source of Funding	:	ICMR
Study Period	:	2015 – 2017

NIRT is the co-ordination site for this multicentre ICMR task force study.

Colloborating Institutes:

1. Regional Medical Research Centre for Tribals (ICMR), Jabalpur.
2. Regional Medical Research Centre, (ICMR), Bhubaneswar.
3. Regional Medical Research Centre (ICMR), Andaman and Nicobar Islands.
4. Pondicherry Institute of Medical Sciences, Puducherry.
5. Rajendra Institute of Medical Sciences (RIMS), Jharkhand.

Background: The tribal population is an integral part of our civilization and improvement of TB care services under RNTCP in tribal populations is required as this population live in areas that have poor access to the health care delivery systems, and this group is known to be socially and economically impoverished and at a disadvantage. As per the 2011 National Census, the tribal population is estimated at 104.28 million, representing 8.6% of the country's total population. Though TB is a major public health problem in India, there is limited knowledge with few area specific studies on the burden of TB among the tribal population. A meta analysis of these studies projected a pooled estimate of 703/100000 with wide variation among tribal groups. Hence, this study is proposed to estimate the burden of TB among the tribal population in the country in terms of prevalence of pulmonary TB, risk factors for TB and the health seeking behaviour of chest symptomatics. It is hoped that the findings of this study will help towards developing a health system model towards effective interventions.

Objectives: (i) To estimate the burden of TB amongst tribal groups in various states of the country

(ii) To determine the health seeking behavior patterns of persons having symptoms suggestive of TB

(iii) To develop a tribal health system model with feasible interventions to improve case finding and compliance for TB treatment through a community based approach

Summary and Progress: This study is to be undertaken in 14 sites in different states of the country. However due to constraints with funding, the study will be carried out in 2 phases. In the first phase, 5 institutes have been selected covering 6 states. They are National Institute for Research in Tribal Health, Jabalpur (Madhya Pradesh & Chhattisgarh); Regional Medical Research Centre, Bhubaneswar (Odisha), Regional Medical Research Centre, Port Blair (Andaman and Nicobar); Pondicherry Institute of Medical Sciences, Puducherry and Rajendra Institute of Medical Sciences, Ranchi (Jharkhand). The tribal districts which were selected were defined as those with >70% tribal population. Districts were randomly allocated to each site and within districts, clusters (villages) were decided for each site using PPS. Each cluster /village had to cover a population of 800 individuals. The sites and cluster have been elaborated in Table 16. The study has been conducted in Phases. Phase 1 included a situational analysis and social mapping (to understand the tribal area using a participatory approach for the social mapping and a guide with a check list for the situational analysis), Phase 2 constituted the qualitative component of the study (Focus Group discussions, Interviews), and Phase 3 covers the quantitative component which includes listing of the households, profiling the members of the household, identifying and interviewing chest symptomatics and sputum collection from them, sending samples to the IRL for sputum and culture and referral of those diagnosed as TB for treatment and follow-up.

Following the orientation and detailed training of all the investigators and field staff on June 2015 at NIRT, the study was rolled out in all six states by July 2015. The study is coordinated and monitored from NIRT by the PI through visits to the sites by PI and study coordinator, regular communication and video conferencing calls. Most sites have completed Phase 1 and Phase 2

activity and the Phase 3 is in progress (Table 16).

Table 16: Study progress- Phases 1 & 2

	Andaman	Chhattisgarh	Jharkhand	Madhya Pradesh	Maharashtra	Odisha	Total
Total No. of Villages/clusters	3	6	9	16	8	6	48
Phase 1							
No. of Situational analysis completed	3	6	9	18	8	6	50
No. of Social mapping done	3	6	9	16	8	6	48
Phase 2 (Qualitative component)							
No. of Health care provider interviews completed	20	25	20	22	13	49	149
No. of Key informant interviews completed	143	22	18	17	12	32	244
No. of TB patients interview completed	9	3	0	12	2	0	26
No. of FGD completed	7	2	6	17	4	12	48

SB-2: Fostering resilience to psychosocial and HIV risk in Indian men who have sex with men

Principal Investigators	:	Beena E Thomas (India PI); Dr. Conall O'Cleirigh (US PI)
Source of Funding	:	National Institute of Mental Health, USA
Study Period	:	2015 – 2020

Background: India has the world's third largest HIV epidemic, and given the population size, has one of the largest populations of men who have sex with men (MSM) in the world. MSM in India have an estimated seroprevalence of 14.7%. HIV prevention efforts for MSM in India are limited to condom distribution and HIV education, with no large scale efficacy trials of interventions and therefore no sufficient evidenced-based interventions in this population.

This proposal is the outcome of successful community-based Indo-US research collaboration between MGH and NIRT. The 2 NGOs dedicated to HIV prevention among MSM are Sahodaran (Chennai) and The Humsafar Trust (Mumbai). We have, in every phase of the development of this proposal engaged the community in developing the research ideas, forming the program of research, designing / refining the studies, and disseminating the results.

Objective: To test the efficacy of the self-acceptance-based intervention in comparison to HIV - VCT on co-primary outcomes: sexually transmitted infection (STI) incidence and reduced episodes of unprotected anal sex among HIV-infected and uninfected MSM in Chennai and Mumbai

Methods: This is a two-arm randomized controlled trial comparing a self-acceptance based HIV risk reduction intervention with voluntary HIV/STI counselling and testing (VCT) among MSM in Chennai and Mumbai. The self-acceptance based HIV risk reduction intervention consists of 4 group sessions and 6 individual sessions and HIV and STI testing (at baseline and 12-months). The STIs would include: Chlamydia infection, gonorrhoea and syphilis. If the participant test positive for HIV/STI they are referred for care and treatment as required. Participants in both arms will be followed for one year, with behavioral and psychosocial

assessments conducted at baseline, 4, 8, and 12 months.

Summary and Progress: The study in Chennai site started after receiving HMSC clearance in May, 2015. During this period of reporting, 74 patients were recruited (Intervention – 37 & control – 37). Thirteen of the 37 participant in the intervention arm have completed all 6 individual and 4 group sessions. The 4th month follow-up for 37 participants (both intervention and control arm) has been completed. After receiving approval, the Humsafar Trust initiated the study on June 23rd 2015. During this period (April 2015-March 2016), 73 participants (Intervention – 37 & Control – 36) have been recruited. Thirteen of 37 participants in the intervention arm have completed all 6 individual session and 4 group sessions. The 4th month follow-up for 40 participants has been completed. The study is ongoing.

SB-3: Investigating pre-treatment loss to follow-up of smear-positive TB patients in the Revised National Tuberculosis Control Program in Chennai city and Tiruvallur district, Tamil Nadu

Principal Investigators	:	Dr. Beena E Thomas (Indian PI) Dr. Ramnath Subbaraman (US PI)
Source of Funding	:	NIHGHES Fellowship via Brigham and Women's Hospital
Study Period	:	2016 (1 year)

Background: Pretreatment loss to follow-up (PTLFU), previously referred to as "initial default" refers to patients who are found to be smear-positive but fail to enroll in treatment**.** Four prior studies on PTLFU have been done in India in Tiruvallur district of Tamil Nadu, Andhra Pradesh, and Uttarakhand. These studies showed a 5-22% prevalence of PTLFU. In Tiruvallur, the rate was 15% for those diagnosed at RNTCP facilities and 24% for those diagnosed in a community survey in 2004-2005. The RNTCP statistics suggest that as many as 100,000 smear-positive cases remain

unaccounted for nationally every year. To investigate this problem, a multi-phase study in Chennai and Tiruvallur districts of Tamil Nadu was initiated.

Aims: (i) To identify the proportion of missing or incorrect phone numbers or addresses in the designated microscopy patient register

(ii) To determine the outcome of all newly smear-positive cases listed in the microscopy register during that time period

(iii) To determine reasons for PTLFU of newly smear-positive individuals using in-depth qualitative interviews

(iv) To understand the perspectives of RNTCP staff regarding this problem, using interviews and focus group discussions

Summary and Progress: The study was initiated on September 2015. Following the NIRT orientation training, the study was rolled out on October 2015. Eighteen Designated Microscopy Centres have been covered thus far. The study is on-going.

SB-4: Community driven health committee – its feasibility and effectiveness in addressing gender issues in public health with special reference to TB

Principal Investigator	:	Dr.E. Thiruvalluvan
Source of Funding	:	Indian Council of Medical Research
Study Period	:	2015-2017

Background: Poverty is attributable to three quarters of the burden of ill-health and infectious diseases particularly TB. Access to TB health services is the right of an individual. However it is widely reported that there are gender disparities in access to health services. This has resulted in exposure of women to risk of TB, poor access to information and poor understanding about TB disease management, prevention and control. There is need for a demand for quality TB services from the community with a bottom –top approach which can be ensured through community driven models of care. This approach has been successful in HIV/AIDS control. It was

therefore felt that this approach needs to be tried in TB control as well.

Objectives: (i) To understand the gender barriers in TB control strategy

(ii) To study the feasibility and effectiveness of community based health committees and its contribution to accessibility, improved quality and gender sensitive TB health care services

(iii) To develop guides and tools to minimize gender disparities in TB programmes

Summary and Progress: The study is being done in phases with a qualitative and quantitative component. This study is being carried out in two districts in Tamil Nadu namely Madurai and Tiruvallur, to assess the difference between women's participation in Health system strengthening in these two regions. The PHC and HSC profiling have been completed in both the regions (Tabe 17).

Table 17: Profiles of PHC & HSC

	Location	Intervention	Control
Madurai	Rural	Karungalakudi	Vellalore
	Urban	T.Kallupatti	Samayanallur
Tiruvallur	Rural	Madarapakkam	Minjur
	Urban	Nemam	Naravarikuppam

The social mapping was conducted in the intervention sites to assess the facilities available within the villages. The entire exercise was carried out along with the participation of the community members. Overall, 36 and 88 key informant interviews were done in Madurai and Tiruvallur districts respectively with different category of population. Totally, 10 focus group discussions (FGDs) and 143 (82 in Madurai and 61 in Tiruvallur) in-depth interviews have been completed. The barriers identified from this interview are challenges in accessing healthcare facilities such as lack of transport facilities, patients' long waiting hours, lack of bed facilities, lack of diagnostic facilities (CT-scan, ECG, X-ray etc.), poor attitude of healthcare providers,

improper disposal of wastes, non-availability of Medical Officers, non-availability of separate toilet for male and female, non-availability of HSC facility in some of the villages, and visits to the villages made by the Village head nurses are only for antenatal check and polio drops**.** The probable final list of forum members has been prepared based on the Interviews and Focus Group Discussions (FGD) conducted in the study areas. Efforts are in progress in finalizing the Community Forum for TB control which will be followed by intense training of the members to conduct the activities as planned**.** The study is ongoing

SB-5: A study on psychosocial issues facing MDR patients to design appropriate intervention strategies to promote drug adherence

Principal Investigator	:	Dr.E. Thiruvalluvan
Co-Investigator	:	Dr. Beena E. Thomas
Source of Funding	:	Intramural project
Study Period	:	2 years

Background: MDR-TB is defined as TB resistant to INH and RMP, two first line drug therapies for TB. Almost 50% of MDR-TB cases worldwide are estimated to occur in China and India. Treatment of MDR-TB is much more complex with 18-24 months of treatment. MDR-TB incidence levels are 3% in new cases and 12%-17% in re-treatment cases.

Objectives: (i) To understand the psychosocial issues facing MDR TB Patients (Depression, stigma, social support)

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

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

Summary and Progress:

Phase I – Qualitative Phase*:* MDR-TB patients registered under DOTS Plus programme during the period of 2013-2014 in Chennai districts, of Tamil Nadu were included for this study. Two trained

professional medical social workers conducted the FGDs at the Government health facilities. The findings revealed that majority of participants were unaware of 'MDR-TB'. Most of the TB patients had not disclosed their TB status, even to their family members. Many patients experienced substantial enacted stigma, leading to breakdown of family relationships and isolation within the family. Patients experienced stigma from health care providers. Overall study findings indicate that there is a 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. This phase helped to prepare the MDR intervention manual.

Phase II – Quantitative Phase: This is an intervention phase study in which all the zones of the Chennai Corporation were selected. MDR-TB patients (>18 years) registered from August 2014 to January 2015, under the RNTCP, were assessed. The intervention consisted of 3 individual counselling sessions (1st, 3rd, and 5th month) conducted by trained interventionists. An intervention manual using a community participatory approach was used to guide the sessions. This involved one to one counselling sessions and visual aids were used to explain facts about TB, MDR-TB and the TB treatment and management. Thirty five MDR-TB patients have been enrolled in this study.

The study is ongoing.

Projects initiated

Strengthening implementation and operational research under the RNTCP in India

Principal Investigator	:	Dr. Beena E Thomas
Source of Funding	:	Global funding through Central TB Division and ICMR

This is an innovative project aimed at strengthening implementation and operational research focusing on the priority areas as defined by the RNTCP in India. There was "Call for Proposals' which required researchers to submit their proposals as teams. These teams needed to include clinicians, social scientists, epidemiologists, statisticians or economists. Responding to this call, 68 concept proposals were received. These proposals were screened by a team of steering committee members who are experts from various disciplines. A total of 15 proposals were shortlisted. The selected teams were invited for a training workshop at NIRT that was scheduled from 30th May to 03rd June, 2016 to develop their concept proposals into full proposals. This workshop was facilitated by Implementation/Operational research experts from the WHO and national experts from India.

Activities having public health importance:

Tribal outreach in Arunkundram and Panchathiriti Village

'Irular' is one of the tribal groups of India, also known as the second largest tribal group of Tamilnadu. They mainly reside in the lower slopes of Western Ghats at present which cover the states of Tamilnadu and Kerala in South India. The Department of Social and Behavioural Research (DSBR), NIRT, Chennai has been working in the Arunkundram and Kunnapattu villages under Kanchipuram district, Tamil Nadu as an outreach activity. The main approach behind this is to adopt a holistic and need based one, and focus on the overall development and access to heath care among the tribal groups in collaboration with NGOs and other public as well as private organizations. This programme was initiated in the year 2014 and has been in progress for two years. We conducted a need assessment survey and social mapping in Arunkundram MGR Nagar, Annanagar and Panchathiriti villages.

The needs identified through these activities were addressed and based on these needs regular activities are in progress. The activities include medical health camps, referral of patients to public and private health care, sensitization programmes on TB, provision of nutrition and clothes, flood relief activities, vocational training programmes, assisting in accessing land deeds (*Patta*); and we are currently working on promoting toilets, and renewable energy support (Solar panel) for the tribal community with the help of State Tribal Welfare, Tamil Nadu.

Outreach program in a slum area – Kannagi Nagar

This slum area houses 15500 tenements. We started with a need assessment in this area and found that alcohol was highly prevalent and a major problem of this area. The DTO had concerns that the case detection rate in the Corporation zone that covered this area was very low. A baseline survey on TB was conducted in this area. It was found out that TB awareness among the community was very low and misconceptions on TB were high. A TB sensitization program was organized with a holistic approach that included a short play on alcohol use, which was followed by various activities on TB sensitization such as short film, *Villupaatu* (folklore) and an interactive session on TB. It was also found that the reason for low detection was the long distances the symptomatics from this area had to travel to access healthcare services. The team appraised the DTO of the possibility of setting up a designated microscopy centre (DMC) in a private health facility in this area and the NIRT provided a sputum microscope to the clinic to facilitate the process. This clinic is now a DMC and DOTS centre for the RNTCP.

The activities in this area in terms of ongoing TB sensitization programs using a holistic approach continues among various groups.

Outreach activities among school children

Students have been an emerging channel in TB control, and one of the many activities that have been undertaken includes TB sensitization programmes among school students. This activity was initiated on March

2015. So far, 7200 students have been covered from 25 government/ corporation schools. The Corporation of Chennai has been very supportive at this activity with education officers, principals and teachers involved in this activity. The Social and Behavioural Research team from NIRT have an interactive package for this sensitization programme which includes folklore (*Villupattu*) that conveyed the basic facts about TB, brief talk on the facts and treatment of TB disease and short film on TB. Apart from this, follow-up sessions in all these schools were also conducted to find out the impact of the programme and the response has been encouraging. Families and relatives of these school children have also been sensitized, referrals of symptomatics achieved, and teachers have been made aware about the need to promote this kind of TB advocacy among school children.

LABORATORY STUDIES

Department of Bacteriology

STUDIES COMPLETED:

(i) (B1): 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 – LDCE Funds
Study Period : 2014-16

Background: Luciferase Reporter phages (LRP) are recombinant mycobacteriophages that specifically infect and replicate in viable *M. tuberculosis* (MTB) organisms. While doing so, the cloned luciferase gene gets expressed and catalyses the reaction between substrate luciferin and cellular ATP resulting in emission of light that can be measured as Relative Light Units (RLU) in a luminometer. This principle is used for measuring the viability of MTB that are exposed to drugs in drug susceptibility tests (LRP DST).

Aim: To evaluate DST by luciferase reporter phage assay

Methodology: Four sites, namely NIRT-Chennai, NITRD – New Delhi, BPHRC - Hyderabad and IRL-Chattisgarh participated in the study. One hundred and fifty clinical isolates from the NIRT repository were tested by LRP DST to Isoniazid (H), Rifampicin (R), Kanamycin (K) and Ofloxacin (O) at concentrations – 0.1, 2, 2.5 and 2 μl/ml respectively by two formats i.e., 24 hrs and 72 hours. Briefly, a standard suspension of each clinical isolate at three weeks of growth on LJ medium, equivalent to 1 McFarland standard was prepared in 7H9 liquid medium and 100 μl was added to 400μl of drug free and drug containing medium. Two such sets were prepared. They were incubated at 37^{o}C for 24 hours or 72 hours. To each vial, 50□l of LRP phAETRC202 at a titre of 10^{9}/ml was added and incubated for 4 hours. To 100μl of the cell-phage mix taken in a cuvette, 100ul of D-luciferin (0.33mM in 0.05M sodium citrate at pH4.5) was added and the light emitted was measured at 10 seconds integration as RLU. A reduction of 50% or more seen in the RLU of drug containing vial compared to drug free control indicated susceptibility of the strain to the drug tested.

The clinical isolates were coded independently and tested at the sites.

The results obtained were decoded and compared with the standard DST results of MGIT 960. MGIT DST was performed only at NIRT as per manufacturer's protocol.

Results: The performance characteristics of LRP DST in comparison with MGIT DST for each format and for each drug are given in table 18.

Table 18: Performance characteristics of LRP DST

Isoniazid (0.1 μg/ml)

H – 24 hours	Overall (N= 326)
Sensitivity	78% (60-91)
Specificity	64 % (42-86)
Agreement	72% (51-85)
PPV	75% (50-88)
NPV	67% (51- 82)

H – 72 hours	Overall (N= 358)
Sensitivity	69% (38-87)
Specificity	76% (52-95)
Agreement	73% (46-90)
PPV	78% (44-92)
NPV	66% (46-87)

Rifampicin (2μg/ml)

R – 24 hours	Overall (N= 326)
Sensitivity	75% (64-84)
Specificity	83% (60-100)
Agreement	79% (62-91)
PPV	81% (52-100)
NPV	77% (66-86)

R – 72 Hours	Overall (N=358)
Sensitivity	71% (45-82)
Specificity	85% (60-100)
Agreement	79% (55-90)
PPV	81% (42-100)
NPV	77% (62-85)

Kanamycin (2.5 μg/ml)

K -24 hours	Overall (N= 326)
Sensitivity	51% (42-66)
Specificity	87% (64-100)
Agreement	83% (64-97)
PPV	41% (20-100)
NPV	91% (89-98)

K -72 Hours	Overall (N= 358)
Sensitivity	44% (20-100)
Specificity	88% (68-97)
Agreement	82% (68-97)
PPV	38% (22-76)
NPV	90% (88-100)

Moxifloxacin (0.5μg/ml)

Mx– 24 hours	Overall (N= 326)
Sensitivity	71% (50-100)
Specificity	45% (11-77)
Agreement	51% (24-73)
PPV	22% (17-28)
NPV	87 % (85-100)

Mx– 72 hours	Overall (N= 358)
Sensitivity	54% (46-100)
Specificity	76% (65-94)
Agreement	72% (62-98)
PPV	36% (26-70)
NPV	87% (83-100)

Moxifloxacin (2.0μg/ml)

Mx– 24 hours	Overall (N= 30)
Sensitivity	96%
Specificity	52%
Agreement	61%
PPV	52%
NPV	98%

Mx– 72 hours	Overall (N= 30)
Sensitivity	79%
Specificity	86%
Agreement	85%
PPV	61%
NPV	95%

Conclusions: The performance characteristics of LRP – DST in comparison with MGIT960 was lower than the expected levels. A few key technical and scientific reasons have been identified for future modifications.

(ii) Purity and *in vivo* efficacy studies on Transitmycin: A novel anti-TB and anti-HIV compound

Principal Investigator : Dr. K. R. Uma Devi (email: umadevi.r@nirt.res.in)
Co-PIs : Dr.R. Balagurunathan – Periyar University
Dr. Mukesh Doble - IIT Madras
Dr Vanaja Kumar – Centre for Drug Discovery, Sathyabama University
Dr. Myneedu – NITRD, New Delhi
Source of Funding : ICMR
Study period : 2015- 2016

Background: In the search for novel anti TB drugs, a yellow pigment producing actinomycetes was isolated from sediment samples collected from the coral reef area of Rameswaram in Tamil Nadu. In a preliminary study conducted with a novel *Streptomyces* sp, R2 fraction isolated from coral reef ecosystem containing Transitmycin (Tr), showed activity against drug resistant TB and HIV. However, In the laboratory conditions, the *Streptomyces* sp R2 was able to produce Tr only in agar surface fermentation – a variant of solid state fermentation, with a yield of 200 mg per gram of crude extract (obtained from 1250 ml of YEME agar medium used) with approximately 95% purity. Hence, there was an urgent need for bioprocess development to increase

the yield and purity of Tr by an improved, economically better fermentation approach.

Aim: To purify Tr with >98% purity and to carry out *in vitro* efficacy studies against 30 *M. tuberculosis* isolates

Methodology: Transitmycin from *Streptomyces* sp R2 was produced using YEME agar plates. Meanwhile different fermentation methods such as solid state fermentation on agar and also mixed phase fermentation on liquid medium containing solid support like synthetic resin was also tested to improve the yield of purified compound. Transitmycin was purified to get 98% purity by TLC, column chromatography and HPLC. Twenty strains exhibiting different drug resistant patterns were randomly selected and coded by a statistician from National Institute for Epidemiology, Chennai as advised by the ICMR Expert Committee. Independent codes were given for isolates to be tested by NIRT and Centre for Drug Discovery and Development (CDDD), Sathyabama University (CDDD – SU). Activity of purified Tr against these 30 clinical *M. tuberculosis* isolates was determined by luciferase reporter phage (LRP) assay as well as by conventional broth dilution method at three different Institutes.

Results:

Purification of Transitmycin to obtain > 98% purity:

Primarily, the yellow pigment from actinomycete strain R2 was produced by fermentation on YEME agar through solid-liquid extraction method using ethyl acetate. The presence of Tr was confirmed using TLC analysis followed by column chromatographic purifications. Five batches [four from solid and one from liquid] of crude samples containing Tr were received from Periyar University. The samples were subjected to further purification using HPLC followed by characterization to obtain > 98% purity at IIT, Chennai, from each batch.

Antimicrobial activity of transitmycin:

Transitmycin of 98% purity was obtained and tested against 30 isolates of *M. tuberculosis* at five different concentrations such as 0.5, 1.0, 10, 50 and 100 µg/ml.

Out of the 30 strains tested, results are available for 28 strains from NIRT and for 29 from CDDD-SU. Inhibition of growth leading to a reduction in relative

light unit (RLU) by 50% in the test vial compared to the growth control vial was defined as the cut-off point. Hence, strains showing reduction of RLU equivalent to or more than the cut-off point i.e. 50% are defined as being susceptible to that particular drug at that concentration. All 28 strains including 8 XDR TB (resistant to 7 drugs), were susceptible to Tr at 10µg/ml. One strain that was sensitive to SHRE exhibited 99% inhibition at 50µg/ml of Tr. Among the 7 XDR TB strains, 6 were found to be inhibited by Tr at 1 µg/ml itself at NIRT and the remaining XDR was inhibited at 10 µg/ml.

At CDDD-SU, 20 strains were found to be resistant to Tr at 10µg/ml. At 50µg/ml concentration, all except one drug sensitive strain were sensitive to Tr. All 29 strains were inhibited at 100µg/ml.

The number of strains showing inhibition up to 50% or more is represented in Table 19.

Table 19: Details of strains showing >50% inhibition

	Percentage of strains showing >50% inhibition				
Centre	TR 0.5 µg/ml	TR 1.0 µg/ml	TR 10 µg/ml	TR 50 µg/ml	TR 100 µg/ml
NIRT	46.4	39	96.4	100	100
CDDD-SU	37.9	20.6	62	89.6	100

At NIRT, a total of 96.4% of the strains were found to be inhibited by Tr at 10 µg/ml, whereas at CDDD-SU 89.6 % of the strains were inhibited at 50 µg/ml and all the strains were inhibited at 100 µg/ml at both sides.

Conclusions: (I) It was possible to produce Tr in bulk using liquid medium.

(ii) By adopting a meticulous approach, the natural compound was purified to obtain 98.32% purity.

(iii) Testing about 30 coded TB isolates with the compound of >98% purity yielded encouraging results in both drug resistant and drug sensitive isolates.

STUDIES IN PROGRESS:

B-2: Multi-centric 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	:	2014-16

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^{TM} 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 being validated at the 4 sites 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 370 patients for the study. The study is ongoing and is likely to be completed by December 2016.

B-3: Validation of an indigenous test for diagnosis of DR-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: 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. Recently WHO endorsed nucleic acid amplification tests, such as Line Probe Assay (LPA) (MTBDRplus from Hains Diagnostics) and Xpert MTB/RIF (Cepheid Inc.), system. The assays can detect *M. tuberculosis* as well as drug resistance simultaneously. With the success of these technologies, many indigenous technologists are introducing their methodologies for rapid detection of DR-TB. One such is the TruNat Rif which is an RT PCR based automated platform for detection of RMP resistance. Another indigenously developed, simple, cost effective, culture based assay – Indigenous Gold Standard kit (IGS), capable of detecting MDR and XDR in 7-21 days has been developed at AIIMS, Delhi. These technologies need to be evaluated stringently before implementation in high burden settings.

Aim: To evaluate emerging indigenous technologies for rapid detection of drug resistance of *M. tuberculosis* 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 for each site separately and cumulatively for all sites in comparison with MGIT960.

Progress: 590 patients have been recruited. The study is ongoing at other sites and is likely to be completed by August 2016.

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	:	2014-16

Background: PZA is an important component of TB treatment regimens. 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^{o}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. Whole Genome Sequencing of the strains to identify mutations in the *pnc*A gene is currently being done to resolve discrepancies.

B-5: Determination of cross-resistance among fluoroquinolones in clinical isolates of *M. tuberculosis* from new sputum smear positive cases

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

Background: Fluoroquinolones (Fqs) such as Ofloxacin (Ofx), Gatifloxacin(Gx), Levofloxacin (Lx) and Moxifloxacin (Mx) are drugs used in the treatment of TB. A recent study on drug resistance surveillance among new smear positive (NSP) cases in the state of Tamil Nadu, showed a high level (10.4%) of resistance to Ofx. This has been attributed to the widespread use of Ofx for the treatment of common respiratory infections in the community. Conventionally, drug susceptibility testing to any Fqs in laboratories is done using Ofx, though it has been replaced by Mx 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 Mx 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. Drug susceptibility testing to Ofx and Mx were set up at a concentration of 2μg/ml for Ofx and 0.5μg/ml for Mx as per the manufacturer's protocol. Twelve among 40 Ofx resistant strains were found to be susceptible to Mx (30%). Whole genome sequencing of the strains to identify mutations in *gyr*A/gyrB genes is currently being done to resolve discrepancies.

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

Principal Investigator	:	Dr.S. Sivakumar (email: shanmugamsiva@nirt.res.in)
Source of funding	:	ICMR-Extramural
Study Period	:	2014-17

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. Development of rapid molecular methods, which can be completed within 1 or 2 days, is important for timely detection.

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

(ii) To genotype the XDR strains

(iii) 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) were collected and were subjected to antimicrobial testing with first line, second line and reserve drugs on solid LJ Medium or the commercial liquid medium based system MGIT960. Genomic DNA is extracted as per standard protocol for genotyping using spoligotyping and MIRU-VNTR, amplification and targeted sequencing of genes, namely, *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 done. Additional SNPs/genes involved in resistance will be looked at if the phenotypic and genotypic resistances do not correlate. Sequencing will be done using Illumina MiSeq. Sequence reads for the isolates will be mapped against the corrected reference genome of *M. tuberculosis* strain H37Rv.

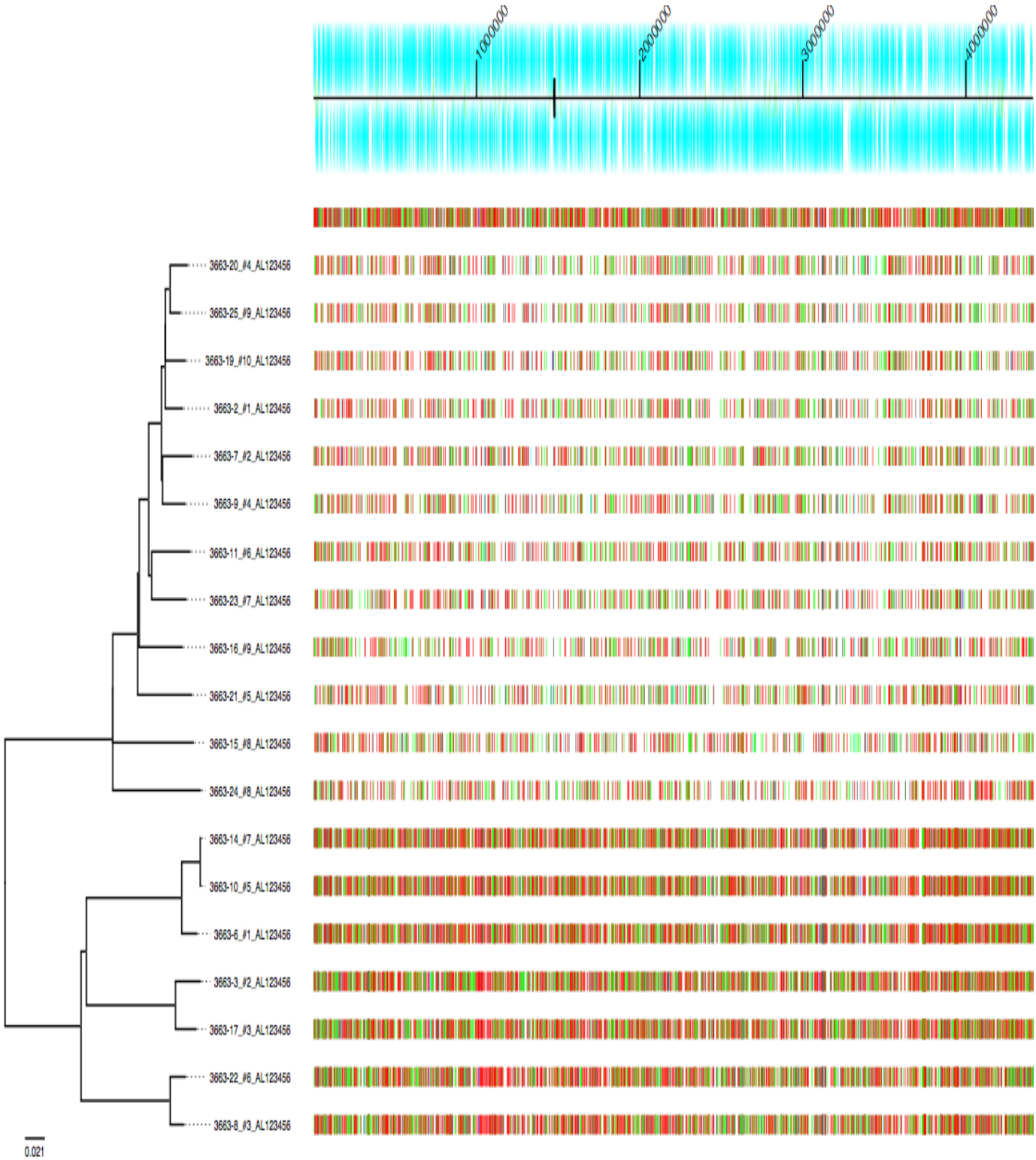
Progress: Spoligotyping has been performed on 19 isolates to determine the hotspots for resistance.

Pyrosequencing for *katG, inhA, ahpC, rrs, gyrA, rpoB* has been completed for 16 XDR TB isolates, 6 MDR-TB isolates and 7 Pan sensitive isolates. Whole genome sequencing has also been performed for 19 XDR/MDR isolates. SNP tree from the whole genome analysis (Fig. 5) of XDR strains show 3 out of 7 ofloxacin resistant isolates (gyrA A90V) are likely to be treatable with MFX.

Among 8 XDR isolates which are phenotypically kanamycin resistant, 5 have mutation (rrs 1401G) that confers cross-resistance to kanamycin, amikacin & capreomycin and the other 3 have mutation (eis -12 C/T promoter) that confer resistance to kanamycin alone and hence offers choice of other aminoglycosides for treatment. One XDR TB isolate harbored a novel mutation in RplC and two isolates had one novel Rrl mutation which is the target for linezolid. The drugs oxazolidinone (AZD5847) and sutezolid (PNU-100480), which are currently in clinical development, are believed to share the same resistance mechanisms. Additional mutations that could be associated with resistance to newer drugs have also been detected. Phenotypic DST is currently standardized to correlate the results for all the reserved drugs including delaminid and bedaquiline.

Conclusion: The study is ongoing but the available data clearly suggests that WGS can be a choice of test in the case of XDR and pre XDR patients to assist in individualized treatment regimen after ruling in the known high confidence resistance mutation.

Fig. 5: Phylogenetic tree for 19 samples and their genomic SNP data. Colours signify the minority type of SNPs (Green non-synonymous SNPs and Red synonymous SNPs).



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 Dusthackeer;
Dr. K. R. Uma Devi (email: azger@nirt.res.in; umadevi.r@ nirt.res.in)

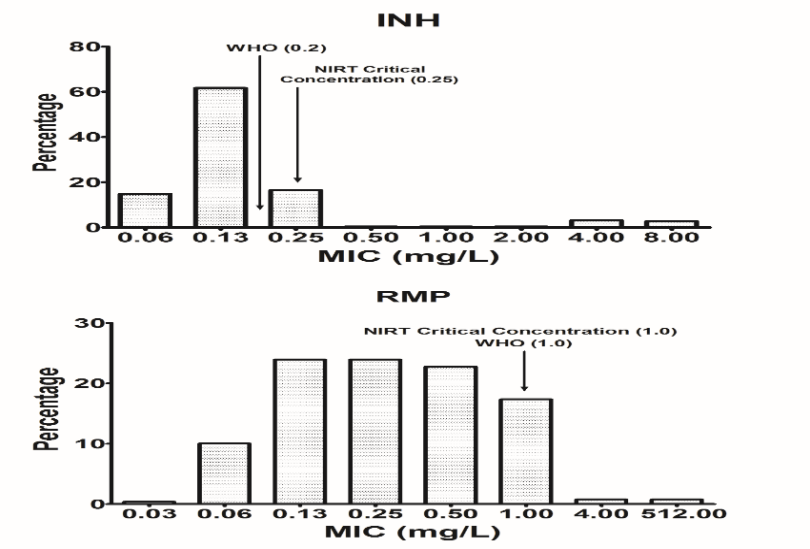
Source of funding : ICMR Intramural
Study Period : 2014-2016

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 pharma-codynamic profiles. The validation of the critical concentrations based on the wild type minimum inhibitory concentration (MIC) distribution and hence of epidemiological cut-off (ECOFF) becomes imperative in every population and might not be the same in every geographical setting. Hence in the present work we attempted to determine the critical concentration level for INH and RMP using the PK/PD data 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 in order to determining susceptibility of the south Indian isolates of *M. tuberculosis*

Current status: The study included 284 clinical isolates of MTB obtained from patients prior to start of treatment. DST by MIC on solid LJ medium was completed for 264 isolates. The observed MIC for INH was 0.2µg/ml and for RMP was 1µg/ml (Fig. 6). The observed MIC in this study for both INH and RMP are identical to the recent updated critical concentration for these drugs by WHO. http://www.stoptb.org/wg/gli/assets/documents/Updated%20critical%20concentration%20table_1st%20and%202nd%20line%20drugs.pdf – as accessed on the Web on 05/08/2016 on solid LJ medium. Currently, determination of MIC using MGIT960 is being carried out.

Fig. 6: Wildtype MIC distribuition in *M. tuberculosis* strains for INH & RMP



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 Dusthackeer (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 *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.

Aims: (i) To investigate the potential of RPF proteins both from the culture filtrate (CF) supernatant of *M. tuberculosis* H37Rv and commercial RPF recombinant proteins to enhance the sensitivity of detection limit of mycobacteria in the sputum samples from patients at various stages of active PTB

(ii) To reduce the time to detection among smear positive-culture positive sputum samples by treating the samples with RPF

Current Status:

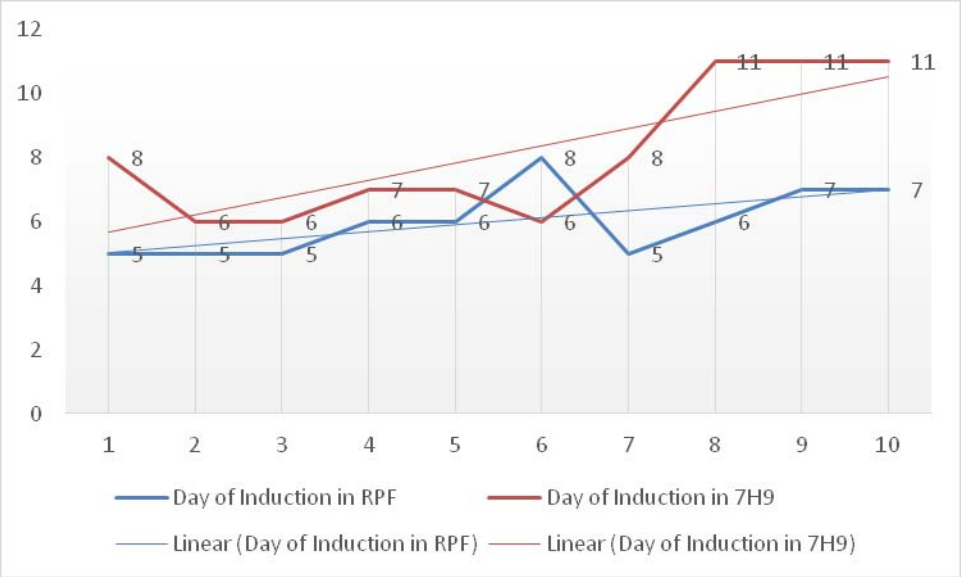
Sputum treatment with RPF: A total of 285 sputum samples were included in the study, of which 268 were smear and culture negative. Among the 268 smear and culture negative specimens, presence of non-culturable cells (NC) by conventional method was observed in 101 specimens. Growth induction was noticed based on increase in the optical density (OD) in liquid culture and additional colonies on 7H11 agar presumably due to growth of resuscitated cells (RC).

Significant increase in OD was seen in 86 cultures that were exposed to CF and 77 of 7H9 (plain growth medium) exposed cultures. In those cultures that had an increased optical density, smear examination was also performed. Presence of AFB was confirmed in all these cultures. The results were compared with their original smear and culture results. Among the CF exposed specimens, 22 resulted in additional colonies due to RCs in comparison with the un-exposed specimen that showed no growth on LJ, while only 5 of the 7H9 exposed cultures resulted in growth of additional colonies due to RCs.

Time to detection: Smear and culture positive specimens were used to measure the time to detection. In general, CF exposed cultures exhibited growth one to four days earlier in comparison with those exposed to liquid medium only (Fig. 7). However, one of the CF exposed specimens and 15 of the 7H9 exposed specimens respectively did not result in RCs.

Further experiments are in progress.

Fig. 7: Role of CF in time to detection (X-axis: specimen and Y-axis: Time in days)



B-9: Accelerating access to quality TB diagnosis for pediatric cases in 9 major cities in India

Site Principal Investigator	:	Dr.K.R. Uma Devi (email: umadevi.r@nirt.res.in)
PI & Collaborators	:	Dr. Neeraj Raizada, FIND, Delhi, Hyderabad, Kolkatta
Source of funding	:	USAID/FIND
Study Period	:	2014-2016

Xpert MTB/RIF is a cartridge based automated nucleic acid amplification test (CBNAAT) for TB and RMP resistant-TB case detection. Since, 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, WHO released a global guidance document 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 can be used for detection of mycobacteria in pediatric specimen

such as gastric lavage, BAL, induced sputum, lymph node aspirates, etc.

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 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. This project offers diagnostic solution with no cost to patient or provider both in private and public sector. Any pediatrician both in public and private sector send the specimens for testing to the lab. The tests are performed on the same day and the results communicated to a referring provider electronically and at the same time notified to RNTCP under Nikshay.

From the time of inception of the project, 7199 pediatric suspects have been screened by this facility. So far, 169 referral sites have been engaged, including referrals from 74 private sectors and 95 public sectors. A total of 4560 samples have been screened in this academic year which included both pulmonary and extra-pulmonary samples. Among the extra-pulmonary cases, Xpert MTB/RIF diagnosed 6% as MTB positives and 3% as positives among pulmonary cases as shown in Table 20:

Table 20: Details of samples received and the percentage positivity

Sl.No	Data	Samples received from March 2015 - April 2016	Samples received from 2014 - April 2016
1	Total no. of Samples received	4560	7199
2	No. of Pulmonary samples received	1882	3610
3	Positivity among pulmonary specimens	58 (3%)	98 (2.7%)
4	Total No. Extrapulmonary samples received	2678	3580
5	Positivity among extrapulmonary samples	162(6 %)	244(6.8%)

Dept. of Biochemistry & Clinical Pharmacology

COMPLETED STUDIES:

(i) *NAT2* gene polymorphisms and plasma INH concentrations in TB patients in south India

Principal Investigators	:	Dr. Geetha Ramachandran; Dr.A.K. Hemanth Kumar (email:geethar@nirt.res.in; hemanthkumarak@nirt.res.in)
Source of funding	:	USAID through WHO-SEARO (Model DOTS project)
Study period	:	2013-2015

Introduction: INH continues to be the most widely used chemotherapeutic agent for the treatment of TB. The primary step in the metabolism of INH is acetylation, catalysed by the enzyme, N-acetyl transferase (NAT), resulting in the formation of acetyl INH. NAT2 gene displays genetic polymorphism, and its activity is expressed at highly variable levels. Several studies have shown that human subjects show a wide degree of variation in their capacity to acetylate INH to acetyl INH in spite of receiving similar prescribed INH doses. Individuals can be distinctly characterised phenotypically as being either slow or rapid acetylators (the concentration of the enzyme being higher in rapid acetylators). Genotyping of the *NAT2* gene using molecular techniques permit identification of three genotypes, namely, rapid, intermediate and slow.

The *NAT2* gene frequency for slow allele differs widely across different racial and ethnic groups. High variation in *NAT2* gene frequencies from different regions in India have been reported.

Aim: To genotype a cohort of TB patients from Chennai, Tamil Nadu for *NAT2* gene polymorphisms and to compare plasma INH concentrations among the different genotypes

Methods: A prospective cohort study design was followed in which adult patients with either pulmonary or extra pulmonary TB receiving anti-TB treatment (ATT) in the RNTCP treatment centres in Chennai Corporation were recruited. Patients received either

category I or category II treatment. Eligible patients were administered anti-TB drugs under direct supervision and about 3ml blood was collected at two hrs post-dosing. The blood was distributed into heparinised and EDTA vacutainer tubes, the former used for INH estimation and the latter for extraction of DNA and genotyping experiment.

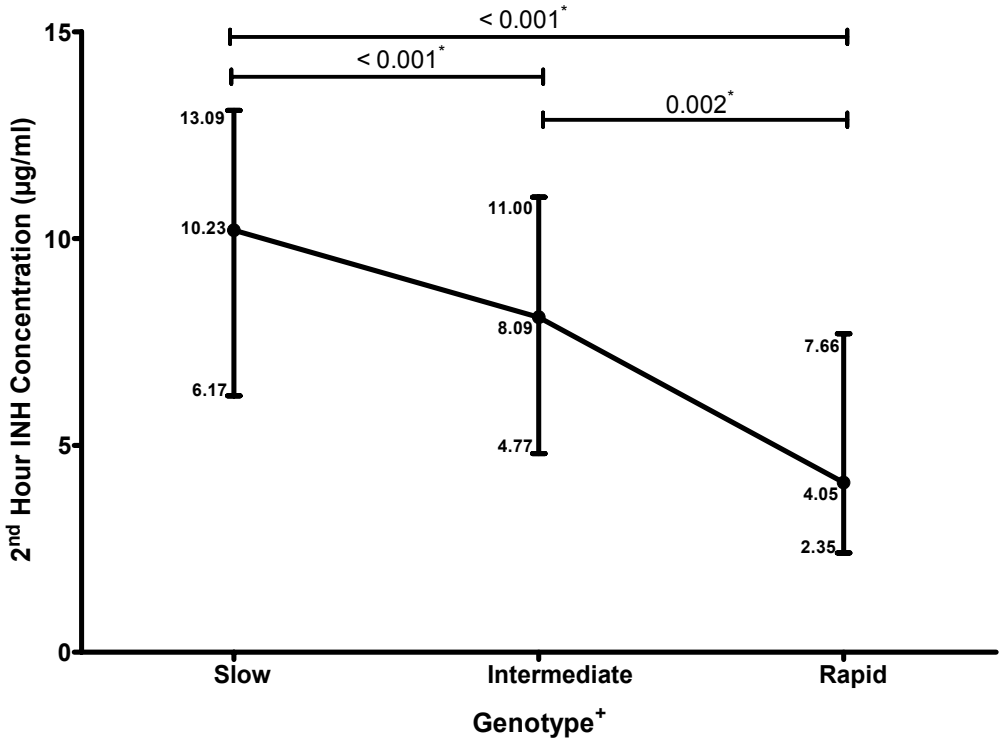
Plasma INH was estimated by HPLC according to a validated method.

NAT2 genotyping was performed by determining six single nucleotide polymorphisms (SNP) (rs1041983, rs1801280, rs1799929, rs1799930, rs1208 and rs1799931) in the *NAT2* gene using Taqman SNP genotyping assays and analysed using Sequence Detection Software. The slow, intermediate and rapid *NAT2* acetylator phenotypes were determined using NAT2PRED Web server. This software allows use of the six polymorphisms in *NAT2* to eventually determine the acetylator phenotype.

Results: A total of 326 patients took part in the study. The number of slow, intermediate and fast acetylators were respectively 189 (58%), 114 (35%) and 23 (7%). The distribution followed Hardy-Weinberg equilibrium. The median 2-hr INH concentrations in slow, intermediate and fast acetylators were 10.2, 8.1 and 4.1µg/ml respectively. The differences in INH concentrations among the three genotypes were highly significant ($p < 0.001$). There existed a significant trend in the INH concentrations among the genotypes; the slow acetylators had the highest concentration, followed by the intermediate acetylators and fast acetylators had the lowest INH concentration (Fig. 8).

Conclusions: We have determined *NAT2* genotype of TB patients in Chennai for the first time. Slow acetylators comprised 58% of the study population. Two-hr INH concentrations differed significantly among the three genotypes.

Fig. 8: Median 2-hour INH concentrations in the different genotypes. The vertical bars denote Inter-quartile range



* Mann whitney test (adjust the p value with the Bonferroni method) was performed at 5% level of significance

+ Kruskal whitney test was performed at 5% level of significance ($p < 0.001$)

(ii) (BCP-2) Pharmacokinetics of thrice weekly RMP, INH and PZA in adult TB patients in India

Principal Investigators	:	Dr. Geetha Ramachandran; Dr.A.K. Hemanth Kumar (email:geethar@nirt.res.in/ hemanthkumarak@nirt.res.in)
Source of funding	:	USAID through WHO-SEARO (Model DOTS project)
Study period	:	2013-2015

Introduction: A majority of persons with uncomplicated TB are treated with a 6-month intermittent regimen in the RNTCP in India. Yet treatment failures, relapses and development of multi-drug resistance strains of *M. tuberculosis* occur and continue to threaten TB control programmes. Although favourable treatment outcomes are achievable in a high proportion of patients, low drug levels may be critical where there is variable drug quality, different disease presentations, malnutrition, HIV co-infection, severe illness and other co-morbidities. While several potential determinants of drug concentration variability are recognised, they are poorly characterised in TB patient populations.

Aim: To study the pharmacokinetics (PK) of RMP, INH & PZA in adult TB patients treated with thrice-weekly regimens in Chennai

Methods: A prospective PK Study was conducted among 101 adult patients with both pulmonary and extra-pulmonary forms of TB. The patients were diagnosed and treated for TB with either Category I or II treatment regimens at the RNTCP centres in Chennai Corporation, India. The PK study was conducted during the first month of ATT, after patients had received a minimum of two weeks of treatment. The anti-TB drugs were administered under fasting conditions and drug administration were observed by an investigator. Blood samples were obtained before and at 2, 4, 6 & 8 hrs after drug ingestion. Estimation of RMP, INH & PZA was undertaken within a week of blood collection according to previously validated and published methods. Non-compartmental analysis with WinNonlin version 6.4 (Certara) was used to compute the peak drug concentration (C_{max}), time to C_{max} (T_{max}) and the area under the curve (AUC_{0-8} & $AUC_{0-\infty}$) and half-life ($t_{1/2}$).

Genotyping of the *NAT2* gene was performed in a sub-group of 88 patients.

Results: HIV co-infected patients constituted 2% of the study cohort, while 22.8% were having diabetes mellitus. Slow acetylators for the *NAT2* gene constituted 64.3% of this cohort. The PK parameters of RMP, INH & PZA are shown in Table 21. The number (%) of patients with RMP C_{max} < 8.0 µg/ml was 89 (88.1%). The corresponding numbers for C_{max} of INH (< 3.0 µg/ml) and PZA (< 20.0 µg/ml) were 1 (1%) and none

respectively. The proportion of patients with measured C_{max} of RMP, INH & PZA at 2 hrs were 83.2%, 97.0% and 92.1% respectively.

The C_{max} and AUC_{0-8} of RMP and PZA was significantly higher in female than male patients. Patients above 60 years had higher C_{max} and AUC_{0-8} of RMP than those aged below 60 years, the difference being significant for AUC_{0-8}. Patients with BMI <18.5 kilogram (kg)/ meter $(m)^2$ had significantly higher C_{max} and AUC_{0-8} of INH and PZA. Smokers had lower PZA C_{max} and AUC_{0-8} than non-smokers. Although C_{max} and AUC_{0-8} of INH and PZA was lower in those with diabetes and TB than those with only TB, the differences were statistically significant for PZA only. There existed significant differences in the C_{max} and AUC_{0-8} of INH among the slow, intermediate and rapid genotypes. Using AUC_{0-8} as the dependent variable, sex (INH & PZA), smoking (PZA), type of TB (RMP), mg/kg dose (INH & PZA), category of ATT (RMP) and *NAT2* slow genotype (INH) stood significant.

Conclusions: Plasma concentrations of RMP, INH and PZA at 2-hr post dosing mimic C_{max}. A high proportion of TB patients in this cohort had RMP C_{max} below the expected range which is a matter of concern. Sub-therapeutic drug levels are likely to pose a problem in a subset of patients.

Table 21: PK parameters of RMP, INH and PZA (n = 101)

RMP	INH	PZA
5.0 (3.8 - 6.9)	11.3 (8.2 - 13.2)	40.2 (34.2 - 43.7)
2 (2 - 2)	2 (2 - 2)	2 (2 - 2)
27.9 (20.1 - 33.9)	41.1 (33.0 - 59.9)	228.0 (194.5 - 252.0)
45.0 (29.8 - 68.0)	53.4 (36.9 – 81.8)	544.8 (407.6 – 712.3)
4.7 (3.5 - 7.4)	3.1 (1.9 - 3.8)	8.6 (7.2 - 11.4)
C_{max} Sub-Therapeutic (n)**		C_{max} Sub-Therapeutic (n)**
89 (88.1%)	1 (1.0%)	0 (0%)
12 (11.9%)	100 (99.0%)	101 (100%)

*Median (IQR); **n (%)
RMP – Rifampicin; INH – Isoniazid; PZA – Pyrazinamide

<denotes Sub-therapeutic Cut-off (< 8.0µg/ml for RMP, <3.0µg/ml for INH and <20.0µg/ml for PZA) C_{max} – Peak Concentration; T_{max} – Time at which peak concentration attained; AUC – Area Under the time concentration curve; $t_{1/2}$ – Half-life

(iii) Anti-TB drug concentrations in TB patients with and without diabetes mellitus

Principal Investigators	:	Dr. Geetha Ramachandran; Dr.A.K. Hemanth Kumar (email:geethar@nirt.res.in; hemanthkumarak@nirt.res.in)
Source of funding	:	USAID through WHO-SEARO (Model DOTS project)
Study period	:	2013-2015

Introduction: There is growing evidence that diabetes mellitus (DM) is an important risk factor for TB and could affect disease presentation and treatment response. Furthermore, TB might induce gluose intolerance and worsen glycaemic control in people with DM. In a setting of the increasing overlap of populations at risk of both diseases, the combination of TB and DM poses a health threat. Few studies have observed lower concentrations of RMP and INH in patients with TB + DM than those with TB.

Aim: To compare two-hr post-dosing plasma concentrations of RMP, INH and PZA between TB patients with and without DM being treated with thrice weekly anti-TB regimens in India

Methods: A prospective study was undertaken in adult patients with either pulmonary or extra pulmonary TB receiving ATT in the (RNTCP) treatment centres in Chennai Corporation. Diagnosis and treatment with either category I or category II ATT was according to RNTCP guidelines. Any patient with a known history of DM, with or without random blood glucose $\geq$ 200mg/dl on the study day was considered diabetic.

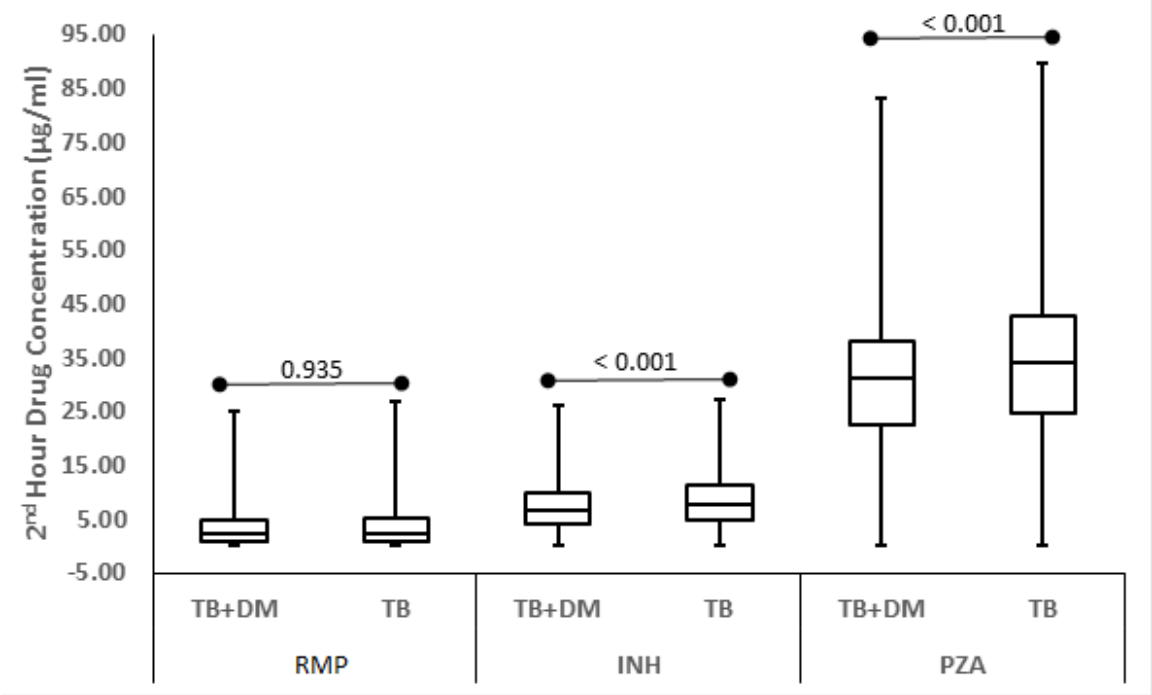
Eligible patients were administered anti-TB drugs under direct supervision and blood was collected at two hours post-dosing. Plasma concentrations of RMP, INH and PZA were estimated by HPLC according to validated methods.

Results: A total of 1912 patients were recruited to the study, of whom 452 patients (24%) had DM. The median (IQR) plasma RMP, INH and PZA concentrations in TB patients with and without DM were 2.3 (0.6 – 5.0) & 2.3 (0.7 – 4.9), 6.6 (3.9 – 10.0) & 7.8 (4.6 – 11.3) and 31.0 (22.3 – 38.0) & 34.1 (24.6 – 42.7) µg/ml respectively. Patients with TB + DM had significantly lower plasma INH and PZA concentrations than those with TB ($p < 0.001$ for both drugs) (Fig. 9).

There existed a significant negative correlation between blood glucose and plasma INH ($r = -0.09$; $p < 0.001$) and PZA ($r = -0.092$; $p < 0.001$). The proportion of patients with 2-hr RMP (<8µg/ml) INH (<3µg/ml) and PZA (<20µg/ml) below normal in TB patients with and without DM were 92% & 91%, 17% & 16% and 19% & 17% respectively; none of the differences were statistically significant.

Conclusions: Two-hour plasma INH and PZA concentrations were lower in patients with TB + DM than those with TB; however, this was unlikely to be clinically significant. A negative correlation between blood glucose and drug concentrations is suggestive of delayed absorption or faster elimination of INH and PZA in the presence of glucose.

Fig. 9: Two-hr post-dosing drug concentrations in TB patients with and without DM



The central line of the box plot denotes median and the lower and upper lines denote 25 and 75 percentiles. The vertical bars denote minimum and maximum values.
RMP – rifampicin; INH – isoniazid; PZA - pyrazinamide

(iv) Variability in plasma RMP and INH in TB patients

Principal Investigators	:	Dr. Geetha Ramachandran; Dr.A.K. Hemanth Kumar (email:geethar@nirt.res.in; hemanthkumarak@nirt.res.in)
Source of funding	:	USAID through WHO-SEARO (Model DOTS project)
Study period	:	2013-2015

Introduction: Low serum concentrations of anti-TB drugs have been associated with poor treatment outcomes. Limited information is available on drug levels prevailing in patients during the course of ATT.

Aim: To study intra-patient variability in plasma RMP and INH concentrations during ATT, that is, at start of treatment, at end of intensive phase (IP) of ATT and at end of ATT in adult TB patients being treated in the RNTCP

Methods: Adult TB patients receiving thrice-weekly ATT in the RNTCP in Chennai Corporation were studied. Two-hr post-dosing concentrations of RMP

and INH were determined during month 1, at end of IP and end of ATT, after directly observed drug administration. Plasma concentrations of RMP and INH were estimated by high performance liquid chromatography. The time at which the patients took their breakfast was noted on each occasion.

Results: A total of 485 patients took part in the study, of whom drug concentrations were available in 357 and 348 patients at end of IP and end of ATT respectively. About 97% of the study subjects took their anti-TB medications after breakfast; 90% of them took the drugs within one hr of taking breakfast. The median (IQR) RMP concentrations during first month, at end of IP and end of ATT were 2.1 (0.4 – 5.0), 2.4 (0.6 – 5.5) and 2.2 (0.5 – 5.3) µg/ml respectively. The corresponding INH concentrations were 7.1 (4.2 – 9.9), 7.2 (3.9 – 10.9) and 6.7 (3.9 – 9.5) µg/ml. Comparison of plasma RMP and INH concentrations between month 1, end of IP and end of ATT in the same patient were carried out using ANOVA; differences were not statistically significant over the time period (Table 22). However, there were significant differences in RMP and INH concentrations between patients ($p<0.001$). There existed a significant correlation between RMP concentrations obtained at different time points, the r values being 0.29, 0.26 and 0.24 for month one vs end of IP, month one vs end of ATT and end of IP vs end of ATT respectively ($p < 0.001$ in all three cases). The corresponding r values in the case of INH were 0.15, 0.19 and 0.23 respectively ($p < 0.001$ in all three cases).

Conclusions: Plasma RMP and INH concentrations in the same patient were not significantly altered during the course of ATT. Hence drug estimations at any convenient point of time during the course of ATT can be carried out.

Table 22: Drug concentration at different time points during ATT (Values are reported as Median & IQR)

Period of ATT		RMP	INH
Month-1	N ( )	484 2.1 (0.4 – 5)	484 7.1 (4.2 – 9.9)
End of IP	N	357	357

	( )	2.4 (0.6 – 5.5)	7.2 (3.9 – 10.9)
End of ATT	N ( )	348 2.2 (0.5 – 5.3)	352 6.7 (3.9 – 9.5)
Significance		ns	Ns

ns – non-significant at 5% level by ANOVA
IP – intensive phase; ATT – anti-TB treatment

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	:	2013-2016

Background: The fluoroquinolone group of drugs has been demonstrated to have significant therapeutic potential in the management of TB. Among the newer generation of fluoroquinolones, MFX is a drug with promising antimycobacterial activity and 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 is a sub-study in an ongoing open-label randomized controlled trial, in which regimens of 3 and 4 months duration using MFX along with first-line drugs are compared with the standard 6-month regimen for efficacy and safety. Newly diagnosed adult, HIV negative, non-diabetic sputum positive PTB patients are being recruited. 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. As on 31st March 2016, 285 patients have been recruited from Chennai and Madurai; of them, 198 have completed treatment. Further recruitment of patients to the study is in progress.

BCP-3: Dose related pharmacokinetics of rifabutin during concomitant ritonavir administration in HIV - infected TB patients: a multicentric study

Principal Investigators	:	Dr. Geetha Ramachandran (email:geethar@nirt.res.in)
Source of funding	:	NACO
Study period	:	2016-2017

Background: TB is the most common opportunistic infection and is an entry point for a significant proportion of HIV-infected patients who are eligible for ART. It is therefore a common scenario that HIV-infected patients eligible for ART also have concomitant TB and require treatment for both infections. Current recommendations are to treat patients with HIV & TB with a regimen including a rifamycin for the full course of ATT. The rifamycin class of compounds is known to be potent inducers of the hepatic CYP450 enzyme system, which is responsible for the metabolism of several drugs. The rifamycins vary in their potential as CYP450 inducers, with RMP being most potent, rifapentine intermediate, and rifabutin (RBT) being much less active. RMP markedly lowers the serum levels of PIs and NNRTIs by inducing the activity of cytochrome P450 CYP3A. It may result in suboptimal antiretroviral activity and, therefore, subsequent acquired drug resistance. RBT has been shown to be as effective against TB as RMP, and has little, if any, effect on the serum concentrations of protease-inhibitors, which are meta-bolized through the CYP3A system. The combination of RBT (if available) with PI-based ART is the preferred form of therapy for patients unable to take NNRTI-based ART. However, ritonavir (RTV), being a CYP3A4 inhibitor markedly increases serum concentrations and toxicity of RBT. The dose of RBT during RTV co-administration remains a matter of debate.

Aim: To study the pharmacokinetics of RBT at two dosing levels (150 mg daily & 300 mg thrice weekly) during concomitant LPV/RTV administration in HIV-infected TB patients

Methods: This has been planned as a prospective observational pharmaco-kinetic study at eight sites in India (four each for 150 mg daily & 300 mg thrice weekly). The pharmacokinetic study of RBT 300mg thrice weekly dose has been initiated. The sites selected for this dose are Government Hospital for Thoracic Medicine, Chennai, School of Tropical Medicine, Kolkata, BJ Medical College & Hospital, Pune and Government Rajaji Hospital, Madurai. The study is conducted in adult patients with HIV and TB being treated for minimum two weeks with standard ATT with RBT-containing regimens and second-line antiretroviral regimens containing RTV. On the study day, the patient will be administered the appropriate antiretroviral/anti-TB drugs under supervision. Serial blood samples at pre-dosing, and at 1, 2, 4, 6, 8, 12 and 24 hrs after drug administration are collected. Plasma RBT is estimated by HPLC according to validated methods.

The estimated sample size from each site is nine patients. As on 31st March, 2016, we have completed the study in 6 patients in Chennai and 8 patients in Kolkata. Further recruitment of patients to the study is in progress.

BCP-4: Pharmacokinetics of second-line anti-TB drugs in MDR-TB patients

Principal Investigators	:	Dr.A.K. Hemanth Kumar (email:hemanthkumarak@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2016-2019

Introduction: MDR-TB has become a significant public health problem in a number of countries and an obstacle to effective TB control. In the RNTCP in India, MDR-TB patients are treated with six drugs including an aminoglycoside and a fluoroquinolone for a period of 24 months. The intensive phase of treatment is for six months with kanamycin, levofloxacin, ethionamide, cyloserine, PZA and EMB daily and the continuation phase of treatment is for 18

months with levofloxacin, ethionamide, cycloserine and EMB daily. Limited information is available on the pharmacokinetics of the drugs used to treat MDR-TB patients and factors that are likely to impact drug levels.

Aims: To study the pharmacokinetics of anti-TB drugs in MDR-TB patients who are receiving treatment with the DOTS plus regimen in the RNTCP in India and to relate drug levels with time to culture conversion

Methods: This is a prospective, cohort study conducted in adult MDR-TB patients treated at the Govt. Hospital of Thoracic Medicine, Tambaram, Chennai. Patients who are found suitable for the study will be explained about the study procedures and informed written consent will be obtained before commencement of the study. On the study day, serial blood samples are collected at pre-dosing and at 1, 2, 4, 6, 8 and 12 hrs after supervised drug administration. Plasma drug concentrations are estimated according to validated methods by HPLC. Based on the plasma concentration of drugs obtained at different time points, certain pharmacokinetic variables will be calculated.

Bacteriological tests: Sputum samples will be collected from all the patients at baseline and every month upto six months of start of treatment. Baseline samples will be subjected to culture by solid media and DST by MGIT. Subsequent samples will be subjected to culture only.

Sample size: The sample size has been calculated as 471 patients who will be recruited over a period of three years.

As on 31st March, 2016, we have recruited 41 patients. The study is in progress.

DEPT. OF HIV

COMPLETED STUDIES:

(i) (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 : Intramural
Study period : 2012-2016

Background: A major focus in HIV vaccine research is to develop a vaccine that can elicit highly potent broadly neutralizing antibodies (bNAbs) such as those seen in the HIV-infected individuals, in order to prevent HIV infection. Several research groups in the world have identified and characterized a number of bNAbs with great breadth and potency, but the subtype C HIV-1 strains are reported to be largely resistant to majority of these antibodies. Hence there is a need to identify bNAbs of known or novel specificities capable of strongly neutralizing subtype C HIV-1 isolates.

Objectives: (i) To screen sera of HIV-infected individuals in the early stage of HIV infection for the presence of broadly neutralizing antibodies (bNAbs)

(ii) To characterize the epitope specificities of NAbs specificities in the different regions of HIV-1 gp120 and gp41

Methodology: A total of 101 HIV-1 infected subjects attending the ART Centre at the Kilpauk Medical College and Hospital, Chennai, were recruited for the study. All study subjects were asymptomatic, naïve to ART and were within 3 years of diagnosis of HIV infection at the time of sample collection. Plasma obtained from these individuals were screened for the presence of bNAbs with a panel of 18 pseudoviruses belonging to different subtypes and classified under tiers 1, 2 and 3, using the standard neutralization assay. The neutralization potency of samples exhibiting broad cross-clade neutralization was explored by determining plasma dilution that neutralized 50% of the infectious viruses (ID50 values). Further, the epitope

specificities of the bNAbs in broadly cross-clade neutralizing sera were characterized by performing ELISA with linear peptides as well as whole proteins and conformational epitopes.

Results: We iidentified 12 samples with broad cross-clade neutralization (BCN) activity with pseudoviruses belonging to all 3 tiers. Ten of the 12 samples also demonstrated good potency for neutralization (ID50>100) with tier 3 pseudoviruses (Table 23). Four BCN samples had CD4- binding site antibodies, 4 samples had N160 and N332 glycan-dependant antibodies, and 4 samples had MPER binding antibodies. Interestingly, 8 samples had antibodies that recognized more than one vulnerable site on the HIV-1 envelope.

Conclusions: This is the first study to identify second-generation broadly cross-clade neutralizing antibodies belonging to the PG9/PG16 and PGT series of antiboies in HIV-1 infected individuals from south India. Further, we also showed that a significant number of HIV-1 subtype C infected individiuals contain neutralizing antibodies targeting multiple vulnerable sites on the envelope of HIV-1.

Table 23: **Neutralization titer of broadly cross neutralizing samples with tier 3 pseudoviruses**

Sample ID	Indian HIV-1 subtype C plasma & tier-3 pseudo viruses							
	Subtype-B viruses		Subtype CRF02_AG viruses					GMT
	PVO-4	TRJO4551.58	33-7	251-18	253-11	257-31	278-50	
NAB001	2731	6362	1430	491.7	876.8	115	93.25	734
NAB016	9457	1065	941.5	120	116.2	76.15	138.7	391
NAB033	417.1	363.7	506.3	220.8	64.07	149.3	275.1	239
NAB046	69.6	2348	840.5	62.4	50.08	120	144.3	185
NAB059	11016	2062	8225	427.5	600	49.48	1271	1170
NAB062	156.7	3029	929.3	49.73	1146	53.14	50.69	253
NAB063	14915	927.2	365.8	175.1	718	165.3	1174	741
NAB065	855	268.1	361.6	296.8	136.6	75.25	100	220
NAB069	5760	251	360	193.8	3060	591.4	402	688
NAB120	47.04	114.1	540	262.3	1902	540	714.2	343
GMT	1162	873	804	184	408	132	254	

(GMT refers to geometric mean titer)

(ii) Variability in V1V2 and PNGs in pediatric HIV-1 viral variant transmitted through the vertical route

Principal Investigator : Dr. Luke Elizabeth Hanna
(email: hanna@nirt.res.in)

Source of funding : Intramural
Study period : 2015-2016

Background: Mother-to-child transmission of HIV represents a good model to study the dynamics of viral envelope diversity in vertically transmitted HIV variants. Characteristic features in the fraternal twin, V1V2 and its kindred, Potential N-linked glycosylation sites (PNGs) have been reported to correlate with mucosal transmission of majority.

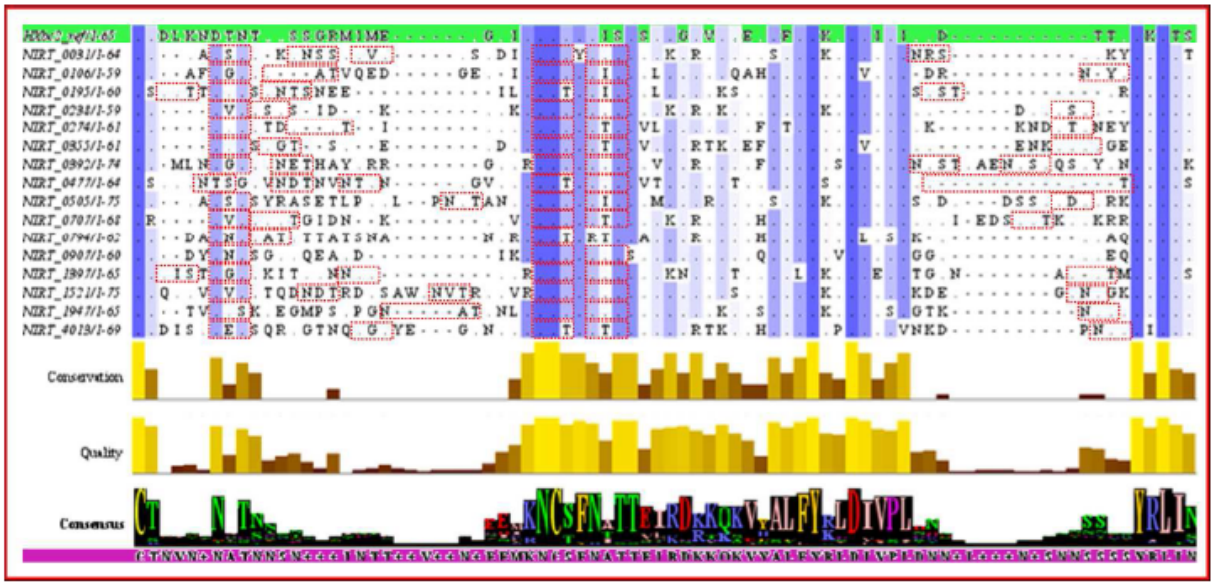
Aim: To analyze the characteristics of the envelope gene in HIV-1 strains transmitted via the vertical route from the mother to the child

Methodology: Sixteen full length HIV-1 envelope genes were amplified from proviral DNA of infants (<2 years) who acquired infection from the mother via the vertical route. Amplicons were sequenced using the 3100 Avant Genetic Analyzer (Applied Biosystems). The envelope sequences were analysed to chatracterize the viral variant at the sequence level.

Results: Through keen analysis, we identified unique features of glycosylation, difference in length of V1V2 region and net charge of the V2 and V3 regions that characterized the unique quasispecies that is responsible for the establishment and spread of HIV infection in the early stages of infection in vertically transmission (Fig.10).

Conclusions: Our findings indicate that vertically transmitted viral variants are unique with respect to the characteristics of their variable regions and glycosylation pattern.

<u>Fig. 10:</u> Representation of distinct features of V1V2 and PNGs in HIV-1 subtype C isolates obtained from children recently infected via the vertical route



Potential N-linked glycosylation sites are boxed in red. The shadow with blue color indicates consensus sequences; the high intensity of color denotes more conservation and less intensity denotes less conservation. Reference sequence, HXB2 and the consensus sequence are shown at the top and bottom of the image and are highlighted in green and violet color respectively. The (.) indicates identity to the consensus sequence and (+/-) indicates insertion/deletion at particular position.

(iii) Identification of potent intertype neutralizing antibodies in a HIV type2 infected individual from India

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2013-2016

Background: HIV-2, a closely related virus to HIV-1, is characterized by slower disease progression and transmission, longer latency period and low or undetectable viremia. The pathogenic difference between the two viruses has always been an intriguing topic of research.

Aim: To understand the contribution of host immunity to the delayed pathogenesis of HIV-2 infection, by screening plasma of HIV-2 infected individuals for the presence of bNAbs capable of intra and intertype cross-clade neutralization

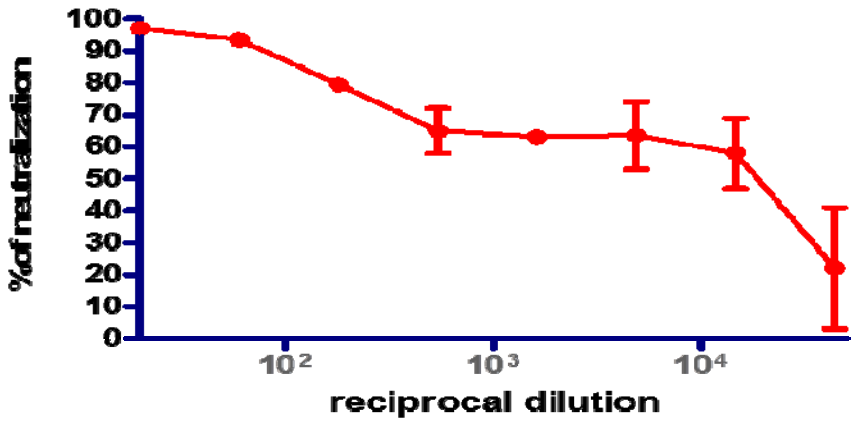
Methodology: Blood was collected from 25 asymptomatic, ART-naïve, HIV-2 infected individuals. Plasma obtained from these individuals was screened for the presence bNAb using the standard neutralization assay with a panel of HIV-2 and HIV-1 pseudoviruses. Samples capable of broad neutralization were subjected to PepScan analysis to elucidate the common epitopes responsible for the neutralization response in the envelope of the viruses. Statistical analyses were performed using GraphPad prism version 5.0.

Results: Only one of the 25 samples showed broad cross-clade and cross-type neutralization with HIV-1 and 2 pseudoviruses. The breadth of neutralization was 94.4% (17/18) (Table 24). The ID50 of the serum sample with a representative tier 3 pseudovirus was 1:4860, indicating that the antibodies in the serum were highly potent (Fig.11).

Table 24: Percentage of neutralization of HIV-2 plasma (68305) against HIV-1 viruses

	HIV-1 Isolate	% of Neutralization
Clade C	ZM197	83
	ZM109	96
	DU156	88
	CAP210	96
	16936	96
	PINDIE	20
	GS015	95
Clade B	SF162	93
	6535	76
	TRO11	93
	PVO.4	92
	TRJO.4	90
Clade A	33.7	95
	278	90
	280	94
Clade AG	251	70
	253	97
	242	89

Fig. 11: ID50 of a representative plasma sample



The ID50 of the serum sample with a representative tier 3 pseudo virus Pvo4

Pepscan analysis was performed on this sample with the complete set of 192 linear peptides (15mers with 11 amino acid overlap) corresponding to the complete sequence of consensus subtype C gp140 envelope protein. Based on the ELISA results, immunoreactivity was found to be targeted against the linear antigenic epitopes in the C1 and V3 regions of the viral envelope.

Conclusion: This study identified an exceptionally distinct HIV-2 plasma exhibiting broadly cross reactive, high titer NAbs that effectively neutralize most heterologous HIV-1 and HIV-2 virus strains. Further characterization of this sample could result in the identification of novel prophylactic targets that can be employed as leads for the design of effective vaccines against HIV.

STUDIES IN PROGRESS:

HIVL-4: Cohort for TB Research by the Indo-US Medical Partnership (C TRIUMPh) study:

Principal Investigator : Dr. C. Padmapriyadarsini
Funding : DBT/NIH
Study period : 2013-2018

In the reporting year, the multicentric study (C-TRIUMPh), Cohort for TB Research by the Indo-US Medical Partnership, has seen tremendous progress in terms of enrollment of study participants. Detailed information on the samples processed and stored under the umbrella of this cohort study are provided in the tables below (Tables 25 - 27):

Table 25: No. of C-TRIUMPh study samples received and processed during the period April 2015 to Mar 2016:

Cohort	A	B	Total
No. of Baseline Samples received	88	169	257
No. of Follow up samples received	511	391	902
Total No. of samples received and Processed	599	560	1159

Table 26: No. of C-TRIUMPh study samples stored during April 2015 to March 2016

Cohort	PBMC	PLASMA	QGIT (PLASMA)	PAXGENE (WHOLE BLOOD)	DNA (WHOLE BLOOD)
A	1022	3864	24	479	119
B	1331	4472	3708	556	168
Total	2353	8336	3732	1035	287

Table 27: No. of samples processed for QGIT (Cohort-B) for C-TRIUMPh study during April 2015 to March 2016:

Visit	Samples Received	Samples Tested
Entry Level	164	138
4^{th} month	105	65
12^{th} month	63	26
Total samples	332	229

HIVL-5: Full length genome amplification of HIV-1 isolates from recently infected pediatric samples

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email:hanna@nirt.res.in)
Co-Investigator	:	Dr. Ujjwal Neogi, Karolinska University, Sweden
Source of funding	:	Intramural
Study Period	:	2016-2017

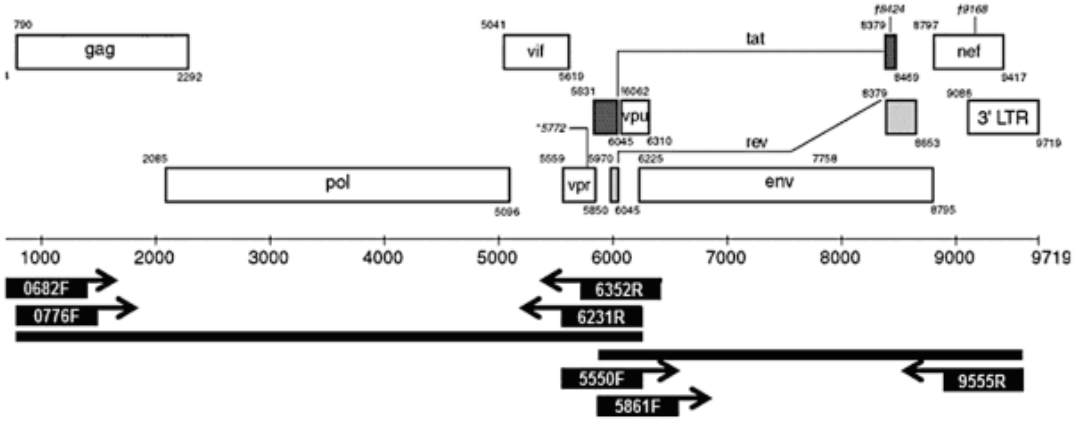
Background: One of the major challenges for developing a successful vaccine against HIV is the enormous diversity within and between the subtypes and there are many questions on this pertinent topic. With technological advances, epidemiological signatures and recombination hot spots, as well as adaptations to escape immune recognition at whole genome level, have been identified. There have been several attempts to develop a protocol for near full-length HIV-1 genome amplification for whole genome sequencing application. However, most of the attempts used proviral DNA from peripheral blood mononuclear cells or co-cultured cells. Data on free virus in the plasma, that is routinely being used in clinical drug resistance testing and represents the most recent viral population in the host, are few.

Aim: To generate near full length genomes of circulating viral isolates by nested PCR for whole genome sequencing

Methodology: Blood samples were collected from 10 HIV positive babies (aged <2 months) whose mothers were reported to be HIV positive at the time of pregnancy. Plasma samples were separated and stored at -80°C until use. Viral RNA was extracted using QIAamp viral RNA kit using manufacturer's instructions. cDNA was synthesized using 6352R and oligo d(T)18 primers. Near full length genome was amplified in two fragments - one amplicon of approximately 5.5 kb (Gag-Vpu) and the other of approximately 3.7 kb (Vif-3'LTR) using nested PCR (Fig, 12). The amplified PCR products were run on a 1% agarose gel at 150 V for 25 minutes to determine the presence of the expected bands. Next generation

sequencing was performed using an Ilumina sequencer at Scigenom, Kochi, India, with HiSeq 2500 run and 250bp PE Rapid Run Mode.

Fig. 12 : Amplification strategy



Current status: Till date, 10 full length HIV-1 genomes have been amplified using the above mentioned strategy.

Some of these genomes have been sequenced. The study is ongoing.

HIVL-6: Analysis of protective humoral and cell mediated immunity in HIV vaccinated individuals

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email:hanna@nirt.res.in)
Source of funding	:	IAVI through India – East Africa Vaccine Design program supported by USAID
Study Period	:	2015-2016

Background: Despite immense efforts, we do not have a safe and effective vaccine against HIV. There is general consensus that an effective HIV vaccine

should induce humoral as well as cellular immune responses that prevent initial infection of the host cells or limit early events of viral dissemination. Antibody responses typically require CD4+ T helper cells, and the major helper subset that is involved in this function are T follicular helper (Tfh) cells. In HIV-infected individuals, high frequencies of Tfh cells correlates with high titers of broadly neutralizing antibodies and reduced viral loads. In addition to humoral responses, several studies have demonstrated that HIV-infected individuals with antigen-specific CD8 T-cells that simultaneously produce multiple cytokines (i.e, IFN-γ, TNF-α, and IL-2) have lower viral loads as compared to individuals with cells making only 1 or 2 cytokines. The ability of a vaccine to elicit the above mentioned responses would therefore contribute to the efficacy of a given vaccine.

Aim: To measure V1/V2 and V3 antibodies and neutralizing antibodies, Tfh cell frequencies and poly-functional T-cells in stored samples of vaccinated individuals

Methodology: Archived plasma samples were screened for the presence of broadly neutralizing antibodies by ELISA. Based on the results of the V1/V2 antibody ELISA, three-time point samples were chosen for the neutralization assay - (i) 3 months+14 days, (ii) 6 months+14 days and (iii) 18 months. The plasma samples were screened with a panel of pseudoviruses belonging to different HIV-1 subtypes selected from the standard pseudovirus panel was obtained from the NIH AIDS Reagent Program. The pseudoviruses were titrated on TZM-bl cells and neutralization assay was performed using 200TCID50 of pseudoviruses and a plasma dilution of 1:20. Frequency of Tfh cells and polyfunctional T-cells was measured using flow cytometry.

Results: We found good antibody responses to the V2 and V3 peptides in majority of the vaccinated individuals 6 months post vaccination. However, none of the samples showed significant levels of neutralizing antibodies. The study is ongoing.

HIVL-7: HIV drug resistance patterns seen in HIV-infected children covered under the National PPTCT/EID programs

Principal Investigator : Dr. Luke Elizabeth Hanna
(email:hanna@nirt.res.in)
Source of funding : Intramural
Study Period : 2015-2017

Background: Parent-to-child transmission (PTCT) of HIV accounts for about 2% of the total HIV infection load in the country. PTCT of HIV can occur during pregnancy, at the time of delivery or through breastfeeding. In India, the prevention of parent/patent to child transmission (PPTCT) program has been in place for many years, and recommends antiretroviral prophylaxis for both HIV+ mother and child.

Aims: (i) To assess the proportion of young children with drug-resistant HIV in a representative population from some of the southern states of India enrolled in the Early Infant Diagnosis (EID) Program of the National AIDS Control Organization (NACO)

(ii) To determine the drug resistance pattern in infants in our settings

Methodology: 243 dried blood spot samples that had a positive HIV DNA test were chosen for the study. DNA was extracted from dried blood spots and subjected to PCR amplification and direct sequencing of HIV-1 Reverse transcriptase (RT) gene by an in-house method. Sequence data was interpreted using the Stanford Drug Resistance data base.

Current status: Out of the 243 samples, RT gene was successfully amplified and sequenced in 115 samples, of which 52 (45.2%) were from male and 63 (54.7%) from female children. The data is being analyzed.

HIVL-8: Study on the prevalence of HIV drug resistance in an ART naïve population from Tamil Nadu

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2015-2016

Background: Studies to assess the baseline, pretreatment drug resistance mutations (DRMs) in HIV-1 subtype C infected individuals are required because of the limited availability of such data on non-subtype B HIV strains. Patients who started their first line ART regimen based on their baseline HIV DR pattern have greater and long lasting viral suppression than those without it. However, this is not feasible in resource limited settings like India, due to the high cost and requirement of sophisticated infrastructure for testing.

Aim: To evaluate the prevalence of transmitted drug resistance in our population and analyzing the pattern of HIV-1 DRMs in the regional level, besides obtaining information on the circulating HIV-1 subtypes

Methodology: This is a retrospective study carried out on samples collected from HIV-1 infected ART naïve individuals who attended the ART Centre at the Kilpauk Medical College and Hospital, Chennai, during the period April 2011 to September 2012. Fifty three HIV-1 infected ART naïve patients were recruited for drug resistance testing. An in-house method was used to amplify the HIV-1 Pol gene encompassing the protease and RT regions and then sequenced. Sequences were interpreted with the help of the Stanford Drug Resistance Database.

Current status: Data analysis is ongoing. The study will report on the prevalence of DRMs for protease inhibitors (PI), nucleoside reverse

transcriptase inhibitors (NRTI), and non-nucleoside reverse transcriptase inhibitors (NNRTI) in the drug naïve populations.

HIVL-9: Analysis of the patterns of HIV DRMs in first line ART failures enrolled under NACO's ART programme in south India

Principal Investigator	:	Mr.K. Ramesh (email:kramesh_76@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2016-2017

Background: HIV patients on ART showing indications of clinical failure are tested for virological failure before moving them from a first line to a second line regimen.

Aim: To utilize a minimum resource strategy to assess HIV DRMs in first line failures

Methodology: This was designed as a cross-sectional study. Venous blood was collected in EDTA from first line ART failures and plasma was obtained and used for genotypic drug resistance testing.

Current status: A total of 57 sequences have been genotyped thus far. The study is in progress.

Department of Immunology

COMPLETED STUDIES:

(i) Variable transcriptional adaptation between the laboratory (H37Rv) and clinical strains (S7 and S10) of *M.tuberculosis* under hypoxia

Principal Investigator	:	Dr. Alamelu Raja (email: alameluraja@gmail.com)
Research Scholar	:	Ms. D. Santhi
Source of funding	:	ICMR Fellowship / Intramural
Study Period	:	2012- 2015

Background: Understanding the biology of persistence is required to develop effective intervention strategies against TB. Oxygen deprivation (hypoxia) is shown as a potential stimulus to study dormancy *in vitro*. Limited information is known about the clinical isolates that are involved during disease outbreaks. Hence, we selected 2 prevalent south Indian clinical strains of *M. tuberculosis* to study gene expression. In general, genes that are down regulated during *in vitro* stress experiments are given less importance without verifying their expression levels in clinical strains.

Aim: To identify differentially regulated genes (down regulated in H37Rv laboratory strain, but up-regulated in both clinical isolates - S7 and S10) under hypoxia

Methods: Aerobic and anaerobic culture methods: H37Rv, clinical isolates S7 and S10 were grown in Middlebrook 7H9 media supplemented with 2% (v/v) glycerol, 10% albumin-dextrose-catalase (ADC) and 0.05% (v/v) Tween 80 at 37°C and 200 rpm for 25 to 30 days to obtain aerobic cultures. For anaerobic cultures, the strains were inoculated in screw capped test tubes (20 x 125mm, with a total fluid capacity of 25.5 ml) containing supplemented Middlebrook 7H9 (MB7H9). For uniform dispersion of cultures and to control the rate of oxygen2 depletion, stirring was done with 8-mm Teflon-coated magnetic stirring bars in the tubes (120 rpm) and incubated at 37°C. Oxygen depletion in the cultures was assessed by adding a sterile solution of methylene blue to the medium at a final dye concentration of 1.5µg/ml. Reduction and decolorization of this dye served as a visual indication of oxygen depletion.

RNA isolation, cDNA synthesis and labelling: RNA from both aerobic and anaerobic cell pellets (10^7 bacterial cells) was used to isolate RNA by standard Trizol

method (Sigma Aldrich, USA). Final purification of RNA and DNase treatment to remove residual DNA was carried out by RNeasy columns (Qiagen, USA). RNA quality was assessed by measuring the ratio of absorbance of total RNA at 260/280 and 260/230 nm.

For cDNA synthesis, 2 µg of RNA from each sample was incubated with WT (Whole Transcript) primers according to manufacturer instruction (Low Input Quick Amp Labeling WT kit, Agilent Technologies, USA). Fluorescently labelled cRNA, transcribed from cDNA, by T7 polymerase transcription master mix (Low Input Quick Amp Labeling WT kit, Agilent Technologies, USA) containing 5X transcription buffer, 0.1M DTT, nucleotide triphosphate (NTP), T7 polymerase and labelled with Cy3-CTP (aerobic cultures of H37Rv, S7, S10) or Cy5-CTP (anaerobic cultures of H37Rv, S7, S10) were incubated for 2 hrs at 40^0C.

Hybridization: A 60mer oligonucleotide based custom array chip was used from Agilent Technologies in 8x15K format. 300 ng of Cy5 labelled cRNA from anaerobic cultures of H37Rv, S7 and S10 was hybridized against 300ng Cy3 labelled cRNA from aerobic cultures of H37Rv, S7 and S10. Following image analysis, feature extraction was performed using Feature extraction tool version 9.5.3.1 (Agilent Technologies, USA).

Results: A total of 10 genes was found in common as over-expressed in both the clinical isolates but repressed in H37Rv under hypoxia. These sets of genes were termed as “differentially regulated genes” and followed throughout the text. These are Rv0812, Rv1463, Rv1544, Rv1582c, Rv1601, Rv2035, Rv2045c, Rv2430c, Rv2483c and Rv3538, their fold expression cluster is given Fig. 13 and Table 28.

Fig. 13: Heat map describing differences in the transcriptional signature between H37Rv and clinical isolates S7 and S10 under hypoxia

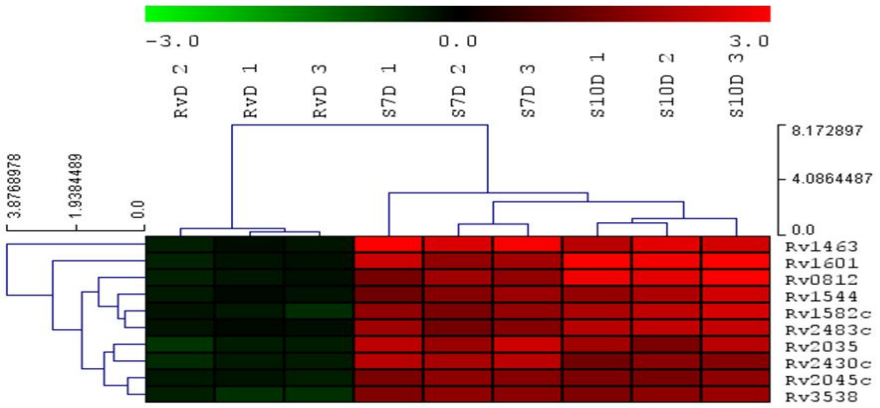


Table 28: Fold expression of the genes

S. No	Rv No	Gene Name	Fold change in H37Rv		Fold change in S7		Fold change in S10		Predicted Function
1	Rv1463		**-0.25**	-1.02	**3.63**	3.50	**2.48**	2.28	Probable conserved ATP-binding protein ABC transporter
2	Rv1601		**-0.27**	-1.46	**2.05**	2.12	**3.40**	2.81	Probable imidazole glycerol-phosphate dehydratase HisB
3	Rv0812	pabC	**-0.28**	-4.46	**1.68**	2.45	**2.85**	2.95	Probable amino acid aminotransferase
4	Rv1544		**-0.21**	-1.18	**1.59**	1.25	**2.10**	1.2	Possible ketoacyl reductase
5	Rv1582c		**-0.35**	-1.04	**1.67**	1.57	**2.30**	1.97	Probable PhiRv1 phage protein
6	Rv2483c	plsC	**-0.16**	-1.22	**1.61**	1.60	**2.28**	1.78	Possible transmembrane phospholipid biosynthesis bifunctional enzyme PlsC
7	Rv2035		**-0.43**	-0.99	**2.18**	1.49	**1.86**	1.14	Conserved hypothetical protein
8	Rv2430c	PPE41	**-0.37**	-0.91	**2.15**	2.76	**1.54**	3.04	PPE family protein PPE41
9	Rv2045c	lipT	**-0.29**	-1.56	**1.59**	1.47	**1.55**	2.61	Carboxylesterase LipT
10	Rv3538	hsd4B	**-0.50**	-0.80	**1.64**	1.77	**1.67**	1.14	Possible 2-enoyl acyl-CoA hydratase

Conclusions: The data presented here show the existence of variations between laboratory and clinical strains of *M. tuberculosis* in terms of gene expression under hypoxia. An understanding of this variation opens the path to identify a set of genes which have altered expression (that is, down regulated in H37Rv but over-expressed in clinical isolates). Gene expression analysis in other clinically relevant *M. tuberculosis* strains during *in vitro* stress experiments could bring highly relevant representative genes that can be targeted for the design of anti-TB drugs and vaccine.

(ii) Use of alternative biomarkers other than IFN-γ in the diagnosis of active TB among HIV co-infected patients

Principal Investigator	:	Dr. Alamelu Raja (email: alameluraja@gmail.com)
Research Scholar	:	Ms. Prabhavathi M
Source of funding	:	CSIR / Intramural
Study Period	:	2013- 2016

Background: Decreased tuberculin activity, reduced sensitivity to acid fast staining and atypical radiographic presentations hinder the diagnosis of TB in HIV co-infected patients. The recently developed Interferon-gamma (IFN-γ) release assays (IGRAs) has suboptimal sensitivity (60%-70%) to detect active TB among the HIV co-infected patients, suggesting that ~1 in 3 HIV-TB co-infected patients will have negative IGRA results. In IGRA test, antigen specific T-cells secrete a plethora of cytokines and evaluation of these secreted cytokines other than IFN-γ, may be a useful approach for diagnosis of active TB among HIV co-infected patients.

Aim: To evaluate diagnostic ability of TB antigen specific cytokines other than IFN-γ in HIV and HIV-TB co-infected patients and to determine whether the diagnostic performance of IGRA could be improved by addition of these cytokines

Materials and methods: We prospectively enrolled 53 HIV positive subjects and 55 HIV-TB co-infected patients from India. IGRA was performed by using QuantiFERON TB-Gold In tube (QFT-GIT) method (Table 29). TB antigen specific IL-1β, TNF-α, IL-2, IL-6, IL-8 and IL-12 levels were evaluated by ELISA in plasma harvested from QFT-GIT tubes.

Results: The TB antigen specific IL-1β levels were significantly elevated in HIV-TB (median 294.2 pg/ml) compared to HIV group (median 778.3 pg/ml) (Fig. 14). To determine the diagnostic performance of antigen specific IL-1β assay, a cut-off point 300 pg/ml was fixed by ROC curve analysis. Of the 55 HIV-TB patients tested, 40 patients were positive for IL-1β test and conferred 73% sensitivity. In case of QFT-GIT, it was positive in 30 out of 55 HIV-TB patients; by considering indeterminate results as negative, QFT-GIT showed 55% sensitivity and by excluding indeterminate results, QFT-GIT exhibited 73% sensitivity.

Of the 53 HIV positive subjects tested, IL-1β assay was negative in 27 subjects and yielded 51% specificity. QFT-GIT assay was negative in 21 out of 53 HIV subjects; hence yielded specificity of 45% (by excluding indeterminate results) and 53% (by considering indeterminate results as negative) respectively.

A combination of smear test + IL-1β assay and smear test + QFT-GIT assay exhibited 95% (52/55) and 93% (51/55) sensitivity respectively. Moreover, unlike IFN-γ, IL- β levels were not influenced by low CD4 counts. The remaining cytokine levels did not differ significantly between HIV and HIV-TB study groups.

Conclusion: Though the specificity of IL-1β has remained similar to that of QFT-GIT, antigen specific IL-1β has showed higher sensitivity than IFN-γ (read out marker of QFT-GIT). Of the 14 HIV-TB patients who gave indeterminate results, 10 subjects were shown to be positive by IL-1β assay. Moreover, IL-1β assay was not influenced by low CD4 counts. Thus, we suggest IL-1β could act as an additional biomarker along with the existing markers for diagnosis of TB among HIV co-infected patients.

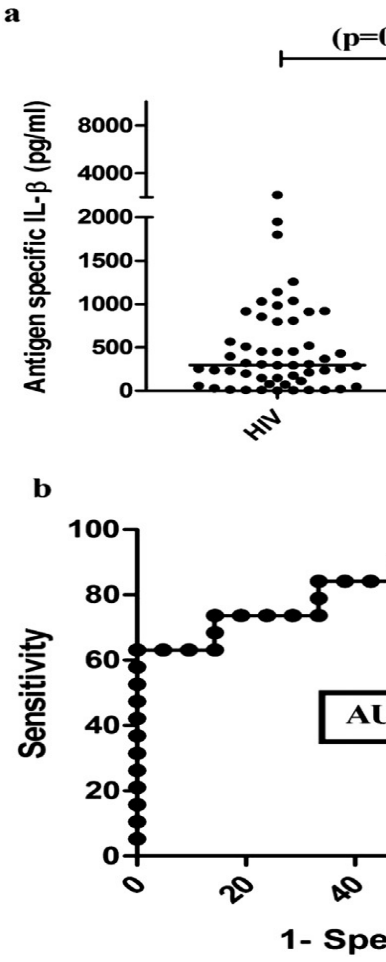
Table 29: Demographic and baseline characteristics of the study groups

Fig. 14: Diagnostic performance of antigen specific IL-1β in HIV & HIV-TB groups

Table 1: Demographic and baseline characteristics of the study groups.

	HIV	HIV-TB
No. of subjects (N)	53	55
Age	19-56	21-52
Sex:		
Male	29 (55%)	38 (69%)
Female	24 (45%)	17 (31%)
HIV strain:		
HIV-I	46 (87%)	51 (93%)
HIV-I &II	7 (13%)	4 (7%)
Smear test status:		
Positive	-	42 (76.36%)
Negative	-	13 (23.63%)
Culture test (available for):	-	19 (34.54%)
smear negative-culture positive, N	-	13
smear positive-culture positive, N	-	6
Chest x-ray (available for)	-	31 (56.36%)
QFT-GIT:		
Positive	25 (47.16%)	30 (54.54%)
Negative	21 (39.62%)	11 (20%)
Indeterminate	7 (13%)	14 (25.45%)
CD4+ Cell Count:		
· 100 cells/µl	12 (22.64%)	22 (40%)
100 to 200 cells/µl	14 (26.41%)	17 (30.90%)
·200 cells/µl	27 (50.94%)	16 (22.09%)

Figure 1. Diagnostic per specific IL-1b in HIV and H



(iii) Evaluation of antigen specific responses for identification of *M. tuberculosis* infection

Principal Investigator	:	Dr. Alamelu Raja (email: alameluraja@gmail.com)
Research Scholar	:	Mr. Balaji Pathakumari
Source of funding	:	ICMR / Intramural
Study Period	:	2013- 2016

Background: Among the *M. tb* infected individuals, about 90% of them show persistent long term infection without developing any symptoms and this condition is said to be latent infection (latent TB infection or LTBI). Approximately 5-10% of LTBI individuals develop active TB due to reactivation and resuscitation of dormant bacilli indicating that persons with LTBI are the largest reservoir of potential future source of active TB which is transmittable. Previously, we found higher IFN-γ response in LTBI individuals compared to active TB patients against immunodominant *M. tb* secreted protein fractions. Further analyses by mass spectrometry revealed a total of 24 novel T-cell antigens in those protein fractions. We have chosen two proteins, Rv2204c and Rv0753c among the 24 for the present study.

Aim: To characterize the immune response of two *M. tuberculosis* antigens in LTBI and active TB and to evaluate the utility of these antigens as potentially useful diagnostic markers for identifying LTBI in high endemic settings

Methods: Two *M. tb* proteins Rv2204c and Rv0753c were cloned, over-expressed in *E. coli* and purified by affinity chromatography. Antigen-specific immune response was evaluated in 39 PTB patients and 35 healthy house-hold contacts (HHC). After whole blood culture for 6 days, the secretion of cytokines (IFN-γ, TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p40, IL-17 and interferon gamma–inducible protein [IP-10] and chemokines (IL-8, monocyte chemotactic protein-1 [MCP-1] and MCP-2) were quantified in culture supernatants using Enzyme linked immunesrbent assay (ELISA). The differences in various cytokine secretions among the study groups

were assessed by Mann–Whitney U test. Cut-off values were determined by Receiver operating curve (ROC).

Results: Though there was a differential expression of cytokines against the antigens, significant difference was observed in the levels of IFN-γ, TNF-α, IL-6, IL-8, IL-12p40, MCP-1 and MCP-2 ($p<0.05$) between the two groups (Fig. 15). Other antigen specific cytokines like *IL-1β, IL-2, IL-10, I*L-17 and IP-10 did not show significant difference between HHC and PTB. Both Rv2204c and Rv0753c antigen specific IFN-γ response showed 86% positivity in HHC; whereas in PTB, these antigens showed 18% and 21% positivity respectively (Table 30). Rv2204c antigen-specific IFN-γ/TNF-α response displayed maximum positivity of 91% in HHC and minimum positivity of 10% (4/39) in PTB. Rv2204c and Rv0753c specific IL-12p40 response showed 89% (31/35) and 86% (30/35) positivity in HHC; whereas in PTB, the positivity was 36% (14/39) and 41% (16/39) positivity respectively *(*Table 30*)*. We further explored whether combination of different antigens will improve the positivity in HHC. For combination analysis, positivity was calculated as number of subjects positive for either of the antigens. In terms of IFN-γ response, among the various antigen combinations, Rv2204c+Rv0753c showed a maximum positivity of 97% (34/35) in HHC and 31% (12/39) positivity in PTB.

Conclusion: Rv2204c and Rv0753c specific IFN-γ response and IFN-γ/TNF-α ratio may provide the basis for the development of diagnostic test, which will be useful for LTBI screening in high TB endemic countries. Since the present study results are preliminary, it has to be evaluated further in a larger number of patients.

Fig. 15: Cytokine and chemokine responses to *M. tuberculosis* antigens

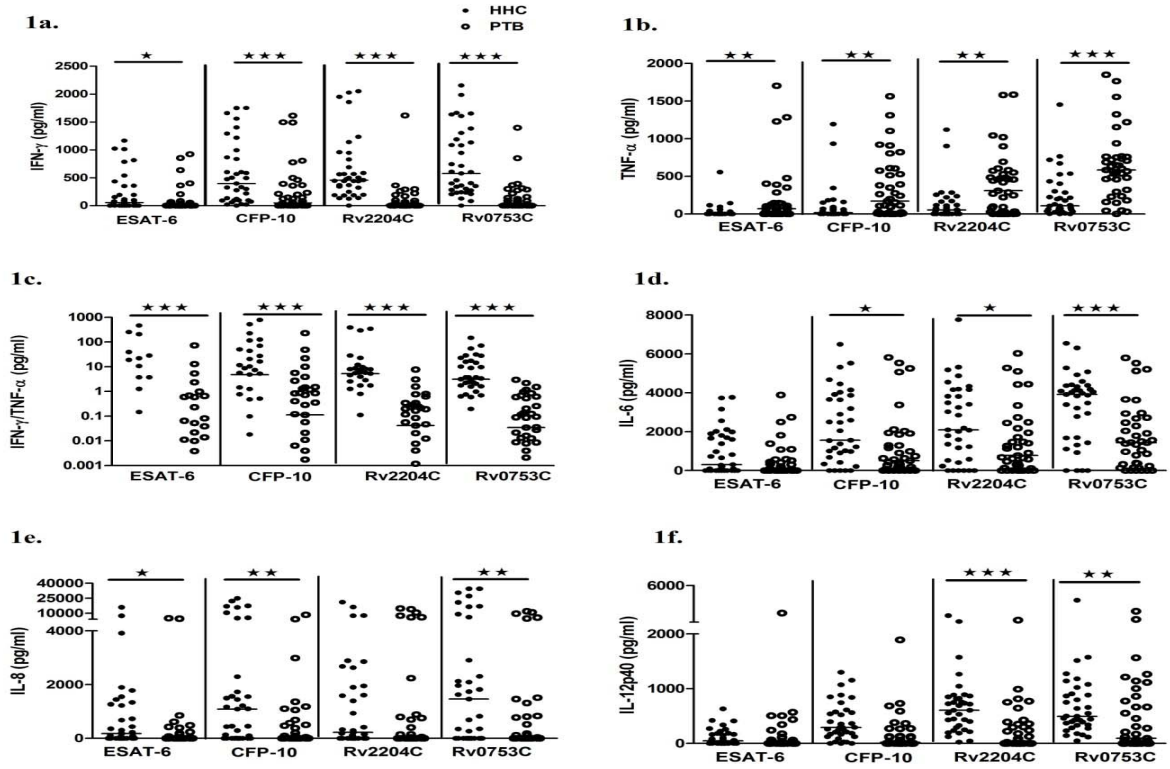


Fig. 15: Cytokine responses following antigen stimulation: The concentration of various cytokines and chemokines were measured by ELISA. Fig 1a) IFN-γ, 1b) TNF-α, 1c) IFN-γ/ TNF-α ratio, 1d) IL-6, 1e) IL-8, 1f) IL-12p40. Horizontal Line indicates median. Closed circle-HHC, open circle-PTB. *= $p<0.05$, **=$p<0.01$, ***=$p<0.001$.

Table 30: Diagnostic potential of cytokines in response to ESAT-6, CFP-10, Rv2204c & Rv0753c

Protein name	% Positivity in HHC (N=35)	% Positivity in PTB (N=39)	Cut off (pg/ml)	Area under the Curve (AUC)
IFN-γ				
ESAT-6	71 (25/35)	46 (18/39)	6.15	0.633
CFP-10	86 (30/35)	46 (18/39)	68.6	0.7612
Rv2204c	86 (30/35)	18 (07/39)	186.4	0.9168
Rv0753c	86 (30/35)	21 (08/39)	227	0.8846
Rv2204c+Rv0753c	97 (34/35)	31 (12/39)	-	-
IFN-γ/TNF-α				
ESAT-6	86 (30/35)	38 15/39)	0.802	0.7883
CFP-10	86 (30/35)	41 (16/39)	0.935	0.8183
Rv2204c	91 (32/35)	10 (4/39)	1.657	0.9289
Rv0753c	91 (32/35)	21 (08/39)	0.625	0.9385

(iv) Molecular characterization of Ffh-FtsY interaction and proteomic identification of their potential substrates in *M. tuberculosis*

Principal Investigator : Dr. Sujatha Narayanan (email: Sujatha.sujatha36@gmail.com)
Co-Investigators : Mr.V. Arunkumar / Dr.P. Kannan (kannanp@nirt.res.in)
Source of funding : ICMR / Intramural
Study Period : 2012- 2015

Background: Universally conserved signal recognition particle (SRP) pathway that mediates the co-translation of membrane and secretory proteins is believed to be essential for eukaryotic and prokaryotic cells. The *M. tuberculosis* SRP pathway consists of two proteins Ffh, FtsY and a 4.5*S* RNA molecule. Although the *Escherichia coli* SRP pathway has been studied well, the understanding of the *M. tuberculosis*

SRP pathway components is very limited.

Objectives: (i) To study the interaction and GTPase activity of Ffh and FtsY

(ii) To construct and characterize the *ffh* and *ftsY* gene knockdown in *M. tuberculosis*

Materials and methods:

Pull down assay: The nickel affinity pull down assay was performed by incubating the recombinant His-Ffh protein with Ni-NTA resin for 2 hrs at 4°C under gentle agitation. The columns were washed with buffer A containing 40 mM imidazole. Similarly, glutathione affinity pull down assay was also performed by incubating recombinant GTS-FtsY and His-Ffh or BSA with Glutathione sepharose resin. The eluted proteins were separated by SDS-PAGE and subjected to western blotting.

GTP hydrolysis assay: The purified GST-FtsY and His-Ffh proteins were incubated in a 10X TMD buffer (50 mM Tris–Cl (pH 7.4), 5 mM $MgCl_2$, 1 mM DTT) with 200 µM unlabeled GTP and 1 µCi of [γ-^{32}P] GTP. The reactions were stopped with 0.5 µl of 10% SDS, and 10 µl of the reaction mixtures were spotted on the polyethyleneimine (PEI)-cellulose coated thin layer chromatography plate and subjected to autoradiography.

Construction of antisense *ffh* and *ftsY*: The full length *ffh* and *ftsY* genes from *M. tuberculosis* were amplified using the respective oligonucleotide primers. The amplified gene products were inserted into pAZI9018b vector to get *ffh* and *ftsY* in antisense orientation (As-ffh-pzi and As-ftsY-pzi). The sequence confirmed clones as well as vector control, pAZI9018b (Rv) were electroporated into *M. tuberculosis* H37Rv electro competent cells and plated on 7H10 agar media supplemented with OADC containing 50 µg/ml hygromycin.

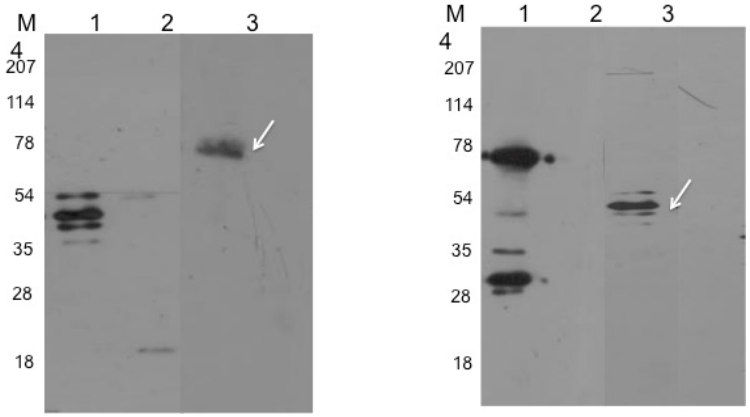
Validation of antisense constructs: Total RNA was isolated from Rv, As-ffh-pzi and As-ftsy-pzi strains at different time points using an RNeasy kit (Qiagen). For determination of relative mRNA concentrations by qRT-PCR, cDNA was synthesized using QuantiTect reverse transcription kit (Qiagen). Analysis of qRT-PCR data was carried out using the comparative CT method.

2D gel electrophoresis: The cytoplasmic lysates from Rv, As-ffh-pzi and As-ftsy-pzi cell extracts were

precipitated using SDS and trichloro acetic acid (TCA)–acetone precipitation. The pellets were air dried and suspended in appropriate volume of 2D-rehydration buffer. Isoelectric focusing (IEF) was carried out using "in-gel rehydration" method. After IEF, the strips were subjected to SDS-PAGE. Differentially expressed proteins were shortlisted using PDQuest software (Bio-Rad, USA). Protein spots that showed increased intensities were selected using mass spectrometry.

Results: *In vitro* interaction between Ffh and FtsY: A Histidine pull down assay with recombinant His-Ffh using Ni-NTA affinity chromatography showed Ffh protein was able to pull down GST tagged FtsY protein (Fig. 16A). Similarly in a reverse reaction, GST pull down assay with recombinant GST-FtsY using Glutathione sepharose chromatography also showed that FtsY protein was able to pull down Histidine tagged Ffh protein (Fig. 16B).

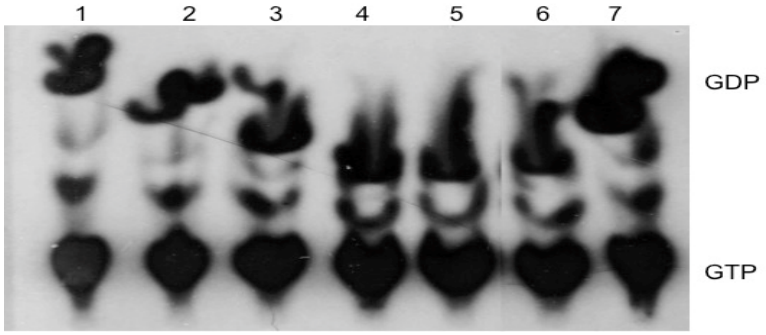
Figs. 16 A & B: Identification of Ffh – FtsY interaction



(A) 6x-His pull-down assay. Lane 1, His-Ffh loads; lane 2, unbound proteins; lane 3, FtsY probed using anti-GST antibody. (B) GST-pull down assay. Lane 1, GST-FtsY loads. lane 2, unbound proteins; lane 3, Ffh probed using anti-His antibody. Control experiments were also performed with BSA as bait protein (A, B lane 4). Lane 1 from A and B shows the bait protein load controls of Ffh and FtsY proteins that are probed against anti-His (A) and anti-GST (B) antibodies respectively.

The rate of GTP hydrolysis was demonstrated upon *M. tuberculosis* Ffh-FtsY complex formation. Equal concentrations of His-Ffh and GST-FtsY was incubated with [γ-^{32}P] GTP, the GTP hydrolysis was observed to be much faster as compared to the dissociation of the complex (Fig. 17).

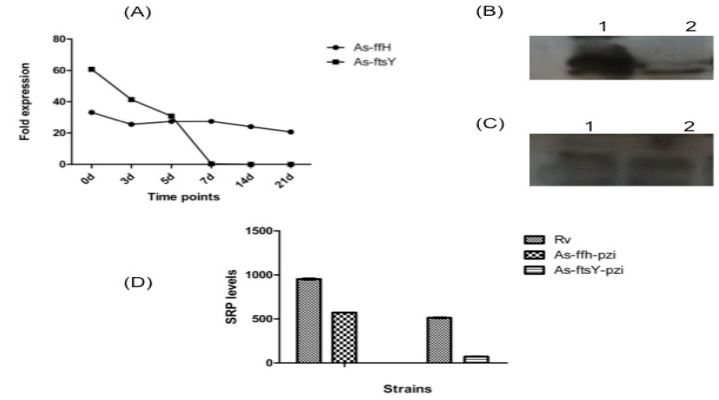
Fig. 17: GTPase activity during Ffh-FtsY interaction



Lane 1, Control reaction without any protein; lane 2, Ffh and (γ-^{32}P) GTP; lane 3, FtsY and (-γ^{32}P) GTP; lane 4-7, incubation of (γ-^{32}P) GTP with Ffh-FtsY for 5, 15, 30 and 60 min respectively.

Antisense inhibition of *ffh* and *ftsY*: The antisense DNA strands of *ffh* and *ftsY* genes were cloned in pAZI9018b vector under the *lacI* promoter. Measuring the levels of Ffh and FtsY at both RNA and protein level validated the antisense mediated gene knockdown (Fig. 18).

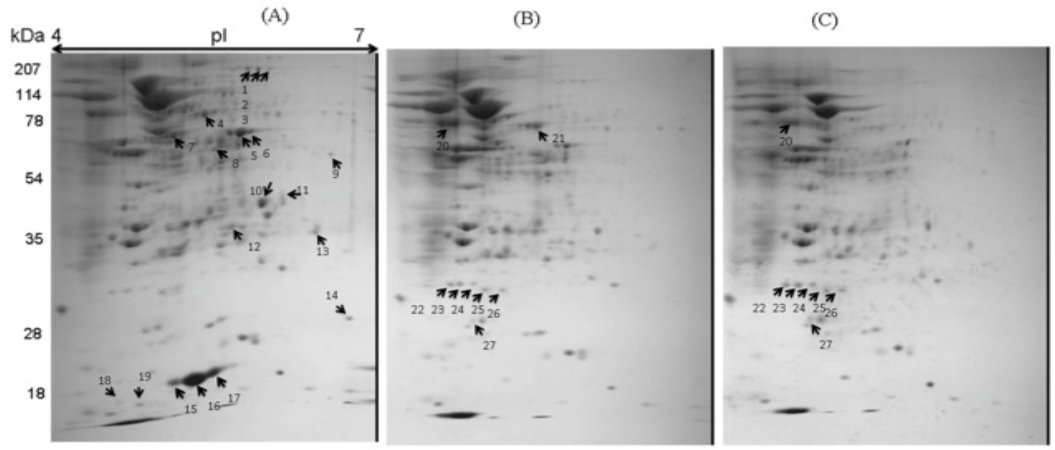
Fig. 18: Quantification of *ffh* and *ftsY* expression in pAZI9018b backbone



(A) qRT-PCR quantification of Ffh and FtsY transcripts using pDVA ffh-As-pzi and pDVA ftsY-As-pzi constructs. (B) FWB analysis for *ffh* knockdown strains. Equal amount of cytoplsmic lysates from 21 d cultures of Rv (lane 1) and pDVA ffh-As-pzi (lane 2) strains were probed with bait proteins and anti-His antibody. (C) FWB analysis for *ftsY* knockdown strains. Equal amount of cytoplsmic lysates from 21 d cultures of Rv (lane 1) and pDVA ftsY-As-pzi (lane 2) strains were probed with bait proteins and anti-His antibody. (D) Quantification of Ffh anf FtsY protein bands from Rv, pDVA ffh-As-pzi and pDVA ftsY-As-pzi strains using densitometer.

2DE/MS analysis of SRP knockdown strains: In order to identify putative target proteins of Ffh and FtsY, the cytoplasmic lysates of Rv, As-ffh-pzi and As-ftsY-pzi strains were separated by 2-D gel electrophoresis. Statistical analysis of the gels detected 27 spots with significant changes between Rv versus As-ffh-pzi and As-ftsY-pzi (Fig. 19). A total of 22 protein spots could be identified using MALDI TOF-TOF MS/MS analysis and categorized into five functional categories according to their biological function. In comparison to Rv, knockdown of *ffh* (As-ffh-pzi) and *ftsY* (As-ftsY-pzi) caused higher abundance of 3 proteins and lower abundance of 11 proteins in knockdown strains.

Fig. 19: 2D gel electrophoresis for As-*ffh* and As-*fts*Y strains



The cytoplasmic cell extracts of pDVA ffh-As-pzi, pDVA ftsY-As-pzi and Rv constructs in *M. tuberculosis* were subjected to 2D gel electrophoresis. The CBB R250 stained gels (A) Rv, (B) As-ffh-pzi and (C) As-ftsY-pzi were shown. The arrow marks represents the difference in spots using PDQuest software were analysed by mass spectrometry.

Conclusion: This work demonstrated the existence of universally conserved active SRP pathway components in *M. tuberculosis*. *In vitro* studies provided evidence that the *M. tuberculosis* Ffh interacts with FtsY in the absence of 4.5*S* RNA. Complex formation resulted in increased GTP hydrolysis because of their reciprocal stimulation. Depletion of *ffh* and *ftsY* resulted in differential expression of 14 proteins that will remain an important subject for future investigation.

(v) Protective efficacy of LpqS gene knock out mutant of *M. tuberculosis* in guinea pig animal model

Principal Investigator	:	Dr. Sujatha Narayanan (email: Sujatha.sujatha36@gmail.com)
Co-Investigators	:	Dr.S. Suba; Dr.P. Kannan
Source of funding	:	ICMR / Intramural
Study Period	:	2012- 2015

Background: Bacterial lipoproteins are a functionally diverse class of membrane anchored proteins. Inactivation of genes coding for individual lipoproteins results in attenuated phenotype of the mutants. LpqS is a lipoprotein highly conserved among slow growing pathogenic mycobacteria. Our previous study has shown that the *lpqS* gene deletion mutant of *M. tuberculosis* (*MtbΔlpqS*) replicates poorly in THP1 derived macrophagic cell line.

Objectives: To study the protective efficacy of *lpqS* gene knock out mutants in guinea pigs

Methods:

Evaluation of the protective efficacy of the *MtbΔlpqS* against *M. tuberculosis:* For evaluation of protective efficacy of *MtbΔlpqS,* two set of experiments were carried out. Guinea pigs (n = 6) were immunized with 5×10^5 CFU of BCG (Pasteur strain) or *MtbΔlpqS* in 100 µl of saline by subcutaneous (S) route or aerosol route (A). In the control group, guinea pigs were immunized with 100 µl of saline (S.). The animals were then challenged 5 weeks post immunization with 50-100 bacilli of virulent *M. tuberculosis* $H_{37}Rv$ *via* the respiratory route in an aerosol chamber. After 5 weeks of infection, animals were euthanized and dissected aseptically. Bacterial enumeration and evaluation of gross and histopathological changes in differentially vaccinated guinea pig groups were carried out as per the standard protocol.

Statistical analysis: Graph pad prism was used for statistical analysis. One way ANOVA was used to analyze the data obtained for bacterial enumeration, gross and histopathological changes and cytokine measurements by qRTPCR.

Results:

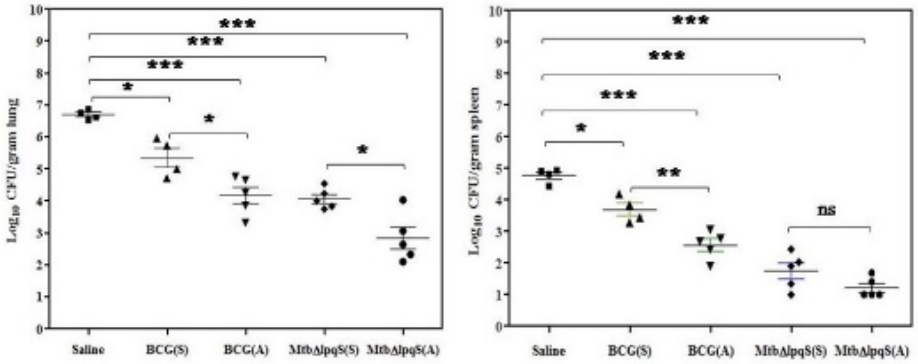
***MTBΔlpqS* offers superior protection than BCG in guinea pigs:** *MTBΔlpqS &* BCG immunization significantly reduced the bacillary load in the animal organs. Compared to saline vaccinated group, BCG immunization reduced the bacillary load in the lung by about 1.4 log and 2.5 log on immunization through subcutaneous and aerosol routes respectively ($p<0.001$) (Fig. 20). *MTBΔlpqS* immunization was more effective than BCG immunization both through subcutaneous and aerosol routes ($p<0.001$). Immunization of guinea pigs with *MTBΔlpqS* through subcutaneous and aerosol routes, caused the bacillary load in the lung to be reduced by about 2.6 and 3.8 log respectively compared to unvaccinated animals ($p<0.001$). A similar trend was observed with bacterial reduction in the spleen of animals immunized with BCG and *MTBΔlpqS*. Immunization through subcutaneous and aerosol routes caused reduction in the splenic bacillary load by about 1.1 log and 2.2 log respectively with the BCG immunized animals and is by about 3 log and 3.5 log respectively with *MTBΔlpqS* immunized animals ($p<0.001$). *MTBΔlpqS* was observed to limit bacillary multiplication better than BCG. Vaccination of guinea pigs with BCG and *MTBΔlpqS* through aerosol route offered superior protection compared to subcutaneous route of immunization. Compared to subcutaneous route of immunization, aerogenic immunization reduced the bacillary load by about 2 log with respect to both BCG and *MtbΔlpqS* in the lung ($p < 0.05$). A similar trend was observed with respect to CFU of the spleen following immunization through aerosol route.

Gross pathological changes: Severe pathological damage was observed in the organs of saline vaccinated guinea pigs. In addition, a marked enlargement of spleen with numerous tubercles was observed with this group (Figs. 21 A & B). In contrast, immunization with BCG and *MTBΔlpqS* significantly reduced the pathological damage that was observed in the organs of unvaccinated animals. BCG vaccinated animals exhibited pulmonary damage that showed few large tubercles and numerous small tubercles. However, lesions were negligible or extremely small and predominantly scanty in the *MTBΔlpqS* vaccinated group compared to BCG

vaccinated group**.** Among the *MTBΔlpqS* vaccinated groups, organs of animals immunized through aerosol route exhibited minimal damage compared to animals immunized through subcutaneous route.

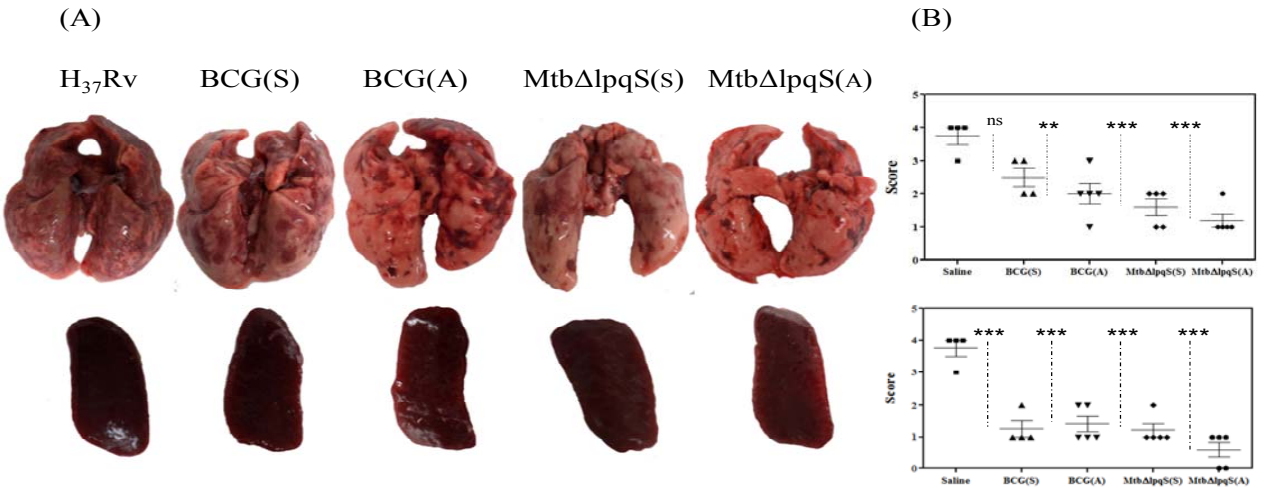
Histopathological changes: Histopathological changes in the organs of BCG, *Mtb*Δ*lpqS* and saline vaccinated (control group) guinea pigs were evaluated after challenging the animals with virulent H_{37}Rv. In the saline-vaccinated group, the lung sections showed granulomatous infiltrations characterized by caseating necrotic multifocal coalescing granulomas spanning the entire lung. The extent of granulomatous infiltration in BCG vaccinated animals was less comparable to that observed in the case of saline treated animals (Fig. 22). Immunization with *Mtb*Δ*lpqS* resulted in a significant reduction in the granulomatous infiltration, with the presence of very few small and discrete granulomas, when compared to both BCG and saline treated animals. The alveolar and bronchiolar structures were well conserved with no sign of active disease compared to BCG and saline vaccinated groups. Surprisingly, vaccination with *Mtb*Δ*lpqS* also prevented hepatic damage. Negligible granulomatous infiltration was observed in the liver sections. These animals showed a normal hepatic tissue organization and visible portal lobules compared to BCG immunized animals. No granulomatous infiltration was observed in the spleen of *Mtb*Δ*lpqS* immunized guinea pigs. In contrast, multifocal coalescing granulomas were observed in the spleen sections of BCG vaccinated and saline treated animals. Thus *MTBΔlpqS* immunization of guinea pigs resulted in fewer or negligible pulmonary, hepatic and splenic lesions compared to both BCG and saline immunized groups.

Fig. 20: Superior protection by *MtbΔlpqS* vaccination against *M. tuberculosis* challenge



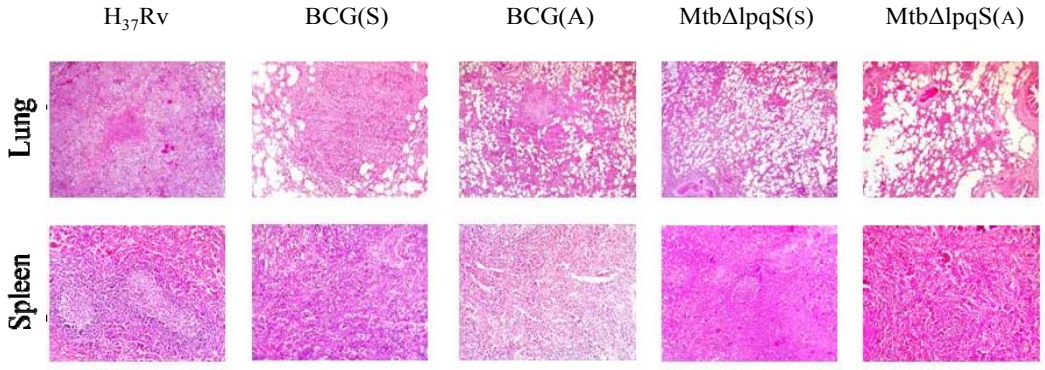
Superior protection by MtbΔlpqS vaccination against *M. tuberculosis* challenge. The figure depicts the bacillary in the immunized and saline treated guinea pigs at 5 weeks post infection with *M. tuberculosis*. Immunization with MtbΔlpqS significantly reduced the bacillary load in the lung and spleen when compared to saline and BCG group. The lower limit of detection was 1.0 Log_{10}CFU/g of tissue and animal with undetectable bacilli were allotted a CFU of 1.0Log_{10}/g. Each point represent the Log_{10}CFU for an individual animal and the bar depicts mean (±SEM) for each group. Missing data points represent animals that succumbed to disease before the time of euthanasia. *,p<0.05; **p< 0.01;***,p<0.001.

Figs. 21A & B: Reduction in gross pathological lesions in MtbΔlpqS vaccinated guinea pigs following *M. tuberculosis* challenge



Reduction in gross pathological lesions in MtbΔlpqS vaccinated guinea pigs following *M. tuberculosis* challenge. (A) The figure depicts the Representative photographs of Lung and Spleen of vaccinated and saline treated Guinea pigs (n=6) euthanized at 5 weeks post-infection. **(B)** Based on the extent of involvement, number and size of tubercles, areas of inflammation and necrosis, gross pathological changes were graded 1-4 and represented graphically.

Fig. 22: Histopathology of guinea pig organs at 5 weeks post challenge



Histopathology of guinea pig organs at 5 weeks post challenge. Representative photographs of the H&E stained lung and spleen tissues of individual animal (n=6) vaccinated with either BCG, MtbΔlpqS or saline and euthanized at 5 weeks after challenge. Lung and spleen of saline vaccinated animals exhibited severe pathology characterized by the presence of numerous large and small sized tubercles. In the case of MtbΔlpqS immunization the pathological damage was minimal compared to BCG vaccination.

Conclusions: The results of this study have established the efficacy of a *M. tuberculosis* mutant in imparting protection against PTB. Superior protection offered by *MTBΔlpqS* compared to BCG might be because of its construction from the human pathogen *M. tuberculosis* that has retained several of the antigens that BCG has lost over time. The aerogenic route of vaccination is more advantageous over other route of vaccination in that it may boost local and systemic immunity. In addition, immunization through aerogenic route results in bacilli rapidly encountering alveolar macropahges and other antigen presenting cells.

(vi) Vitamin D receptor gene polymorphisms and treatment outcome in PTB

Principal Investigator	:	Dr.P. Selvaraj (email: P.selvaraj53@yahoo.com)
Co-Investigators	:	Dr.P. Paul Kumaran; Dr.M. Harishankar; Dr.C. Ponnuraja; Dr.K. Chandrasekar
Source of funding	:	ICMR / Intramural
Study Period	:	2013- 2016

Background: Vitamin D_3 exerts its activity through vitamin D receptor (VDR), a nuclear hormone receptor Polymorphic variants of VDR gene have been shown to be associated with faster sputum conversion during ATT.

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

Study subjects: One hundred and eighteen 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 were available for the time points - pretreatment, 15 days, 30, 45, 60, 90, 120, 150, and 180 days of treatment were included for this study.

Methodology: Polymerase Chain Reaction (PCR) with Allele specific primers and Restriction fragment length polymorphism (RFLP) – methods were used to assess following VDR gene polymorphisms:

1. 5' Regulatory Region: Cdx2 & A-1012G
2. Coding Region: Fok I
3. 3' Untranslated Region: BsmI, ApaI & TaqI

The data collected were correlated with genotype frequencies of VDR gene polymorphisms to find out the role in sputum conversion during ATT (Table 31).

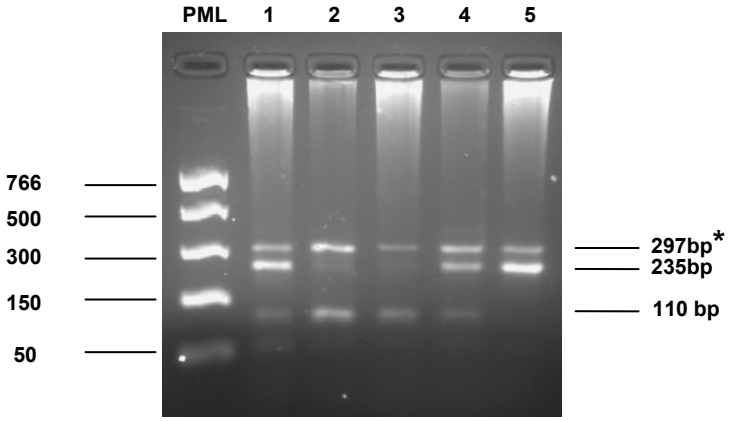
Table 31: Demographic details of the PTB patients

Patients recruited	Sex		**Total**
	Male	Female	
Number of patients	85	33	**118**
Age Groups			
<=30	26	24	50
31-40	26	06	32
41-50	25	01	26
=>51	08	02	10
Total	**85**	**33**	**118**

Results: 1. Genotyping of VDR gene polymorphisms: Based on the PCR and PCR-RFLP method, the genotype was assigned which is shown in Figs. 23 & 24. In Cdx-2 polymorphism, the homozygous genotype of the frequent allele 'G' yielded a 110 base pair (bp) size, whereas the homozygous genotype of the infrequent allele 'A' a 235 bp size and the internal control fragment t 297 bp. The heterozygous GA genotype yielded 2 bands of 110 bp and 235 bp size. For genotyping of *Bsm*I polymorphism, the homozygous genotype of the infrequent allele 'b' containing the restriction site yielded 2 bands of 650 bp and 175 bp sized. The homozygous genotype of the frequent allele 'B' which lacks the restriction site yielded a single band of 825 bp size. The heterozygous genotype Bb yielded 3 bands of 825 bp, 650 bp and 175 bp sized. In TaqI genotyping, the homozygous genotype of the frequent allele 'T' yielded 2 bands of 495 bp and 245 bp size and the infrequent allele 't' yielded 3 fragments of 290 bp, 205 bp and 245 bp size. The heterozygous

genotype Tt yielded 4 bands of 495 bp, 290 bp, 245 bp and 205 bp size.

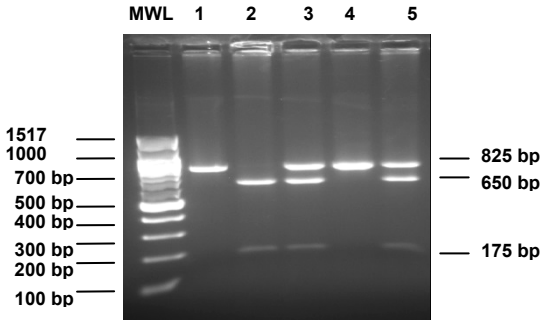
Fig. 23: Cdx-2 genotyping



PML - PCR marker ladder [New England Biolabs (NEB)]
Lanes 2&3 - Homozygous genotype for frequent allele (GG)
Lanes 1&4 - Heterozygous genotype (GA)
Lanes 5 - Homozygous genotype for infrequent allele (AA)
*Internal control

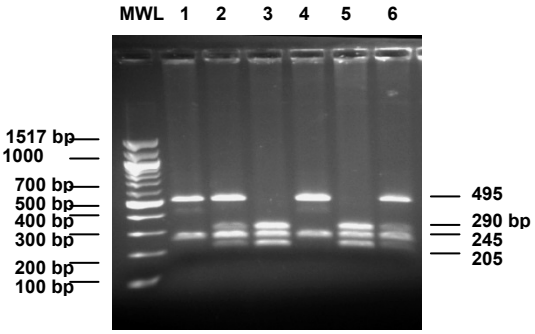
Fig. 24:

Genotyping of *Bsm*I polymorphism



MWL - 100 bp Molecular weight Ladder (NEB)
Lanes 1&4 - Homozygous genotype for frequent allele (BB)
Lanes 3&5 - Heterozygous genotype (Bb)
Lanes 2 - Homozygous genotype for infrequent allele (bb)

Genotyping of *Taq*I



MWL - 100 bp Molecular weight Ladder (NEB)
Lanes 1&4 - Homozygous genotype for frequent allele (TT)
Lanes 2&6 - Heterozygous genotype (Tt)
Lanes 3&5 - Homozygous genotype for infrequent allele (tt)

2. VDR gene polymorphisms and time to sputum conversion: The genotypes of different VDR promoter and 3' UTR polymorphisms were studied with time to sputum convertion which is shown in Table 32 In general, the VDR

polymorphic variants associated with time to sputum conversion, but the results were not significant. The 3' UTR polymorphisms BSMI "bb" (48 days), ApaI "aa" (48 days) and TaqI "tt" (52 days) genotypes were associated with faster culture convertion compared to "BB" (52 days), "AA" (53 days), and "TT" (55 days) genotypes. In translation initiation region polymorphism, FokI "ff" was associated with faster convertion (50 days) compared with FF (55 days) genotype. In the two promoter polymorphisms studied, Cdx-2 "GA" (52 days) genotype and GG genotype (48 days) of A1012G were associated with faster culture convertion compared to "AA" genotype (Cdx-2: 58 days; A1012G: 55 days).

Table 32: VDR genotypes associated with faster sputum conversion in PTB

VDR polymorphism	Genotype	Culture conversion day
3' UTR polymorphisms		
BsmI (B/b)	bb	48
ApaI (A/a)	aa	48
Taq1 (T/t)	tt	52
Translation initiation polymorphism		
FokI (F/f)	Ff	50
Promoter region polymorphisms		
Cdx-2 (G/A)	GA	52
A1012G	GG	48

Conclusions: The VDR polymorphism genotypes "bb", "aa", "tt", "ff", "GA" and "GG" were associated with faster sputum conversion during ATT. Future studies with larger sample size will be helpful to gain more knowledge to confirm this study finding.

(vii) Neutrophil mediated innate immune responses in TB: *In vitro* studies to understand the interaction of *M. tuberculosis* strains with neutrophils

Principal Investigator	:	Dr.D. Sulochana (email: sulochanadas@gmail.com)
Research Scholar	:	Ms. Nancy Hilda J.
Source of funding	:	ICMR / Intramural
Study period	:	2013-2016

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

Aims: (i) To compare the differential capacity of clinical MTB strains (S7, S10) and laboratory strain H37Rv to activate and enhance neutrophil functions

(ii) To understand the changes occurring in neutrophils during TB

Methods: Purified neutrophils were infected with *M. tuberculosis* strains at MOI of 3 and the activation was studied at early time point of 4 hrs and late time point of 20 hrs. Uninfected neutrophils served as negative control and PMA stimulated cells were used as positive control. Cell phenotyping was done by flow cytometry. The cytokines like IL-1β, IL-8, TNF, MCP-1 and MIP-1α were measured in infected neutrophil culture supernatants (Nu sups) using commercial ELISA kits.

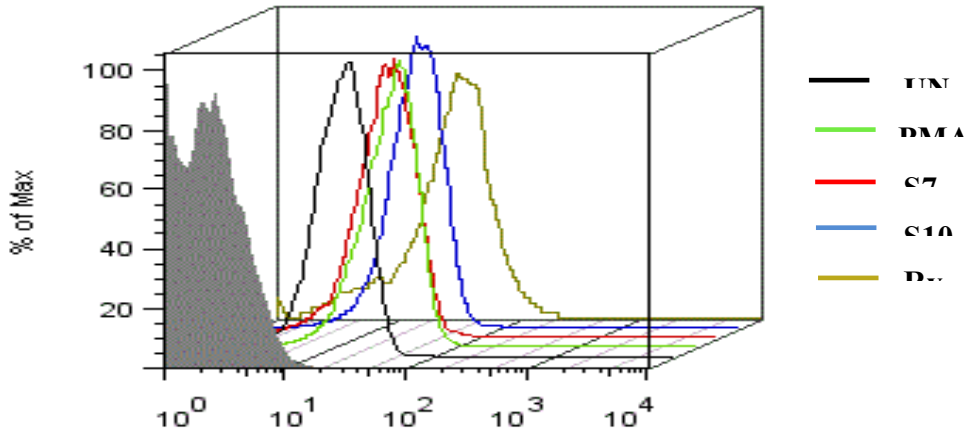
Results: Neutrophils being initial immune cells to encounter MTB, they are naturally expected to undergo changes in their pathogen recognition receptors after exposure. As expected, neutrophils from newly diagnosed PTB patients (PTB neutrophils) showed significantly increased expression of TLR 1, 2 and 4 compared to neutrophils from healthy volunteers (Normals) (Figs. 25 - 27). At early time point, all MTB strains were effective in altering the

expression of TLR 1, 2 and 4 in both the groups (normals and PTB neutrophils). But, at later time point only H37Rv was effective on TLR expression of neutrophils. Similar to TLR expression, the PTB neutrophils showed a significant increase in release of cytokines compared to normals (Figs. 28, 29). The secretion of chemokine MIP-1α was increased by S10 and H37Rv only in PTB neutrophils whereas all the strains increased the release of TNF. Only H37Rv increased the secretion of IL-1β and IL-8 in both the groups.

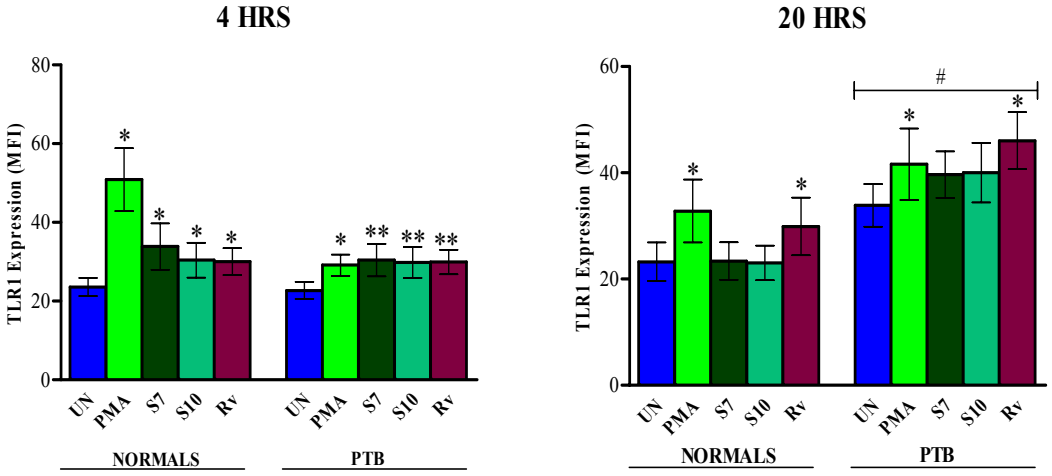
Conclusion: H37Rv is more effective in activating neutrophils. Neutrophils undergo immunological modulation as a result of TB infection.

<u>Fig. 25:</u> Expression of TLR1 by M.tb infected neutrophils

(a) Representative scatter gram showing the expression of TLR1



(b)



Un- Uninfected

* Compared with respective UN neutrophil

*P<0.05, **P<0.001

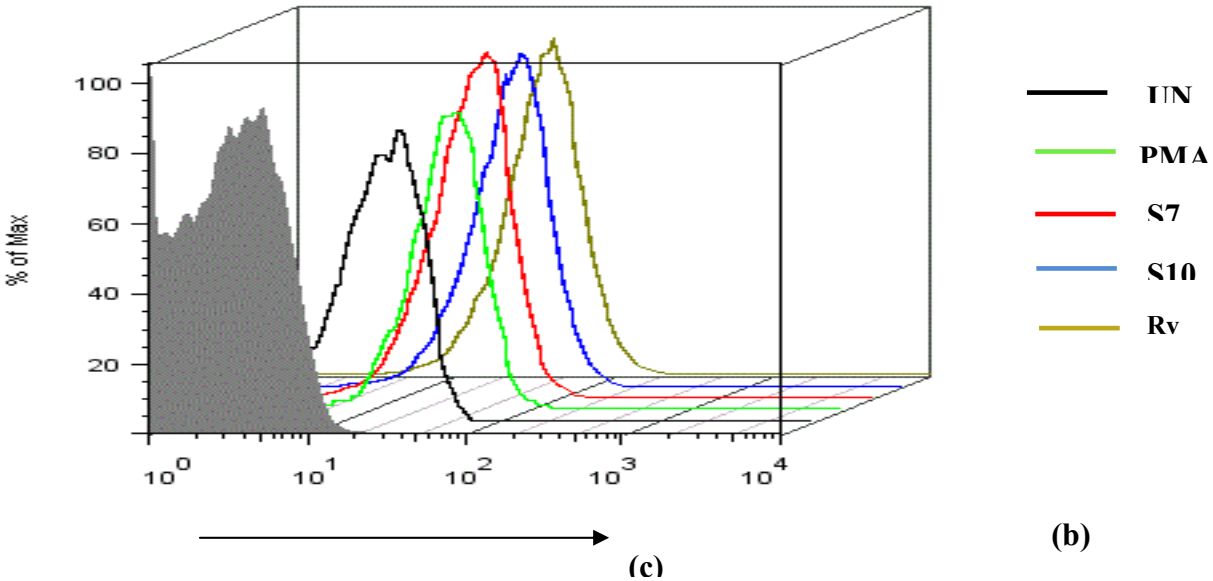
\# Compared with respective Normal neutrophil

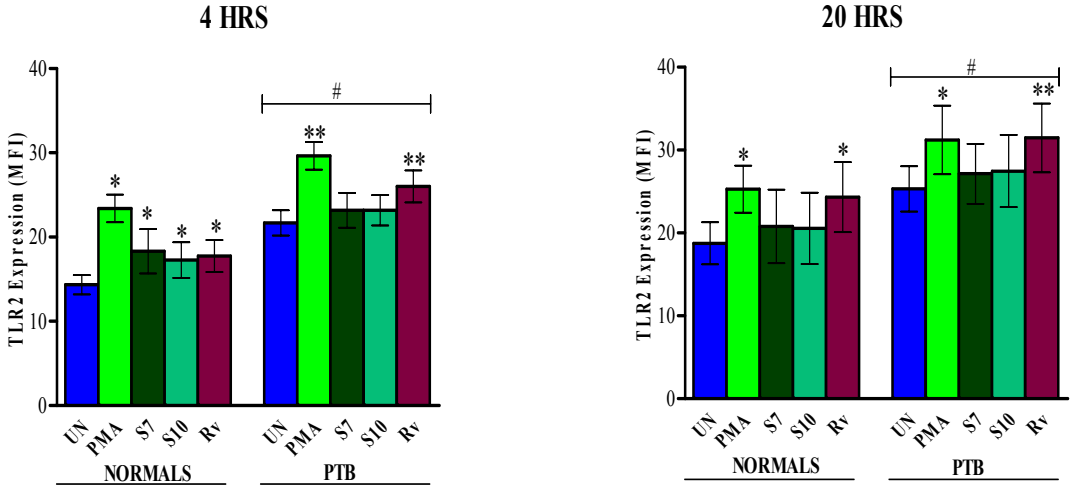
# P<0.05

The expression of TLR1 by normal (N=19) and PTB neutrophils (N=18), stimulated with PMA and infected with S7, S10 and H37Rv at a MOI of 3 is shown in figure. A representative staggered histogram showing the expression of TLR1 is given in (a), with filled peak representing unstained cells. The MFI values for surface expression of TLR1 by neutrophils at 4h (b) and 20h (c) are represented as Mean ± SEM in bar graphs. P<0.05 is considered to be statistically significant.

Fig. 26: Expression of TLR2 by M.tb infected neutrophils

(a) Representative scatter gram showing the expression of TLR2





Un- Uninfected

* Compared with respective UN neutrophil

\# Compared with respective Normal neutrophil

*P<0.05 , **P<0.001

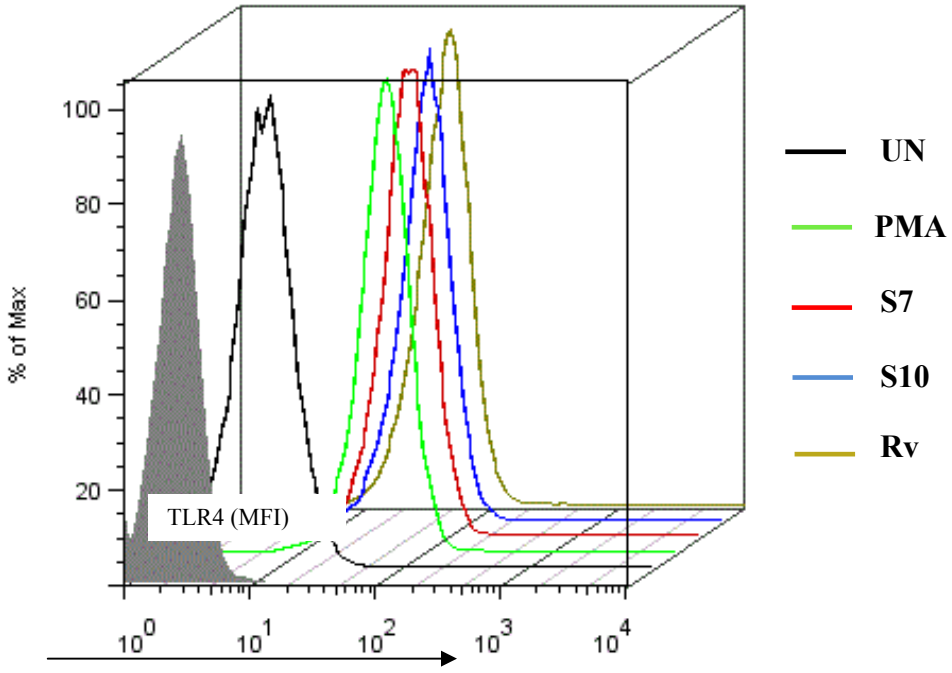
# P<0.05

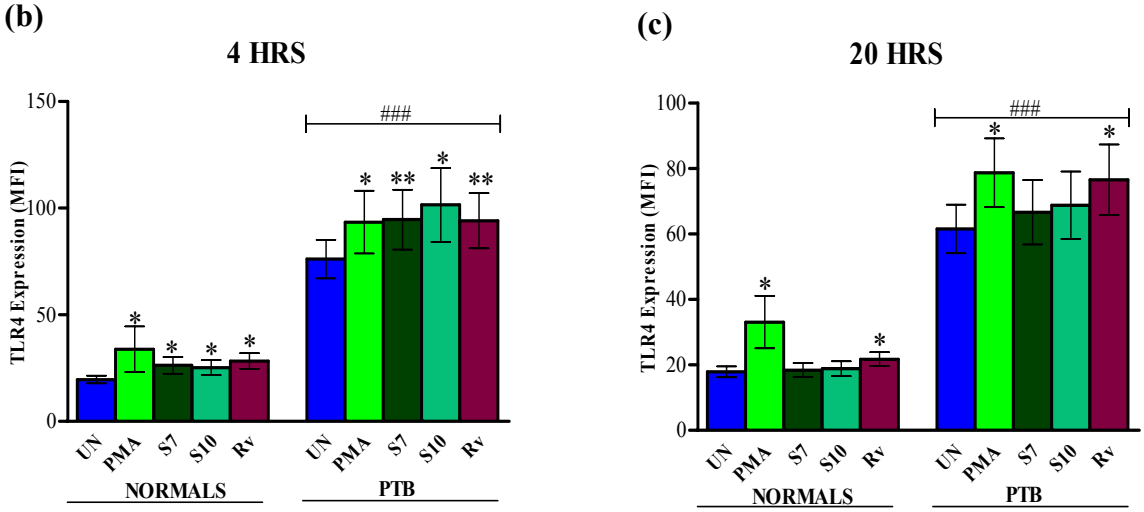
The expression of TLR2 by normal (N=19) and PTB neutrophils (N=18), stimulated with PMA and infected with S7, S10 and H37Rv at a MOI of 3 is shown in figure. A representative staggered histogram showing the expression of TLR2 is given in (a), with filled peak representing unstained cells. The MFI values for surface expression of TLR2 by neutrophils at

4h (b) and 20h (c) are represented as Mean ± SEM in bar graphs. P<0.05 is considered to be statistically significant.

Fig. 27: Expression of TLR4 by M.tb infected neutrophils

(a) Representative scatter gram showing the expression of TLR4





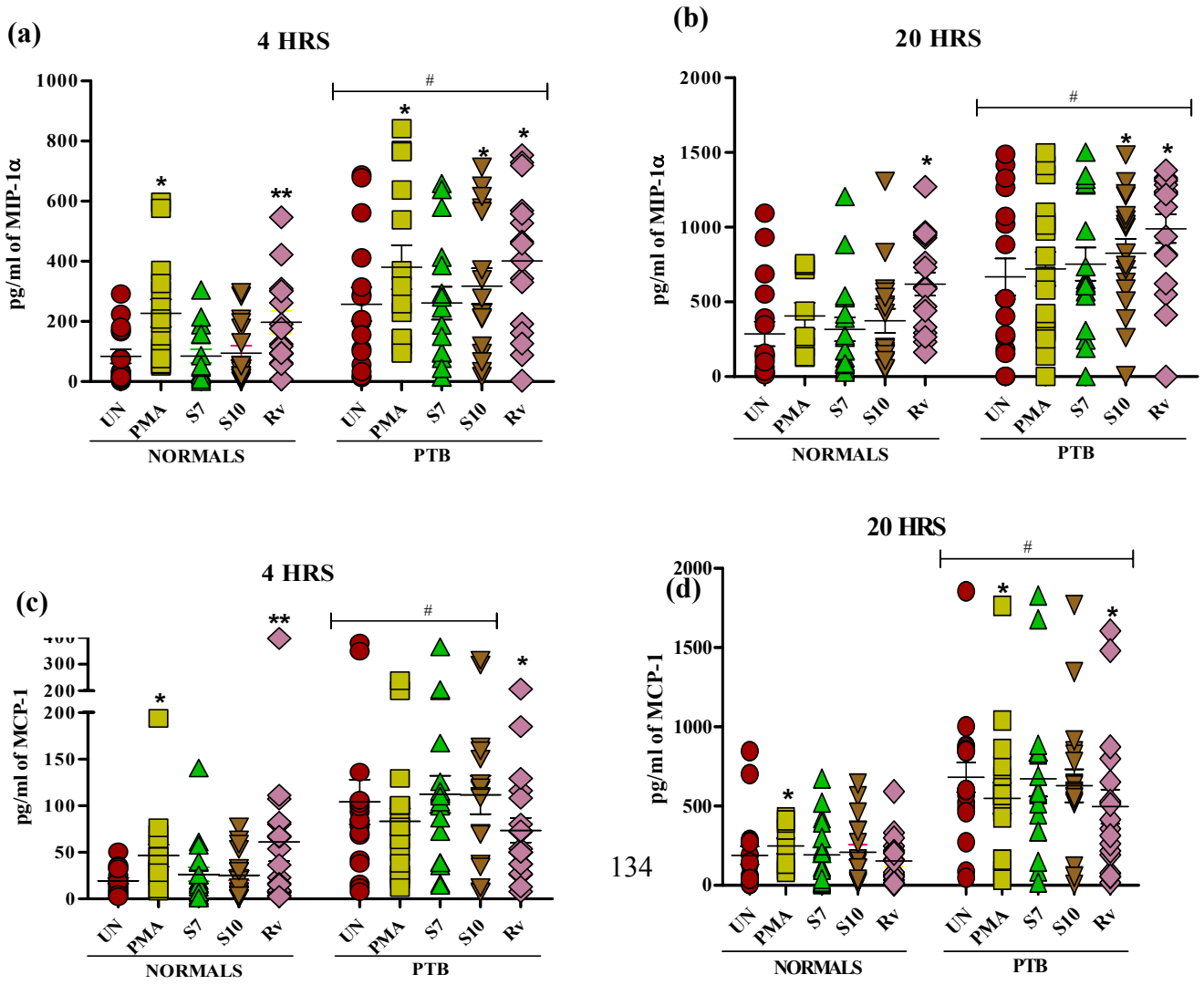
Un- Uninfected

* Compared with respective UN neutrophil # Compared with respective Normal neutrophil

*P<0.05 , **P<0.001 ### P<0.0001

The expression of TLR4 by normal (N=19) and PTB neutrophils (N=18), stimulated with PMA and infected with S7, S10 and H37Rv at a MOI of 3 is shown in the figure. A representative staggered histogram showing the expression of TLR4 is given in (a), with filled peak representing unstained cells. The MFI values for surface expression of TLR4 by neutrophils at 4h (b) and 20h (c) are represented as Mean ± SEM in bar graphs. P<0.05 is considered to be statistically significant.

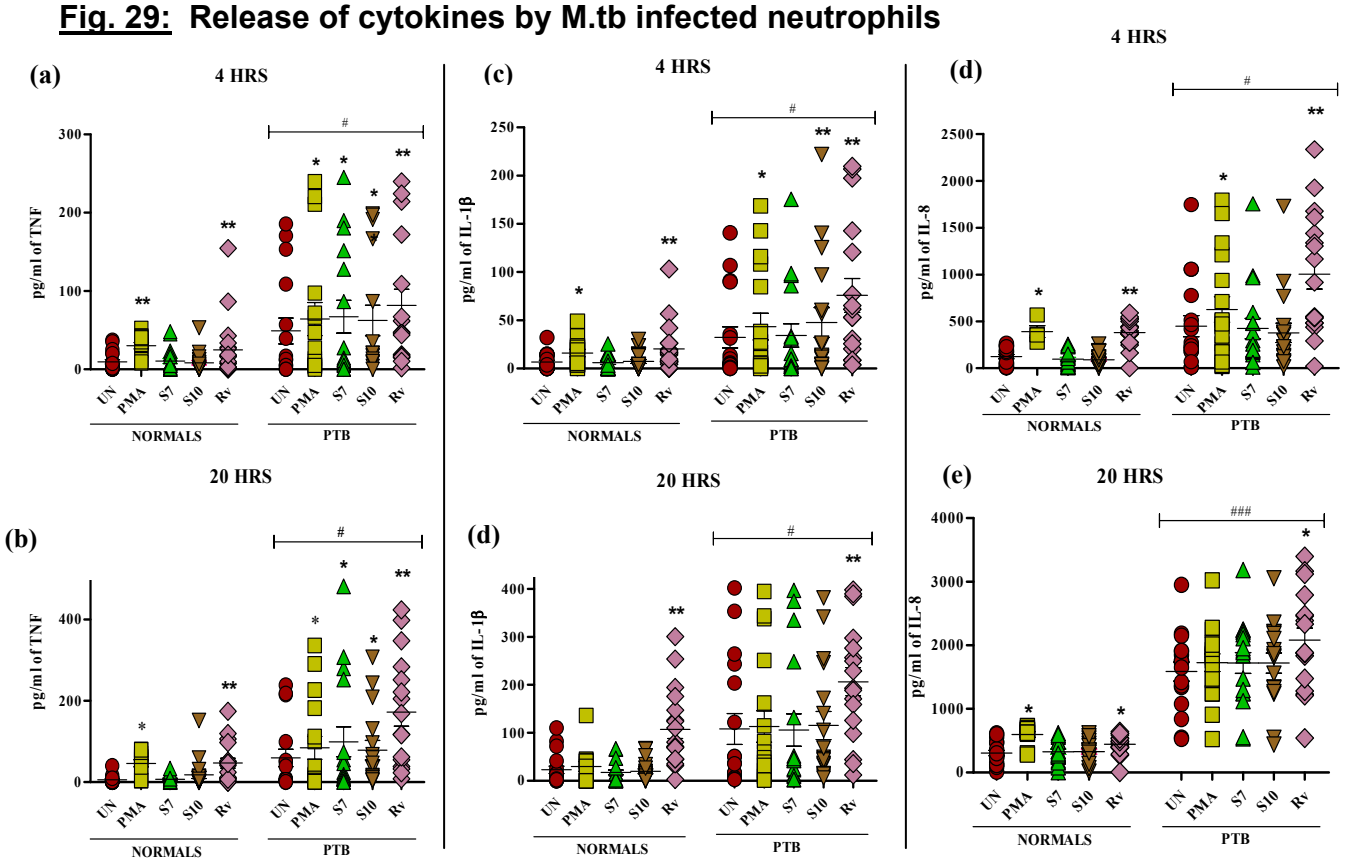
Fig. 28: Release of monocyte attracting chemokines by M.tb infected neutrophils



Un- Uninfected
* Compared with respective UN neutrophil # Compared with respective Normal neutrophil
*P<0.05, ** P< 0.001 # P<0.05

The figure shows secretion of MIP-1α and MCP-1 by normal (N=19) and PTB (N=18) neutrophils stimulated with PMA and infected with S7, S10 and H37Rv at a MOI of 3 for 4h and 20h. The culture supernatants were used to find the secretion pattern of MIP-1α (a,b) and MCP-1 (c, d) by infected neutrophils at 4hrs and 20hrs. The levels of MIP-1α and MCP-1 are represented as vertical scatter plots with mean ± SEM values.

Fig. 29: Release of cytokines by M.tb infected neutrophils



Un- Uninfected
* Compared with respective UN neutrophil # Compared with respective Normal neutrophil

The figure shows secretion of TNF (a, b), IL-1β (c, d) and IL-8 (e, f) by normal (N=19) and PTB (N=18) neutrophils stimulated with PMA and infected with S7, S10 and H37Rv at a MOI of 3 for 4h and 20 hrs. The culture supernatants were used to find the secretion pattern of cytokines by infected neutrophils at 4 hrs and 20 hrs. The levels of TNF, IL-1β and IL-8 are represented as vertical scatter plots with mean ± SEM values.

STUDIES IN PROGRESS:

I-1: Structural characterization of three essential genes from *M. tuberculosis*

Principal Investigator	:	Dr. Alamelu Raja (email: alameluraja@gmail.com)
Research scholar	:	Ms.G. Akilandeswari
Source of funding	:	Intramural / Inspire Fellowship
Study Period	:	2012-2017

Background: Latent TB pose risk in the society as it may lead to outbreak of the active TB disease during the life time of an infected person. Most of the metabolic pathways are shut down during dormancy, the non-replicative stage of *M. tuberculosis*, while few genes are expressed or over-expressed for the survival of the bacilli. Thus, studying the dormancy associated genes and antigens would aid us to understand the biology of dormant bacilli and devise any prophylactic and therapeutic intervention. In this connection, we have initiated a study where over-expressed proteins and genes are characterized under hypoxia. We have used H37Rv, the laboratory strain, and the two south Indian prevalent strains of *M. tuberculosis*-S7 & S10).

Aims: (i) Cloning of three dormancy associated antigens (Rv1294, Rv2949c, Rv2515c) in pET28a

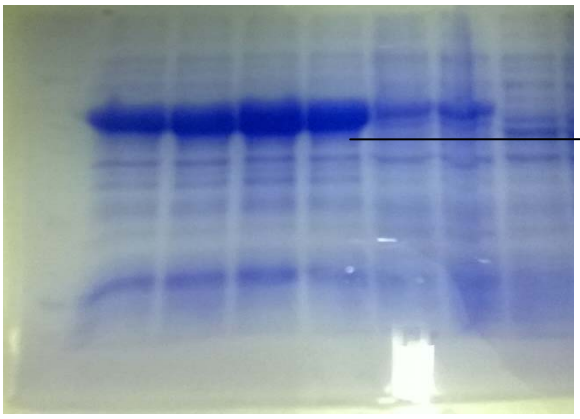
(ii) Expression of these three genes in *E. coli* and purification of the gene products using Ni-NTA Agarose

(iii) Crystallization and X-Ray diffraction studies on the proteins purified

Methodology: The sequence confirmed recombinant plasmids of the respective genes of interest (Rv1294, Rv2949c, Rv2515c) were transformed into an expression strain, BL21 DE3. The overnight culture was then inoculated into a fresh LB medium, grown at 37ºC for 3 to 4 hrs till the OD of the culture reaches 0.5. The culture was then induced with IPTG and time kinetics was done at various temperatures (37ºC, 30ºC, 23ºC) and different time points (4-6 hrs, 8-10 hrs, 12-24 hrs respectively) (Fig. 30). At each time point, samples were drawn and run on a 12% SDS-PAGE. The condition at which there was maximum expression of the protein was considered for further expression and purification (Fig. 31).

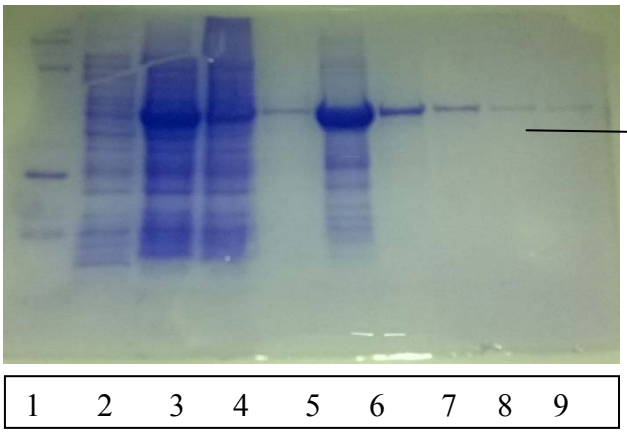
Results:

Fig. 30: Time kinetics of Rv1294 recombinant protein at 23°C



1. Protein mol weight marker
2. 23°C 9hrs post induction
3. 23°C 12hrs post induction
4. 23°C 14hrs post induction
5. 23°C 16hrs post induction
6. 23°C 18hrs post induction
7. 23°C 24hrs post induction
8. Uninduced

Fig. 31: Purified protein fractions of Rv2515c after induction (37ºC, 3hrs)



1. protein mol weight marker
2. Uninduced
3. Crude lysate
4. Flow through
5. Wash1

6-10 Eluted protein fractions

Once the condition of expression is standardised, intensive purification of the proteins has to be done as the quality and quantity of the protein should be good enough for crystallizing the proteins.

Future directions:

- Crystallization and X-Ray diffraction of all the proteins.
- Modelling, docking and MD simulation of these proteins using bioinformatics tools for latent TB drug targeting.

I-2: Cytokine gene polymorphisms in HIV and HIV-TB

Principal Investigator	:	Dr.P. Selvaraj (email: selvarajp@nirt.res.in)
Co-Investigators	:	Dr.M. Harishankar; Dr. Soumya Swaminathan
Source of funding	:	Intramural
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. 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 TB are meagre in Indian population. In the present study, various cytokine gene polymorphisms are being carried out in HIV and HIV-TB co-infection in south Indian population. This study will be carried out in 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 TB (HIV+TB-), (n= 150)
2. HIV-1 seropositive patients with TB (HIV+TB+), (n=115)

3. HIV-1 seronegative patients with PTB (HIV-PTB+) (n=150)
4. Healthy controls (n=150)

Cytokine gene polymorphisms are being studied by allele specific PCR method using stored DNA samples extracted from the patients and controls (Table 33).

The study is in progress.

Table 33: Cytokine gene polymorphisms in controls & HIV-infected patients with or without TB

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
5. IFN-γ +874 (A/T)	148	106	150	150
6. IP-10 -1447 (A/G)	148	106	150	150
7. IP-10 -872 (G/A)	148	106	150	150
8. IP-10 -135 (G/A)	148	106	150	150

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

I-3: Prevalence of TB (*M. tuberculosis* & *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; Dr Sujatha Narayanan (chandrakumar.d@nirt.res.in; baskar.d@nirt.res.in; sbalaji@nirt.res.in; sujatha.sujatha36@gmail.com)
Colloborators	:	Dr. Dhinakar Raj; Dr.Maroudham, Tamil Nadu 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, 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 transmit the infection to cattle and humans. Similar to animal-human transmission, human–animal transmission occurs at locations of active animal-human interaction. This is posing a formidable challenge in controlling and eradicating myco-bacterial 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 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* from 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. 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.

Spoligotyping: Direct locus - DRa (0.2 µmol/µl) and DRb (0.2µmol/µl) primers were used for spoligotyping. The spacers between the direct repeats in the target region were amplified by using two 18-nucleotide primers (primer 5'-CCAAGA GGGGACGGAAAC-3' and biotinylated primer 5'-GGTTTTGGGTCT GACGAC-3'). The PCR products were then hybridized to a Biodyne C membrane (Isogen Bioscience, Maarsen, The Netherlands). This membrane contains immobilized synthetic oligomeric spacer sequences derived from the direct-repeat region of *M. tuberculosis* H37Rv and *M. bovis* BCG. Hybridized DNA was detected by using an enhanced chemiluminescence kit (Biobasic, Israel), with exposure to X-ray film producing a pattern or profile reminiscent of a bar code. The hybridization pattern was analyzed using SPOTCLUST (http://tbinsight.cs.rpi.edu/run_spotclust.html) database as per the standard procedure.

Results: A total 271 animal handlers were screened, of whom 6 were positive for TB. A total of 207 cattle were screened by comparative tuberculin skin testing, of whom 23 animals were positive for TB infection. Spoligotyping was perfomed using DNA samples from tissues, milk and nasal swab of animal origin and *M. tuberculosis* isolates from animal handlers. Results indicated the presence *M. tuberculosis* in bovine; majority belonging to Manu 1, EAI_5 EAI_3 IND and orphan types. The *M. tuberculosis* isolates from animal handlers belonged to EAI_3 and

EAI_2 spoligotypes. These results indicate the presence of same strains in ruminants and human in the same geographical region. This was suggestive of the existence of an active 'spill-over' mechanism of *M. tuberculosis* infection in bovines. The study is in progress.

DEPARTMENT OF STATISTICS

COMPLETED STUDIES:

(i) Performance accuracy of classifiers in sustaining disease conversion parameters using clinical trial TB data: Data mining approach

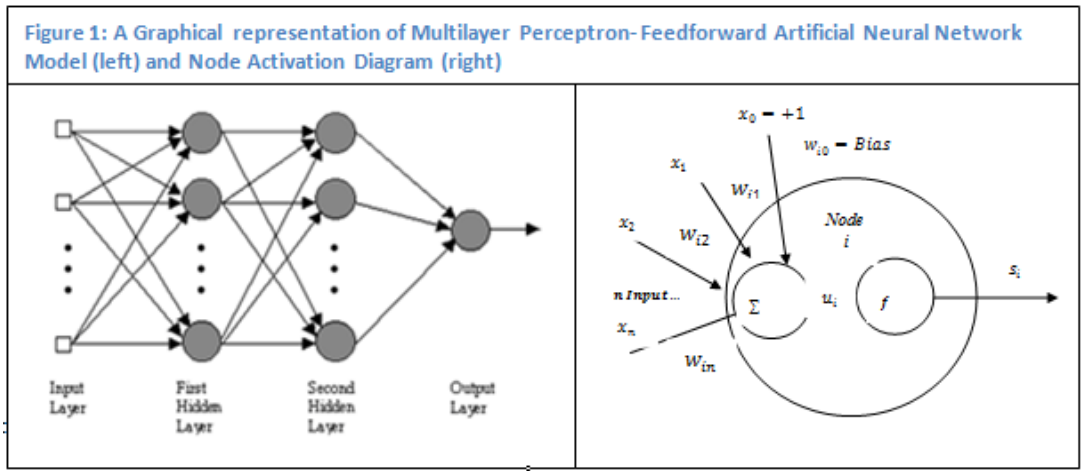
Principal Investigator : Dr. C. Ponnuraja
(email: cponnuraja@nirt.res.in)
Funding : Intramural
Study period : 2015-2016

Background: Data mining applications can greatly benefit all stakeholders involved in the healthcare industry. It can help physicians to identify risk factors, guide effective treatments and best practices in health care industry which can help patients to receive better and affordable healthcare services. The huge amounts of data generated by healthcare transactions are too complex and voluminous to be processed and analyzed by traditional methods. Data mining is the only method which provides the technology to transform mounds of data into useful information for decision making.

Aim: To compare various classification techniques with respect to performance accuracy in order to develop predictive modelling with clinical trial TB data

Methods: The classification of TB patients is of substantial importance in TB disease conversion. During the last few years, many algorithms have been proposed for this task. This project reviewed different supervised machine learning techniques and their performance accuracies for classification with an original dataset and carry out a methodological comparison using “WEKA” software. We used the C4.5 (J48) tree classifier, Iterative Dichotomiser- 3 (ID3), a Multilayer Perceptron (MLP) (Fig.32) and a naive Bayes classifier over a large set of TB data.

<u>Fig. 32:</u> A graphical representation of multilayer perceptron-feed forward artificial neural network model (left) and node activation diagram (right)

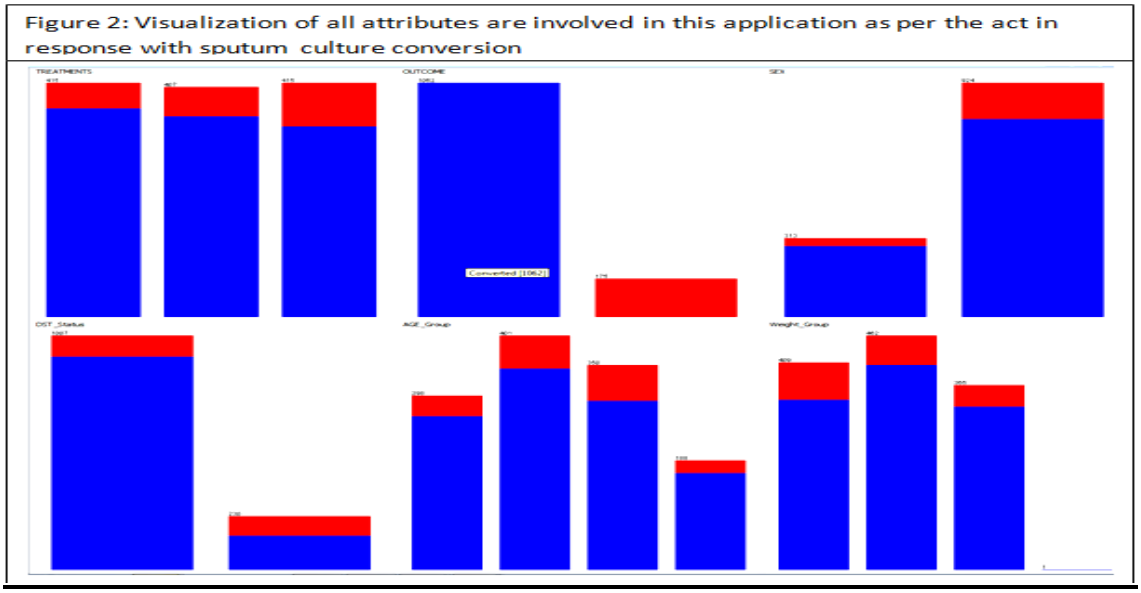


Clinical Trial TB Data: The data used in this work was the randomized controlled clinical trial PTB data from NIRT. Eligible patients were randomly allocated into three different regimens including the standard RNTCP regimen as control with two more trial regimens of six months duration each [Tuberculosis Research Centre (2004)]. All patients were assessed clinically and bacteriologically every month up to six months. In this application there were 1237 patients with variables such as *age* (years) and *weight* (kg) *sex* (male/female)at baseline DST (DST: sensitive to all drugs and resistant any one drug) sputum culture conversion ("converted" and "not converted"). The treatment groups were three – Treatment A Treatment B and control (Table 34 & Fig. 33).

Table 34: Attributes of sputum culture conversion in Randomized controlled clinical trial PTB data

Attributes	Converted	Not Converted
	1062(86%)	175(14%)
Regimens		
Treatment A	369	46
Treatment B	338	77
Control	355	52
Sex		
Female	281	32
Male	781	143
Pre-Treatment DST		
sensitive	916	91
resistant	146	84
Age groups		
Less & Equal 24	263	35
25-34	344	57
35-45	289	60
More & Equal to46	166	23
Weight groups		
Less & Equal to 38	336	73
39-44	404	58
More & Equal to44	322	44

Fig.33: Visualization of attributes responsible for sputum culture conversion



The results suggested that the naive Bayes classifier has more accuracy compared to ID3 and J48 classifiers but MLP is almost close to naive Bayes (Table 35). ID3 has the lowest model performance accuracy compared to all others classifiers. The Kappa statistic value is closer between naive Bayes and MLP. The values of Mean Absolute Error, Root Mean Squared Error, Root Absolute Error, Root Relative Squared Error for naive Bayes were comparatively lower when compared to the values of MLP, ID3 and J48. This suggests that the naive Bayes and MLP algorithms are suitable for prediction of disease conversion for TB. However the other two algorithms were likely to be close to naive Bayes and MLP algorithms. According to the ROC area, naive Bayes and MLP algorithms have similar values.

Table 35: Overall performance accuracy between classifiers

Stratified cross-validation summary	**Naïve Bayes**	**Multi layer Perceptron**	**J48**	**Id3**
Total Number of Instances	1237	1237	1237	1237
Correctly Classified Instances	**86.2 %**	**86.0%**	**84.8%**	**84.3%**
Kappa statistic	0.08	0.07	0.16	0.09
Mean absolute error	0.2154	0.2151	0.2096	0.214
Root mean squared error	0.329	0.328	0.348	0.351
Root relative Absolute error	88.48 %	88.38 %	86.13 %	88.25 %
Root relative squared error	94.46 %	94.35 %	100.01 %	101.09%

Table 36: Performances accuracy between classifiers with their confusion matrices for the status of sputum culture conversion

Sputum Culture Status	***Predicted (→)***		***TP (%)***	***FP (%)***	***ROC***	***Precision%***
Actual (↓)	Converted **(Negative)**	Not Converted **(Positive)**				
Naive Bayes						
Converted **(Negative)**	**TN**=1055	**FP**=7	99.3	94.3	71.8	86.5
Not Converted **(Positive)**	**FN**=165	**TP**=10	5.7	7.0	71.8	58.8
Weighted Average			86.1	81.0	71.8	**82.6**
Multilayer Perceptron						
Converted **(Negative)**	**TN**=1022	**FP**=40	96.2	86.3	64.6	87.1
Not Converted **(Positive)**	**FN**=151	**TP**=24	13.7	3.8	64.6	37.5
Weighted Average			84.6	74.6	64.6	**80.1**
J48						
Converted **(Negative)**	**TN**=1058	**FP**=4	99.6	99.4	52.4	85.9
Not Converted **(Positive)**	**FN**=174	**TP**=1	6.0	4.0	52.4	20.0
Weighted Average			85.6	85.4	52.4	**76.6**
ID3						
Converted **(Negative)**	**TN**=1029	**FP**=33	96.9	89.1	61.6	86.8
Not Converted **(Positive)**	**FN**=156	**TP**=19	10.9	3.1	61.6	36.5
Weighted Average			84.7	77.0	61.6	**79.7**

Table 36 demonstrates precision based on sputum culture conversion status through confusion matrices. The confusion matrix in predictive analytics is a table with two rows and two columns that reports the number of false positives (FP), false negatives (FN), true positives (TP), and true negatives (TN).

TP is the proportion of positive cases that are correctly identified and TN is the proportion of negative cases that are correctly identified. FP and FN are also called type I error and type II error respectively. TP (%) and FP (%) are the rates of TP and FP respectively. ROC examines the performance of classifiers as an additional way besides the confusion matrix. It examines with the false positive rate and the true positive rate. A non-parametric classifier is represented by a single ROC point, corresponding to its pair of *FP*, *TP*. The point (0,1) is the perfect classifier: that classifies all positive cases and negative

cases correctly and the point (1,0) is the classifier that is incorrect for all classifications. Naive Bayes and Multilayer Perceptran are identified as the better classifier based on the percentage of precision (82.6% and 80.1% respectively) and ROC area (71.8 and 64.6 respectively) on the criteria of "Weighted Average" approach.

Discussion & Conclusion: The study was done with a dataset from patients infected with TB; we got different results for each classifier. Experiments were performed mainly to identify the best classifier for predicting patients with TB. These classifiers, for instance, Naive Bayes, ID3, J48 and MLP were used for providing incomparable impression as well as to identify relatively appropriate classifiers among the four. Naive Bayes and MLP classifiers were performing well especially in correctly classifying the instance for sputum culture conversion. It was also observed that these two approaches performed better in many ways when compared to other two methods. In the aspects of correctly classified instances, accuracy, precision and area of ROC are the evidence for identifying better performance among classifiers. According to these criteria, Naive Bayes and MLP possessed consistency as well as individuality in all aspects. Moreover, the Naive Bayes and Multilayer Perceptron classifiers were remarkably effective and showed a good performance for this dataset. We propose these two approaches are the better to predict the performance based on sputum culture conversion among patients with TB by means of data mining classification technique.

STUDIES IN PROGRESS:

S-1: Systematic review with Meta-Analysis: success, failure and relapse rates with regimens in TB trials

Principal Investigator	:	Dr.C. Ponnuraja (email: cponnuraja@nirt.res.in)
Co-Principal Investigator	:	Dr.P. Paul Kumaran
Study period	:	2015-2016

Aim: To assess treatment patterns using clinical trial data for suggesting therapeutic recommendations

Methodology: We identified all SCC randomised controlled clinical trials performed on TB patients at NIRT, Chennai, India. Each reported results from numerous trials over a period between 1974 and 2012. All these trials have been categorized into two groups based on their treatment segment. This will also make comparisons with similar trials reported globally.

Status: Data extraction from different sources is in progress

S-2: Bayesian survival models for longitudinal TB data

Principal Investigator	:	Dr.C. Ponnuraja (email: cponnuraja@nirt.res.in)
Study period	:	2015-2016

Aims: To develop a new method for longitudinal responses with respect to survival modelling, to account for heterogeneity, and to develop an equivalent Bayesian approach which could support the above two for time to culture conversion

Methodology: The main source of data was from existing NIRT clinical trials of TB. This was used to compare empirically the survival difference between two or more groups irrespective of all categorical covariates like sex, treatment etc. under various model assumptions. All covariates collectively are considered for survival modeling techniques under various model assumptions. The primary event of interest is time to sputum conversion during treatment period. The covariates considered for both categorical and continuous types were age, sex, treatment groups, weight at baseline, pre-treatment drug susceptibility pattern), sputum culture grade at baseline (low grade and high grade), smoking status, alcoholic status, CXR etc. Clustered covariate, age group will be considered mainly for the applications of frailty model.

Status: The final stage of Bayesian analysis and its interpretation are in progress.

DEPARTMENT OF EPIDEMIOLOGY

COMPLETED STUDIES:

(i) 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.

According to the RNTCP Annual Report, 2011, it is estimated that nearly 8% are known to be HIV-infected among all TB patients tested. Also, treatment outcomes among HIV-infected TB patients treated under programmatic conditions show low failure rates but high case fatality.

Despite having relatively well performing national programs in place, there is inadequate data on HIV-TB co-infection and risk factors for poor treatment outcomes among these patients in India. 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.

To address this, we conducted a retrospective cohort study to determine the TB treatment outcomes among patients with HIV-TB co-infection at the end of ATT. This study was identified possible factors associated with such outcomes.

Study Objectives:

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

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

Methodology: The study was a retrospective record review of the TB-

HIV co-infected patients initiating TB treatment between January – December 2012.

Population: TB-HIV co-infected patients ≥ 15 years from ART centres in Tamil Nadu initiating on treatment between January – December, 2012 and having complete record of treatment outcomes were included in the study.

Data collection and management: Data was collected using data extraction format prepared in Microsoft Excel. The format was developed by considering variables to be studied which were found in the ART card of the patients. Exact information from the card was recorded in the Microsoft Excel format by the concerned staff at each ART centre and the data was checked for completeness by the principal investigator.

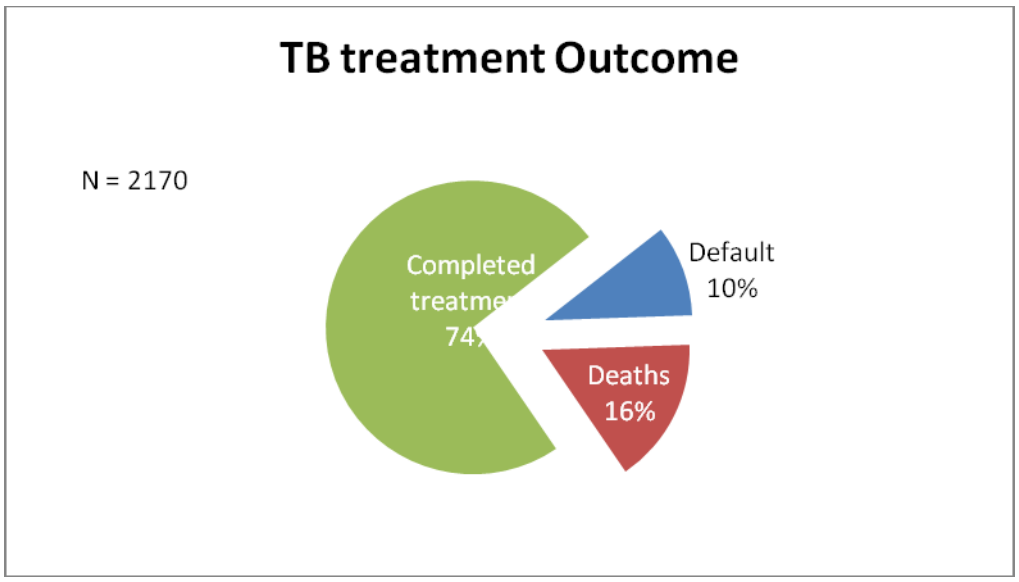
Data analysis: Data was entered, cleaned in Microsoft Excel 2013 and analysed using SPSS version 16. Descriptive statistics aimed to summarize patients' characteristics aross different categories of the independent variables were calculated.

To assess the relation of each predictor variable and the treatment outcome, univariate logistic regression model was used. Those variables with a p-value of less than 0.05 were included in the multiple logistic regression model by backward stepwise entry method. A chi-square test was also performed to assss the association between the dependent and individual variables and combination of them to identify the significance of predictor variables.

Ethical considerations: Ethical clearance was obtained from the Institutional Ethics Committee of the NIRT. Supporting letters were obtained from National AIDS Control Organizations.

Main findings: Data from 49 of 52 ART centres of Tamil Nadu were received. Information of 2340 TB-HIV co-infected patients were obtained. After excluding subjects <15 years old and all those who did not have information on the TB treatment outcome, the number available for analysis was 2170.

Fig.34 : TB treatment outcome data in 2170 patients



650 (31.0%) TB-HIV patients were diagnosed with TB while on ART. 990 (47.2%) initiated ART within 90 days of TB treatment, 214 (10.2%) initiated ART after 90 days of initiation of TB treatment and 245 (11.7%) had not yet started ART (Fig. 34).

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

Unfavourable TB treatment outcome was defined as those who had died, failed or defaulted during TB treatment.

Univariate regression analysis showed that being male, having low BMI and low education level were significant factors contributing to an unfavourable TB treatment outcome. Also, smear positive PTB patients on Category II treatment were at more risk for unfavourable TB treatment outcome.

Low CD4 count at the initiation of TB treatment and not being on ART at the initiation of ATT are possible risk factors for unfavourable TB treatment. TB being diagnosed within two years of HIV diagnosis was also a risk factor for unfavourable TB treatment outcome.

Multiple logistic regression was done to determine the adjusted effects of the independent variables on unfavourable TB treatment outcome. Low BMI, low educational level, habitual smoking, poor functional status at baseline and being on Category II TB treatment were

significant risk factors in the multiple logistic regression.

Conclusion: There was high rate of death (16%) or overall unfavorable treatment outcome (death, defaulting and failure = 26%) among TB/HIV co-infected. This is a serious public health concern that needs to be addressed.

STUDIES IN PROGRESS:

E-1: Prevalence survey of PTB in Tiruvallur district

Principal Investigator	:	Dr.C.K. Dolla (email: chandrakumar.d@nirt.res.in)
Source of fundnig	:	Model DOTS Project and Intramural
Study Period	:	2015 - 2017

Objective: The primary objective of the survey is:

(i) To estimate current prevalence of bacteriologically positive TB among adults aged ≥ 15 years in Tiruvallur district;

(II) To determine the prevalence of TB infection among children aged < 15 years in TB affected households.

Methods: We are doing a community based cross-sectional survey in the villages/urban areas in five blocks of Tiruvallur district where the impact of SCC and DOTS is being monitored over a period of 15 years under Model DOTS project. Our sample size is 84,000 population and we are implementing a cluster design for this survey. All adults above 15 years undergo digital Chest X-rays to screen for pulmonary lesions. Sputum samples are being collected from those who are symptomatic or who have abnormal chest X-ray. Smear, culture and drug sensitivity for all the sputum samples are being carried out. TB cases identified through the survey will be referred to the nearest RNTCP centre for initiating treatment. Household contacts (<15 years) of TB cases will also be screened for TB.

Data collectIon: From July 2015 to May 2016 we have covered a population of 35,521. Among them 26,475 eligible adults, 21,590 (82%) subjects have been X-rayed and a total 4259 sputum specimens have been collected from 2426 eligible individuals based on both symptomatic and X-ray confirmation. Seventy two (72) participants have been diagnosed as TB based on the sputum results and were referred to RNTCP for starting on TB treatment. The study is ongoing.

BIOMEDICAL INFORMATICS

STUDIES COMPLETED:

(i) *In silico* analysis of rifampicin resistance in *M. tuberculosis*

Principal Investigator: Dr. Luke Elizabeth Hanna
(email: hanna@nirrt.res.in)
Source of funding : Intramural
Study period : 2014-2016

Background: Rifampicin (RIF) is an important drug used to treat TB. RIF binds to the β-subunit of DNA-dependent RNA polymerase (RNAP), close to the RNA/DNA channel and inhibits RNAP of bacterial but not of mammalian origin, by physically blocking the elongation of the growing RNA chain. 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). 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 27 amino acids. It is of interest to study the interactions between the clinical mutants (MTs) of RpoB and RIF to see 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 RMP and investigate the basis of RIF resistance

Methodology: A homology model was generated with the crystal structure of Rif 2A68 (with 3 domain structure) for the WT, and D516V, L521M, H526D, H526Y, H526R, S531L and L533P mutant structures generated using 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: Docking of RIF with WT and MTs showed higher values with WT as compared to all MTs, 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. Molecular docking simulation for WT RpoB protein with RIF gave a binding energy of -22.88 kcal/mol.

(ii) TB – Drugs: Database of drugs for TB

Principal Investigator : Dr. Jagdish Chandrabose
(email: sjcbose@gmail.com)
Source of funding : ICMR Biomedical Informatics Project
Study period : 2015-2016

Background: The successful control of TB is being threatened by the emergence of drug resistant forms of *M. tuberculosis*. WHO (2015) estimated that a total of 111 000 people were started on MDR-TB treatment in 2014, which is an increase of 14% compared to 2013. Further, the burden of TB in the form of XDR-TB is also emerging, with cases reported from 105 countries till the end of 2014 (WHO 2015). Various combinations of drugs are being used for the treatment of different forms of TB. Currently there are about 28 different drugs in use for TB treatment, and over 10 drugs in various stages of the drug discovery pipeline. However, the present situation warrants more new and effective drugs for the successful management of TB. In this context, there is also a need for a unique database for anti-TB drugs, which can provide all relevant information on the existing anti-TB drugs as well as those in trial, to researchers and medical professionals.

Aim: To develop an online database called, TBDRUGS (Database of Drugs for TB), to provide pharmacological, clinical, and molecular information on all approved anti-TB drugs as well as those in the research pipeline

Methodology: Pharmacological, clinical and molecular information on all anti-TB drugs have been collected from various sources and provided in the database. In addition, the database also describes the mechanism of action and targets for the drugs and provides links to other useful and relevant databases.

Results: The present version of the TBDRUGS database (v. 1.0) contains information on 40 anti-TB drugs, including 28 approved drugs and 12 candidate drugs, which are in different phases of the drug discovery and development pipeline. In addition, the database also describes the mechanism of action and targets for the drugs and provides links to other useful and relevant databases. The TBDRUGS can be accessed from http://bmi.icmr.

org.in/tbdrugs/ and http://bic.icmr.org.in/tbdrugs/.

Conclusion: Primarily, the database would be a useful resource for physicians. Besides, it can prove to be a useful tool for TB researchers. Based on the analysis of the data stored in the database, we found that 35 out of 40 TB drugs target either the cell wall biosynthetic or information pathways of *M. tuberculosis*. This finding highlights the importance of targeting these paths for the discovery of more drugs. We speculate that this kind of an approach would enhance the success rate of the drug discovery process.

(ii) Pharmacophore analysis, molecular docking and molecular dynamics simulation for the identification of Lpd inhibitors of *M. tuberculosis*

Principal Investigator : Dr. Sameer Hassan
(email: sameerhassan1@gmail.com)

Source of funding : ICMR Biomedical Informatics Project
Study period : 2015-2016

Background: Lipoamide dehydrogenase (Lpd), belongs to the oxidoreductase family that contains redox centre which can oxidise or reduce adjacent enzymes in the cascade. The cascade can be represented as NADH → Lpd → DlaT → AhpD → AhpC → ROOH. In this cascade, Lpd redox centre is invigorated directly from NADH and also participates in pyruvate dehydrogenase biosynthesis and peroxidase activities of *M. tuberculosis* (Mtb). Any perturbation in Lpd could negatively impact electron flow and thereby render the organism more susceptible to oxidative and nitrosative stress. Thus, Lpd could be a potential drug target in Mtb.

Aim: To identy potent inhibitors of Mtb Lpd

Methodology: Based on structure and ligand based pharmacophore analysis, Tyr-16, Leu-42 and Leu-100 (as mapped to 4M52) were identified as

critical residues. Molecular docking analysis was carried out with a set of drugs retrieved from LIDAEUS and PDB databases.

Results: The drugs named 26SPH1-044-766 and glutathionyl spermidine disulphide were identified as potent inhibitors of Mtb Lpd. The ability of 26SPH1-044-766 to bind to Arg-93 along with Tyr-16, Leu-42 and Leu-100 identified it as a specific drug against Mtb Lpd enzyme. Molecular docking simulation studies revealed the stability of receptor-ligand complex.

Conclusion: The study has identified some possible inhibitors for Lpd enzyme of Mtb, which need to be confirmed by *in vitro* testing.

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(iii) Understanding the structural stability and dynamics of PknI bound inhibitors – A molecular simulation analysis

Principal Investigator	:	Dr. Sameer Hassan (email: sameerhassan1@gmail.com)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2015-2016

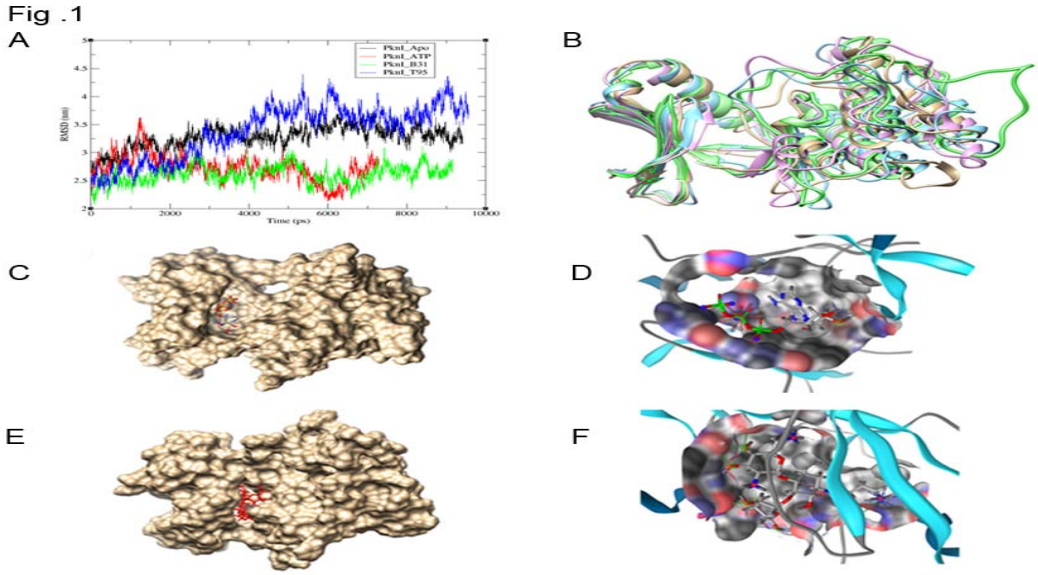
Background: *M. tuberculosis* survives under various environmental stress conditions for several decades by adaptive responses modulated by 11 serine/threonine protein kinases (STPKs). Among the 11 STPKs, five of them namely PknI, PknE, PknG, PknH and PknK, have been reported to support intra-macrophage survival. From our previous observations, we found that PknI plays a role in cell division and virulence of Mtb. We also identified two potential drugs (T95 and B31) for PknI based on virtual screening for molecules having the highest GOLD score and interaction with all the identified critical amino acids.

Aim: To analyse the stability and dynamic behaviour of PknI of Mtb

Methodology: The stability and dynamic behavior of PknI in its apo form, complexed with the cofactor ATP, and drugs such as B31 and T95 was tested using molecular dynamic simulation.

Results: Our simulation results suggest that B31 molecule is more stable within the PknI pocket and PknI-B31 complex has a similar conformation to that of PknI-ATP complex, indicating that B31 would be a better drug candidate than T95, since the T95 molecule was found to move away from the binding pocket during molecular dynamics simulation experiments (Fig. 35). .

Fig. 35: PknI structure analysis



A) RMSD plot for PknI in apo form (black), complexed with ATP (red), B31 (green) and T95 (blue). B) Structural superimposition of the final structure after 10 ns of simulation from each simulation experiment. C) Surface view of PknI and the binding pose of ATP (represented in stick form) within the active site of PknI protein after 10 ns of molecular simulation. D) Close view of ATP within the active site represented using electrostatic properties. E) Surface view and binding pose of B31, represented in stick form (red in color) as analysed after 10 ns of simulation. F) The interaction of B31 with active site residues of PknI is shown in close view, represented using electrostatic properties.

(iv) Understanding the evolutionary dynamics of HIV for prioritizing epitopes for immunogen design

Principal Investigator: Dr. Jagdish Chandra Bose
(email: sjcbose@gmail.com)
Source of funding : ICMR Biomedical Informatics Project
Study period : 2015-2016

Background: The hallmark challenge posed by HIV is its genetic diversity. The diversity of HIV is attributed mainly to the lack of proof reading mechanism (ie. the ability to confirm that the DNA transcript made is an accurate copy of the RNA code), followed by recombination. This can shuttle mutations between viral genomes resulting in the formation of quasi species within each host. Furthermore, the prevalence of HIV-1 subtypes differs from region to region; for example, while more than 90% of the infection in India is caused by HIV-1 subtype C, subtype B dominates in North America, Western and Central Europe. This diversity is a major challenge that has made the development of a HIV vaccine unsuccessful. We hypothesize that a better understanding of the evolutionary dynamics of HIV would throw light on ideal candidate epitopes, ie. epitopes that are largely conserved across subtypes and across the evolutionary time scale.

Aim: To understand the evolutionary dynamics of HIV in the context of identifying conserved epitopes across HIV-1 subtypes, that can be useful for immunogen design

Methodology: Phylogenetic analysis was undertaken on a total of 2133 sequences of HIV-1 representing six different clades (HIV-1 clades A, B, C, D, G, CRF_AE and CRF_AG) that were reported to have been sequenced during the period 2000 to 2016 (downloaded from the HIV Database). A list of 543 CTL epitopes from Gag that have been reported as immunogenic was obtained from the HIV Database (Los Alamos). These epitopes were examined for their degree of conservation over the years and across the clades in the 2133 sequences.

Results: It was found that 21 epitopes were conserved in 90% of the sequences, and another 89 were conserved in 80% of the sequences. Ten of these epitopes were conserved in majority of the clades. These results indicate that the above approach could play a useful role in shortlisting potential candidate epitopes for HIV vaccine design.

STUDIES IN PROGRESS:

B-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: Drug resistant forms of TB such as multidrug resistant (MDR) and extensively drug resistant (XDR) TB, have become an increasingly seirous problem throughout the world.

Aim: To develop an integrated database comprising of clinical, laboratory and molecular data on MDR and XDR-TB cases

Methodology: Detailed demographic data, molecular data, laboratory investigations, microbiological data, drug resistance pattern, X-ray reports, treatment history, etc. of patients with MDR and XDR-TB, at baseline and at every subsequent monthly visit during the course of treatment are captured and presented in the database.

Results: In its present form, the database contains data for 200 variables for 34 XDR patients and 56 MDR patients registered and treated at the N I R T. The data in DDTRB can be effectively utilized to obtain important information on drug resistance and sensitivity pattern, course of emergence of resistance, relationship between DR-TB and co-morbid diseases, smear and culture conversion rates, etc. The database has classified the DR-TB cases as Pure XDR, Poly resistant and Pre-XDR, and MDR.

Present status: The database is ready for internal validation. Feedback on physician friendliness will be obtained from the clinicians in the Institute. Subsequently, the database will be launched online and expanded to include data from other sites. It is planned to develop this into a national database for DR-TB.

B-2: *In silico* analysis of the variable loops of HIV gp120 protein to aid in the differentiation of R5, X4 and R5X4 HIV viruses

Principal Investigator : Dr. Luke Elizabeth Hanna
(email: hanna@nirt.res.in)

Source of funding : ICMR Biomedical Informatics Project
Study period : 2014-2017

Background: A huge challenge in the study of rapidly evolving viruses is the development of tools that can manage the huge diversified complex genetic data. HIV-1 mutates its genome rapidly with excess heterogeneity enabling it to infect various cell types by using different chemokine receptors expressed on the cell surface. R5 viruses use CCR5 as co-receptor for viral entry, while X4 viruses use CXCR4. Some viruses, known as dual-tropic or R5X4 viruses, are capable of using both kinds of co-receptors. Co-receptor usage of HIV isolates is not only useful for predicting disease progression in HIV-infected individuals, but has also been recognized as important drug targets. The significance of co-receptor usage has led to the development of several *in silico* tools to predict tropism based on V3 loop sequences.

Aim: To investigate the significance of each of the 5 variable regions (V1-V5) in the HIV envelope in distinguishing between the three tropic groups of viruses

Methodology: Full length gp120 sequences of the three groups of viruses, R5-tropic (n=1260), X4-tropic viruses (n=650) and R5X4-tropic viruses (n=493), were obtained from published literature and databases. Hidden Markov Model based alignment was performed with the V1-V5 variable loop region sequences. Sequences were then coded for features such as hydrophobicity, bulkiness and polarity for discriminant analysis. One-way ANOVA was performed using the total charge of the protein, length and glycosylation sites.

Results: Discriminant statistical analysis of the V1-V5 regions revealed a few significant differences between the three tropic groups (R5, X4, R5X4). While the V3 and V1 loop sequences could distinguish between R5 and X4

viruses, the V2 loop sequence appeared to be the best for distinguishing between all three kinds of viruses with high probability. On the other hand, V4 and V5 loop sequences were not significantly different between the three groups. One-way ANOVA analysis performed for all the loop regions took into consideration features such as total charge, glycosylation pattern and length of the loop. A significant difference was observed in the mean length of loops of V1, V2, V3 and V4 between the three groups. Multiple pair-wise comparisons revealed that each pair-wise comparison was also significant for all three viral groups. A significant difference was also observed in the median charge of the V1, V2, V3 and V4 regions between the three groups. Multiple pair-wise comparisons revealed that pair-wise comparison were significant for V4 loop. A significant difference was observed in the median glycosylation content of the V1, V2, V3 and V4 loops between the three groups. Multiple pair-wise comparisons revealed that pair-wise comparison were significant for V3 loop.

ELECTRONIC DATA PROCESSING DIVISION

Electronic Data Processing

(Contact person: Ms. Basilea Watson; email: basilea@nirt.res.in)

The Electronic Data Processing division provides data entry/verification support for the research undertaken in the epidemiological unit, clinical division, laboratory and other operational research studies. It plays a key role in the conduct of Epidemiological surveys. In addition to data entry and verification, it also processes the data for errors and discrepancies, and generates data tabulations, analysis and helps in publication of research work.

Maintenance of IT equipments: The maintenance of the IT equipments is managed by the EDP division. All break-down calls of computers and its peripherals are dealt under comprehensive annual maintenance contract. This includes managing the installation and ensuring that the computers are maintained and kept up to-date. In the past year, 39 outdated computers were replaced with new advanced PCs.

The division helps in providing audio-visual system for presentation of research materials during conferences, meetings and training programmes held at the centre.

Management of LAN: The management of the LAN facility is carried out with the support given by NIH-ICER project. In the past year, login facility on the network system was created for 72 new users and added to the existing user account database. Also, the storage was upgraded to 20TB during this period. Federated Services with single authentication to access the NIH Library to Research Team in NIRT was initiated.

Data entry/verification work: The quantum of data records of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2015 to March, 2016 is given below -

No. of records entered: 33, 808

No. of records verified: 33, 119

A total of 15, 264 records were processed for the Thiruvallur prevalence survey undertaken by the Epidemiology unit of NIRT.

Research work: The results of a population-based survey on general and tuberculosis mortality in two states of India, namely, Andhra Pradesh and Orissa were published this year. Also a secondary analysis of data from the BCG trial to investigate the relationship between BCG and TB disease, and between BCG and positive tuberculin skin test was published as a short communication.

INTERNATIONAL CENTRE FOR EXCELLENCE IN RESEARCH

COMPLETED STUDIES:

(i) Host immune responses in filarial infection and strongyloidiasis: A. Modulation of $CD4^+$ and $CD8^+$ T-cell function in filarial infections by IL-19 and IL-24

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);
Study Period	:	2015-2016

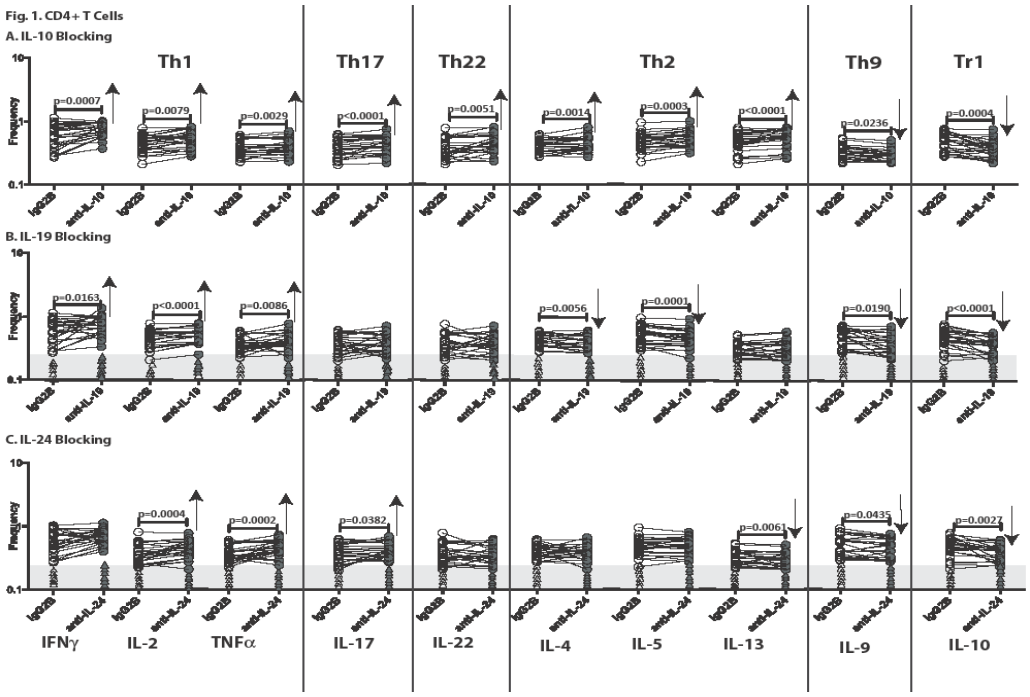
Background: IL-19 and IL-24 are cytokines highly expressed in filarial infections.

Aim / Methodology: To study the role of IL-19 and IL-24 in regulating T-cell responses, we examined the frequency of Th1/Tc1, Th2/Tc2, Th9/Tc9, Th17/Tc17, Th22/Tc22 and Tr1 cells in 26 filarial-infected individuals stimulated with filarial antigen following IL-19 or IL-24 neutralization

Results: IL-19 or IL-24 neutralization resulted in significantly enhanced frequencies of Th1/Tc1 and/or Th17/Tc17 cells and signfiicnatly reduced frequencies of Th2/Tc2, Tr1 and/or Th9/Tc9 ells (Fig. 36).

Conclusion: We demonstrated that IL-19 and IL-24 are associated with the modulation of T-cell responses in filarial infections.

<u>Fig. 36:</u> Altered frequencies of CD4$^+$ Th1, Th17, Th22, Th2, Th9 and Tr1 cells following neutralization of IL-10, IL-19 and IL-24.



The BmA-stimulated frequencies of CD4$^+$ Th1, Th2, Th9, Th17, Th22 and Tr1 cells were measured by flow cytometry following neutralization of IL-10 (A), IL-19 (B), IL-24 (C) or isotype control antibody in filarial infected individuals (n=26). The data are represented as line diagrams with each line representing a single individual.

(i) Host immune responses in filarial infection and strongyloidiasis:

B. IL-10 and TGFβ-mediated Th9 responses in human helminth 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)
Study Period	:	2014-2016

Background: Th9 cells are a subset of $CD4^+$ T-cells that express the protoypical cytokine, IL-9. Th9 cells are known to effect protective immunity in animal models of intestinal helminth infections. However, the role of Th9 cells in human intestinal helminth infections has never been examined.

Aim / Methodology: To examine the role of Th9 cells in *Strongyloides stercoralis* (Ss), a common intestinal helminth infection, we compared the frequency of Th9 expressing IL-9 either singly (mono-functional) or co-expressing IL-4 or IL-10 (dual-functional) in Ss-infected individuals (INF) to frequencies in uninfected (UN) individuals

Results: INF individuals exhibited a significant increase in the spontaneously expressed and/or antigen specific frequencies of both mono - and dual functional Th9 cells as well as Th2 cells expressing IL-9 compared to UN. The differences in Th9 induction between INF and UN individuals was predominantly antigen - specific as the differences were no longer seen following control antigen or mitogen stimulation. In addition, the increased frequency of Th9 cells in response to parasite antigens was dependent on IL-10 and TGFβ since neutralization of either of these cytokines resulted in diminished Th9 frequencies. Finally, following successful treatment of Ss infection, the frequencies of antigen - specific Th9 cells diminished in INF individuals, suggesting a role for the Th9 response in active Ss infection. Moreover, IL-9 levels in whole blood culture supernatants following Ss antigen stimulation were higher in INF compared to UN individuals.

Conclusions: Ss infection is characterized by an IL-10- and TGFβ dependent expansion of Th9 cells, an expansion found to reversible by anti-helmintic treatment.

(i) Host immune responses in filarial infection and strongyloidiasis: C. Systemic cytokine profiles in *Strongyloides stercoralis* infection and alterations following treatment

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)
Study Period	:	2014-2016

Background: *Strongyloides stercoralis* (Ss), is a soil transmitted helminth infection, that infects ~ 50 - 100 million people worldwide. Despite its widespread prevalence, very little is known about the immune response that characterizes human Ss infection.

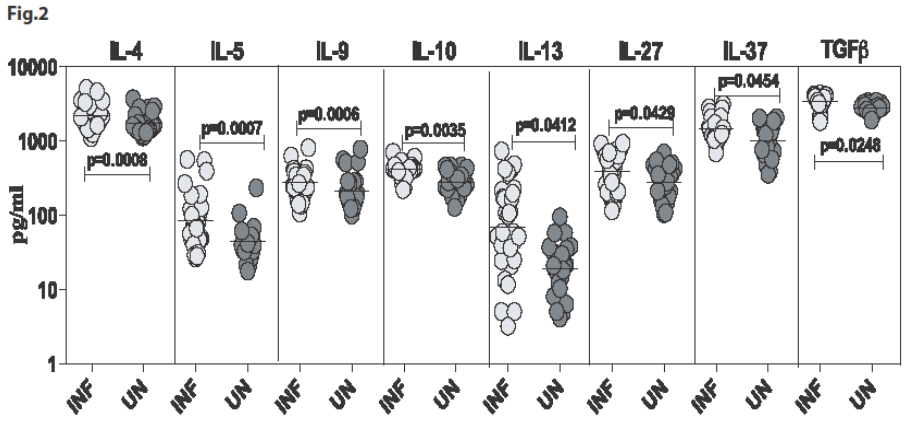
Aim / Methodology: To study the systemic cytokine profile characteristic of Ss infection, we measured the circulating levels of a large panel of pro - and anti - inflammatory cytokines in asymptomatic, infected (INF) individuals (n=32) and compared them to uninfected (UN), endemic controls (n=24)

Principal Findings: INF individuals exhibited significantly lower circulating levels of pro - inflammatory cytokines (IFNγ, TNFα and IL-1β) and significantly higher levels of anti - inflammatory cytokines (IL-4, IL-5, IL-9, IL-10, IL-13, IL-27, IL-37 and TGFβ). Moreover, treatment of Ss infection resulted in a significant reversal of the cytokine profile with increased levels of pro - inflammatory (IFNγ, TNFα, IL-2, IL-17A, IL-17F, IL-22, IL-23 and IL-1β) and decreased levels of anti - inflammatory (IL-4, IL-5, IL-9, IL-10, IL-

13, IL-27, IL-37 and TGFβ) cytokines following treatment (Fig. 37).

Conclusions: Thus, Ss infection is characterized by alterations in the levels of systemic cytokines, reflecting major alterations in the underlying immune response to this chronic helminth infection.

<u>Fig. 37:</u> Ss infections are associated with heightened plasma levels of anti - inflammatory cytokines at homeostasis



The plasma levels of anti - inflammatory cytokines (IL-4, IL-5, IL-9, IL-10, IL-13, IL-27, IL-37 and TGFβ) - were measured by ELISA in INF (n=32) or UN (n=24) individuals. The results are shown as scatter plots with each circle representing a single individual and the bar representing the GM. P values were calculated using the Mann-Whitney test with Holm's correction for multiple comparisons.

(ii) Immunology of TB and its co-morbidities: A. Circulating angiogenic factors as biomarkers of disease severity and bacterial burden 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)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2014-2016

Background: Angiogenesis and lymphangiogenesis are classical features of granuloma formation in PTB. In addition, the angiogenic factor - VEGF-A is a known biomarker for PTB.

Aim / Methodology: To examine the association of circulating angiogenic factors with PTB, we examined the systemic levels of VEGF-A, VEGF-C, VEGF-D, VEGF-R1, VEGF-R2 and VEGF-R3in individuals with PTB, latent TB (LTB) or no TB infection (NTB)

Results: Circulating levels of VEGF-A, VEGF-C andVEGF-R2 were signi-ficantly higher in PTB compared to LTB or NTB individuals. Moreover, the levels of VEGF-A, VEGF-C and VEGF-R2 were significantly higher in PTB with bilateral and/or cavitary disease. The levels of these factors also exhibited a significant positive relationship with bacterial burdens in PTB. ROC analysis revealed VEGF-A and VEGF-R2 as markers distinguishing PTB from LTB or NTB. Finally, the circulating levels of all the angiogenic factors examined were significantly reduced following successful chemotherapy.

Conclusions: Our data demonstrated that PTB is associated with elevated levels of circulating angiogenic factors, possibly reflecting vascular and endothelial dysfunction. In addition, some of these circulating angiogenic factors could prove useful as biomarkers to monitor disease severity, bacterial burden and therapeutic responses.

(ii) Immunology of TB and its co-morbidities: B. Coincident diabetes mellitus modulates $CD4^+$ Th1, Th2 and Th17 cell responses in LTB in an IL-10 and TGFβ dependent manner

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.P. Paul Kumaran; Dr.C.K. Dolla
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2014-2016

Background*:* Type 2 diabetes mellitus (DM) is a risk factor for the development of active TB, although its role in the TB-induced responses in LTB is not well understood. Since Th1, Th2 and Th17 responses are important in immunity to LTB, we postulated that coincident DM could alter the function of these $CD4^+$ T-cell subsets.

Aim: To examine mycobacteria–induced immune responses in the whole blood of individuals with LTB-DM and compared them with those without DM (LTB-NDM)

Results*:* LTB-DM was characterized by diminished frequencies of mono – and dual – functional $CD4^+$ Th1, Th2 and Th17 cells at baseline and/or following mycobacterial - antigen stimulation. This modulation was at least partially dependent on the regulatory cytokines - IL-10 and TGFβ, since neutralization of either cytokine resulted in significantly increased frequencies of Th1 and Th2 cells but not Th17 cells in LTB-DM but not LTB individuals (Fig. 38).

Conclusion*:* LTB-DM is therefore characterized by diminished frequencies of Th1, Th2 and Th17 cells, indicating that DM alters the immune response in latent TB leading to a defective induction of protective $CD4^+$ T-cell responses, thereby providing a potential mechanism for increased susceptibility to active disease.

<u>Fig. 38:</u> Frequency distibution of mono - and dual - functional Th1, Th2 and Th17 cells in LTB-DM and LTB-NDM

Figure S4. Frequency distibution of mono - and dual - functional Th1, Th2 and Th17 cells in LTB-DM and LTB-NDM. The antigen (PPD, ESAT-6, CFP-10, Flu PP) as well as the PMA/ Ionomycin stimulated frequencies of mono – and dual – functional CD4+ Th1 cells in LTB-DM and LTB-NDM (A) Th2 cells in LTB-DM and LTB-NDM (B) and Th17 cells in LTB-DM and LTB-NDM individuals (C) are shown. Data are represented as pie charts with each piece of the pie representing the geometric mean percentages of the mono - or dual - functional CD4+ T cell subsets in each group and under each condition with total cytokine producing CD4+ T cells serving as 100%.

The antigen (PPD, ESAT-6, CFP-10, HIV Gag) as well as the PMA/ Ionomycin stimulated frequencies of mono – and dual – functional $CD4^+$ Th1, Th2 and Th17 cells in LTB-DM (A) and LTB-NDM individuals (B) are shown. Data are represented as pie charts with each piece of the pie representing the geometric mean percentages of the mono - or dual - functional $CD4^+$ T cell subsets in each group and under each condition with total cytokine producing $CD4^+$ T cells serving as 100%.

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)
Co-investigators	:	Dr.C.K. Dolla; Dr.M. Satiswaran
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 70 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. The study recruitment and follow-up are over and the immunological studies are being carried out. .

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-helmintic therapy. Patient recruitment is ongoing.

ICER-4: Host immune responses inTB 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-PTB, 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 peripheral blood. We have recruited 70 out of the 100 patients needed for this study.

CONTRIBUTION TO THE NATIONAL PROGRAMMES

(1) HIV Laboratory services:

(i) Early Infant Diagnosis Program

(Contact person: Dr. Luke Elizabeth Hanna; email: hanna@nirt.res.in)

Source of Funding: National AIDS Control Organization (NACO)

The HIV/AIDS department continues to support NACO's Early Infant Ddiagnosis (EID) programme by serving as a Regional Reference Laboraotry and centralized testing facility for the states of Tamil Nadu, Pondicherry, Andhra Pradesh and Kerala. A total of 2685 DBS samples were received and tested during the reporting period, of which 92 (3.42%) samples were found to be HIV-1 positive.

(ii) Viral Load testing for the Second Line ART program

(Contact Person: Dr. Luke Elizabeth Hanna; email:hanna@nirt.res.in)

Source of Funding: National AIDS Control Organization (NACO)

The HIV/AIDS department has continued to support NACO's second line ART program by undertaking viral load testing for the states of Tamil Nadu, Kerala and Pondicherry. As part of this activity, 1171 samples were received and tested during the reporting period.

2. Quality Assurance activities for testing services

(Contact Person: Dr. Luke Elizabeth Hanna; email: hanna@nirt.res.in)

The HIV/AIDS laboratory has continued its excellent record in quality assurance during this year. The laboratory paticpated successfully in various EQA programmes: (i) NIH/VQA and RCPA QAP for HIV-1 viral load testing; (ii) NIH/VQA and CDC GAP for HIV1 DNA PCR; (iii) WHO and NIH/VQA for HIV drug resistance genotyping; (iv) EQAS programs offered by NARI for CD4/CD8 count testing and HIV serology (Rapid). The laboratory received good scores and continued to maintain its accrddited / certified status.

3. Laboratory support for clinical trials, research studies and patient care

(Contact Person: Dr. Sudha Subramanyam; email: sudhas@nirt.res.in)

The routine lab in the HIV department performs a number of tests including serological testing for HIV, HBSAg, HCV, CD4/CD8 cell count estimation, complete blood count, blood grouping and Rh typing, sample processing and storage for various research studies and clinical trials. The laboratory also extends its testing services for screening and confirmation of syphilis and for molecular diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhea* (CT/NG).

III. Bacteriology Lab services:

(i) RNTCP activities

Contact person	:	Dr.K.R. Uma Devi (email: umadevi.r@nirt.res.in)
Source of Funding	:	Ministry of Health and Family Welfare, Central TB Division, New Delhi

The Department of Bacteriology at NIRT designated as one of the National Reference Laboratories (NRL) is responsible for monitoring 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 RNTCP in India. The main responsibilities of NRLs is to monitor EQA of smear microscopy as well as culture and DST for both first line and second line drugs (FLD, SLD) by both phenotypic and genotypic methods. NIRT provides training for laboratory personnel and is responsible for certifying state level laboratories, e.g., the IRLs, Medical Colleges and private laboratories for culture and DST for diagnosis of DR-TB. Second line DST services are being provided 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 seven DR-TB centers in Tamil Nadu, one in Andaman and Nicobar Island and also for the states of Telengana and West Bengal for PMTD services. Under RNTCP, a total of 13 laboratory personnel from 4 different institutions have been trained for both first and second line DST liquid (MGIT) media. Totally 16 laboratories has been certified for solid culture, liquid culture and/or molecular diagnosis for DR-TB till date. Certification process is in progress for 9 Medical colleges/ Private laboratories for FLD and for 2 laboratories for SLD including IRLs of Andhra Pradesh and Telangana. Pre-certification assessment visit has been conducted for two medical Colleges/culture & DST labs/NGOs in Tamil Nadu. Certiication of previously certified 16 laboratories has been renewed based on seventh round proficiency testing.

For PMDT services, a total of 446 follow-up cultures and 1083 patient samples have been received for XDR diagnosis from presumptive of XDR under Cat-IV treatment and XDR diagnosis at base line respectively, and XDR diagnosed details are presented in Table 37. On-site evaluation of smear microscopy was conducted for Kerala. A total of 11 sets of 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 37: Details of XDR diagnosis for PMDT services in RNTCP at NIRT (2015-16)

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 in Cat-IV treatment					
446	88	358	27	114	30
XDR Diagnosis at Baseline (Three DR-TB centres of Tamil Nadu)					
1083	219	864	14	141	18

*Contamination, MTB not detected and culture negatives

TRAINING:

As part of Supra National Reference Laboratory (SNRL) activity, the Department of Bacteriology is committed to provide training facility for students of various education and/or research institutions in the country. Between April 2015 and March 2016, a total of 16 M.Sc., Microbiology students, 58 B.Sc., Microbiology students, 27 M.Sc., Medcial Microbiology students, 18 MD Microbiolgoy students and 30 Diploma in Medical Lab Technology students were trained for mycobacterial procedures. In addition, 36 students underwent summer internship training for mycobacterial culture and DST. During the period, 3 M.Tech. (Biotechnology), 5 B.Tech. (Biotechnology) students from Anna University and 1 M.Sc., (Biotchnology) student from Bharathidhasan University successfully completed their final year dissertation work.

Update on MDR-TB diagnosis by LPA and Gene Xpert for the period between 1st April, 2015 and 31st March 2016

Diagnosis of MDR-TB by LPA was done from smear positive specimens of 3899 patients during the period between 1st April, 2015 and 31st March 2016 as part of service to the Tamil Nadu PMDT activities of RNTCP. Of the 3899 patients, 178 were found to have MDR-TB, 57 were resistant to RMP only, 384 were resistant to INH only and 3171 were sensitive to both INH and RMP. In the case of 2134 patients in whom sputum smear was negative, 746 samples were tested by MGIT and 1388 were tested by Gene Xpert system. Using the two systems, 9 were found to have MDR, 6 were mono resistant to H, 2 were mono resistant to R and 13 were found to be resistant to R by Xpert. The service is being offered for 3 districts of Chennai (North, South and Central) and Kanchipuram. In addition, 937 follow-up samples were processed by MGIT960 system.

LIBRARY AND INFORMATION CENTRE

Library and Information Centre

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

During the last fifteen years the Library has made significant investments in acquiring e-resources including e-archives. For providing access to the e-resources, a customized **Digital Library** portal has been established in 2001. 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 (NPL) supported by ICMR. It enhances a simplified integrated 24hours access facility (intranet) to our users.

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 administration
- 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.1) 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.

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 international tuberculosis institutes.

NIRT Web site

The NIRT Web Site (http://nirt.res.in) is designed, developed and being maintained by the library.

APPENDICES

SUMMARY SHEET OF PUBLICATIONS FROM NIRT (2015-2016)

Sl.No.	Publishing journals	No. of papers	Impact factor	Total Impact Factor
BACT. DEPT				
1	J Infect	1	4.441	4.441
	PLOS ONE	1	3.234	3.234
	Bioorg Med Chem Lett	1	2.42	2.42
	J Mole Graph Model	1	1.722	1.722
	Indian J Med Res	2	1.446	2.892
	Indian J App Microbiol	1	-	-
	TOTAL	**7**		**14,709**
BIO-INFORMATICS CELL				
2	J Mol Model	1	1.736	1.736
	TOTAL	**1**		**1.736**
CLINIC				
3	LANCET	3	45.217	135.651
	Lancet Infect Dis	1	22.433	22.433
	PLOS Med	1	14.429	14.429
	Clin Infect Dis	3	8.886	26.658
	Clin Pharmol Therapy	1	7.903	7.903
	Curr Opin HIV AIDS	1	4.68	4.68
	PLOS ONE	2	3.234	6.468
	BMC Pediatr	1	-	-
	BMJ Open	1	-	-
	J Acquir Immune Def Synd	1	-	-
	Public Health Action	1	-	-
	TOTAL	**16**		**218.222**
CLINICAL PHARMACOLOGY				
4	Clin Pharmol Therapy	1	7.903	7.903
	Int J Tuberc Lung Dis	1	2.315	2.315
	TOTAL	**2**		**10.218**
SOCIO BEHAVIORAL RESEARCH				
5	PLOS ONE	2	3.234	6.468
	Indian J Med Res	1	1.446	1.446
	Asian J Res Social Sci Humanities	1	-	-
	J Public Helath (Oxf)	1	-	-
	TOTAL	**5**		**7.914**
EPIDEMIOLGOY				
6	Indian J Tuberc	2	-	-
	Int J Tuberc Lung Dis	1	2.315	2.315

	TOTAL	**3**		**2.315**
HIV DIVISION				
7	Indian Pediatrics	2	-	-
	Int J Biol Macromolecules	1	2.858	2.858
	TOTAL	**3**		**2.858**
ICER/NIRT-NIH				
8	J Clin Invest	1	13.215	13.215
	J Infect Dis	2	5.997	11.994
	J Immunology	2	4.922	9.844
	PLOS Neg Trop Dis	2	4.446	8.892
	Eur J Immunol	1	4.034	4.034
	Immunoloy	2	3.795	7.59
	Infect Imm	1	3.731	3.731
	PLOS ONE	2	3.234	6.468
	Tuberculosis	2	2.711	5.422
	Cytokine	2	2.664	5.328
	Immune Inflamm Dis	1	-	-
	TOTAL	**18**		**76.518**
IMMUNOLOGY				
9	J Infect	1	4.441	4.441
	Vaccine	1	3.624	3.624
	Mol Immunol	1	2.973	2.973
	J Biotechnology	1	2.871	2.871
	Tuberculosis	2	2.711	5.422
	Cytokine	1	2.664	2.664
	Int Immunopharmacol	1	2.472	2.472
	Hum Immunol	1	2.138	2.138
	J Mole Graph Model	1	1.722	1.722
	Indian J Med Res	1	1.446	1.446
	Indian J Med Microbiol	1	0.9	0.9
	Can J Physiol Pharmacol	1	-	-
	Clin Exp Med	1	-	-
	Med Microbiol	1	-	-
	Macrophage	1	-	-
	TOTAL	**16**		**30.673**
STATISTICS				
10	Indian J Med Res	1	1.446	1.446
	Sci Res J	1	-	-
	TOTAL	**2**		**1.446**

LIST OF PUBLICATIONS

Publications in Journals	**:**	**73**	
Accepted	**:**	**19**	

Published	i) International	:	61
	ii) National	:	12
Books		:	1
Accepted	i) International	:	17
	ii) National	:	2

International:

1. Amudha P, Hassan S, Prabuseenivasan, Shainaba AS, Hanna LE, Kumar V. Andrographolide: A potent antituberculosis compound that targets Aminoglycoside 2'-N-acetyltransferase in *Mycobacterium tuberculosis*. *J Mol Graph Model.*2015;61:133-140.

2. Andrade BB, Pavan Kumar N, Amaral EP, Riteau N, Mayer-Barber KD, Tosh KW, Maier N, Conceição EL, Kubler A, Sridhar R, Banurekha VV, Jawahar MS, Barbosa T, Manganiello VC, Moss J, Fontana JR, Marciano BE, Sampaio EP, Olivier KN, Holland SM, Jackson SH, Moayeri M, Leppla S, Sereti I, Barber DL, Nutman TB, Babu S, Sher A. Heme Oxygenase-1 regulation of matrix metalloproteinase-1 expression underlies distinct disease profiles in tuberculosis. *J Immunol.*2015;195:2763-2773.

3. Anuradha R, Munisankar S, Dolla C, Kumaran P, Nutman TB, Babu S. Parasite antigen-specific regulation of Th1, Th2, and Th17 responses in strongyloides stercoralis infection. *J Immunol.*2015;195:2241-2250.

4. Aravindhan V, Mohan V, Arunkumar N, Sandhya S, Babu S. Chronic endotoxemia in subjects with Type-1 diabetes is seen much before the onset of microvascular complications. *Plos One*.2015;10:e0137618.

5. Arunkumar V, Sameer H, Kannan P, Sujatha N. *In silico* and experimental validation of protein-protein interactions between PknI and Rv2159c from *Mycobacterium tuberculosis*. *J Mol Graph Model.*2015;62:283-293.

6. Balaji P, Prabhavathi M, Raja A. Evaluation of cytokine and chemokine response elicited by Rv2204c and Rv0753c to detect latent tuberculosis infection. *Cytokine***.**2015**;**76:496-504.

7. Balaji P, Anbarasu D, Parthasarathy RT, Alamelu R. PpiA antigen specific immune response is a potential biomarker for latent tuberculosis infection. *Tuberculosis.*2015;95:736-743.

8. Banurekha VV, Thomas BE, Dina N, Kannan T, Suma P, Sathyapriya K, Harivanzan V, Meenachi C, Shivakumar SV, Lavanya J, Ashok J, Swaminathan S. The usefulness and feasibility of mobile interface in tuberculosis notification (MITUN) voice based system for notification of tuberculosis by private medical practitioners - A pilot project. PLoS One 2015;10:e0138274.

9. Banurekha VV, Dina N, Ramalingam S, Perez-Velez CM, Becerra MC, Swaminathan S. Setting priorities for a research agenda to combat drug-resistant tuberculosis in children. *Public Health Action.*2015;5:222-235.

10. Baskaran D, Mohan Kumar P, Srividya A, Chandrasekaran V, Vijayaraj 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* 2015;10(2015) e0124260.

11. Ganga Devi NP, Ajay KM, Palanivel C, Sahu S, Selvaraj M, Valan AS, Rewari BB, Soumya S. Implementation and Operational Research: Epidemiology and Prevention: High loss to follow-up among children on Pre-ART care under National AIDS Program in Madurai, south India. *J Acquir Immune Defic Syndr.* 2015;69:e109-114.

12. GBD 2013 DALYs and HALE Collaborators, Murray CJ, Barber RM, Foreman KJ, Ozgoren AA, Abd-Allah F, Abera SF, Aboyans V, Abraham JP, Abubakar I, Abu-Raddad LJ, Abu-Rmeileh NM, Achoki T, Ackerman IN, Ademi Z, Adou AK, Adsuar JC, Afshin A, Agardh EE, Alam SS, Alasfoor D, Albittar MI, Alegretti MA, Alemu ZA, Alfonso-Cristancho R, Alhabib S, Ali R, Alla F, Allebeck P, Almazroa MA, Alsharif U, Alvarez E, Alvis-Guzman N, Amare AT, Ameh EA, Amini H, Ammar W, Anderson HR, Anderson BO, Antonio CA, Anwari P, Arnlöv J, Arsenijevic VS, Artaman A, Asghar RJ, Assadi R, Atkins LS, Avila MA, Awuah B, Bachman VF, Badawi A, Bahit MC, Balakrishnan K, Banerjee A, Barker-Collo SL, Barquera S, Barregard L, Barrero LH, Basu A, Basu S, Basulaiman MO, Beardsley J, Bedi N, Beghi E, Bekele T, Bell ML, Benjet C, Bennett DA, Bensenor IM, Benzian H, Bernabé E, Bertozzi-Villa A, Beyene TJ, Bhala N, Bhalla A, Bhutta ZA, Bienhoff K, Bikbov B, Biryukov S, Blore JD, Blosser CD, Blyth FM, Bohensky MA, Bolliger IW, Başara BB, Bornstein NM, Bose D, Boufous S, Bourne RR, Boyers LN, Brainin M, Brayne CE, Brazinova A, Breitborde NJ, Brenner H, Briggs AD, Brooks PM, Brown

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55. Kumar NP, Moideen K, George PJ, Dolla CK, Kumaran P, Babu S. Impaired cytokine but enhanced cytotoxic marker expression in *Mycobacterium tuberculosis* - induced CD8+ T cells in individuals with Type 2 diabetes and latent *Mycobacterium tuberculosis* infection. *J Infect Dis.*2016;213:866-870.

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60. Thomas BE, Banurekha VV, Kannan T, Dina N, Barman SB, Jayabal L, Ovung S, Swaminathan S. Perceptions of private medical practitioners on tuberculosis notification: A study from Chennai, south India. *PLoS One*.2016; 11:e0147579.

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National:

1. Azger D, Gomathi S, Shambhavi C, Kumar V, Mehta P, Swaminathan S. Drug resistance among extrpulmonary TB patients: Six years experience from a supernational reference laboratory. *Indian J Med Res.*2015;142:568-574.

2. Azger D, Radhakrishnan R, Nagarajan P, Ponnuraja C, Kumar V, Selvakumar N. Phosphate buffered saline for transport of *M. tuberculosis* cultures. *Indian J App Microbiol.*2015;18:1-4.

3. Desikan S, Narayanan S. Genetic markers, genotyping methods & next generation sequencing in *Mycobacterium tuberculosis. Indian J Med Res.* 2015;141:761-774.

4. 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* 2015;33:267-270.

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6. Lakshmi R, Ranjani R, Ravi Kumar D, Syam Sundar A, Radhika G, Rahman F, Selvakumar N, Kumar V. Revisiting the susceptibility testing of *Mycobacterium tuberculosis* to ethionamide in solid culture medium. *Indian J Med Res*.2015;142:538-542.

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8. Senthil S, Neha M, Pratibha K, Rahul G, Achhelal P. Acceptability and utilization of female condom among couples attending family welfare clinics in Mumbai. *Asian J Res Social Sci Humanities*;2015:5:54-61.

9. Srinivasan R, Venkatesan P. Bayesian spatio-temporal model for tuberculosis in India. *Indian J Med Res.*2015;141:478-480.

10. Subramani R, Datta M, Swaminathan S. Does effect of BCG vaccine decrease with time since vaccination and increase tuberculin skin test reaction? *Indian J Tuberc*.2015;62:226-229.

11. Thomas BE, Srividya A, Manogaran C, Swaminathan S. Pulmonary tuberculosis among tribals in India: A systematic review & meta-analysis. *Indian J Med Res*.2015;614-623.

2016:

12. Kolappan C, Subramani R, Swaminathan S. General and tuberculosis mortality in two states of India: A population-based survey. *Indian J Tuberc* 63(2016) 28-33.

Chapters:

1. Seema Sahay, Verma A, Shewale S, Murugesan P. Child Safety, Welfare and Well Being, Chapter: Child Rights in the Context of HIV/AIDS, Publisher: Springer India 2016, Editors: Deb S, pp.175-187.

2. Azger D, Selvakumar N. Contributed a review on Biosafety in Mycobacteriology which appeared in the WHO TDR training manual on "Biosafety for human health and the environment in the context of the potential use of genetically modified mosquitoes (GMMs)", published in 2015. http:www.who.int/tdr/publications/biosafety-gmm/en/

Accepted:

International:

1. Anuradha R, Munisankar S, Bhootra Y, Kumar NP, Dolla CK, Kumaran P, Babu S. Coexistent malnutrition is associated with perturbations in systemic and antigen –specific cytokine responses in latent tuberculosis infection. *Clin Vaccine Immunol.*

2. Ashokkumar M, Tripathy SP, Hanna LE. Variability in V1V2 and PNGs in pediatric HIV-1 viral variants transmitted through vertical route. *AIDS Res Hum Retroviruses.*

3. Dinesh Kumar P, Hasssan S, Narayanan S. Multifaced PknE: Apoptosis inhibition, HIV co-infection, host signalling crosstalk and in orchestrating the physiology of *Mycobacterium tuberculosis*. *J Microb Biochem Technol.*

4. Hemanth Kumar AK, Sudha V, Leelavathi A, Geetha Ramachandran. A rapid isocratic high performance liquid chromatography (HPLC-UV) method for the quantification of ritonavir in human plasma. *Int J Pharm Pharmaceut Sci.*

5. Hemanth Kumar AK, Kannan T, Chandrasekaran V, Sudha V, Vijayakumar A, Ramesh K, Lavanya J, Swaminathan S, Ramachandran G. Pharmacokinetics of thrice-weekly rifampicin, isoniazid and pyrazinamide in adult tuberculosis patients in India. *Int J Tuberc Lung Dis*

6 Jagadish Chandrabose Sundaramurthi, Hanna LE, Sriram S, Sridharan Brindha, Joel Gnanadoss J, Savariar V, Harpreet S, Swaminathan S. TB-DRUGS - Database of Drugs for Tuberculosis. *Tuberculosis.*

7. Kumar NP, Moideen K, Viswanathan V, Kornfeld H, Babu S. Effect of standard tuberculosis treatment on naive, memory and regulatory T cell homeostasis in tuberculosis-diabetes co-morbidity. *Immunology*.

8. Mave V, Chandanwale A, Kinikar A, Khadse S, Kaga, A, Gupte N, Suryavanshi N, Nimkar S, Koli H, Khwaja S, Bharadwaj R, Joshi S, Horng H, Benet LZ, Ramachandran G, Dooley KE, Gupta A, Gandhi M. Isoniazid hair concentrations in children with tuberculosis: a proof of concept study. *Int J Tuberc Lung Dis.*

9. 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 Sex Behav.*

10. Narendran G, Swaminathan S. TB-HIV co-infection: a catastrophic comradeship. *Oral Dis.*

11. Ramachandran G, Kumar AK, Kannan T, Bhavani PK, Kumar RS, Gangadevi NP, Banurekha VV, Sudha V, Venkatesh S, Ravichandran N, Kalpana S, Mathevan G, Sanjeeva GN, Agarwal D, Swaminathan S. Low serum concentrations of Rifampicin and Pyrazinamide associated with poor treatment outcomes in children with tuberculosis related to HIV status. *Pediatr Infect Dis J.*

12. Ramesh K, Hemanth Kumar AK, Vijayalakshmi R, Sudha V, Bharathiraja T, Lavanya J, Swaminathan S, Ramachandran G. *SLCO1B1* gene polymorphisms do not influence plasma rifampicin concentrations in a south Indian population. *Int J Tuberc Lung Dis.*

13. Shainaba SA, Sameer H, Hanna LE, Uma Devi R, Vanaja K. Homology modeling, substrate docking, and molecular simulation studies of mycobacteriophage Che12 lysin A. *J Mol Model*.

14. Srivastava S, Pasipanodya JG, Ramachandran G, Deshpande D, Shuford S, Crosswell HE, Cirrincione KN, Sherman CM, Swaminathan S, Gumbo T. A long-term co-perfused disseminated tuberculosis-3D liver hollow fiber model for both drug efficacy and hepatotoxicity in babies. *EBioMedicine.*

15. Swaminathan S, Jotam G. Pasipanodya, Ramachandran G, Hemanth Kumar AK, Srivastava S, Deshpande D, Nueremberger E, Gumbo T. Drug concentration thresholds predictive of therapy failure and death in children with tuberculosis: bread crumb trails in random forests. *Clin Infect Dis.*

16. Thomas B, Closson EF, Biello K, Menon S, Navakodi P, Dhanalakshmi A, Mayer KH, Safren SA, Mimiaga MJ. Development and Open Pilot Trial of an HIV-Prevention Intervention Integrating Mobile-Phone Technology for Male Sex Workers in Chennai, India. *Arch Sex Behav.*

17. Unissa AN, Hassan S, Indira Kumari V, Revathy R, Hanna LE. Insights into RpoB clinical mutants in mediating rifampicin resistance in *Mycobacterium tuberculosis. J Mol Graph Model.*

National:

1. Hemanth Kumar AK, Ramesh K, Kannan T, V Sudha V, Hemalatha H, Lavanya J, Swaminathan S, Ramachandran G. *NAT2* gene polymorphisms and plasma isoniazid concentrations in tuberculosis patients in south India. *Indian J Med Res.*

2. Hemanth Kumar AK, Chandrasekaran V, Kumar K, Kawasakar M, Lavanya J, Swaminathan S, Ramachandran G. Food significantly reduces plasma concentrations of first-line anti-TB drugs. *Indian J Med Res.*

Awards/Honours

- Dr.N. Saravanan – "Best Participant Award" in DST sponsored "15th Foundation Training Programme for Scientists and Technologists" at Indian institute of Public Administration, New Delhi held during Nov 2015 – Feb 2016.

- Dr.C. Padmapriyadarsini – ":Lupin-TAI Oration" at the Annual National Conference on Tuberculosis and Chest Diseases "NATCON 2015" at the King George Medical University, Lucknow during Feb 2016.

Special Assignments

Dr. Uma Devi

Membership of Professional Societies

- Life Member, Indian Immunology Society

Other assignments:

- DBT Nominee on IBSC committee for KMS Healthcare Pvt. Ltd - 2nd IBSC meeting on 14th Jan 2015.
- Scientific advisor for proposal on Developing a rapid multiplex test to diagnose Extensively drug resistant (XDR) TB and Multidrug resistant (MDR) TB: BIRAC funded project for Yathum Biotech.

Reviewer for International Journals:

- Plos One and Vaccine

Reviewer for International Journals:

- Tuberculosis Association of India

Assignments related to research activities:

Student Advisory Committee:

- External Member in Doctoral Committee for Ph. D at JIPMER, Pondicherry University.
- Member, Doctoral committee at Department of Environmental Biotechnology, Bharathidasan University/
- Member, Doctoral committee for Ph.D, Venkateswara College of Engineering, (Anna University).
- As a Examiner to the Public Viva voce Examination in University of Madras, Taramani.
- As a External expert of the Doctoral Committee.

Dr.N.S. Gomathi

- N ominated to attend the Leadership Meeting for RePORT International /RePORT India at Boston Medical University, between 23rd and 25th of September, 2015.
- N ominated by Ministry of Health and Family Welfare, Government of India to monitor hygiene and clean practices in JIPMER and NIMHANS under the KayaKalp Scheme between 3.2.2016 to 6.2.2016

Reviewer for National Journals:

- I nvited to be reviewer for articles in Current Science in March 2016

Dr. Azger Dusthackeer:

- External examiner nominee for Ph. D Public Viva-Voce examination at Sathyabama University on 27th July, 2015
- Conducted a Doctoral committee review meeting for Ph. D students at Sathyabama University on 3rd, July, 2015

Reviewer for National Journals:

- Reviewer for two of the articles for Plos One and one for Asia Pacific journal for tropical disease

Dr.M. Makesh Kumar:

Membership in Expert Committees

- Member, Tamilnadu Medical Council, Chennai.
- Member, Indian Medical Association - Madurai Branch.
- Member, National Foundation for Infectious Diseases (NFID), Bethesda, Maryland, USA.

Dr. Beena E. Thomas, Scientist 'E'
Membership in Expert Committees & Special Assignments

- Member of the Scientific Working Group (SWG) WHO-TDR for research capacity strengthening and knowledge management unit (RCS/KM)
- Member of the Technical Resource Group(TRG) for MSM- NACO
- Member of DR TB Counseling Committee, CTD
- Editorial Board Member for Journal of Health Sciences
- Member of National Advisory Committee on Advocacy Communication Social Mobilization (ACSM)
- Member of International Association of Schools of Social Work (IASSW)
- Member of International Federation for Social Workers (IFSW)
- Member of the Board of studies – University of Madras – Social work

Mr. Senthil Sellappan

- Life member of Professional Social Worker's Association

Mr. P. Murugesan

- Life member of Professional Social Worker's Association

Dr. N. Saravanan:

- Served as a Member in the Doctoral Committee at the School of Bioscience & Technology, Vellore Institute of Technology, Vellore.

Dr. Geetha Ramachandran:

- Invited speaker at the winter symposium organized by Christian Medical College, Vellore during Feb 2016 - Geetha Ramachandran.

- Invited speaker at the Nutrition guidelines workshop organized by Yenepoya University, Mangalore during Feb 2016 - Geetha Ramachandran.

Capacity Building

- Dr.P. Kannan & Dr.N. Saravanan – DST sponsored "15th Foundation Training Programme for Scientists and Technologists" at Indian institute of Public Administration, New Delhi held during Nov 2015 - Feb-2016.

- Dr.M. Makesh Kumar - Participated in the workshop on "Capacity Building of Ethics Committees for Clinical Research in India" Organized at Pondicherry Institute of Medical Sciences, Puducherry during April 2015.

- Dr.M. Makesh Kumar - Participated in a one day session for the 'software launch' of the "five software"s and training on the CReATE software solutions which held at PGIMER, Chandigarh during Oct 2015.

- Dr. N. Saravanan - Qualified as a Nominee for "The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)" a statutory body under the Ministry of Environment, Forest and Climate Change Govt. of India after attending a rigorous training programme conducted at National Institute for Animal Welfare (NIAW), Haryana held during Sep 2015

- Dr. AK Hemanth kumar - Residential training programme on "Accountability and Responsiveness in Scientific Organizations" conducted by Department of Science & Technology (DST), at Vadodara during October 2015 .

- Dr. M. Muniyandi, Mr. S. Azhagendran and Mr. Nanda Kumar - Workshop on 'Instrument Development' in Chennai organized by Samarth, Chennai during November 2015

Participation in Conferences / Seminars / Workshops

1. Guest lecture given - “Newer TB Diagnostic modalities of Drug Resistant Tuberculosis” in a CME organized by IMA south Chennai, held at Tiruvellore, Chennai during April 2015 – N.S. Gomathi.

2. Guest lecture given - “Newer TB Diagnostic modalities” in a CME organized by IMA
   South Chennai, held at Rajan Eye Care Hospital, Chennai during April 2015 - N.S. Gomathi.

3. Workshop on “Next generation sequencing – Bioinformatics and data analysis” held at AUKBC, Chennai during Mya 2015 – Sameer Hassan.

4. Participated in TB research methods course and advanced TB diagnostic research course at McGill University, Montreal, Canada during July 2015 – Dina Nair.

5. Participated in the **“**8th International AIDS Society (IAS) conference on HIV pathogenesis, treatment and prevention” held at Vancouver, Canada during July 2015 - Beena E. Thomas.

6. Relational Sequencing TB Data platform project workshop held at Geneva during July 2015 - K.R. Uma Devi.

7. 2nd WHO-National technical consultation meeting to prevent, detect and treat hepatitis on World Hepatitis day, held at Institute of Liver and Biliary Sciences, New Delhi during July 2015 – Syed Hissar.

8. Invited speaker in the 29th South Pedicon conference and 40th Annual State IAP TNSC at V I T, during August 2015 – Dina Nair.

9. Workshop on Technical and Operational guidelines held at National Institute for TB and Respiratory Disease, New Delhi during August 2015– Dina Nair.

10. Lecture on ‘Clinical trials in Ayurveda, Siddha and Unani medicine’ in CME for ASU faculty members / postgraduates on Good clinical practice’ held at Tamil Nadu Dr MGR Medical University during August 2015 – Syed Hissar.

11. Expand TB Symposium held at New Delhi during Sep 2015 - K.R. Uma Devi.

12. RePORT International Leadership Meeting held at Boston, USA, during Sep 2015 - Luke Elizabeth Hanna, N.S. Gomathi.

13. Participated in a 3 day workshop on SLD DST by LPA organized by Central TB Division, held at NTI, Bangalore during Sep 2015 – N.S. Gomathi.

14. Workshop on Special programme for Research and Training in Tropcial Diseases on the development of a global core competency framework for clinical research held at Geneva, Switzerland during Sept 2015 - Padmapriyadarsini C.

15. Participated in The Union's "National operational research course" – SORT IT held at Chennai during Sept 2015 – Dina Nair.

16. Participated in Consultation meeting on Modernizing TB treatment Adherence Monitoring & Support, organized by BMGF held at New Delhi during Oct 2015 – Beena Thomas.

17. First Medical Technology Steering Committee meeting held at Directorate of Medical Education, Kilpauk during Oct 2015 - K.R. Uma Devi.

18. Oral abstract presented at the Keystone Symposia on "Human Nutrition, Environment & Health held at Bejing, China during Oct 2015 – Padmapriyadarsini C.

19. UK-India Workshop on 'New diagnostics and therapeutics to tackle antimicrobial resistance' organized by UK Science & Innovation Network, British High Commission held at New Delhi during Oct 2015 – Syed Hissar.

20. Invited talk on "Infectious diseases and women" at the International Conference on Women and Health held at the Indian Institute of Science, Bangalore, during Nov 2015 - Luke Elizabeth Hanna.

21. Training programme on Global Health Security Agenda held at New Delhi during Nov 2015 - K.R. Uma Devi.

22. Lab Biosafety training held at NTI, Bangalore during Nov 2015 - K.R. Uma Devi.

23. Invited as a temporary advisor to the "Biregional Expert consultation on advancing implementation research on HIV/AIDS" held at Tokyo, Japan during Nov 2015 – Beena E. Thomas.

24. Poster presentation at National Conference on "Frontiers in MS technology and emerging applications" organized by Indian Institute of Chromatography and Mass Spectrometry held at Chennai during Nov 2015 – A.K. Hemanth Kumar.

25. National workshop on 'Economic Evaluation in Health Care' in Post Graduate Institute of Medical Education and Research, Chandigarh Organised by School of Public Health, PGIMER, Chandigarh in collaboration with Public Health Foundation of India held at New Delhi during Nov - Dec 2015 – M. Muniyandi.

26. Invited as a temporary advisor to the World Health Organization (WHO) in TDR consultation on promoting implementation / operational research in countries

receiving Global Fund grants, held at Geneva, Switzerland during Dec 2015 – Beena E. Thomas.

27. Workshop on "Recent developments in Medical Biotechnology and structure based drug design" at IIT, Guwahati, during Dec 2015 – Gayathri Devi.

28. WHO-Childhood TB subgroup meeting held Cape Town, South Africa during Dec 2015 – Syed Hissar.

29. Oral-e-poster presentation on "The role of the chest x-ray in the diagnosis of intra-thoracic childhood tuberculosis" in 46th Union World Conference on Lung Health meeting held at Cape Town, South Africa during Dec 2015 – Syed Hissar.

30. Asia Pacific Association for the study of liver – single theme conference on "Hepatitis C Virus infection and disease & recent advances in liver diseses' organized by Institute of Liver and Biliary sciences held at New Delhi during Dec 2015 – Syed Hissar.

31. Short course on 'Clinical trials – design, analysis, interpretation and reporting' organized by Dept of Biostatistics, CMC in collaboration with University of North Carolina, Chapel Hill, USA held at CMC, Vellore during Dec 2015 – Syed Hissar.

32. Workshop on "Systems biology of antimicrobial resistance" held at New Delhi, organized by ICMR-NIAID during Jan 2016 – Jagdish Chandra Bose.

33. Workshop on "Sequencing and data analysis" organized jointly by NIRT, India and NIH, USA, held at NIRT during resistance" at New Delhi, organized by ICMR-NIAID during Jan 2016 – Jagdish Chandra Bose, Sameer Hassan, Gayathri Devi.

34. Participated in Tr@inforPed HIV- TB Scientific Workshop held at Pune, during Jan 2016 - Dina Nair, Bella D, Angeline G, Poorana Gangadevi N.

35. Poster Presentation at Keystone Symposia "Tuberculosis Co-Morbidities and Immunopathogenesis" Keystone, held at Coloradao. USA during Jan 2016 - N Pavan Kumar, Anuradha R.

36. Symposium on "TB vaccines and immunity" held at Les Diablerets, Switzerland during Jan - Feb 2016 - S. Sivakumar

37. Participated in the 5th Annual Joint Leadership Group Meeting and 14th Symposium on advances in TB research, held at CMC Vellore, organized by RePORT India, RePORT International Consortium during Feb 2016 – N S Gomathi.

38. Oral presentation at the 70th National Conference on Tuberculosis & Chest Diseases (NATCON 2015) held in Lucknow during Feb 2016 – Geetha Ramachandran, N.S. Gomathy, Prabu Seenivasan, Radhakrishnan R, Joel Klinton.

39. Participated in the “Sequencing and Data Analysis in Bioinformatics Workshop” held at NIRT, during Feb 2016 - N. Pavan Kumar, Anuradha R.

40. Participated in “Bio-specimen and FreezerPro® Workshop” held at N I R T during Feb 2016 - N. Pavan Kumar, Anuradha R, Kadar Moideen.

41. Indo-Dutch Meeting organized by IAVI held at New Delhi, during Feb 2016 - Luke Elizabeth Hanna.

42. Guest Lecture on “Past, present and future of tuberculosis research” held at Institute of Basic Medical Sciences, Madras University, Chennai, during Feb 2016 - Luke Elizabeth Hanna.

43. First quarterly GHSA review meeting held at New Delhi during Feb 2016 – K.R. Uma Devi.

44. Meeting of the Technical Resource Group(TRG) for MSM, organized by NACO held at New Delhi during Feb 2016- Beena Thomas.

45. Sensitization Project workshop “Accelerating access to quality TB diagnosis for pediatric cases in Chennai, Hyderabad, Kolkata and New Delhi” held at NIRT, during Feb 2016 - K.R. Uma Devi.

46. Meeting of DR TB Counseling Committee, organized by CTD held at New Delhi during Feb 2016 – Beena Thomas.

47. Workshop on Biospecimen and FreezerPro organized by National Institute of Allergy and Infectious Diseases held at NIRT during Feb 2016 – A.K. Hemanth Kumar, V. Sudha.

48. Poster presentation at the conference on Retroviruses and Opportunistic Infections organized by International Antiviral Society, USA held at Boston, USA, during Feb 2016 – A.K. Hemanth Kumar.

49. Invited to present a lecture on ‘Advances in TB diagnostics” organized by Saveetha Medical College, held at Thandalam, Chennai during March 2016 - N.S. Gomathi.

50. Invited to present a lecture on “Phages in TB diagnosis” organized by Department of Microbiology, held at AIIMS, New Delhi during March 2016 - N.S. Gomathi.

51. Attended a pre-conference workshop in “Bioinformatics: Basics and Insights in NGS data analysis” held at PGIMER, Chandigarh during March 2016 – V.N. Azger Dusthackeer.

52. Participated in the International symposium on “Integration of genetics and genomics in laboratory medicine” held at PGIMER, Chandigarh, during March 2016 - V.N. Azger Dusthackeer.

53. Invited talk at the International Conference on Innovations in Diagnostics and Therapeutics organized by the Centre for Drug Discovery, Sathyabama University held at Chennai during March 2016 - Luke Elizabeth Hanna.

54. Workshop on "Antibiotics and Antimicrobial Resistance in Water Systems-Prevalence and impacts" held at IIT Madras during March 2016 - K.R. Uma Devi.

55. ‘Indiaclen’ Annual conference held at SRM University, Kancheepuram during March 2016 – Ramesh Kumar.

56. Participated in the Stop-TB Partnership for the M & E and research discussions, organized by Duke centre for International Development, Sanford School of public policy, Duke University held at New Delhi during March 2016 – Beena Thomas.

Workshop(s) / Symposia/ Other Events

1. **World Driver's day.**
   Date: Sept 17, 2015
   Venue: National Institute for Research in Tuberculosis, Chennai.

The "World Driver's Day 2015" was celebrated for the first time in NIRT on the 16th September 2015 that was organized by the drivers of the institution. It was a well-attended function where staff at all levels - scientific, technical and administrative participated with enthusiasm. The drivers had also invited dignitaries who participated in making this day a memorable one.

All the speakers expressed their appreciation to drivers and especially drivers of NIRT. Their commitment, hard work and enthusiasm to work was commended and greatly appreciated. In a research center like NIRT where there was a lot of focus on patients and their follow up for treatment. Drivers play a major role in case holding.

2. **Workshop on ‘’Sequencing and data analysis in Bioinformatics”.**
Date: Feb 9 – 13, 2016.
Venue: National Institute for Research in Tuberculosis, Chennai.

A workshop on “Sequencing and Data Analysis in Bioinformatics” was conducted jointly by NIH-NIAID during Feb 9 to 13, 2016 at the National Institute for Research in Tuberculosis. The major objective of the workshop was to train participants on analysis of large data generated through Next Generation Sequencing. The participants were trained in different bioinformatics tools and softwares for analysis and interpretation of large datasets. Some of the topics covered in the workshop included Mapping and De-novo Assembly, ChIP-seq, Microbiome Data Analysis and Microbial Ecology, Variant Calling and Exome Analysis, RNA-seq Data Analysis and R language for Next Generation Sequencing Data Analysis. The faculty included computational biology and structural biology specialists from the NIAID Bioinformatics and Computational Biosciences Branch (BCBB). The workshop was attended by 25 scientists and research scholars from various ICMR and non-ICMR organizations in the country.

3. **Workshop on ‘’Pharmacovigilance & Pharmacoepidemiology in RNTCP’’.**
Date: March 4-5, 2016.
Venue: National Institute for Research in Tuberculosis, Chennai.

A workshop on ‘’Pharmacovigilance & Pharmacoepidemiology in RNTCP’’ was conducted at NIRT during 4th and 5th March, 2016. The workshop was funded by the USAID through WHO-SEARO, New Delhi through the Model DOTS project. The objectives of this workshop were two-fold – firstly, to gain a better understanding of the ongoing pharmacovigilance programme and the challenges faced by the adverse drug reaction (ADR) monitoring centres in reporting ADRs, and secondly, to develop an operational research protocol related to daily anti-TB treatment in RNTCP.

We were fortunate to have experts from diverse disciplines - clinicians, program managers, microbiologists, clinical pharmacologists, health economists, statisticians, public health experts etc. The workshop participants comprised of programme managers representing the Central TB Division, WHO, Pharmacovigilance programme of India, Directors and their representatives from ICMR institutes, Pharmacology professors from Medical Colleges across India, STOs, DTOs, epidemiologists, clinicians in TB Patient Care etc.

There were presentations by experts on topics such as, Pharmacoepidemiology, RNTCP’s perspective on ADR Management and Pharmacovigilance, Role of medical colleges in Pharmacovigilance, Pharmacokinetics of anti-TB drugs, Pharmacoeconomics and Experience from RNTCP, ART and PvPI centres. ICMR institutes highlighted their role in the Pharmacovigilance/Pharmacoepidemiology and the future directions.

Dr Soumya Swaminathan, Secretary, Dept of Health Research & Director General, ICMR emphasised the importance of the Pharmacovigilance programme and the need for ICMR to participate and strengthen it.

The participants were divided into groups, which worked on different components of the operational research proposal. Each group presented their component which was further discussed.


Research Centre)
for Tuberculosis Research & Training
Dr. NA KSHIRSAGAR
04.03.2016 09:32


Introduction
Dr.(Mrs.) Nilima. A . Kshirsagar
National Chair (Clinical Pharmacology),
ICMR, New Delhi, Govt. of India,
NIRRH, ICMR, Parel, Mumbai-12
Email Id:- kshirsagarna@yahoo.in
covigilance & Pharmacoepidemiology in
RNTCP
Chennai
Date : 4th -5th March 2016
04.03.2016 09:41


National Institute for Research in Tuberculosis
(Tuberculosis Research Centre)
WHO Collaborating Centre for Tuberculosis Research & Training
Dr. PARVEEN SHARMA
04.03.2016 09:45

4. **Dissemination workshop titled "HIV prevention through mobile phone technology among male sex workers in India".**
Date: March 5, 2016
Venue: National Institute for Research in Tuberculosis, Chennai.

A dissemination meeting was held at the **National Institute of Research in Tuberculosis (NIRT) – ICMR** on the **5th March, 2016** to share the findings of the Intervention study using mobile phone technology for HIV prevention. The study was undertaken by the Department of Social and Behavioural Science Research (DSBR) in collaboration with Massachusetts General Hospital/Harvard Medical School (Boston, USA). The work shop was attended by around 100 members which included representatives from the state HIV prevention programme (TANSACS), NACO, CBO representatives, MSM community members and representatives from NIRT.

Dr. Soumya Swaminathan, Director General, ICMR welcomed the gathering and delivered the inaugural address. The importance of disseminating the findings which are key to translating research to policy was highlighted by her. Dr Swaminathan emphasized that impact in public health can be brought only by understanding the behaviour and the need for socio behavioural research.

The findings of the study were presented by Dr Beena Thomas (Indian PI) and Dr.Mathew Mimiaga (US-PI). The findings reflected the effectiveness of mobile phone technology in sexual risk reduction and as a feasible and acceptable HIV prevention strategy. Mr.Vivek Anand-CEO of Humsafar, a community based organisation (CBO) shared their experiences using technology through the internet as another strategy for those MSM who used the internet for risky sexual behaviours. Dr Thanikachalam, the joint Project Director of TANSACS appreciated the study and said that this was an intervention that needed to be advocated as a powerful HIV prevention strategy. The discussions from the floor were interactive and lead by a panel which include Dr Shyamala Nataraj founder of SIAAP (NGO), Jeya from Sahodaran (CBO) and Sankari from Neerankal (CBO). Dr Shyamala complimented the team for undertaking such a challenging study and commented on the professional expertise that the team had displayed in reaching out effectively through an innovative intervention to the MSM hard to reach community.


HPMP Dissemination
HPMP Dissemination

5. **ICMR-NIAID Joint Symposium "Advancing TB Vaccine Agenda in India & Beyond".**

Date: February 8 & 9, 2016

Venue: Venue: National Institute for Research in Tuberculosis, Chennai.

The NIRT organized a joint symposium on "Advancing TB Vaccine Agenda in India & Beyond" in association with ICMR and NIAID during February 8 & 9, 2016 at NIRT, Chennai. Dr Soumya Swaminathan, Secretary, Department of Health Research and DG, ICMR delivered the Presidential address. Dr Vijayaraghavan, Secretary, DBT was the Chief guest. Mr. Philip Min, US Counsel General, Chennai delivered a special address. The meeting was attended by researchers engaged in TB vaccine research across the globe. NIRT, Chennai, is planning a multi-centric post-treatment vaccine for TB in the near future.

International Yoga Day Celebrations on June 21 2015. Ms Nrithya Jagannathan from Krishnamachari Yogamandiram, Chennai, gave yoga lessons and demonstrated asanas. Children from neighbouring schools participated.

A meeting of experts was held to discuss the situation and future of Biorepositories in the country in July 2015.

A gathering was held to mark the passing away of our beloved ex-President Dr.APJ. Abdul Kalaam on July 29 2015.

A workshop on Meta Analysis and Systematic Reviews was held from Aug10 -14 2015

Annual Day Celebrations on Aug13 2015

Dr.V.M. Katoch, former DG ICMR was the chief guest. He was gifted a collage of Dr. Wallace Fox's photographs

Lab automation was inaugurated in the Bacteriology Department

Video-conferencing link with Madurai unit of NIRT was also inaugurated

A cultural event on the same day

Felicitations to Dr Soumya Swaminthan, Director, NIRT on becoming Director General, ICMR, on Aug.14 2015.

A gathering was held on March 12 2016 to condole the sudden and sad demise of Dr.V. Kumaraswami

Photos of departed National leaders were unveiled on March 17 2016

World TB Day was observed on 24 March 2016

Patients actively participated

Staff of NIRT performed a skit to dispel misconceptions regarding treatment of TB

Vigilance Awareness Week was observed between Oct 26-31, 2015

Ph.D. Scholars

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

Sl. No.	Name of the candidate	Title of the Ph.D. thesis	Part time / Full time	Supervisor/ Guide
1.	Mr. Brijendra Singh	Chemokine gene polymorphism and chemokine expression in PTB	Full time	Dr.P. Selvaraj
2.	Mr.K.Srinivasan	Role of *M. tuberculosis* PknI, a serine / threonine protein kinase and DacB2 a pencillin binding protein in the pathogenesis of TB	Full time	Dr. Sujatha Narayanan
3.	Ms.R. Lakshmi	Drug resistance in ethionamide and its association with resistance towards isoniazid in *M. tuberculosis*	Full time	Dr. Vanaja Kumar
4.	Mr.S. Sivakumar	Molecular epidemiology of tuberculosis from Tiruvallur, south India	Part-time	Dr. Sujatha Narayanan

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

Sl.No.	Name of the candidate	Title of the Ph.D. thesis	Part / Full time	Supervisor/ Guide
1.	Mr.M. Harishankar	Studies on the effect of 1,25-Dihydroxy-vitamin D_3 on chemokine expression and the regulatory role of variant vitamin D receptor genotypes on vitamin D_3 modulated chmokines in PTB	Full t ime	Dr.P. Selvaraj
2.	Mr.K. Afsal	Effect of 1,25-Dihydroxyvitamin D_3 on innate and adaptive immunity in PTB	Full time	Dr.P. Selvaraj
3.	Ms. Nancy Hilda J.	Chemokine gene polymorphism and chemokine expression in PTB	Full time	Dr.D. Sulochana
4.	Mr.P. Jovian George	Helminth Immunology	Full time	Dr. Luke E Hanna
5.	Ms.D. Santhi	Novel subunit vaccine targets from *M. tuberculosis*	Full time	Dr. Alamelu Raja
6.	Ms. Maddineni Prabhavathi	Comparative genomics and pathogenesis of TB	Full time	Dr. Alamelu Raja
7.	Mr.P. Pugazhvendhan	Immunoproteomic identification of B-cell antigens of *M. tuberculosis*	Full time	Dr. Alamelu Raja
8.	Mr.V. Arunkumar	Gene regulation of mycobacteria	Full time	Dr. Sujatha Narayanan
9.	Ms. Ahmed Kabir Refaya	Mycobacterial transcriptional regulators in pathogenesis	Full time	Dr. Sujatha Narayanan
10.	Ms. Vasantha M.	Structural equation modeling	Part-time	Dr.P. Venkatesan
11.	Ms.A.S. Shainaba	Phage based drug target identification and anti-mycobacterial drug discovery	Full time	Dr. Vanaja Kumar

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

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. thesis	Supervisor/Guide
1.	Mr.S. Balaji	ICMR	Diagnostic evaluation of novel T-cell (Rv2204c, Rv2394) antigens of *M. tb*	Dr. Alamelu Raja
2	Ms.G. Akilandeswari	INSPIRE FELLOW	Structural characterization of 3 essential genes from *M. tb*	Dr. Alamelu Raja
3.	Mr. Narayanaiah Cheedarla	UGC	Comparative studies between HIV-1 and HIV-2 cases in India	Dr. Luke E. Hanna
4.	Ms. Vidya Vijayan	INSPIRE FELLOW	Immunopathogenicity of HIV-2 infection	Dr. Luke E. Hanna
5.	Ms.B. Hemalatha	CSIR	Understanding pathogenesis of HIV infection	Dr. Luke E. Hanna
6.	Mr. Ashok Kumar	ICMR	Identification and biological characterization of Founder/Transmitted virus in early HIV-1 Clade-C infection in India	Dr. Luke E. Hanna
7.	Mr.S. Sivasankaran	DST	Evaluation of mucosal immune responses to HIV infection in discordant couples: Templates for a vaccine	Dr. Luke E. Hanna
8.	Mr.C. Yuvaraj	INSPIRE Fellow	Characterization of three intermediatory metabolic enzymes (DlaT, Icl-1 and GlnA 1) in the laboratory strain H37Rv and clinical isolates of MDR-TB	Dr.K.R. Uma Devi
9.	Ms. Gunapati Bhargavi	INSPIRE Fellow	Functional characterization of oxidoreductases of *M. tuberculosis*	Dr.P. Kannan

Staff (Part-time) registered for their Ph.D. programme with 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

OBITUARY

NIRT staff who passed away during the period 2015-2016

Dr.R. Prabhakar, Director 1983 -1995	–	04.10.2015
Dr.V. Kumaraswami, Director-in-charge 2008-2010	–	04.03.2016
Mr.P. Philipose	–	24.04.2015
Mr.K.R. Madhavan	–	30.06.2015
Mrs.J.L. Monga	–	August 2015
Mr. Appe Gowda	–	14.10.2015
Mr.V.K. Venkatesan	–	03.12.2015
Mr.R. Vijayakumar	–	17.02.2016
Mr. Stanly Jones Rajasingh	–	13.03.2016
Mr.D. Dakshinamurthy	–	30.03.2016

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS, CHENNAI

STAFF LIST

(As on 1st April 2016)

SCIENTIST 'G' & Director

1. Dr. Sanjay Mehendale, MBBS, MD, MPH

SCIENTIST 'G'

1. Dr. Alamelu Raja, M.Sc., Ph.D.
2. Dr. Srikanth Prasad Tripathy, MBBS, MD

SCIENTIST 'F'

1. Dr. Mohan Natrajan, MBBS, Ph.D., Dip. in Der.

SCIENTIST 'E'

1. Dr. P. Paul Kumaran, MBBS, MPH
2. Dr. Pradeep Aravindan Menon, MBBS, PGDPM
3. Dr. C. Padmapriyadarsini, MBBS, DNB, MS
4. Dr. D. Baskaran, MBBS, PGDTCD
5. Dr. Sudha Subramanyam, M.Sc., Ph.D.
6. Dr. Geetha Ramachandran, M.Sc., Ph.D.
7. Dr. Beena E Thomas, M.A., Ph.D.

SCIENTIST 'D'

1. Dr. KR Uma Devi, M.Sc., Ph.D.
2. Dr. S. Ramesh Kumar, MBBS
3. Dr. G.Narendran, MBBS, DTRD, DNB
4. Dr. C. Ponnuraja, M.Sc., Ph.D.
5. Dr. Luke Elizebath Hanna, M.Sc., Ph.D.
6. Dr. C.K. Dolla, MBBS, MPH
7. Dr. P. Kannan, M.V.Sc., Ph.D.
8. Dr. V.V.Banurekha, MBBS, PGDPH
9. Dr. K. Rajendran, M.Sc.

DBT-Ramalingaswami Fellowship

1 Dr.A.R. Anand, M.Sc. Ph.D.
2 Dr.B. Ramalignam, M.Sc., Ph.D.

SCIENTIST 'C'

1. Dr. A. Sheikh Iliayas, MBBS
2. Dr. P.K. Bhavani, MBBS, PGDPH
3. Dr. N. Saravanan, M.Sc., M.Phil., Ph.D.
4. Dr. S. Syed Hissar, MD, MPH
5. Dr. Dina Nair, MBBS, PGDPH
6. Dr. N. Poorana Ganga Devi, MBBS, PGDPH
7. Dr. A.K. Hemanth Kumar, M.Sc., Ph.D.
8. Dr. M. Makesh Kumar, MBBS
9. Dr. D. Bella Devaleenal, MBBS, PGDOL, MPH
10. Dr S Sriram, MBBS, MPH
11. Dr Angeline Grace G, MBBS, APGDCRMW, MPH
12. Dr. N.S. Gomathi, M.Sc., Ph.D.
13. Dr. M. Muniyandi, M.A., M.Phil., MPS, Ph.D.

SCIENTIST 'B'

1. Mr. S. Sivakumar, M.Sc., Ph.D.
2. Mr. P. Murugesan, M.A., PGDC, BDA
3. Dr. R. Priya, M.Sc., Ph.D.

Sr. Library & Information Officer

1. Dr. R. Rathinasabapati, MLIS, Ph.D.

Nur **Nursing Superintendent**

1. Ms. A. Gunasundari, M.Sc.

1. **Asst. Nursing Superintendent**

1. Ms. G. Mangalambal, M.Sc.

Nursing Sister

1. Ms. Valarmathi Nagarajan, M.Sc.
2. Ms. C. Kavidha, B.Sc.
3. Ms. S. Chellam
4. Ms. K. Sureswari
5. Ms. Shyamala Gopu
6. Ms. A. Komathi, B.Sc.

Staff Nurse

1. Ms. V. Revathy
2. Ms. R. Valarmathy
3. Ms. R. Manimegalai

4. Ms. V. Farthimunnisa
5. Ms. Shakila Shankar
6. Ms. V. Indirani
7. Ms. K. Porselvi, B.Sc.
8. Ms. A. Selvi
9. Ms. S. Stella Mary
10. Ms. A. Stella Mary
11. Ms. R. Saraladevi
12. Ms. P. Pandeeswari
13. Ms. M. Rathinam
14. Ms. S. Theensuwai, B.A.
15. Ms. P. Kowsalya
16. Ms. M. Mohana
17. Ms. R. Vetrichselvi, M.A.

Junior Staff Nurse

1. Ms.OR Vijayalakshmi
2. Ms.A.Vijayalakshmi
3. Ms.A.Poongkodi
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. Mr. Krishna Yadav Kattagoni, B.Sc.
10. Ms.J.Jemima, B.Sc.
11. Mr.N.Lokeswaran, B.Sc.
12. Ms.R.Selvi
13. 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. Mr. K. Sankaran, M.Sc.
2. Mr. M. Ponnambalam, B.Sc.
3. Dr. E. Thiruvalluvan, M.A., Ph.D
4. Mrs. Chandra Suresh, M.A.
5. Mrs. D.Kalaiselvi, M.A.
6. Mr. M. Rajasakthivel, M.A.
7. Mr. S. Manoharan, B.Sc.

Technical Officer – A

1. Mr. R.K. Rajendran
2. Ms. K. Silambu Chelvi, M.Sc., M.Phil.
3. Dr. L. Sekar, M.Sc., Ph.D.
4. Dr. K. Chandrasekaran, Ph.D.
5. Mr. E. Kirubakaran
6. Mr. T. Gowri Shankar
7. Dr. R. Srinivasan, M.Sc., Ph.D.
8. Ms. M. Vasantha, M.Sc., M.Phil.
9. Ms. S. Sivagama Sundari
10. Mr. K. Ramesh, M.Sc.
11. Mr. M Harishankar, M.Sc.
12. Mr. S. Anbalagan, M.Sc.
13. Ms. Lucia Precilla, M.Sc., M.Phil.
14. Mr. S. Senthil, M.A., M.Phil
15. Ms. V.M. Girijalakshmi, M.Sc.
16. Ms. A. Deepalakshmi, M.A.
17. Dr. K. Ramakrishnan, M.Sc., Ph.D.
18. Ms. D. Saraswathi, M.Sc., M.Phil.
19. Dr. S. Balaji, M.Sc., Ph.D.
20. Dr. D. Anbarasu, M.Sc., M.Phil., Ph.D.
21. Mr. S. Murugesan, M.Sc.
22. Mr. M. Anandan

Technical Assistant

1. Mr. C. Thirukumar, B.A.
2. Mr. M. Asokan
3. Mr. M Tamizhselvan, M.Sc.
4. Mr. D Thangaraj
5. Ms. K. Devika, M.Sc.
6. Mr. M. Baskaran, B.Sc.
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10. Mr. V. Thiyagarajan, M.Sc., M.Phil.
11. Mr. D. Ravikumar, M.Sc.
12. Ms. K. Sumathi, B.Sc.
13. Mr. S. Govindarajan, M.Sc.
14. Mr. K. Ramakrishnan
15. Mr. M. Michel Prem Kumar, M.Sc.
16. Ms. B. Pricilla Rebecca, M.A.
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18. Ms. A. Dhanalakshmi, M.A.
19. Ms. Senthanro Ovung, M.S.W.
20. Ms. V. Mythily, M.Sc.
21. Ms. M. Muthu Vijayalakshmi, M.Sc., M.Phil., B.Ed.
22. Mr. P. Palaniyandi, M.Sc., M.Phil.
23. Dr. C. Manogaran, M.A., M.Phil., Ph.D.
24. Ms. G. Radhika, B.Sc.
25. Ms. H. Hemalatha, M.Sc.
26. Ms. Devi Sangamithrai, M.Sc.

27. Ms. K. Jeyasree, B.Sc.
28. Mr. A. Madheswaran, B.Sc.
29. Mr. S. Venugopalan, M.Sc.

Technician – C

1. Mr. D. Madhavan, M.Sc.
2. Mr. RK Syed Nisar, M.Sc.
3. Ms. B. Brindha, M.Sc.
4. Mr. P. Nagarajan, M.Sc.
5. Mr. P. Sivaraman, M.Sc.
6. Ms. V. Sudha, M.Sc., M.Phil.
7. Ms. R. Nithya, B.Sc.
8. Ms. Rohini Puvaneshwari, B.Sc.
9. Mr. R. Rajkumar, B.Sc.
10. 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. P. Chandran
2. Mr. M. Kawaskar
3. Mr. Santhana Mahalingam, B.Sc.
4. Mr. T. Bharathiraja, B.Sc.
5. Mr. S. Mangaiyarkarasi, M.Sc.
6. Mr. M. Pandidurai
7. Mrs. R. Sathya, B.Sc.
8. Mr. T.M. Loganathan
9. Mr. A. Vijayakumar, M.Sc.

Technician – A (Engg.Support)

1. Mr. P.Johnson Kennedy
2. Mr. Harihara Ganapathi Subramanian

ADMINISTRATION

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.

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.
5. Ms. M.N. Raadha, M.C.S.
6. Mr. A. Lakshmanan
7. Ms. L. Vijayakumari
8. Mr. B. Durai Raj
9. Ms. P Kavitha, MA, MBA

Personal Assistant

1. Ms. PS Shanthi
2. Ms. A.L. Rajalakshmi, B.Sc., MLIS, MBA
3. Mr. H. Krishna Kumar, B.Com.

Senior Tel. Opr.-cum-Recept.

1. Mrs. V.Shailaja Devi

Upper Division Clerk

1. Mr. S. Anandaraj
2. Ms. S.Nirmala, MA
3. Ms. J.Suguna, B.Com.
4. Ms. T.Sheela
5. Mr. A.Gopinathan, BA
6. Mr. V. Velmurugan
7. Mr. R. Hariharan, B.Com.

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3. Ms. G.H. Jyothipriya, B.E.
4. Mr. S. Sasikumar, BCA
5. Ms. K. Thiriveni, B.Tech., M.Phil.

Lower Division Clerk

1. Mrs. K.Sumathi, B.A
2. Mr. M.Mohan Shankar
3. Ms. D Tamilselvi, BBA, MBA
4. Mr. D. Sukumar
5. Ms. S Sundari, M.Sc.

Senior Record Sorter

1. Ms.Molly Joseph
2. Mr.K.Ganesan
3. Mr.J.Loganathan

Staff Car Driver (Special Grade)

1. Mr. R. 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
8. Mr. K. Sridhar

Multi Tasking Staff

1. Mr. G.Moshe
2. Mr. C.Nagaraju
3. Mr. P.Vijayakumar
4. Mr. V. Mohan
5. Mr. A.Annamalai
6. Mr. R.Damodharan
7. Mr. D. Bose
8. Mr. N.Murali
9. Mr. C.K.Chittarasu
10. Mr. M.Jayaraj
11. Mr. A.Rajavarman
12. Mr. J.Venkatesan
13. Mrs. R.Ankamma
14. Mr. R. Ankaiah
15. Mr. R.Ravichandran
16. Mr. V.Adikesavan
17. Mr. V.Sundarajan
18. Mr. K.Kuttappan
19. Mr. J.Selvam
20. Mr. G.Easwaran
21. Mr. N.Ankaiah
22. Mrs. Kasiammal
23. Mrs. P.Arul Mani
24. Mr. C.Uthra Bahadur
25. Mrs. S.Lakshmi
26. Mr. Keshbraj Paudel
27. Mrs. D.Sharadha
28. Mrs. B.Nageswari
29. Mr. G.Durai
30. Mr. B. Venkateswaralu
31. Mrs. J.Neelavathy
32. Mrs. H.Ponrose
33. Mrs. T.Thilakavathy
34. Mr. G.Nithyanandam
35. Mr. S.Venkatesan
36. Mr. R.Mohanraj
37. Mr. J.Santhakumar
38. Mr. S.Anjaiah
39. Mr. P.Senthilvelan
40. Mr. P.Kosalaraman
41. Mr. S. Nagarajan
42. Ms. P. Hemalatha
43. Mrs. R.Sakila
44. Mr. M. Manikandan
45. Mr. KN Thirumalai
46. Ms. K. Kamatchi
47. Mr. D Rajasekaran
48. Mrs. P Pandiselvi
49. Mrs. V. Amudhavalli
50. Mr. JV Mohanraj
51. Mr. T. Kumar
52. Mr. D. Ravichandran

N I R T – EPIDEMIOLOGY UNIT, CHENNAI

STAFF LIST

(As on 1st April 2016)

Scientist 'B'

1. Dr.V.N. Azger Dusthackeer, M.Sc., Ph.D.
2. Dr.S. Devarajulu Reddy, M.B.B.S.
3. Ms.R. Mahalakshmi, M.Sc.,

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.D. Sargunan, M.A.,
3. Mr.M. Kalyanaraghavan, M.Sc.,
4. Mr.S. Egambaram, M.A.,
5. Mr.A.S. Tholkappian, M.Com.,
6. Mr.S. Vijayaraj, M.Sc.,
7. Dr. Gomathi Sekar, M.Sc., Ph.D.,
8. Mr.J. Devan, M.Sc.,
9. Mr.T. Nataraj, M.Sc.,
10. Mr.G. Komalesswaran, M.Sc.,
11. Mr.V. Partheeban, M.A.,
12. Mr.K. Balakaliyan, B.Sc.,
13. Mr.S. Nambirajan, M.Sc, M.L.T.,
14. Mr.Senthil Kumar K, M.Sc, M.Phil.,
15. Mr. Radhakrishnan A, M.Sc.,
16. Mr. Ranganathan K, B.Sc.,
17. Mr.P. Munivarathan, B.Sc.,
18. Mr.D. Nithyakumar, M.Sc.,
19. Mr.B. Senthil Kumar, M.Sc.,
20. Ms.D. Kalaivani, M.Sc.,
21. Ms. Thangam (a) Meenakshi, B.Sc.,
22. Mr.V. Ramesh Babu , C R A
23. Mr.A.M. Ramesh, M.A.,
24. Mr.P.K. Venkataramana, B.Com.,
25. Mr.N. Premkumar, B.Sc.,
26. Mr. Venkatesan S, M.A, B.Ed.,

Technical Assistant

1. Mr.S.V. Joseph Rajkumar
2. Mr. Basilea Watson, M.Sc.,
3. Ms. Suganthi C, M.Sc, D.M.L.T.,
4. Ms. Malathi M, M.Sc, C.L.T.,
5. Mr. Lakshmikanthan N, M.A.,
6. Mr.T. Thangaraj M.A, B.Ed.,
7. Ms. Mahizhaveni B, M.Sc, D.M.L.T.,
8. Ms. Vadivu G, M.Sc, D.M.L.T.,
9. Mr. John Arokiya Doss Y, M.Sc, DMLT
10. Mr.K. Rajaraman, M.Sc, C.L.T.,
11. Mr.A. Vasudevan, M.Sc.,(Bio-Chem.) M.Sc., (Clinical Microbiol.)
12. Mr.M. Mahesh Kumar, M.Sc, D.M.L.T.,
13. Mr.K. Anbarasan, M.Sc., M.Phil.,
14. Mr.M. Karthikesan, M.Sc.,
15. Mr.P. Chandrasekaran, M.Sc, M.B.A,
16. Mr.P. Kumaravel, M.A, D.M.L.T.,
17. Mr.B. Ananda kumar, M.Sc, M.B.A.,
18. Mr.S.S. Jeganathan, B.Sc.,
19. Ms.P. Devi Bhagavathy, BCA, MBA.,
20. Mr.T. Kannan, M.Sc., M.Phil.,
21. Ms.R. Vijayalakshmi, M.Sc.,
22. Mr.T.K. Bharath, M.Sc.,
23. Mr.C. Saravanan, BLIS, M.A.,
24. Mr.P. Sathyamurthi, M.Sc.,
25. Dr. Angadi Kiran Kumar, M.Sc., Ph.D.
26. Mr.M. Kannan, B.Sc.,
27. Mr. Manohar Nesa Kumar, M.Sc.,PGMLT
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29. Mr.P. Balaji, M.A., B.Ed.
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Technician – C (Eng. Support)
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2. Mr.B. Vijayakumar
3. Mr.R. Balu

Technician - B
1. Mr.L. Venkatesan
2. Mr.W. Wilkingson Mathew
3. Mr.R. Krishna Bahadur

Technician – B (Eng. Support)
1. Mr.K. Poongavanam

Technician – A (Eng. Support)
1. Mr.J. Ravi

Technician - A
1. Mr.R. Purushothaman
2. Mr.N. Srinivasan

Staff Car Driver Spl Gr
1. Mr.J. Prakash

Staff Car Driver (Grade-I)
1. Mr.P. Soundararajan
2. Mr.V. Thanigaivel
3. Mr.M. Manogaran
4. Mr.K. Saravanan
5. Mr.A.S. Dayalan

Staff Car Driver (Grade-II)
1. Mr.P. Subbaiah
2. Mr.B. Sureshkumar
3. Mr.K. Thulasingam
4. Mr.K. Jagadeesan
5. Mr.J. Loganathan
6. Mr.V. Babu
7. Mr.V.S. Senthilkumar

Staff Car Driver Ord. Grade
1. Mr.G. Vasu
2. Mr.S. Doss
3. Mr.L. Gunalan
4. Mr.M. Pushparaj
5. Mr.M. Anbalagan
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