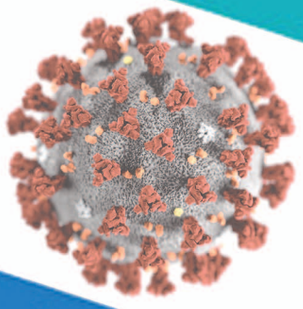
ICMR | NIRT

INDIAN COUNCIL OF MEDICAL RESEARCH

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS

ANNUAL REPORT



2019-20

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

ICMR-NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS

Research Activities

April 2019 – March 2020

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PREFACE

I am pleased to introduce this edition of ICMR-NIRT's Annual Report because 2019 was a very satisfying year for ICMR-NIRT with many new beginnings that have potential to bring a change in the history of Tuberculosis. This edition highlights the Institute's continued commitment and collective efforts to provide high-quality research to support the Nation's TB Elimination Program.

Our priority has always been to identify regimens that can be used in the national and global programs in the control of TB. During the year under review, our efforts to identify patient-friendly shorter regimens for DS- and DR-TB continued. This included shorter regimens for TBLN, MDR- and XDR-TB, repurposing of drugs, and higher doses of drugs for DS-TB, NTM, and pediatric TB management. This year also saw the initiation of a landmark trial in TB prevention – Vaccine for prevention of TB among the household contacts of TB patients. Closely linked to the evaluation of chemotherapy and TB vaccine, studies of behavioral aspects of patient and health systems continue.

The Epidemiology Division continues to carry out the National Survey of the State-wise prevalence of pulmonary TB in the country as well as the sentinel surveillance for TB burden in high-risk groups for TB.

The Bacteriology Department completed validation of indigenous kits that are simple, rapid, and reliable to diagnose TB and recommended for inclusion in the diagnostic algorithm of National TB programs working towards TB elimination. The department continues to provide expert services to not only DS- and DR-TB but also to non-tuberculosis mycobacteria. This year also saw the full automation of the Bacteriology Department of ICMR-NIRT.

The Clinical Pharmacology Department standardized serum estimation of newer drug levels including bedaquiline. They continue to work with drug level estimation of repurposed drugs like verapamil, metformin, sulphonylurea besides the I-line and II-line anti-tuberculosis drugs.

This year also saw ICMR-NIRT's Department of Biochemistry initiate work with the AYUSH system of medicine evaluating and characterizing potential therapeutic leads as adjuvant therapy during anti-tuberculosis treatment.

Our labs continue to successfully run the PBMC Cryopreservation program and assess the viability and recovery of the PBMC at other labs participating in this program. Activities related to the early antibacterial activity of anti-TB drugs, whole-genome sequencing, transcriptomics, and immunoproteomic analysis continue to progress in our labs. We have also started a prospective observational cohort for HIV infected adults under the National HIV Cohort Program this year.

Other important research activities of the institute include Statistics, Electronic Data Processing, and Health Economics. The institute continues to work in the field of Health Technology Assessment and a project on Hepatitis B & C is currently ongoing. The institute continues to be recognized for its expertise in the field of TB research and training for which

it is recognized as a WHO collaborating centre and also as an International Centre of Excellence in Research (ICER) by the National Institute of Health. The ICER laboratory continues its work on TB including extra-pulmonary TB and TB in pregnancy besides the TB comorbidities including helminth infection, diabetes mellitus.

The Department of HIV/AIDS at ICMR-NIRT has been serving as one of the Regional Reference Laboratories to the National AIDS Control Organization's Early Infant Diagnosis Program and ART Programs. The Department is enrolled in external quality assurance programs offered by the best agencies for each of the diagnostic services it provides for ICMR-NIRT's clinical trials and research studies

The studies carried out in this institute fully support the national and international TB elimination program and the members of the institute play significant roles in strengthening the TB elimination program globally.

I place before you this annual report that represents the combined efforts of all the staff members of ICMR-NIRT and invite your valuable suggestions to help us improve our efforts towards TB elimination.

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National Institute for Research in Tuberculosis
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Rasta Peth, Pune 411 011

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Head, Dept. of TB & Respiratory Diseases
National Institute of TB & Respiratory Diseases
Sri Aurobindo Marg
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Dr. Sanjay Pujari
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Institute of Infectious Diseases
Business Court, D Wing
Mukundnagar, Pune 411 037

Dr. Rajesh S Gokhale
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National Institute of Immunology
Aruna Asif Ali Marg
Jawaharlal Nehru University
New Delhi 110 067

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Dept. of Microbiology and Cell Biology
Indian Institute of Science
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All India Institute of Medical Sciences,
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Veer Savarkar Marg
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Dr. Raj Kumar
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North Campus, Vijay Nagar Marg
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Dr.K. Sachdeva
DDG (TB),
Room Noi. 522-C, NirmanBhawan
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Porur, Chennai 600 116

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11th Main Road, Anna Nagar,
Chennai 600 040

Dr. Arun Kumar
Vice Principal and Professor of Pharmacology,
Chettinad Hospital and Research Institute
Plot No. 93, Mahalaxmi Nagar part 6, Nandivaram,
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"Samarth", New No.100,
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Dept. of Clinical Pharmacology

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Dept. of Clinical Pharmacology

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Dept. of Statistics

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Liaison Officers **:**
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SC/ST – Dr.P. Kannan (Phone: 2836 9627 / 9500)

ABBREVIATIONS

ART	Anti-retroviral treatment
ATT	Anti-TB treatment
ATP	Adenosine Triphosphate
BDQ	Bedaquiline
BMI	Body mass index
cART	Combinational antitretrovial therapy
CD	Crohn's disease
CHV	Community Health Volunteers
CRT	Co-receptor tropism
CSF	Cerebrospinal fluid
CVL	Cervicovaginal lavage
DBS	Dried blood spot
DEL	Delamanid
DM	Diabetes mellitus
DMC	Designated microscopy center
DR-TB	Drug resistant-TB
DST	Drug susceptibility testing
EBA	Early bactericidal activity
EID	Early infant diagnosis
EMB	Ethambutol
FDC	Fixed dose combination
FGDs	Focus group discussions
GIS	Geographical Information System
HC	Healthy controls
HDTs	Host-directed threrapies
HHC	Healthy household contacts
HTS	High throughput sequencing
I-CVI	Item level content validity index
IDI	In-depth interviews
IGRA	Interferon Gamma Release Assay
INH	Isoniazid
JD	Johne's disease
LAM	Lipoarabinomann
LAMP	Loop mediated isothermal amplification assay
LIMS	Laboraotry information management system
LLR	Log likelihood ratio
LTBI	Latent TB infection
LRP	Luciferase reporter phage
MAP	*M. avium* subspecies *paratuberculosis*
MDR-TB	Multi-drug resistant TB
MOX	Moxifloxacin
MMP	Matrix metalloproteinases
MSM	Men having sex with men
NAA	Nucleic acid amplification
NACO	National AIDS Control Organization
NGS	Next generation sequencing
NHP	National health policies
NT	Non-transmitted

OF	Ofloxacin
OSE	Onsite evaluaiton
PBMC	Peripheral blood mononuclear cells
PBTS	Peripheral blood transcriptional signature
PCR-RFLP	Polymerase chain reaction based restriction fragment length polymorphism
PK	Pharmacokinetic
PMDT	Programmatic management of drug-resistant TB
PPS	Probability Proportional to Size
PPV	Positive predictive value
PTB	Pulmonary tuberculosis
PZA	Pyrazinamide
QALYs	Quality adjusted life years
RBT	Rifabutin
RCT	Randomized clinical trials
RePORT	Regional prospectrive observational research in TB
RMP/RIF	Rifampicin
RNTCP	Revised national TB control programme
SNPs	Single nucleotide polymorphisms
STI	Sexually transmitted infection
TAT	Turnaround time
TBM	Tuberculous meningitis
TBDM	TB and DM
TDM	Therapeutic drug monitoring
TF	Transmitted founder
Tr	Transitmycin
V3	Envelope
VAP	Vaccine action program
VCT	Voluntary counselling & testing
VDBP	Vitamin D binding protein
VFDB	Virulence factor database
WGS	Whole genome sequencing

CLINICAL STUDIES

DEPARTMENT OF CLINICAL RESEARCH

STUDIES COMPLETED:

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
Source of funding	:	USAID (Model DOTS Project)
Study period	:	2013-2020

Background: The lack of a gold standard for diagnosis is a major obstacle for 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.

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.

Objectives:
(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 and

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

Methodology: All children aged < 15 years attending the pediatric out-patient department with any of the following were screened for the study - (a) cough (b) weight loss/ failure to thrive (c) persistent unexplained fever (d) persistent, unexplained lethargy or reduced playfulness. Symptom screening, a detailed general and clinical evaluation was done. Chest X-ray, TST, collection of gastric lavage /induced/expectorated sputum for Xpert® MTB/RIF, AFB smear, culture, and DST if culture positive, were done.

In addition, in infants (i.e. aged < 1 yr), stools was collected for 2 consecutive days which was examined by Xpert® MTB/RIF for AFB smear, culture and DST if culture positive. Blood investigations and FNAC were done if needed. TB diagnosis in children was classified into the following groups based on smear result, chest radiograph and TST as confirmed TB, probable TB and others. Follow-up was done at 2nd week, 4th week, 8th week and at the end of treatment.

Results: The study was initiated in August 2013. The study enrolled

children from five centres, namely Govt. Stanley Hospital (GSH), Chennai; Institute of Child Health (ICH), Chennai; Govt. Vellore Medical College (GVMC), Vellore; Christian Medical College (CMC), Vellore and Govt. Rajaji Hospital (GRH), Madurai. As of March 2020, we screened 3823 children, and out of which 2416 children were successfully enrolled. The baseline characteristics of the enrolled children are detailed in Table 1. Overall, 8.2% of children were bacteriologically positive by smear and/or Xpert and/or MGIT/LJ culture. Children positive only for AFB smear were 2.2% and children positive only for Xpert Mtb were 4.3% (Table 2). The study has been completed.

Table 1: Baseline characteristics of children (n = 2357)

		No. of children*
		N (%)
Age	0-1 yr.	95 (4.0%)
	2-5 yrs.	943 (40%)
	6-10 yrs.	973 (41.3%)
	11-15 yrs.	346 (14.7%)
Gender	Male	1274 (54.1%)
	Female	1083 (45.9%)
TST	Positive	582 (24.7%)
	Negative	1775 (75.3%)
Previously treated with ATT	Yes	142 (6.0%)
	No	2215 (94.0%)
Contact with TB case	Yes	1087 (46.1%)
	No	1270 (53.9%)
Chest X-ray abnormality (n = 1969)	Yes	489 (24.8%)
	No	1480 (75.2%)
HIV	Yes	36 (1.5%)
	No	2321 (98.5%)

* Table shows the data entered in the database

Table 2: Bacteriology results

	Total (n = 2254)
No. of children positive for smear and/or LJ and/or MGIT and/or Xpert No. of children negative for all microbiological investigations	185 (8.2%) 2070 (91.8%)
No. of children with smear results available No. of children with positive smears	2253 50 (2.2%)
No. of children with at least one available LJ culture results No. of children with LJ culture positive	2253 106 (4.7%)
No. of children with at least one available MGIT culture results No. of children with MGIT culture positive	2169 127 (5.8%)
No. of children with at least one available Xpert results No. of Xpert positive cases No. of RIF resistant cases	1988 85 (4.3%) 5 (5.9%)

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

Principal Investigator	:	Dr.M. Makeshkumar
Source of funding	:	ICMR - Intramural
Study period	:	2013-2018

Background: Diabetes has a major impact on the epidemiologic dynamics of TB and poses several challenges for TB control in a resource country like India. Diabetes/TB burden can be brought under control by the 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 on control of TB, and the current gaps in knowledge related to diagnosis, prevention and treatment of TB among diabetics in the Indian population, this cohort study, which was first of its kind to be done in a representative population to establish the incidence of TB among Type 2 diabetes mellitus 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 the 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

Methodology: This is a Multicentric prospective cohort study among Type 2 diabetic patients to study the incidence of TB. Study participants were recruited from patients who attended Diabetic OPD at Govt. General Hospital Chennai, Govt. Rajaji Hospital Madurai and MV Hospital for Diabetes, Royapuram, Chennai.

Study progress: The study recruitment was stopped in June 2017. The recruitment details are shown in Table 3.

Table 3: Demographic details

	Chennai	Gender	Madurai	Gender	Total
Screening	495	M -109	258	M- 73	753
		F - 386		F - 185	
Admission	406	M-83	170	M- 44	576
		F - 323		F- 126	
Referred Back	89		88		177
Discharged Cases	349		156		515
Drop out	57		14		61

M - Male, F- Female

Details of patients recruited to the study are shown in Table 4.

Table 4: Anthropometric measures and diabetic status of recruited participants

Parameter	Value
Mean age with SD	50.46 (±8.60)
Mean weight with SD	61.62 (±10.01)
HBA1C	8.18 (±1.63)
Fasting blood glucose	166.6 (±65.06)
Post-prandial blood glucose	276 (±91.20)

Two participants were smear-positive during screening and they were referred to RNTCP for further management.

Four cases were smear-positive during follow-up. They were evaluated further their sputum smears and x-ray were found to be normal and their sputum cultures were negative.

The study was reviewed by the Pre SAC of NIRT in July 2017. The committee recommended that as not a single case of confirmed TB was detected among hundreds of diabetes followed up for 2 years, the study might be discontinued. All the follow-up visits for the recruited patients were completed and the data analysis is in progress.

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

Principal Investigator	:	Dr. Dina Nair
Source of Funding	:	ICMR-Task Force
Study period	:	2015-2018 (extended for two years)

Background: The transmission, dynamics of DR TB bacilli among the household contacts of MDR-TB patients is uncertain. In India, the data on screening and prevalence of LTBI among child contacts of MDR-TB is sparse. The value of contact tracing and the prevalence of LTBI requires more exploration in India. We proposed a study and understand the risk of transmission among household contacts of DRTB patients.

Methodology: The eligible MDR-TB index cases with their pediatric contacts (<15yrs) were enrolled into the study. Symptom screening, TST, Chest radiograph were done for all contacts. Three groups identified were i) LTBI group - asymptomatic, TST positive ii) TB exposed group – asymptomatic, TST negative iii) Confirmed TB group. The LTBI and TB exposed groups were followed up once in 3 months for 24 months. Those with confirmed TB were referred to the RNTCP for initiation of treatment.

Results: The study was completed in NIRT on 30th March 2020. A total of 151 index cases and 296 of their paediatric household contacts who were less than 15 years were recruited into the study. Among the index cases, 73.5% were males, mean age was 39 years, and the mean number of paediatric household contacts less than 15 years was 2. Almost, 72% had bilateral lung involvement and 49% had cavities in their X-ray Chest. Around 69% of them had a previous history of ATT. Among the 296 enrolled children, 50% (n=149) were males. At baseline, the median age of the children was eight years and the median duration of contacts with the index case was eight years. Among them, 0.7 % (n=2) were HIV positive, 0.7% (n=2) had previous history of INH prophylaxis and 2% had a previous history of ATT. Almost 21% (n=61) were TST Positive (>=10mm). X-ray chest was normal in 89%. At baseline, the number of children with latent TB infection was 19% (n= 56), TB exposed was 81% (n= 239) and TB diseased was one (DS-TB). At 12 months of follow up, the number of children with latent TB infection was 28%, TB exposed were 72% and TB diseased was null. At 21st month follow up, one child was diagnosed with TB. Around 251 paediatric household contacts completed 24 months follow up. At 24 months of follow up, the number of children with latent TB infection was 33%, TB exposed were 67% and TB diseased were none.

Conclusion: Almost one third of the paediatric household contacts of the DRTB patients have latent TB infection. Contact screening and options for prophylaxis for DRTB are the need of the hour to control the spread of DRTB.

CL-16: A study on the effectiveness of food supplement on treatment outcomes and nutritional status of adults with PTB on a retreatment regimen

Principal Investigator	:	Dr. Devarajulu Reddy
Source of Funding	:	Intramural
Study period	:	2017-2019

Background: TB remains one of the major infectious causes of morbidity and mortality worldwide. Diabetes mellitus, undernutrition,HIV, immunosuppression, smoking, alcohol, etc. are a few known high-risk factors for the disease. Undernutrition is a common co-morbid condition for people with active TB and is associated with an increased risk of mortality and poor treatment outcome. Nutrition supplementation to TB patients has not only shown weight gain but also shorter time to sputum conversion, higher cure rate, better quality of life and functionality. We conduct a study to understand the effectiveness of food supplement on the treatment outcomes and loss to follow-up, among adults on retreatment regimen for PTB, attending RNTCP centres in 7 TU'S in Vellore district, Tamil Nadu.

PTB patients aged >18 years, with sputum smear-positive for AFB (treatment failure/relapse/default for ATT/MDR), are screened and clinical examination is done. Sputum smear reports are collected from RNTCP cards at 0, end of 3rd & 8th months. The supplement, given fortnightly as 500gm pack of enriched flour, was distributed to all the tuberculin units of Vellore in a phased manner. The flour consisted of finger millet (ragi), rice flakes, ground nut and roasted Bengal gram with an advice to cook and consume 30gm per day with milk or hot water, which will give an additional 99.35 calories. ATT was given as per the national programme guidelines. Study staff performed surprise home visits to check adherence to the supplement and to ATT.

Sample size: 435

Results: 425 retreatment adult PTB patients (79% males, 12% unemployed and 48% illiterate) were enrolled to the study with 293 receiving and 132 not receiving the nutritional supplement. At the end of treatment, there was no significant difference between the two groups in weight gain [48.3 (42.8 - 58) vs. 49.1 (43 - 57), p=0.643], or the proportion of smear conversion [60 (45 – 79) vs. 82 (45 – 118), p=0.810] or quality of life [55.5 (46.1 – 62.3) vs 56.4 (46.1 – 65.3), p=0.590] between the groups. But treatment completion [30(11%) vs 4(3%), (p= 0.011)] and favorable outcomes [191(67%) vs 74(56%), (p= 0.028)] was significantly higher in the intervention group. There was also a difference noticed in the mid-arm circumference and waist circumference between the groups.

Conclusion: Nutritional supplement though may not cause weight gain or faster sputum smear conversion, acts as a treatment enhancer and improves treatment completion and favorable outcomes among re-treatment patients.

Table 5: Baseline Characteristics

Variables	Control	Intervention	Total	P-value
Smear Results				
Negative	21 (16%)	113 (40%)	134 (32%)	<0.001
Positive	110 (84%)	170 (60%)	280 (68%)	
Outcome				
Favourable	74 (56%)	191 (67%)	265 (64%)	0.028
Unfavourable	57 (44%)	92 (33%)	149 (36%)	
Outcome (Specific)				
Bacteriological Cured	70 (53%)	161 (57%)	231 (56%)	0.024
Rx Completed	4 (3%)	30 (11%)	34 (8%)	
Death	7 (5%)	17 (6%)	24 (6%)	
Incomplete Rx	38 (29%)	56 (20%)	94 (23%)	
Bacteriological Failure / Recurrence	12 (9%)	19 (7%)	31 (7%)	

CL- 18: Cambridge-Chennai Centre Partnership on antimicrobial resistance in TB: Focus on novel diagnostics and therapeutics

Principal Investigator : Dr. Mohan Natrajan
Source of Funding : Global fund through CTD and DBT
Study period : 2016-2019

Background: The sixth component of the WHO Stop TB Strategy emphasizes the promotion of research into new diagnostics, drugs and vaccines. In line with this, five different but closely related projects have been undertaken by partners in NIRT, Chennai, and University of Cambridge, with the overall goal of understanding the pathways and mechanisms of antimicrobial resistance in TB and identifying potential diagnostic and therapeutic targets.

Objectives:
(i) To establish and maintain 2 longitudinal prospective cohorts -- adults with Drug Resistance-PTB (DR-TB Cohort / Cohort-I) and drug-sensitive PTB (DS-TB Cohort / Cohort-II) to generate a clinical database and provide specimens to all the five projects;
(ii) To create a database of *M. tuberculosis* genomes for isolates from India and to define the accuracy and clinical applicability of genetic DST;

(iii) To study the structural and functional implications of novel mutations identified from whole-genome sequencing (WGS) of Indian strains for protein structure and function;
(iv) To develop assays to detect bacteria that express efflux pumps in patients' sputa and to assess directly the effect of verapamil over sputum bacteria singly or in combination with anti-tuberculous treatment;
(v) To identify the T-cell co-stimulatory pathways responsible for controlling exhaustion in DR and DS-TB patients and to perform *in silico* screening for existing pharmaceuticals that limit or reverse exhaustion at the transcriptional level.

Clinical Cohorts:

Principal Investigator : Dr. Mohan Natrajan /
Dr. C. Padmapriyadarsini

Clinical Cohorts & Sample size
COHORT I - 50 patients with pulmonary MDR-TB
(with and without additional drug resistance)
COHORT II - 100 patients with newly diagnosed DS- PTB (DS-TB)

Patient enrolment, sample collection / processing & follow-up: Two new longitudinal prospective cohorts were recruited in order to support all the five proposed projects. Following recruitment, all patients in the Cohort II were followed up for a period of 30 months (24 months of treatment and 6 months of post-treatment follow-up) and all patients in the Cohort II were followed up for a period of 12 months (6 months of treatment and 6 months of post-treatment follow-up). Clinical examination and 2 sputa were collected every month. Blood samples were collected at specific time points (Cohort-I – 0, 2, 6, 12, 18, 24, 30th month; Cohort-II – 0, 2, 6, 12th month). Two sputa and blood samples were collected in the event of failure or relapse.
Sputum samples were utilized in projects 1 to 4 and blood samples were utilized in project 5. Sputum samples were subjected to smear, culture by LJ/MGIT, phenotypic DST by MGIT and whole-genome sequencing for baseline samples only. The baseline blood samples from eligible patients were subjected to, safety lab tests, antigenic stimulation with different peptides, flow cytometry analysis of different markers of T-cell anergy, flow sorting, RNA isolation from specific T-cell subsets, etc.

Progress in recruitment and follow-up: After establishing both cohorts, the 6-month follow-ups of cohort II was completed with 10% default, 14% failure, 3% death, and 5% relapse respectively. In Cohort-I, 50 patients completed 30 months follow-up (24 months of treatment and 6 months of post-treatment follow-up) with 12% default, 20% failure, 8% death, and 4% relapse respectively.

CL-22: A randomized trial of therapy shortening for minimal TB with new WHO-recommended doses/fixed-dose-combination of drugs in African and Indian HIV+ and HIV- children (SHINE Study, SHorter treatment for mINimal TB in childrEn)

Principal Investigator	:	Dr.S. Syed Hissar
Source of funding	:	DFID-UK, Wellcome Trust, MRC-UK and TB Alliance
Study period	:	2017 – 2020

Background: TB in children is frequentlycompared with historical pediatric and adult paucibacillary and non-severe forms of PTBPK data (PK sub-study 1)
are common. Evidence for TB treatment in
children is largely extrapolated from adult**Methodology:** This is a multicentric, open-studies. Trials in adults with smear-negativelabel, parallel-group, non-inferiority,
TB suggest that treatment can berandomized controlled, two-arm trial
effectively shortened from 6 to 4 months.comparing a 4-month vs the standard 6-
New pediatric, fixed-dose combination anti-month regimen using revised WHO
TB treatments have recently beenpediatric anti-TB drug doses. Children aged
introduced in many countries, making theless than 16 years with non-severe TB, with
implementation of WHO-revised dosingor without HIV infection will be tested. The
recommendations feasible. The safety andprimary efficacy and safety endpoints are
efficacy of these higher drug doses haveTB disease-free survival 72 weeks post-
not been systematically assessed in largerandomization and grade 3 or 4 adverse
studies in children, and theevents. Nested PK studies will evaluate
pharmacokinetics across childrenanti-TB drug concentrations and will provide
representing the range of weights and agesmodel-based predictions for optimal dosing.
should be confirmed. Socio-economic analyses will evaluate the cost-effectiveness of the intervention and social science studies will further explore the acceptability and palatability of these new paediatric drug formulations.

Aims:
(i) To determine whether the standard 6-month regimen (8 weeks HREZ followed by 16 weeks HR) can be reduced with similar efficacy, to 4 months (8 weeks HREZ followed by 8 weeks HR), in HIV-infected and uninfected African and Indian children with minimal TB, using recently revised dosing guidelines for anti-TB drugs;
(ii) To determine whether the higher WHO-recommended doses of daily first-line anti-TB drugs, given as new WHO-recommended, fixed-dose combination (FDC) dispersible tablets, and prescribed according to weight bands, result in appropriate drug exposures when

Current status: This study was initiated in August 2017. The study enrolled children from two centres, Govt. Stanley Hospital (GSH), Chennai and Institute of Child Health (ICH), Chennai. As of March 2020, we had screened 101 children and out of which 63 participants were enrolled successfully in the study.
All study participants have completed 72 weeks of follow up and the study has been completed.

STUDIES IN PROGRESS:

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 tuberculosis.

Principal Investigator	:	Dr. D. Baskaran
Source of funding	:	Intramural
Study period	:	2013-2020

This Clinical trial compares a 4-month Ofloxacin containing ATT regimen with a 6-month of ATT (Control regimen) in terms of response to treatment and relapse upto 24-months post treatment in patients with TB lymphadenitis.
The study is being conducted in Govt. Stanley Hospital, Govt. Rajiv Gandhi Medical 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 301 patients have been enrolled to trail. The study is ongoing.

TBL STUDY ANALYSIS

NUMBER OF CASES ADMITTED IN STUDY = 301 (as on March, 2020)

Regimen

2EHRZ3/4RH3 - 150 (105 Females & 45 Males)
2OHRZ7/2OHR3 - 151 (103 Females & 48 males)

Table 6: No of cases analyzed = 278 (patients completed 6 months period)

2EHRZ3/4RH3 (138 CASES)	DIED	1
	FAV	134
	UNFAV	3
	Total	138
2OHRZ7/2OHR3 (140 CASES)	DIED	-
	FAV	136
	UNFAV	4
	Total	140

Table 7: DST Results

DST results available cases = 211

Characteristics	Test Regimen (N=117)	Control regimen (N=94)	Total (N=211)
Sensitive to H,R,E,O	99	86	185
Resistant	18	08	26
EMB_OF	01	-	01
INH	05	03	08
RIF_OF	01	-	01
OF	11	04	15
RIF	-	01	01

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

Principal Investigator	:	Dr. C. Padmapriyadarsini
Source of Funding	:	Department of Biotechnology
Study period	:	2013-2020

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 the 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 be made available to investigators of this partnership on request. The study has completed the enrolment of participants and is currently following the patients and their contacts. As of March 2020, 395 TB patients (Cohort A) and 551 household contacts (Cohort B) have been enrolled and are being followed up in the study.

Table 8: SUMMARY CHARACTERISTICS OF THE ACTIVE TB COHORT

Update as of March 2020			
Baseline characteristics	**PTB (n=508)**	**EPTB (n=197)**	**Pediatric TB (n=194)**
Age, Median (IQR)	35 (23 - 48)	29 (22 - 38)	09 (06 - 12)
Male sex, n (%)	321 (63%)	94 (47%)	92 (50%)
BMI, Median (IQR)	17.40 (15.74 - 20.09)	19.97 (17.17 - 23.71)	14.01 (13.03 - 15.36)
Anaemia, n (%)	269 (53%)	85 (42%)	-
HIV, n (%)	32 (6%)	26 (13%)	8 (4%)
DM, n (%)	123 (24%)	12 (6%)	-
Pre-DM, n (%)	135 (26%)	49 (24%)	-
HbA1c, Median (IQR)	5.8 (5.2 - 6.5)	5.4 (5.1 - 5.9)	-
Smoking, n (%)	79 (15%)	9 (4%)	-
Alcohol, n (%)	220 (43%)	48 (24%)	-
CXR Score, Median (IQR)	57 (25 - 80)	10 (00 - 30)	15 (00 - 50)
Cavitation, n (%)	191 (37%)	15 (7%)	29 (16%)
Smear +ve,n (%)	318 (62%)	11 (5%)	12 (6%)
MGIT +ve,n (%)	368 (72%)	31 (15%)	24 (13%)
Relevant outcomes	**PTB (n=461)**	**EPTB (n=153)**	**Pediatric TB (n=126)**
Failure,n (%)	52 (11%)	3 (2%)	4 (3%)
Relapse,n (%)	33 (7%)	5 (3%)	8 (6%)
Death,n (%)	31 (7%)	8 (5%)	0 (0%)

CL- 9: An open-label, non-randomized, two-stage, dose-finding study of Verapamil tablet formulation in adult TB patients in the continuation phase of anti-TB treatment

Principal Investigator	:	Dr.C. Padmapriyadarsini
Funding Agency	:	ICMR
Study period	:	2019-2022

This is a phase 2 open-label dose finding PK 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., verapamil when added to standard TB therapy will accelerate sputum clearance of *M. tb*. Stage 2 of the study is being funded by ICMR and will be done at two sites – Kilpauk Medical College, Chennai and Regional Medical Research Centre, Bhubaneswar. The protocol was amended to version 3.3 with the addition of a new site in Bhubaneswar. NIRT and RMRC IEC approvals have been obtained and the changes have been notified to DCGI, Govt. of India. Awaiting regulatory approvals to initiate the stage 2 of the study.

CL- 11: Species identification and response to the appropriate treatment of symptomatic pulmonary non-tuberculous mycobacterial disease among patients treated for TB in Tamil Nadu

Principal Investigator : Dr.C. Padmapriyadarsini
Funding Agency : ICMR Task Force
Study period : 2013-2020

This is a descriptive study to identify the various species of pathogenic NTM causing symptomatic pulmonary disease and to evaluate their response to treatment, initiated based on American Thoracic Society guidelines among patients with symptomatic pulmonary NTM disease in Tamil Nadu. The study is currently enrolling patients diagnosed with pulmonary NTM from Chennai and Kanchipuram district. NTM species are identified in sputum and appropriate treatment is based on the species identified. As of March 2020, 48 patients have been recruited to the study. All patients are on treatment as per ATS guidelines. The Study is ongoing. Table 9 describes the different species of pulmonary NTM identified in our cohort.

Table 9:

Speciation at baseline		Frequency
FEMALE	M.abscessus	1
	M.abscessus,M.fortuitum	1
	M.avium	1
	M.intracellulare	7
	M.intracellulare, M.fortuitum,M.absessus	1
	M.kansasii	3
	M.Kyorinense	1
	Total	**15**
MALE	M.abscessus	3
	M.avium,M.Gordanae	1
	M.avium,M.intracellulare,M.Malmonese	1
	M.fortuitum,M.peregrinum,M.abscessus,M.intracellulare	1
	M.intracellulare	5
	M.kansasii	19
	M.kansasii,M.abscessus	1
	M.kansasii,M.intracellulare,M.malmoense	1
	M.simiae	1
	Total	**33**
Total		**48**

<u>CL-19:</u> Optimizing treatment to improve TBM outcomes in children: The TBM-KIDS trial

A Phase I/II Randomized, Open-label trial to evaluate the pharmacokinetics, safety, and treatment outcomes of multidrug treatment including high dose RMP with or without Levofloxacin versus standard treatment for pediatric tuberculous meningitis

Principal Investigator	:	Dr. Bella Devaleenal
Source of Funding	:	The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
Study period	:	2016-2021

Background: Paediatric tuberculous meningitis (TBM) is associated with high mortality and severe morbidity. At the current recommended doses, CSF concentrations of RMP barely exceed the MIC against M. tb. In this trial, we are evaluating the pharmacokinetic (PK), safety and treatment outcomes of regimens containing higher dose of RMP with or without levofloxacin for the treatment of paediatric TBM. The functional and neurocognitive outcomes are also being assessed. This is a multi-centric trial done in Pune, Malawi and Chennai.

Objectives:

Primary objectives

(i) To characterize the PK [plasma and cerebrospinal fluid (CSF)] of RMP given at model-derived optimal daily doses and levofloxacin in children aged 6 months to 12 years of age with TBM;

(ii) To assess the relationship between RMP concentrations and longitudinal functional outcomes, adjusting for factors known to affect treatment response, such as stage at presentation, leukotriene A4 hydrolase genotype, levofloxacin co-administration;

(iii) To evaluate the safety of TBM treatment over eight weeks, by Arm

Secondary objectives:

(i) To describe neurocognitive outcomes among children aged 6 months to 6 years of age treated for TBM, longitudinally over 18 months, by Arm;

(ii) To describe TBM treatment outcomes at 12 months

Methods: Open-label, randomized clinical trial in three treatment groups. Children with TBM will receive INH and PZA at standard doses for 8 weeks. Arm 1 participants will receive high-dose RMP for 8 weeks plus EMB at standard doses. Arm 2 participants will receive high-dose RMP plus levofloxacin for 8 weeks. Arm 3 participants will receive RMP plus EMB at standard doses for 8 weeks (control Arm). Patients will receive 8 weeks of study treatment in the intensive phase and then will complete 10 months of standard continuation phase. PK sampling will be performed within the first week and at 6 (+/- 2) weeks following treatment initiation. Participants will have scheduled follow-up visits to assess safety, clinical status, functional and neuro-cognitive outcomes up to 12 months.

Progress: Study was initiated in NIRT in June 2017. As of 30th March 2020, 1891 children with neurological signs and symptoms were approached for study eligibility, 518 children were pre-screened for the study. 24 children were screened and 10 children were enrolled in study from NIRT. Study is now closed for recruitment. Sub-study “Clinical profile

and CSF bio repository of the pediatric suspected meningitis cases' includes storing of CSF samples collected for TBM diagnosis and collection of clinical details for diagnosis as a part of prescreening for the main study.

CL-20: A phase IIB Open-Label Randomized trial to evaluate the antibacterial activity, pharmacokinetics, safety and tolerability of Metformin when given along with RMP, INH, PZA, and EMB in adults with newly diagnosed sputum positive PTB: an 8-week study

Principal Investigator	:	Dr.C.Padmapriyadarsini (email:padmapriyadarsinic@nirt.res.in)
Funding Agency	:	India TB Research Consortium
Study period	:	2018-2020

This is a phase 2b open-label randomized clinical trial with an aim to evaluate the anti-bacterial activity of Metformin, by measuring the time to sputum culture conversion in liquid media, when given daily for 8-weeks along with standard first-line anti-TB treatment in adults with newly diagnosed sputum positive PTB. This is a multicentric trial and will enroll 320 new sputum smear-positive PTB patients in New Delhi, Pune and Chennai. As of March 31, 2020, 322 patients have been enrolled from all three sites. The study is ongoing and all recruited patients are on follow-up.

Table 10: As of from April 2019 to March 2020 (Enrolled: 187)

Variables	Overall (n = 187)	Centre		
		NIRT (n=89) n(%)	NARI (n=52) n(%)	AIIMS (n=46) n(%)
Gender				
Female	47 (25.1)	22 (24.7)	14 (26.9)	11 (23.9)
Male	140 (74.9)	67 (75.3)	38 (73.1)	35 (76.1)
Regimen				
2EHRZ7/4RHE7	93 (49.7)	46 (51.7)	24 (46.2)	23 (50.0)
2METHRZE7/4RHE7	94 (50.3)	43 (48.3)	28 (53.8)	23 (50.0)
Age (in years)*	32.60± 11.46	36.71 ± 12.00	29.79 ± 9.69	27.85 ± 9.44

* Mean (SD)

CL-21: The evaluation of a standardized treatment regimen of anti-TB drugs for patients with MDR-TB- STREAM Stage II

Principal Investigator	:	Dr. G. Narendran
Source of funding	:	The United States Agency for International Development (USAID); UK Medical Research Council (MRC) / Department for International Development (DFID); Janssen Research & Development, LLC; Liverpool School of Tropical Medicine, UK
Study period	:	2018-2023

Background: The prolonged duration of the current regimen (24-27 months) used to treat MDR-TB results in multiple and cumulative side effects and high loss-to-follow-up rates. This results in further amplification of resistance to additional drugs. The STREAM-II study aims to test a more effective regimen that reduces the treatment duration to six-to-nine months, which could significantly enhance the treatment success rate among MDR-TB patients. This could potentially save thousands of lives and reduce the MDR-TB burden in India.

Primary objective:
(i) To assess the efficacy of shorter treatment regimens at 76 weeks and to compare safety during treatment and follow-up up to 132 weeks

Methodology: The STREAM study is an international, multi-center, parallel-group, open-label, randomized, controlled trial, enrolling patients with MDR-TB including patients with RMP-resistant and INH-sensitive TB without resistance to quinolones or amino-glycosides at baseline.
Trial interventions include three arms enlisted below and allocated in the ratio of 1:1:1
Regimen B (control regimen):
Regimen B consists of Clofazimine, EMB, MFX and PZA given for 40 weeks, supplemented by INH, kanamycin, and prothionamide in the first 16 weeks (intensive phase).
Regimen C:
Regimen C is a 40-week all-oral regimen consisting of Bedaquiline (BDQ), Clofazimine, EMB, levofloxacin and PZA given for 40 weeks supplemented by INH and prothionamide for the first 16 weeks (intensive phase).
Regimen D:
Regimen D is a 28-week regimen consisting of BDQ, Clofazimine, levofloxacin, and PZA given for 28 weeks supplemented by INH and kanamycin for the first 8 weeks (intensive phase).

Sample size: Approximately 600 cases (globally).

Study progress: The study stopped enrollment as the global sample size was achieved. Over 2000 patients were pre-screened out of which 130 participants were suitable for further screening and forty-nine patients were randomized from NIRT and 148 from India. The study had migrated to protocol version.8 where there will be a direct comparison of two 9 months' regimens, Regimen B containing the injectable with the all oral regimen (Reg C) containing Bedaquiline. This has been approved by the FDA and DCGI recently. The study provides crucial evidence for a shift to an all oral regimen in the DR-TB programme both in India as well as globally.

With India contributing to nearly a quarter of the global sample size, the results would be highly translatable to the Indian population. Meticulous follow-up is ongoing.

<u>CL- 23:</u> Phase II b open label, parallel, randomized controlled clinical trial to evaluate the safety, tolerability, pharmacokinetics and anti-bacterial activity of high dose RMP versus conventional dose of RMP along with standard anti-tubercular therapy in drug sensitive adult patients of PTB (HICON-R) -- ITRC - (00038-2017)

Principal Investigator	:	Dr.P.K. Bhavani
Source of funding	:	ITRC (India TB Research Consortium)
Study period	:	2018 – 2021

Background: *In vitro*, animal, and human studies from different regions & ethnic populations suggest - higher daily doses of RMP may be safely and successfully used to shorten the 6 -month TB treatment. But there is sparse data on safety and PK of high dose RMP among our population. Hence this clinical trial proposes to investigate the effect of increased dose of RMP in terms of safety, antibacterial activity and PK of High dose RMP (35 /25mg/kg body weight) in comparison with the conventional dose of 10 mg/kg when given along with other anti-TB drugs.

Methodology: Multi-centric Phase II b open label, parallel, randomized controlled clinical trial conducted at NIRT, Chennai, PGI, Chandigarh, KGMCU, Lucknow, NITRD, New Delhi, and Bhagwan Mahavir Hospital, Hyderabad, with a sample of 350 new smear positive adult PTB patients.

Objectives:

(i) To assess of RMP in new sputum smear and culture positive PTB participants receiving various doses of RMP (10 or 25 or 35 mg/kg/day) during 8 weeks high dose phase of ATT;

(ii) To study the steady state pharmacokinetics (PK) of RMP in terms of AUC, C max, T max and T1/2(half-life) in a sub-group of participants in all treatment arms;

(iii) To compare the following between intervention arms vs control arm:

a. Proportion of participants with sputum culture conversion at 8 weeks & at end of treatment;

b. Time to sputum culture conversion;

c. Rate of change in time to sputum culture positivity

(iv)To identify predictive blood biomarkers for drug induced liver injury

Translational Potential: If proved that high dose RMP is safe among our population it could be recommended to the programme and also be adopted in shortening the duration of TB treatment in future.

Study Progress: Totally 333 patients were recruited at 5 sites across India and as on March 2020,89 have completed 18 months of follow up. The study is on-going.

Table 11: As on March 2020

	N = 333	Completed 18 months of Follow up(n=89)
NIRT Chennai	113	43
NITRD, New Delhi	83	22
KGMCU Lucknow	34	6
BMMRC Hyderabad	57	6
PGI Chandigarh	46	12

CL-24: Predictors of resistance emergence evaluation in MDR-TB patients on treatment – PREEMPT

Principal Investigator : Dr.C.Padmapriyadarsini
Source of funding : NIH (RO1)
Study period : 2019-2022

This is a multicounty study between India and Brazil in DR-TB to determine whether low serum antimycobacterial drug concentrations are associated with the clinical emergence of drug resistance in MDR-TB patients, to determine the contribution of increased DNA mutation to clinical emergence of drug resistance in patient isolates and also to determine the earliest time at which mutations responsible for drug resistance can be detected during treatment. In India, the study will be done in Chennai, Pondicherry and Pune. The study has been initiated in January 2019 and currently enrolling the participants.

Table 12: As on March 2020

PREEMPT study (till 31st March 2020)					
S.No	Site	Screened	Enrolment (Target 90)	Gender	
				Male	Female
1	NIRT (Initiated on Feb '19)	37	37	27	10

CL - 25 : Evaluation of cause of death among adult TB patients registered for treatment under the Revised National TB Control Programme in Chennai District, Tamil Nadu using Verbal autopsy

Principal Investigator : Dr.P.K. Bhavani
Source of funding : ICMR (Extramural Adhoc)
Study period : Jan 2020 – Dec 2021

Background: With the available practice of Death reporting, we hypothesize that the TB deaths are misclassified (both over estimated and under estimated). The purpose of this study is to correctly classify the deaths among TB patients who were registered and treated at RNTCP centres in Chennai district using Verbal Autopsy.

Methodology: A Prospective Observational study will be conducted to identify the cause of death among Adult TB patients registered for treatment under the RNTCP programme in Chennai District using Verbal autopsy. Known details of risk factors for death among Tuberculosis patients will be collected from NIKSHAY and RNTCP records (treatment cards).As soon as the death information is received from RNTCP ,the study team will contact the concerned family and get appointment for conducting the Verbal Autopsy. Verbal Autopsy will be conducted within 15 days of the occurrence of the event.

Objectives:
Primary:
(i) To identify the cause of death among TB patients registered for treatment under the RNTCP in Chennai using Verbal autopsy
Secondary:
(ii)To compare the cause of death done using Verbal Autopsy with that of the RNTCP Death Audit report
(iii)To determine the risk factors for death among TB patients registered for treatment under the RNTCP programme in Chennai

Translational Potential: This study will generate evidence on
(i) Actual cause of death whether it is TB related or unrelated
(ii) Will serve as a potential improved source of TB mortality estimation for Chennai District
(iii) Whether conducting Verbal Autopsy yields additional information on the cause of death

Study Progress: As on May 2020, 69 deaths were notified and we have completed Verbal Autopsy for 57 deaths.
Reasons for not conducting VA (n=12) = (Not willing: 2, Out Station: 3, COVID Hot Spot: 4, Unable to contact: 2, MDR Patient: 1)
The study is on-going.

CL - 26 : Improving Airborne Infection Control (AIC) practices in health care facilities involved in the management of Tuberculosis in Chennai.

Principal Investigator	:	Dr. D. Bella Devaleenal
Source of funding	:	ICMR Extramural
Study period	:	2019 – 2021

Background: AIC, though a most important strategy in preventing the transmission of TB, is the least practiced. The Guidelines for AIC was introduced in India in 2010 and one study had evaluated its implementation in three states. Our study aims to study AIC practices based on administrative, environmental control and personal protective environments in facilities and among the health care workers (HCWs) and patients. The identification of strengths and gaps of the AIC practices

would help to strengthen the Programme towards the goal of TB elimination.

Objectives:
Primary Objective

- To describe the AIC practices at Urban Primary Health Centres (UPHCs) and centres providing TB care services in Chennai between 2019 and 2020.
- To describe the awareness and practices of AIC among health care providers in UPHCs involved in TB care in Chennai
- To describe the awareness and practices of AIC among patients who are diagnosed with TB and registered in RNTCP in Chennai
- To train the HCWs in AIC practices and develop AIC plan in the identified institutes involved with the management of TB patients.

Secondary Objective
To assess the impact of interventions to improve the TB AIC practices in Chennai.

Methods: Quasi Experimental Study Design (Pre and Post intervention)
Pre intervention and post intervention AIC assessments:
Health care facilities: UPHCs providing TB care services in eastern division of Chennai Corporation will be included in the Survey. This will be done using an observation check list.
Health care workers: HCWs in the above UPHCs will be included in the Survey. This will be done using a pretested validated questionnaire.
Patients: The patient population will be also recruited from the same UPHCs. The minimum required sample size will be 260 patients during pre and post intervention assessment. This will be done using a pretested validated questionnaire.
Interventions

- Facility wise report and recommendations based on the baseline AIC practices assessment.
- Facility wise development of AIC implementation plan.
- Capacity building on AIC practices to the health care workers - Training programmes.

- Information, Communication and Education (IEC) to patients about AIC- Counselling sessions, IEC materials and Pamphlets.

Progress: The study was initiated in NIRT since October 2019.
After necessary approvals and sensitization of the staff in the UPHCs, the pre intervention assessments were initiated. As on 30th March 2020, pre intervention assessment of 20 UPHCs, 230 HCWs interview and 130 patients interview were completed.

CL - 27 : Time to initiation of tuberculosis treatment among children with central nervous system (CNS-TB)

Principal Investigator	:	Dr. G. Prathiksha
Source of funding	:	ICMR Intramural
Study period	:	Oct 2019 – Apr 2022

Background: CNS TB constitutes only a small proportion out of the total TB cases (around 1%), the amount of suffering in terms of morbidity and mortality especially

in young children is very high. Early diagnosis remains a substantial challenge because of non-specific signs and symptoms at the initial stage of the disease. Early diagnosis and management of TBM and other CNS TB is important, as delay in diagnosis leads to poor outcomes such as death, neurological sequelae and neurocognitive disorders. This study aimed to assess the time for treatment initiation among children with CNS TB and to investigate its determinants.

Objectives:

Primary objectives:

- To estimate the duration of time for tuberculosis treatment initiation among children with CNS TB seeking care in a tertiary hospital
- To determine the factors associated with the of time for tuberculosis treatment initiation among children with CNS TB seeking care in a tertiary hospital.

Secondary objective:

To measure the household economic burden due to CNS-TB in terms of direct, indirect and total costs for diagnosis and treatment until ATT initiation.

Methods: All children diagnosed as CNS TB and started on ATT in Institute of child heath, Chennai are recruited for the study if they are willing .The study design is a mixed method design. Qualitative data will be collected using a structured in depth interview guide. This will be followed by quantitative cross sectional study using a structured questionnaire to estimate the time for TB treatment initiation. The study recruitment period is for 24 months.

Status of the study as on March 2020: 18 Children were enrolled till March 2020.

CL - 28 : A Phase III, Randomized, Double-blind, Three arm Placebo controlled study to Evaluate the Efficacy and Safety of two vaccines VPM1002 and Immuvac (M*w*) in Preventing Tuberculosis (TB) in Healthy Household Contacts of Newly Diagnosed Sputum Positive Pulmonary TB patients.

Principal Investigator	:	Dr. V.V. Banu Rekha
Source of funding	:	ICMR - ITRC
Study period	:	2019 – 2023

Background: Household contacts are at increased risk of contracting tuberculosis (TB) from sputum smear positive index pulmonary TB (PTB) patients. There is a need to evaluate the efficacy of vaccines in the prevention of TB among household contacts of PTB patients. VPM 1002 is a recombinant BCG vaccine from Serum Institute and Immuvac is a heat killed suspension of Mycobacterium W from Cadila Pharma. The objective of this multicentric, Phase III, double blind, randomized clinical trial is to evaluate the efficacy of VPM1002 and Immuvac by comparing the incidence of TB over a 3-year period among healthy household contacts of newly diagnosed sputum positive PTB patients in comparison to placebo.

Methodology: The trial is ongoing in ICMR-NIRT sites in Chennai, Thiruvallur, Tambaram and Madurai. Household contacts aged $\geq$ 6 years, HIV sero-negative, without prior or current anti-TB treatment and with no evidence of TB disease are randomized to receive intra-dermal VPM1002, Immuvac or

placebo. The first dose (0.1ml) is administered in both upper arm at baseline and the second single dose is given in right or left arm at one month. Participants are followed up once fortnightly during initial 2 months and once in 4 months thereafter for a period of 3 years. Solicited and unsolicited adverse events are documented. The immune responses are studied at baseline, 2 months and 6 months in a sub-set of participants.

Results: The trial was initiated in October 2019. A total of 1002 household contacts have been screened and 773 are vaccinated. The trial is ongoing.

CL - 29 : Evaluation of the Efficacy and Safety of a Combination regimen of Bedaquiline, Delamanid, Linezolid and Clofazimine in Adults with Pre-extensive (Pre-XDR) and Extensively Drug-resistant Pulmonary Tuberculosis (XDR-TB): Prospective Cohort Study (BEAT Study)

Principal Investigator	:	Dr. C. Padmapriyadarsini
Source of Funding	:	National Institute for Research in Tuberculosis, ICMR, India TB Research Consortium; USAID
Study period	:	2019-2022

This is a prospective cohort study with an aim of evaluating the efficacy of a new treatment regimen of 6-9 months consisting of Bedaquiline (BDQ), Delaminid (DLM), Linezolid (LZD), clofazimine (CFZ) in adult patients with Pre XDR and XDR pulmonary tuberculosis. This is a multi-centric trial and will enroll 165 pulmonary Pre XDR and XDR patients in New Delhi, Gujarat, Mumbai and Chennai. As of March, 2020, 80 participants have been enrolled from all five sites. The study is ongoing and all recruited patients are on follow-up.

Table 13: Summary of XDR TB study (till 31st March 2020)

S.No	Site	Screened	Enrolment (Target 165)	Gender	
				Male	Female
1	**NIRT (Initiated on Apr '19)**	20	12	8	4
2	**NITRD (Initiated on Apr '19)**	50	30	20	10
3	**BJMC (Initiated on Jun '19)**	24	20	13	7
4	**RBIPMT (Initiated on Sept '19)**	28	12	6	6
5	**GTB (Initiated on Nov '19)**	24	6	1	5
Total		**146**	**80**	**48**	**32**

DEPARTMENT OF SOCIO BEHAVIORAL RESEARCH

STUDIES COMPLETED:

SB-1: Estimate the burden of TB among tribal population and develop 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	:	March 2015 – March 2020

Background: Though TB is a major health problem among the tribal communities, studies conducted among them is rather limited and concentrated only in a few isolated groups mostly from central India. Moreover, until now there is no concrete evidence to estimate the overall prevalence of PTB among the tribal populations in a developing country like India. A meta-analysis done on the existing studies reported a prevalence of 703/10000. However, there was a lot of heterogeneity in the groups studied which was a huge limitation against this background this study was carried out to

1. To estimate the burden of TB amongst tribal groups (TGs) in various states of the country
2. To find out the health seeking behavior patterns of persons having symptoms suggestive of TB

Methods: The study was conducted between April 2015 to March 2020 amongst individuals (tribal) aged ≥15 years in randomly selected tribal villages (clusters) of India. A multistage cluster sampling design without replacement was adopted. The entire country was divided into 6 zones with two or more states: East, West, North, South, Central and North East. In each zone, villages (clusters) with >70% tribal population were enlisted and that formed the sampling frame. From each zone, villages (with >70% tribal population) were selected based on PPES in stage II. To achieve the sample size of 63480, a minimum of 800 individuals were surveyed from each selected village. A total of 88 villages were selected from 17 states of India. The study followed a mixed-method design which included a quantitative and qualitative component. The qualitative component included focus group discussions and interviews with various individuals: key influential persons and groups representing age and gender from the tribal villages. The quantitative survey was conducted among persons >15 years of age in 88 clusters/villages from 17 States. Persons having symptoms suggestive of PTB or history of anti-TB treatment (ATT) were eligible for sputum examination by smear microscopy for Acid Fast Bacilli and culture for Mycobacterium tuberculosis; two sputum samples were collected from each eligible person. Persons with one or both sputum specimen positive on microscopy and/or culture were labelled suffering from PTB. Prevalence was estimated after using the correction factor for X-ray screening. Imputation was also done to gain insight into the prevalence by correcting for bias introduced by the incompleteness of data. However, we present the prevalence as per the observed prevalence correcting for X-ray screening as the prevalence of TB among the tribal population

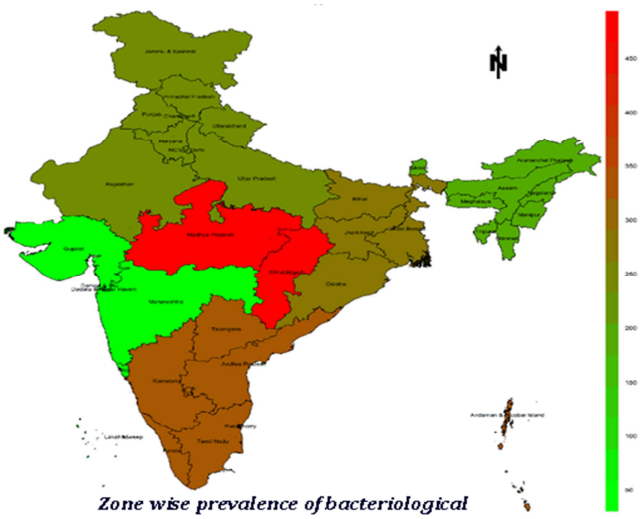
Results: Findings from the qualitative study pointed to structural, socio-cultural, personal and healthcare-

related issues. These included lack of accessibility to health care facilities with challenges in distance, poor roads, and lack of transportation and non-availability of health care personal. There was also lack of awareness and misconceptions on TB, lack of basic facilities and indoor air pollution with people and cattle residing under the same roof, lack of nutrition. Alcohol and smoking risk factors and the dependence on traditional healers and in many cases quacks. With regard to the quantitative survey, total of 92038 eligible individuals (≥15 years of age) were enumerated across 88 villages of India. Out of this, 74532 (81.0%) were screened for symptoms suggestive of TB and 2675 (3.6%) were found to have at least one of the TB symptoms. Of 2675 a total of 2401 (89.8%) produced sputum samples out of which 163 (6.8%), 95 (4.0%) and 193 (8.0%) individuals were found to be smear-positive, culture-positive and bacteriologically positive respectively.

Fig 1:

The prevalence of smear-positive, culture-positive and bacteriologically positive PTB were estimated to be 244 (95% CI 212 – 276), 142 (95% CI 118 – 166) and 289 (95% CI 254 – 323) per 100,000 population, respectively. Since X-ray screening was not done a correction factor of 1.7 was used and the corrected TB prevalence of bacteriologically positive PTB was 490 per 100000 population. The corrected PTB prevalence per 1 lakh population was the highest 791 [95% CI: 676 – 906] in the central zone and least 99 [95% CI: 33 – 166] in the west zone.

Fig 2:



Zone wise prevalence of bacteriological positive PTB among tribal population

Among the 17 states that were covered in this study, Odisha recorded the highest prevalence of 1002 [95% CI: 740 – 1264] and Jammu and Kashmir the lowest 72 [95% CI: 0 – 170] per 100000 population. It was observed that PTB prevalence significantly increased with age ($\chi 2$ for linear trend = 78.70, P value<0.001). Those in the age group of 56 – 65 years and more than 65 years had a very high prevalence of bacteriological positives i.e. PTB estimated at 1000 (95% CI 792 – 1208) and 1163 (95%CI 852 – 1474). Comparison of overall prevalence of PTB between genders showed that males had a significantly higher ((0.4%) prevalence compared to females (0.2%) ($\chi 2$ = 31.66, P value<0.001). Age ≥35 years, BMI

<18.5 Kgs /m^2, a history of anti−TB treatment, and being a smoker and consuming alcohol were significantly associated with the occurrence of smear, culture and bacteriologically positive TB, after adjusting for factors like gender, smoking, alcohol, age and BMI. There were gender differences with regard to symptoms that prompted care seeking. Among females, it was blood in sputum whereas, for the male, weight loss, shortness of breath along with blood in sputum significantly influenced care seeking.

Conclusion: There is a need for innovative focused gender and age-sensitive tribal friendly holistic interventions to reach the tribal population. This may include community engagement and community sensitization on TB using tribal community volunteers (Tribal youth, traditional healers apart from ASHAs) nutrition interventions and mobile vans equipped with sputum microscopy and digital X-ray with CBNAAT/TRUNAT to promote active TB case finding among the tribal population.

SB-2: Fostering resilience to psychosocial and HIV risk in Indian MSM

Principal Investigator	:	Dr. Beena E Thomas
Source of Funding	:	National Institute of Mental Health (NIH), USA
Study Period	:	2015-2020

Background: Men who have sex with men (MSM) in India are a key population at risk for HIV acquisition and transmission. They are also extremely marginalized and stigmatized, facing immense psychosocial stressors and socio-cultural challenges. Current HIV prevention interventions in India do not address resilience to these concerns

Objectives: 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, India.

Methods: 608 MSM with sexual risk for HIV acquisition or transmission were recruited from Chennai and Mumbai, India. They were randomized equally to one of two treatment arms: 1) a resilience-based psychosocial HIV prevention intervention, consisting of group (4-session) and individual (6-sesson) counseling as well as HIV/STI voluntary counseling and testing (VCT); or 2) a standard of care (SOC) control consisting of HIV/STI VCT alone. HIV and bacterial STI testing were conducted at baseline and 12-months; participants completed a psychosocial and behavioral assessment battery at baseline, 4-, 8- and 12-months. The primary outcomes were number of condomless anal sex

(CAS) acts during the past month and number of incident STIs. Chi-square tests were used to examine group differences in STI incidence at 12-months, and negative binomial and linear mixed effects models were performed to examine group differences in longitudinal changes in CAS acts and the hypothesized mediators, respectively.

Results: The intervention arm had a 56% larger reduction in CAS acts from baseline to 4-month follow-up (95%CI=35%-71%, p<.0001) and 72% larger reduction from both baseline to 8- (95%CI=56-82%, p<.0001) and 12- (95%CI=53%-83%, p<.0001) month follow-up compared to the control. Bacterial STI or HIV incidence did not differ across treatment groups.

Conclusions: A resilience-based psychosocial intervention for MSM in India was efficacious in reducing sexual risk through CAS which is crucial for HIV prevention and control among this group. HIV prevention programs for MSM in India should consider addressing mental health resilience as a way to augment reductions in sexual risk for HIV.

STUDIES IN PROGRESS:

SB-13: Utilization of School Students as Ambassadors in TB Sensitization in Chennai city

Principal Investigator : Mrs. Priscilla Rebecca
Source of funding : The United Nations Office for Project Services
Study Period : 2017-2019

Background: One of the important Stop TB strategies was TB education and empowerment of communities to take appropriate TB care. Evidences from several studies carried out in many developing countries show that involving school children in health education have proven to be effective and successful. With this background the current intervention aims to improve the TB specific health literacy in an urban poor community by engaging school students.

Objectives:

1. To promote health literacy among school students about TB and equip them as TB advocates in the community.
2. To develop an intervention model to engage school students as TB advocates to impart TB health literacy in an urban poor community.
3. To study the feasibility of this intervention by the students in the community

Methodology: This is an intervention study among students of classes 7 to 9 from 57 Chennai corporation schools in 5 zones. The study is being carried out in three phases. First phase is the formative phase done to equip the students through intensive TB intervention training sessions. Prior to the intervention training, baseline assessments were done. During this phase, the student TB ambassadors were selected to be actively involved in the TB sensitization activities among the student community within their respective schools, which is the second phase of the study. Finally in the third phase an end line assessment was done to increase the level of TB literacy among the students and the impact and feasibility of the interventions.

Results / Progress of the study: The study was initiated after obtaining permission from the authorities and the selection of schools from the Chennai Corporation Zones. Intervention tools and other IEC materials for primary and secondary interventions were designed and finalized. It includes children friendly animation movie on TB for 8 minutes and a documentary film on TB. Other intervention tools included several materials such as book marks, caps, pens, badges and stickers, posters. Data collection was completed in 20 schools that covered 920 students. Quantitative assessments with the families of the students were also done covering 600 family members. A total of 520 student ambassadors have been selected in the 57 schools, primary and secondary intervention has been completed covering approximately 12,500 students. These student ambassadors have been actively involved in conducting TB awareness programs in their respective schools for the other students. Ambassadors with the community outreach volunteers have organized awareness programmes and summer camps during the vacation. The data entry and data analysis is completed. Manuscript preparation is under progress.

SB-15: Patients' perception on Quality of Care in Tuberculosis Care Settings in Chennai

Principal Investigator : Mr. P. Murugesan
Source of Funding : The United Nations Office for Project Services
Study Period : 2017-2019

Background: Quality of Care plays a vital role in the TB control, which influences early and timely diagnosis, treatment adherence and treatment compliance. Quality of TB care in general context and in India, is conventionally assessed in the context of treatment outcome measures like mortality and microbiological cure and has not focused on patients' expectations and preferences such as satisfaction with care. The patient centric quality is crucial in influencing clinical and treatment outcomes of the patients.

Objectives:

1. To explore and understand patients' perception on the quality of care in TB care settings.
2. To identify the reasons for choice of providers, reasons for shift of providers.
3. To develop a patient specific quality of care tool in TB care settings

Methodology: This is a sequential exploratory mixed method study that includes both qualitative and quantitative design following a sequential approach implemented in a phased manner. The study was conducted in 36 TB units in 15 zones of Chennai Corporation. Probability proportion to size sampling method was used in enrolling the participants for the study. TB patients above 18 years and those registered under the RNTCP and Public – Private Mix (PPM) were included in the study. We have collected data from 714 TB patients.

Phase I: Qualitative Assessment: A total of 72 in-depth interviews were completed among TB patients who are continuing TB treatment in government (n=18) or private (n=18) health facilities and those who have shifted from a government to private health facility (n=18) or vice versa (n=18). A total of 10 Focus Group Discussions (FGDs) were completed. The qualitative data were coded and grouped into themes. The themes emerged from the qualitative data were categorized in to three domains namely, patient factors, health care provider's attitude and health system factors.

Phase II: Quantitative Assessment: The process we adopted in this tool development was multilayered. We first conducted an extensive review of the literature on the existing tools to measure the quality of TB care services. This was followed by in-depth interviews and (FGDs) with TB patients. The purpose of the in-depth interviews was to extract themes to develop the patient-centered quality of TB care tool. This helped us in conceptualizing and listing the gaps to be addressed. After that, using multiple iterations with experts and study staff, the final interview schedule consisting of 32 items was developed. Cognitive interviews, tests, and re-tests were conducted for reliability.
The tool was tested with 640 TB patients both in public and public and private mix (PPM) settings in Chennai,

India. Exploratory factor analysis was done and duly interpreted to explore the underlying phenomena. The tool with 14 items focused predominantly on four dimensions: satisfaction with doctor's care, information given by the health care provider, health visitors, and TB care services. This tool could help strengthen the quality of TB care services and enable the emergence of a more patient-centered approach.

Way Forward:

Two Research papers in Progress:

1. Quality of tuberculosis (TB) care services in Chennai: A qualitative investigation of patients' perceptions
2. Development of a tool to measure Patients' Perception of Quality of Tuberculosis Care.

LABORATORY STUDIES

DEPARTMENT OF BACTERIOLOGY

STUDIES COMPLETED:

B-15: Bioprocess development and preclinical evaluation of novel anti-TB antibiotic, Transitmycin isolated from marine *Streptomyces* sp. MTCC 5597

Principal Investigator	:	Dr. Rajesh Mondal
Source of Funding	:	ICMR-ITRC
Study period	:	Feb 2019 to Apr 2020

Background: Transitmycin (Tr), the novel molecule against TB isolated from the novel producer strain *Streptomyces* sp. MTCC 5597 has been successfully purified up to 98.3% purity and the proposed work will complete the preclinical studies.

Objectives (Briefly):

- Scaling up and Purification for the bulk production of Tr from marine *Streptomyces* sp. MTCC5597;
- *In vitro* efficacy of Transitmycin and its analogues;
- *In vivo* efficacy studies on Tr; *In vivo* toxicological studies viz, lethal dose (LD50) and maximum tolerable dose (MTD) in rats and guinea pigs;
- Pharmacokinetics in guinea pigs; Transitmycin target and mechanism of action.

Results:

Scaling up and Purification for the bulk production of Tr from marine *Streptomyces* sp. MTCC5597 was completed. *In vitro* efficacy of Tr was determined in comparison with Isoniazid, Rifampicin, Kanamycin, Moxifloxacin, Bedaquiline and Delamanid for 55 isolates of MTB of different drug susceptibility including MDR and XDR-TB isolates which showed MIC ranging between 5 and 10 µg/ml (Table. 14) for majority of isolates and minimal bactericidal concentration data is shown in table 14. The results of *In vitro* efficacy for the first and second batch of Tr analogues against *M. tuberculosis* H37Rv by strand displacement assay showed that the intercalation with DNA in all the 4 Tr analogues. Anti TB activity is either absent or very minimal in analogues. Pharmacokinetic values of Tr in uninfected guinea pigs were given table 15.

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***In vivo* toxicological studies viz, LD_{50} and MTD in rats and guinea pigs and invivo efficacy studies:**

The dosage had been tittered as the study progressed based on the mortality of animals. The product induced local necrosis on intravenous (IV) routes. The elevated enzyme levels were dose dependent and suggestive of liver and renal toxicity. At 0.45 mg/kg, the compound was toxic and mortality was observed in mice by IV route, whereas at 0.15 and 0.3 mg/kg, mice survived till 30th day, but liver enzymes were elevated in all the doses. The molecule was toxic with mortality and elevated enzyme levels when used through intraperitoneal route at 0.1, 0.2 and 0.4 mg/kg. There was no mortality among rats when given through IV route, however there was reaction at the injection site at 0.025, 0.05 and 0.1mg/kg. Mortality was observed among rats when given though intramuscular route at 0.1mg/kg on 10th day. Rest of the rats which were given 0.025 and 0.05 mg/kg were alive upto 16th day. Mortality was observed among guinea

pigs when 0.025 mg/kg was given through intraperitoneal route.

Transitmycin effect on bacterial burden in *M.tb* infected guinea pigs

Transitmycin at 0.02 and 0.04 mg/kg dose range did not show any significant activity in the lungs and spleens until the 3rd dose and all animals either died or had to be sacrificed at 6th day post treatment initiation. After 5th dose (i.e. 12th days post treatment) of transitmycin at 0.01 mg/kg, there was significant reduction in bacterial count in guinea pigs ($4.9\log_{10}$) but reduction was found to be similar to that in the INH+RIF control group (Fig.3).

Transitmycin Target Identification and Elucidation of the Mechanism of Action

DNA Intercalation of Transitmycin:

Machine learning and cheminformatics analysis of the transitmycin DNA intercalators conducted showed that the Transitmycin are DNA intercalators and the intercalation was caused due to the interaction of NH2 and =O with the G-C base pair of planar ring structure of the chromophore. The compounds having highest similarity with Transitmycin had the following targets: Nucleic acid - DNA (G-C base pairs), recA - recombinaseA, Methionine Aminopeptidases.

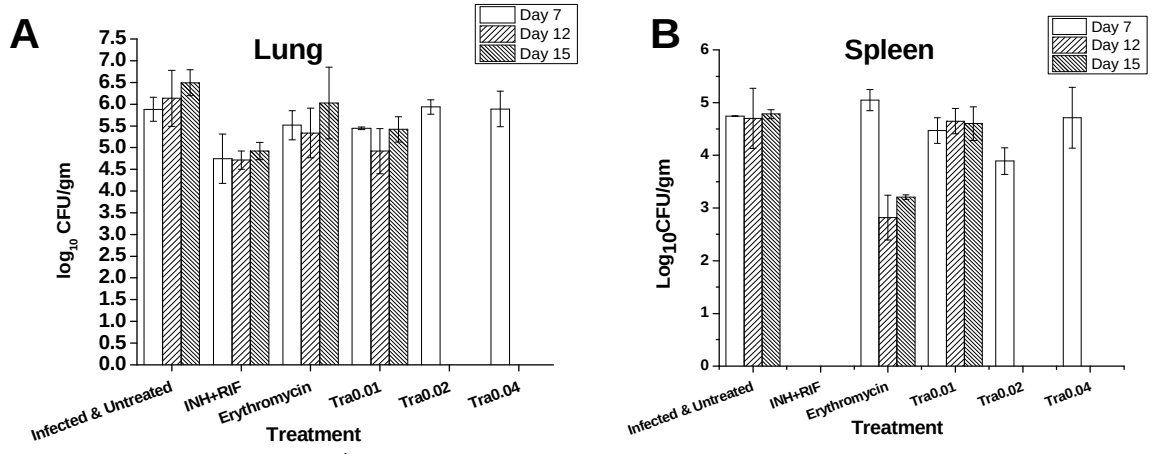
Table 14: Minimum Inhibitory and Bactericidal Concentration of transitmycin: Sample Size: n=55

Transitmycin Concentration	0.312 µg/ml	0.625 µg/ml	1.25 µg/ml	2.5 µg/ml	5 µg/ml	10 µg/ml	20 µg/ml	40 µg/ml
No. of Isolates having MIC_{90}	1	2	6	9	19	15	2	1
No. of Isolates being MBC	-	-	3	13	9	10	10	10

Table 15: Pharmacokinetic values of Transitmycin in uninfected guinea pigs at a dose of 1mg/kg body weight IM:

S. no	Lab No	ug/ml
1	Sample 1 PK (0hrs)	-
2	Sample 2 PK (1hr)	0.05
3	Sample 3 GP (4hrs)	0.21
4	Sample 4 GP (6hrs)	0.10
5	Sample 5 GP (8hrs)	0.06
6	Sample 6 GP (12hrs)	0.10
7	Sample 7 GP (24hrs)	0.09

Fig.3:



Viable bacterial numbers in whole lungs (A) and spleens (B) of *M. tuberculosis*-infected guinea pigs treated with INH+RIF, Erythromycin or Tr @ 0.01mg/kg, 0.02mg/kg, and 0.04mg/kg respectively at 7th , 12th and 15th days post treatment (n=2 per group at each time point except for guinea pigs treated with Tr @ 0.02mg/kg, and 0.04mg/kg respectively for which, n=4 per group)

B-18: Re-analysis of TB recurrence data to assess the added value of Whole Genome Sequencing in differentiating relapses from re-infection

Principal Investigator	:	Dr. S Sivakumar
Source of funding	:	AISRF, Australia
Study period	:	2019 - 2020

Short Description: TB recurrence among patients previously treated for TB pose a major problem, since these cases may reflect sub-optimal functioning of the TB control program and have increased rates of drug resistant disease. Addressing this problem requires enhanced understanding of the underlying mechanism leading to TB recurrence, which requires careful differentiation of relapse (endogenous reactivation of the same strain previously treated, but not eliminated) and re-infection (infection with a new strain unrelated to the previous disease episode). In this study, we performed WGS on the same isolates used in the earlier publication to compare the robustness of our findings using older techniques like RFLP, Spoligotyping and MIRU compared to WGS.

Main findings:

1. Among HIV-negative patients with paired samples, 19/21 (90%) experienced TB relapse. The opposite was observed in HIV-positive patients where the vast majority of TB recurrences (16/20; 80%) resulted from reinfection; the difference in frequency of reinfection was statistically significant (p=0.0001). Two paired isolates with 9 and 7 SNPs respectively were classified as re-infection using a 5 SNP cut-off, despite identical spoligotype and MIRU-12 profiles.

2. Among HIV-negative cases reinfection was rare (2/21). Pre-existing mutations associated with drug resistance were documented in four relapse cases. These were mostly *katG/inhA/FabG1* genes mutations associated with high-level isoniazid resistance. In two instances, drug

resistance amplification was observed with newly acquired rifampicin resistance conferring mutations in the *rpoB* gene (S450L and H445R). One isolate acquired high-level isoniazid resistance via a *katG* gene mutation (S315I).

Conclusion: WGS based analysis confirmed previous differences observed in the relative frequency of relapse and reinfection, depending on HIV status. However, it provided enhanced insight into drug resistance acquisition and spread, with major concern related to potential MDR-TB transmission in HIV care settings.

Future plan: Whole genome approach would be a better tool and used for differentiating relapse vs. reinfection. Also will simultaneously detect resistance to all the possible antituberculosis drugs.

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<u>B-19:</u> Host directed therapy through autophagy stimulation a sub study under: Cambridge –Chennai Centre Partnership on antimicrobial resistance in TB: Focus on novel diagnostics and therapeutics

Principal Investigator	:	Dr. S Sivakumar
Source of funding	:	MRC-DBT
Study period	:	2016 - 2019

Introduction: Increasing level of multidrug resistance has raised concerns about TB control using conventional antituberculous chemotherapy. Host-directed therapy has emerged as a promising novel strategy to improve protection, promote bacterial clearance, and potentially overcome conventional drug resistance. There is, therefore, an urgent need to understand the pathogenic mechanisms of *Mtb* and the host immune response in order to identify and validate novel therapeutic targets. In the pre-chemotherapy era, rest, nutrition and sunlight were frequently successful in arresting PTB, thus indicating a substantial capacity for self-cure that adjunctive host-directed therapies (HDTs) might harness.

Objectives:

(i) Identify and refine potential therapeutics enhancers of autophagy;

(ii) Define the genetic determinants regulating autophagy induction and resistance.

Conclusion: Both the shortlisted compounds (Fig 4A, 4B) lead to decrease in the colony count of *M.tb* Strains varying from 0.5 log to 0.7 log. These compounds could be further tested in large collection of strains before the animal trials as adjunct therapy along with the standard treatment for tuberculosis. We have observed differential activation of autophagy by the clinical strains of *M.tb* isolated from the cohort.

Future: Confirmation of genetic hits: Genes implicated from both the bacterial GWAS of sequenced clinical isolates and the mutant library screen will be specifically tested by generating knockout, knock in, and complemented bacterial mutants and testing their behavior *in vitro* and *in vivo.*

Fig 4A:

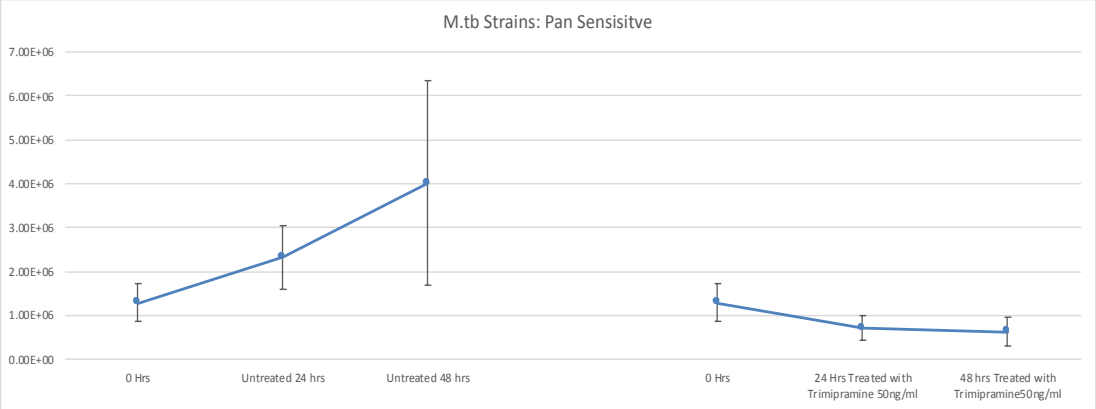


Fig 4B:

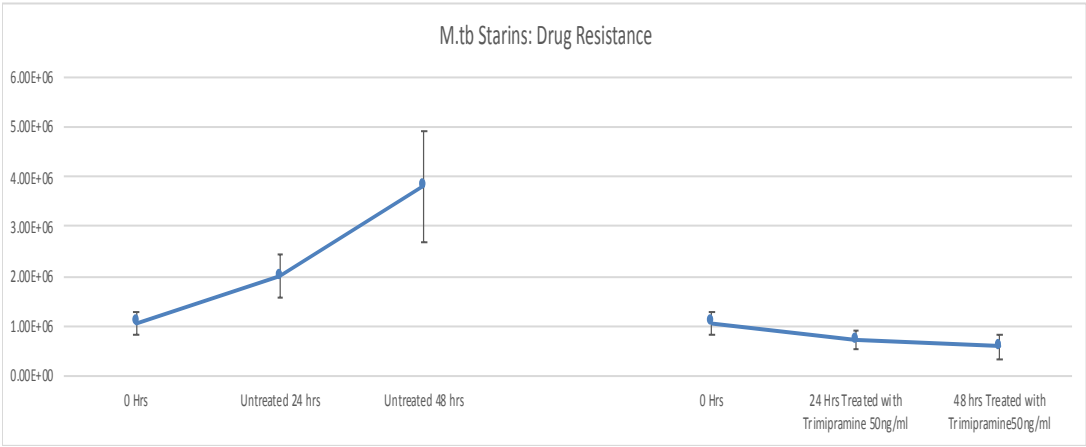


Fig 4A and 4B: Shows the effect of Trimipramine on the in-vitro growth rate on the drug sensitive and drug resistant *M.tb* strains. The growth rate was determined for both treated and untreated cells and found that the treatment with Trimipramine showed significant decrease in growth rate in time dependent manner.

B – 16: Multi-centric validation of indigenous kit 'TB-Detect' for the diagnosis of TB

Principal Investigator	:	Dr. Srikanth Tripathy & Dr. N. S. Gomathi
Source of funding	:	ITRC – ICMR
Study of Period	:	2018 – 2019

Background: The National TB Elimination Programme (NTEP) depends also on direct smear microscopy for case detection. There are several efforts to improve the performance of smear microscopy. We aim to achieve a higher rate of case detection by using the new indigenous field adoptable 'TB Detect' kit which concentrates the sputum specimen by filtration prior to smear preparation.

Aims and Objectives

To evaluate the performance of 'TB-Detect' with reference to direct smear (Zeil Neilson (ZN) & LED-Fluorescent Microscope (LED FM)) and MGIT liquid culture.

Sites: Six sites – NIRT Chennai, NITRD New Delhi, AIIMS New Delhi, BMHRC Bhopal, RMRC Bhubaneshwar, NJILOMD Agra – participated in the study.

Sample Size: 2046 presumptive Pulmonary TB (PTB) patients - based on 85% power, alpha of 5% and pooled 26% and 22% positivity of TB Detect and iLED-FM with 341 patients per site.

Method: One spot sputum sample from 341 presumptive PTB patients was collected after obtaining informed consent at the DMC. At the DMC, two smears were taken. One was stained by ZN method and the other by FM method and read as per NTEP guidelines. A small portion of sputum was used to prepare one of the TB Detect Filter device (F1) as per protocol. One smear was subsequently made from the device and stained by FM. The remaining portion of the sample was sent to the laboratory where one more TB Detect Filter device (F2) was prepared. This was used for inoculating one MGIT culture tube. The remaining sample was processed by NALC – NaOH method and used for raising liquid culture in a second MGIT tube.

Results:
The performance of 'TB Detect' kit was compared to the existing NTEP endorsed smear microscopy tests (ZN & LED-FM). The combined positivity of 'TB Detect' kit at 6 sites was 20% (n=417/2086) vs. 16.1% (n=337/2086) and 16 % (n=333/2086) of LED-FM and ZN smear, respectively (Table 16).

Table 16: Performance of 'TB Detect' kit vs. LED-FM and ZN smear microscopy

Site	TB Detect			LED-FM			ZN		
	Pos*	Neg	Positivity (%)	Pos*	Neg	Positivity (%)	Pos*	Neg	Positivity (%)
Total (n=2086)	**417**	**1669**	**20.0**	**337**	**1749**	**16.1**	**333**	**1753**	**16.0**
NIRT, Chennai (n=358)	54	304	15.1	36	322	10.0	33	325	9.2
RMRC, Bhubaneshwar (n=353)	58	295	16.4	57	296	16.1	54	299	15.3
BMHRC, Bhopal (n=341)	41	300	12.0	41	300	12.0	37	304	10.8
AIIMS, New Delhi (n=345)	97	248	28.1	93	252	27	104	241	30.1
NITRD, New Delhi (n=348)	74	274	21.2	46	302	13.2	41	307	11.8
NJILOMD, Agra (n=341)	93	248	27.2	64	277	18.7	64	277	18.7

The increment in positivity of 'TB Detect' kit compared to LED-FM and ZN smear microscopy was significant ($p<0.001$). It was also observed that the 'TB Detect' kit showed an overall 6.3% increment over ZN smear and 15.7% increment over LED-FM smear in smear grade status. This is likely to decrease the time required to examine each filter slide.
The combined sensitivity of 'TB Detect' kit at 6 sites was ~55% vs. 52% (iLED) and 50.9% (ZN) against MGIT liquid culture. The increment in sensitivity of

'TB Detect' kit over ZN smear was significant ($p<0.05$), but not over LED-FM smear ($p= 0.144$). The 'TB-Detect' kit was found to be bio-safe for sputum processing at all 6 sites. Only one out of 2086 samples was culture positive.

Conclusion:
The indigenous kit described here is simple and has the potential for rapid and reliable TB diagnosis and to increase case detection. The kit 'TB Detect' is expected to be a better alternative to direct smear microscopy and will find use in resource-limited laboratories and in microscopy centres all over the country. Further multicentric validation need to be carried out to check the feasibility of use in different settings.

B - 24: Prospective, multicentre study to assess the diagnostic accuracy of the Truenat MTB and RIF assays in intended settings of use.

Principal Investigator	:	Dr. N. S. Gomathi
Source of funding	:	ITRC – ICMR, BMGF (International sites)
Study Period	:	2019 - 2020

Background
An estimated 3.6 million TB cases go undiagnosed globally each year, leading to substantial morbidity and mortality. In most countries smear microscopy remains the only option for rapid diagnosis of TB, though it detects only 45% of infections. For these reasons, new molecular TB diagnostics that can be instituted at the field level are a research and implementation priority.
The Truenat MTB assay and the RIF reflex assay (Molbio Diagnostics; Bangalore, India) utilize chip-based real-time micro PCR for detection of TB and RIF resistance from DNA extracted from sputum samples. A pilot study conducted in India found the assay to achieve 91% sensitivity and 100% specificity against a composite reference standard. However, further evidence for the performance and operational characteristics of the Truenat MTB assay, as well as the Truenat RIF Reflex assay, is needed prior to recommending it for diagnostic purposes at the field level. .

Study objectives
Assess the diagnostic accuracy and patient outcome of the Truenat MTB and RIF reflex assays for the detection of TB and RIF resistance in diverse settings of intended use.

Sample Size and Population
1110 adult presumptive pulmonary tuberculosis or presumptive DR-TB patients attending designated microscopy centres (DMCs) from India and 556 patients from international sites.

Study sites
Three Reference Labs (NIRT Chennai, Intermediate Reference Lab Ahmedabad and Intermediate Reference Lab Guwahati) with 3 DMCs attached to each and one laboratory (Hinduja Hospital, Mumbai) from a private centre in India and sites in Peru, Papua New Guinea and Ethiopia globally.

Method

Adults with presumptive TB infection attending outpatient clinic settings at DMC's were screened for eligibility and enrolled after informed consent. Information on signs/symptoms and TB history were obtained. Four sputum samples (2 samples on day 1 and 2 samples on day 2) were collected from the patients. Samples S1 and S2 were transported to the reference laboratory and pooled after individual smears were taken. From the pooled sample, Xpert and Truenat were performed. For Truenat assay, DNA was extracted from sputum by the Trueprep Auto device and tested by the Truenat MTB chip and MTBplus chips, which were read by the TruelabUnoDx real-time PCR analyser independently. All DNA samples testing positive by the MTB/MTBplus assay were subsequently tested by the Truenat RIF reflex assay (chip) and also read by TruelabUnoDx analyser. The remaining sample was decontaminated and cultured on LJ and MGIT 960. Growth on LJ and MGIT were confirmed by MPT64 Ag based lateral flow immunochromatographic test. MGIT DST for SIRE/R was done from positive MGIT culture. In addition, Truenat MTB/MTBplus assays and Xpert were performed on the decontaminated sample.

Sample S3 was transported to the reference lab, decontaminated and processed for culture by LJ and MGIT. Growth was confirmed by ICT and MGIT DST for SIRE /R were done from positive MGIT culture. S4 was processed for Truenat MTB/MTPplus and MTB Rif (if positive) in the DMC.

Results

Valid results for all tests were available from 1654 global participants including 1110 from India. In the clinical setting (DMC), the sensitivity of Truenat MTB and Truenat MTBplus in comparison with MGIT for detection of MTB, was 73%, and 80% while the specificities were 98% and 97% respectively.

In the reference laboratory, the sensitivity of Truenat MTB, MTBplus and Xpert in comparison with MGIT for detection of MTB, were 84%, 87% and 85% while the specificities were 97%, 95% and 97% respectively.

For detection of rifampicin resistance, the sensitivity of Truenat MTB Rif and Xpert were 82% and 84% in comparison with MGIT DST while specificity was 98% for both assays.

The overall initial error rates with Truenat MTB and Truenat MTBplus assays were 6.2% and 9.2% respectively of which 87.5% resolved by a single retesting. The initial error rates for Xpert and Xpert Ultra were 2.6% and 0.0%.

Conclusion

The findings show that Truenat MTB and MTB plus assays meet the criteria recommended by WHO and hence have the potential to be considered as a smear replacement test at the DMC and higher labs. The findings are crucial for inclusion of the assay in the diagnostic algorithm of National TB programs working towards elimination of TB.

STUDIES IN PROGRESS:

B-17: Prediction of treatment failure among diabetes-TB patients by peripheral blood transcriptional signature

Principal Investigator	:	Dr. S Sivakumar
Source of funding	:	DBT
Study period	:	2018 – 2021

Short Description: India currently has 61 million people with diabetes mellitus (DM) in the world and will soon become the "diabetic capital" of the world. Diabetes in Asian Indians is characterized by younger age of onset, lower body mass index and increased insulin resistance which has been described as "Asian-Indian Phenotype". The current pandemic of DM is accelerating in a world where approximately one-third of the population is latently infected with *M. tb.* The consequences of these converging epidemics are likely to be substantial. Several studies indicate that patients with TB who have DM, present a higher bacillary load in sputum, delayed mycobacterial clearance, and higher rates of multidrug-resistant infection. Recent epidemiological surveys have clearly shown the possibility of diabetes-TB (DM-TB) nexus in near future. Thus, these issues require urgent attention.

Hypothesis: Can peripheral blood transcriptional signature (PBTS) predict sputum smear conversion after intense phase of anti-TB treatment among subjects with diabetes and TB?

Progress: All the approvals for the study have been obtained and we have started the recruitment of patients into the study. Till date we have recruited 20 patients and the study is ongoing.

B – 22: Identification of inhibitors for multidrug efflux pumps of *M. tuberculosis* from medicinal plants, using *in silico* high-throughput virtual screening and *in vitro* validation

Principle investigators	:	Dr. V. N. Azger Dusthackeer& Dr. S. Christy Rosaline
Source of funding	:	ICMR
Study period	:	June 2017 – June 2020

Background: Increased efflux activity can occur in response to prolonged exposure to sub inhibitory concentrations of anti-TB drugs. One possible method to prevent resistance by efflux pump is to use efflux pump inhibitors (EPI) which in turn increases the activity of the antibiotics. In this proposal, we will be focusing on overcoming drug efflux in MDR-TB clinical isolates by identifying natural compounds from plants.

Objectives

1. *In silico* high throughput virtual screening and validation to identify lead compounds from plants to inhibit Rv1819c, Rv2209, and Rv1218c;
2. Over-expression of *M. tuberculosis* efflux pumps proteins in *M. smegmatis*;
3. *In vitro* validation for the shortlisted lead compounds against the efflux pumps Rv1819c, Rv2209 and Rv1218c

Progress:
In this study, the shortlisted efflux pump inhibitors via *in silico* screening showed the minimal inhibitory concentration (MIC) ranging between 700 to 1000 µg/mL by broth dilution method against MDR and XDR clinical isolates. The MIC of RIF along with the presence of EPIs is effectively reduced by 4- to 8-fold, leading to 50 to 100 percentage of inhibition. To further validate the efflux inhibition potential of the shortlisted lead compounds over-expression of efflux pump genes was carried out in DH5α. Q-PCR method was used to analyze the transcription levels. Furthermore, MICs of the shortlisted lead inhibitors against recombinant *M. smegmatis* remained high, but that of RMP in combination with the small molecular inhibitors reduced from 8 µg/mL to 0.025 and 0.5 µg/mL for Methyl stearate and Myo inositol respectively in the recombinant, demonstrating the potential nature of these inhibitors. The efficiency of the lead compounds was validated by ethidium bromide accumulation assay

B – 23: Prevalence of non-resolving pneumonia in children suspected with TB

Principal Investigator	:	Dr. R. Priya
Source of funding	:	ICMR- Intramural
Study period	:	2018 – 2021

Back ground: Pneumonia is a major cause of morbidity and mortality in infants and children worldwide, with most cases occurring in TB-endemic settings. It has been reported that pneumonia in infants and young children have underestimated the contribution of TB as a direct cause or co-morbidity of acute community-acquired pneumonia in children because of the difficulties of microbiological confirmation in this age group, especially in resource-restricted TB endemic settings. There is an urge for an extensive study on the association of TB with community acquired pneumonia in children by strengthening the microbiological confirmation methods in this age group which in turn would help in improving clinical management. In this study, we hypothesize that among children with respiratory diseases, simultaneous diagnosis of TB and other pneumonia causing organisms will depict the factual representation of the etiology and help in appropriate treatment.

Primary objectives:
(i) To estimate the prevalence of non-resolving pneumonia and co-infection of TB and bacterial/viral agents of pneumonia in children treated with ATT (in Group- A)

Secondary objectives:
(i) To determine the bacterial and viral cause of pneumonia in children (in Group-B);
(ii) To estimate the sensitivity and specificity of the molecular detection of pneumonia against the conventional technique;
(iii) To estimate the prevalence of bacteriologically confirmed and un-confirmed pediatric PTB

Methods:
Smear and culture identification is done for MTB and other bacteria. Xpert MTB RIF testing is done for MTB alone. DNA is extracted from the processed sputum and real time PCR is carried out for detection of a wide variety of bacteria and viruses.

Progress: We have received 45 paediatric pulmonary samples so far and the standardized multiplex real time PCR for detection of bacteria was carried out on 24 samples. The bacterial multiplex PCR have detected a wide panel of organisms in these samples that includes *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Streptococcus pyogenes, Hemophilus influenzae, Moraxella catarrhalis, Burkholderia species and Mycobacterium spp.* The viral multiplex PCR was also standardized and 12 samples have been screened so far. All 12 samples were positive for the genes that target Parainfluenza type I, II and III. Discrimination of these subtypes will be done by a separate PCR panel if necessary. Out of these 45 samples screened for TB, one sample was positive for *M. tuberculosis* by Gene Xpert and culture.

B-25: A Study on the Strain specific Modulation of Tuberculosis granulomatous reaction using *in-vitro* 3D granuloma model

Principle investigators	:	Dr.V. N. Azger Dusthakeer & Dr. R. Sam Ebenezer
Source of funding	:	ICMR
Study period	:	2020 – 2023

Background of the Study:

Granuloma is the hallmark of TB infection. The granuloma contains mostly blood-derived macrophages, epithelioid cells (differentiated macrophages) and multinucleated giant cells (also known as Langhans giant cells), surrounded by T lymphocytes. This microenvironment limits the replication and spread of TB bacilli. In certain cases the granuloma leads to spreading of the infection inside the host called as disseminative granuloma. Hence it affects the treatment process also. A mature granuloma is often characterized by caseous center necrotic lesions with low oxygen level, low pH, and limited nutrients. Chemokines and cytokines play a crucial role in granuloma formation and its type. The chemokines binding to the CCR2 receptor (CCL2/MCP-1, CCL12, and CCL13) are important for the early recruitment of macrophages. Osteopontin, produced by macrophages and lymphocytes, promotes the adhesion and recruitment of these cells. CCL19 and, possibly, CCL21 are involved in the recruitment and priming of IFN-γ-producing T cells. CXCL13 is involved in B-cell recruitment and the formation of follicular structures (Khader et al., 2009). The expression of the CC and CXC chemokines is deregulated at the transcriptional level in TNF-deficient mice, and the lack of these chemokines prevents the recruitment of macrophages and CD4+ T cells, accounting for the critical role of TNF-α in granuloma formation. Block in the TNF-α regulation leads to the down-regulation of Th1, Th17 and Treg cytokines, which further causes the impairment of granuloma.

Objectives:

1. To study the strain specific modulation of TB granuloma formation using in-vitro 3D granuloma model by stimulating the PBMCs isolated from the healthy volunteers.
2. To analyze the pattern of granuloma formation in TB patients using in-vitro 3D

granuloma model by stimulating the PBMCs and to look for any correlation with the disease progression and treatment response.

3. To explore the available immune modulators through ex-vivo studies to direct the host immune response towards the formation of protective granuloma.

Work progress:

The study has been initiated with sub culturing the clinical isolates, which has been approved by SAC and IEC of NIRT.

Bacteriology Lab services:

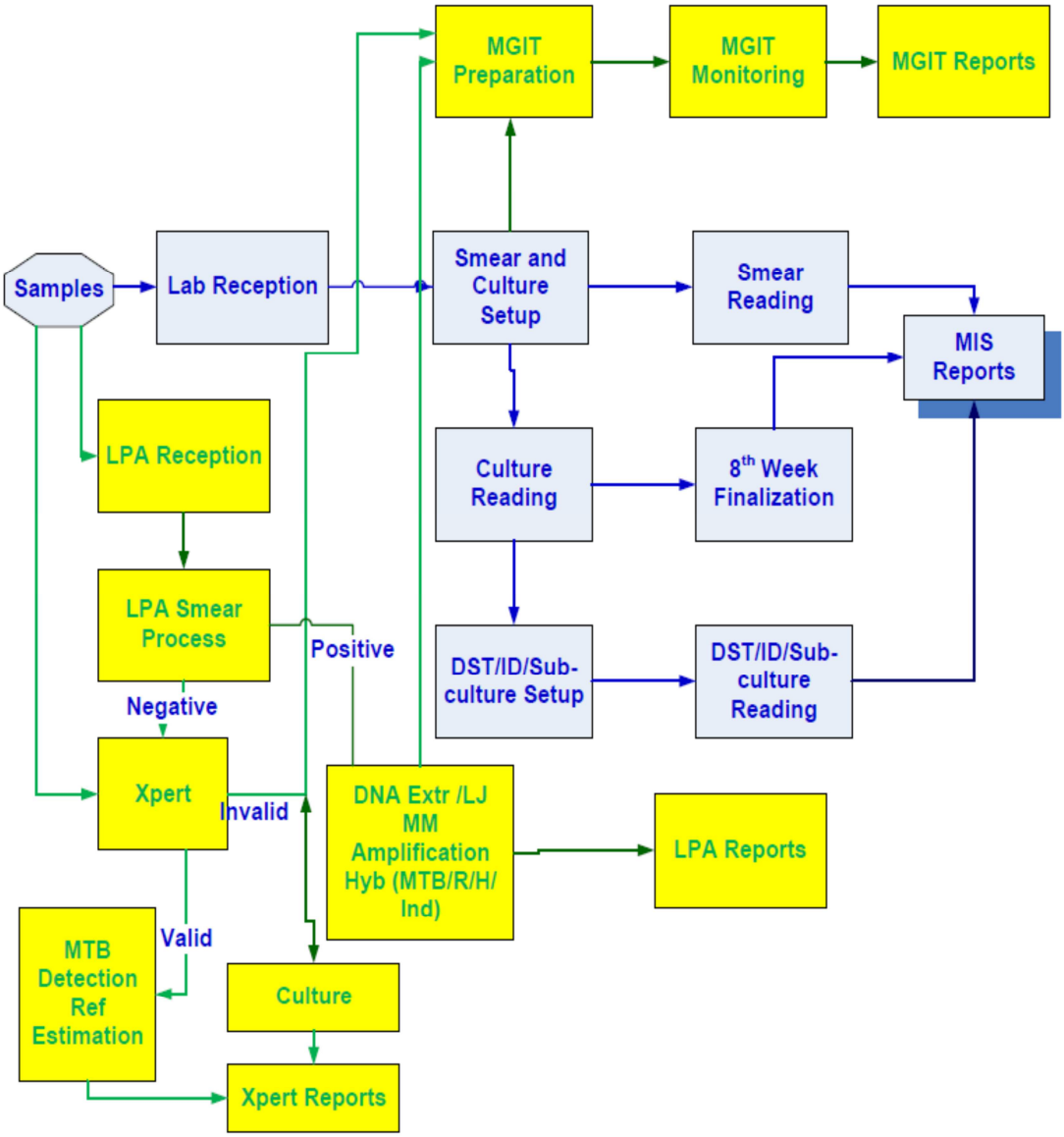
NIRT Bacteriology Automation System

Contact person : Dr. Rajesh Mondal & Dr. Rajendran

Background:
Laboratory investigation is the prime means of diagnosis in modern medical practice. The process includes:
(a) Registering of patient
(b) Sample collection
(c) Stages of investigation
(d) Result entry
(e) Result validation and
(f) Final reporting
The Laboratory Information System (LIS) provides a framework for the systematic completion of these activities. Considering the importance of automating the above process, NIRT engaged a software company (M/s ETPL, Trivandrum), through a public tendering process to specially develop and implement a business process re-engineering of the existing LIS to one using Wi-Fi enabled laptop computers and bar codes. The requirement specification for this automation project was drawn up in 2013, implementation was done in mid 2015. The system was made operational by last quarter of 2015 and is fully operational now. This year 2019-2020, the system was shifted to the local server by the EDP department. The existing workflow is illustrated in Fig. 6 which is functional in all the sections by the use of specific barcode readers and laptops provided in all the section. Interlinking all the sections is the backbone network. This is a CAT6 cable link interlinking all the systems to a Server class DELL machine set up in the computer centre. This system runs all day and contains the DBMS. All systems access the DBMS to place the results and access needed data. It also provides reporting facility that may be used by the clinicians and statisticians to get any required report from the system. The administrative functions are coordinated from the Head of the Departments room with the laptop provided. Various MIS reports are available here.

Status: The automation system was validated by STQC, Thiruvanmiyur and EDP department of NIRT is building it as an in house system. When the original requirements were drawn up, many of the currently existing facilities (MGIT, LPA, and GeneXpert) were not included in currently existing system (Phase I). Due to this, these important systems are not currently incorporated in the existing automation system. Hence, there is a serious gap in the existing automation system and the future extension project aims to close this gap, so that the current system becomes a comprehensive one involving all analysis streams in the bacteriology laboratory. Phase II automation system will be initiated in near future by EDP department after successful completion of in house building of the system.

<u>Fig. 5:</u> Workflow in the bacteriology lab in NIRT. The boxes in blue are part of the currently running automation system (Phase I). The boxes in yellow are proposed to be included in Phase II automation system



DEPARTMENT OF CLINICAL PHARMACOLOGY

STUDIES COMPLETED:

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

Principal Investigator	:	Dr. A.K. Hemanth Kumar
Source of funding	:	Intramural
Study period	:	2016-2019

A pharmacokinetic (PK) study of drugs used to treat multi drug resistant TB (MDR TB), namely levofloxacin (LFX), ethionamide (ETH), cycloserine (CS), pyrazinamide (PZA), moxifloxacin (MFX) and isoniazid (INH) was undertaken in adult MDR TB patients who were treated according to the RNTCP guidelines in India. Factors influencing drug PK and end-of-intensive phase (IP) status were also determined. We recruited 350 MDR TB patients receiving anti-TB treatment (ATT) under NTEP in south India. At steady state, serial blood samples were collected, after supervised drug administration. Status at the end of IP was noted from the programme records. Of the 303 patients for whom end-of-IP status was known, 214 were culture negative (responders), while 45 patients were either culture positive or required change of regimen or had died before completion of IP (non-responders). The median C_{max} (2.0 vs 2.9µg/ml; p = 0.005) and AUC_{0-12} (12.2 vs 17.0µg/ml.h; p = 0.002) of ETH were significantly lower in non-responders than responders at IP. In multivariate logistic regression analysis, after excluding defaulters and adjusting for confounders, AUC_{0-12} of ETH significantly influenced end-of-IP status (aOR - 1.065; 95% CI: 1.001 - 1.134; p = 0.047). Drug doses used currently in the programme produced optimal drug concentrations in majority of patients (Table 17). ETH played a major role in the MDR TB combination regimen and was a key determinant of end-of-IP status. Manuscript prepared and submitted for publication.

Table 17: Pharmacokinetic parameters of drugs

Pharmacokinetic parameters of drugs (Values are given as Median & Inter quartile range)							
PK Parameters	**Levofloxacin n = 252**	**Ethionamide n = 259**	**Cycloserine n = 235**	**Pyrazinamide n = 300**	**Moxifloxacin n = 69**	**Isoniazid (300/600mg dose) n = 40**	**Isoniazid (900mg dose) n = 23**
Cmax, µg/ml	11.6 (9.3 - 14.4)	2.5 (2.0 - 2.5)	37.5 (28.3 - 47.9)	48.9 (38.7 - 60.3)	5.6 (3.9 - 8.1)	10.5 (7.8 - 15.9)	11.8 (7.2 - 19.5)
Tmax, h	2 (1 - 4)	2 (2 - 2)	2 (2 - 4)	2 (2 - 4)	2 (2 - 4)	2 (1 - 2)	2 (1 - 2)
AUC $_{0-12}$, µg/ml.h	88.6 (69.7 - 111.4)	15.2 (10.5 - 15.2)	344.3 (263.8 - 454.6)	453.9 (336.4 - 576.6)	43.1 (30.1 - 64.9)	58.3 (40.0 - 102.4)	74.6 (36.7 - 113.1)
$T_{1/2}$, h	8.0 (6.2 - 11.2)	3.4 (2.2 - 3.4)	16.3 (11.6 - 24.1)	12.7 (9.6 - 16.8)	7.0 (5.7 - 8.8)	4.8 (3.6 - 6.3)	4.7 (3.5 - 6.6)
No (%) of Patients with Cmax							
Below therapeutic Range	34 (13.5%)	71 (27.4%)	16 (6.8%)	10 (3.3%)	7 (10.1%)	3 (7.5%)	6 (26.1%)
Within therapeutic Range	126 (50.0%)	171 (66.0%)	83 (35.3%)	211 (70.3%)	22 (31.9%)	3 (7.5%)	11 (47.8%)
Above therapeutic Range	92 (36.5%)	17 (6.6%)	136 (57.9%)	79 (26.3%)	40 (58.0%)	34 (85.0%)	6 (26.1%)

Therapeutic Range: LFX: 8 – 13 µg/ml; ETH: 2 – 5 µg/ml; CS: 20 – 35 µg/ml; PZA: 20 – 60 µg/ml; MFX: 3 – 5 µg/ml; INH: 3 – 6 µg/ml; C_{max} = Peak Concentration; T_{max} = Time at which peak concentration was attained; AUC = area under the time concentration curve; T1/2 = half-life

<u>BCP – 10:</u> Estimation of the efficacy of novel RMP analogues

Principal Investigators	:	Dr. Geetha Ramachandran, Dr. A.K. Hemanth Kumar & Dr. Srikanth Prasad Tripathy
ICMR – Post-doctoral fellow	:	Dr. P. Saravanan
Source of Funding	:	ICMR-PDF fellowship
Study Period	:	2016-2019

Great efforts have been made to find both synthetic compounds and natural products to battle MDR- and XDR-TB as there is a urgent need to discover new antitubercular compounds. We have synthesized various Rifampicin based novel molecules and estimated its efficacy by *in-vitro* method and some promising molecules were further studied for cytotoxicity assay. Novel Gold metal nano cluster were also synthesized, characterized and studied by in-vitro method. Some of the promising studies are (i) Synthesis and in-vitro studies of novel Rifampicin and Clofozamine (RIF-CFZ) hybrid drug; (ii) Synthesis and in-vitro studies of novel RIF-MET (Metformin) Hybrid drug; (iii) Synthesis and in-vitro studies of RIF-Imidazole scaffold; (iv) Synthesis of novel Gold- (dppf) metal nano cluster and in-vitro studies.

Manuscript under review in Scientific Reports journal: Title: Discovery of Highly Potent Novel Rifampicin analogue through Hybrid of Precursors of Rifampicin and Clofazimine anti-mycobacterial drugs.

STUDIES IN PROGRESS:

BCP – 8: Pharmacokinetic drug-drug interactions between first line anti-TB and anti-diabetic drugs

Principal Investigator	:	Dr. A.K. Hemanth Kumar
Research Scholar	:	Ms. Mary Rebecca
Source of funding	:	Intramural
Study period	:	2017 – 2020

Introduction:

Diabetes mellitus (DM) is a risk factor for TB with prevalence rates among TB patients ranging from 10 to 30%. Cohort studies and a recent meta analysis provide further convincing evidence that TB is more common in patients with diabetes. Type 2 diabetes mellitus seems to be associated with a less favourable response to TB treatment. A study from Chennai observed delayed sputum conversion and high failure rates in new smear positive PTB patients with DM. Limited information is available on the pharmacokinetic drug – drug interactions between first-line anti-TB and anti-diabetic drugs.

Aims:

i. To study the effect of anti-diabetic drugs (biguanides and sulphonlyurea) on the pharmacokinetics of first line anti-TB drugs;

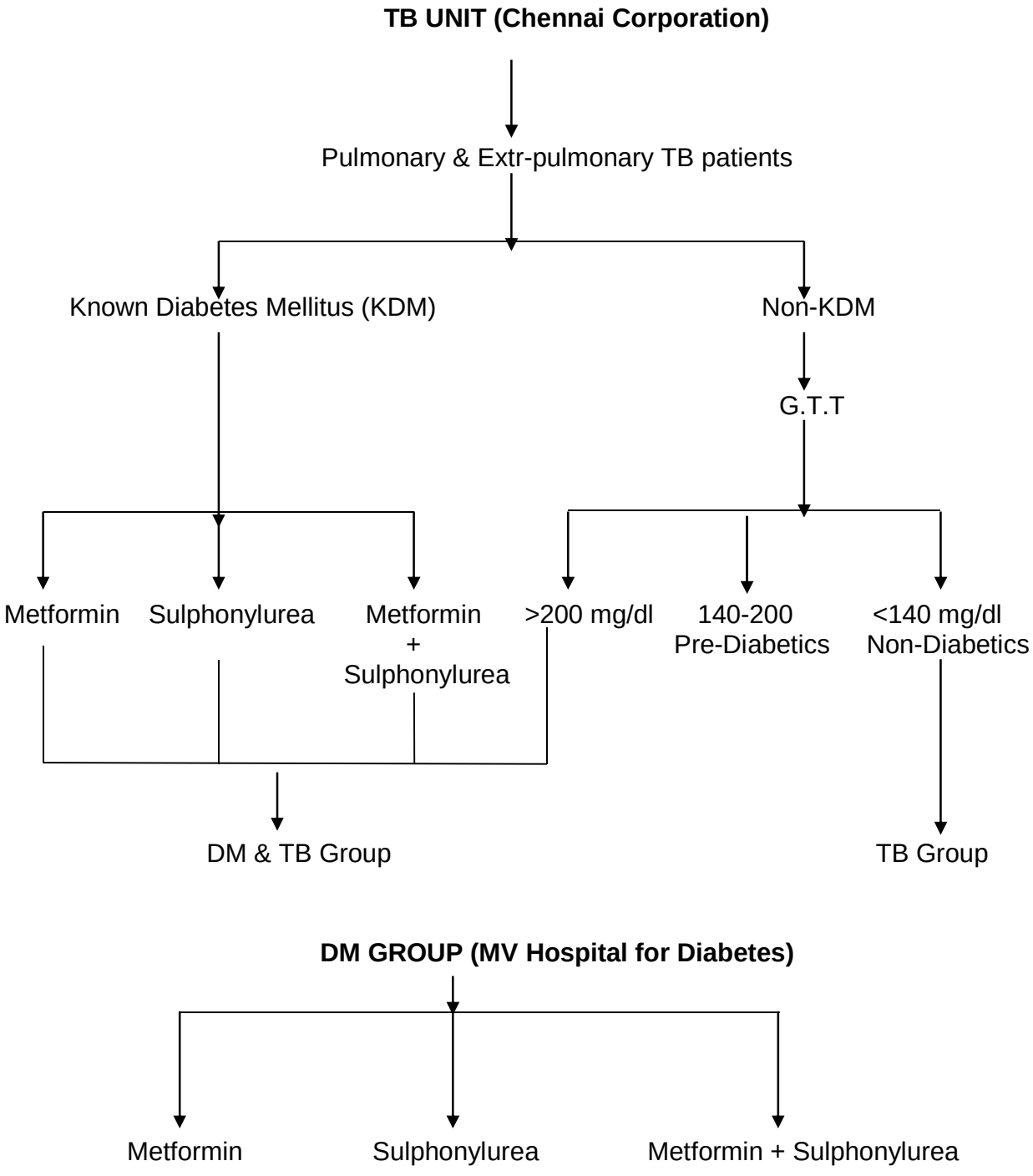
ii. To study the impact of first line anti-TB drugs on the pharmacokinetics of these anti-diabetic drugs.

Methods:

This prospective, observational pharmacokinetic study is being conducted in three groups of patients, namely, TB, DM & TB and DM. Patient recruitment is represented in the fig 6.

A pilot study has been initiated. The required sample size is 12 in the TB group and 36 each in DM and DM &TB groups respectively. The study is in progress.

Fig 6: Schematic representation of patient recruitment



BCP – 11: Therapeutic Drug Monitoring in drug sensitive, non-responding pulmonary TB patients

Principal Investigators	:	Dr. A.K. Hemanth Kumar
Source of funding	:	NTEP
Study period	:	2018 – 2020

Introduction: Therapeutic drug monitoring (TDM) has been used to ensure optimal dosing for maximizing the therapeutic benefit while minimizing toxicity. TDM is not routinely done for the active management of TB. Although published reports describe the attribution of low drug levels to slow response or no response, to question remains about how best to implement TDM on a programmatic scale. Definitions of slow response vary and recommendation for which medication to prioritize for TDM are lacking. However, identification of patients at risk for slow response is critical for improving the treatment outcomes. TDM may be useful in TB management, but programmatic implementation has been less studied.

Aims:

1. To estimate plasma concentrations of first line anti-TB drugs (RMP, INH, PZA) in pulmonary TB patients not responding or slowly responding to treatment under RNTCP after ruling out drug resistance in these patients by culture or molecular methods.

2. In those patients detected to be having sub-therapeutic anti-TB drug levels, to assess the clinical, radiological and bacteriological response by increasing doses of drugs

Methods: This is a prospective study conducted in the Department of Pulmonary Medicine, Goa Medical College. The study participants will comprise of adult pulmonary TB patients who are receiving TB treatment in the NTEP centres in Goa. Patients with positive smear at 2 months of ATT were identified and therapeutic drug monitoring (TDM) was performed at this point of time. The sub-therapeutic cut-off values will be taken as <8µg/ml for RMP, <3µg/ml for INH and < 20µg/ml for PZA. If any of the drug concentrations are found sub-therapeutic, the dose of only those drugs will be increased for a month, after which TDM will be repeated. If the repeat smear is negative, the increased drug doses will be continued till end of ATT. It is expected to enrol 20 patients during this study period. The study is in progress.

BCP – 12b: Impact of pregnancy on Tuberculosis

Principal US Investigator	:	Jerrold J. Ellner, MD
Principal Indian Investigator	:	Sonali Sarkar, MD
NIRT Co-Investigators	:	Dr. A.K. Hemanth Kumar
Study Period	:	2017 - 2020

Introduction: Standard doses of first line anti-TB drugs are recommended by the World Health Organization to treat pregnant women with active TB. Although the available data do not suggest any significant adverse

maternal-fetal effects or need for dose adjustment in pregnancy, the PK of TB drugs in pregnant women has not been systematically studied, even for drugs that comprise the basic first-line regimen

Study Aims:

Aim 1: To determine the impact of pregnancy on diagnostic biomarkers of LTBI and on host biomarkers that may predict risk of progression to TB.

Aim 2:

a. To determine the risk of TB in pregnancy and
b. To describe the severity of disease, the response to treatment and pregnancy outcomes.

Aim 3: To evaluate the pharmacokinetics of TB drugs during pregnancy and assess exposure of the fetus to anti-tuberculous TB drugs.

Methods: The profile of antituberculous drug metabolism between pregnant and non-pregnant TB patients will be compared by conducting PK studies every 2 months during the TB treatment. This will include drug level measurements (at 1 hour, 2 hours and 6 hours post-drug administration) and determine whether, if any dose adjustments are needed for pregnant women. The study is in progress.

<u>BCP – 14:</u> Pharmacokinetics of second-line anti-TB drugs in children and adolescents with MDR TB

Principal investigators	:	Dr A K Hemanth Kumar, Ph.D.,
Source of Funding	:	India TB Consortium, ICMR
Study Period	:	Three years (2019 – 2022)

Introduction: Drug-resistant TB (DR TB) is a continuing threat and is an issue in children. There is paucity of pharmacokinetic data of ATT in children with MDR TB. A prospective, observational study to determine the pharmacokinetics of Levofloxacin (LFX), Pyrazinamide (PZA), Ethionamide (ETH) and Cycloserine (CS) in 25 children with MDR TB treated according to NTEP guidelines at the Sarojini Naidu Medical College, Agra was conducted. The study results showed that the second line anti TB drug levels were within the therapeutic range. The study was limited by the modest sample size, and stressed the need to undertake more studies on the safety and PK of second-line ATT in children with MDR TB.

Aim: To study the pharmacokinetics of certain second line anti-TB drugs (cycloserine, ethionamide, levofloxacin, pyrazinamide, moxifloxacin, kanamycin, amikacin) in children with MDR TB.

Methods: A prospective, cross-sectional PK study is being conducted at Institute of Child Health, Chennai, BJ Wadia Hospital for Children, Mumbai, Lady Hardinge Medical College, New Delhi and National Institute for Tuberculosis and Respiratory Diseases, New Delhi. Sample size is 200 children. Presently, 66 children were recruited and the study is in progress.

DEPARTMENT OF BIOCHEMISTRY

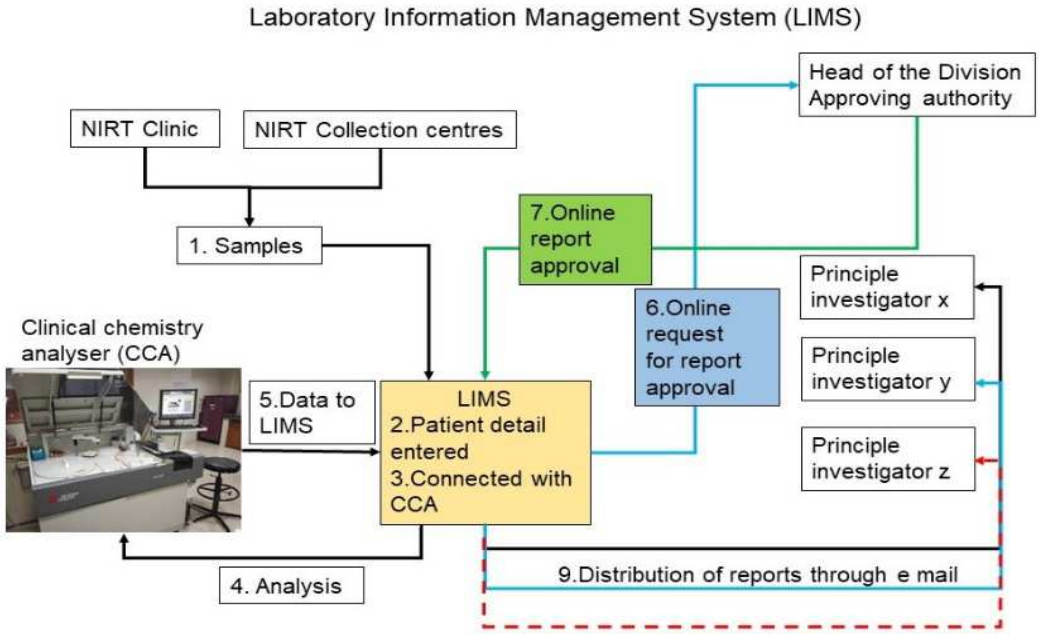
STUDIES IN PROGRESS:

BC-1: Clinical Biochemistry Data Reporting System: Post launch operations

Co-ordinator	:	Dr. N Saravanan
Source of Funding	:	ICMR-Intramural
Study Period	:	2019-20

Clinical Biochemistry Laboratory, Department of Biochemistry has launched the Laboratory Information Management System (LIMS) the month of Aug-2019 to ensure accurate and rapid dissemination of quality data to the respective project investigators within 2 to 4 hours after the receipt of the sample for the effective critical care and health management of their volunteers/research subjects. With the help of fully functional LIMS it is ensured that the clinical chemistry data of an individual subject of different time points in a day are integrated as one report. The report is received and approved by the approving officer in time through local area network. Once approved, the report is delivered to the respective Principle Investigators through email/ online delivery system from LIMS. This process has improved the turnaround time (TAT) exorbitantly and avoided unnecessary delay (24 hours) in reporting the clinical chemistry data which usually happens during conventional reporting procedures. The PIs are now getting the clinical biochemistry status of their patients with in 2 to 4 hours after the submission of samples to the Clinical Biochemistry Laboratory. After e-reporting the data, a hard copy also sent to the respective PIs for further data management.

Fig-7. Workflow of Laboratory Information Management System (LIMS) at Clinical Biochemistry Lab, Department of Biochemistry



BC-3: Vitamin D concentrations in adult tuberculosis patients

Principal Investigator	:	Dr. N Saravanan
Source of Funding	:	ICMR (Intramural)
Study Period	:	2018-2021

Vitamin D is known to play a crucial role in the immune regulation by improving cell-mediated immunity and the phagocytic capacity of macrophages by increasing the production of antimicrobial peptide cathelicidin. Many reports suggest an association of Vitamin D deficiency with the increased prevalence of the TB. It is known that antibiotics are involved in the diminution of vitamin D levels as there is a trigger of pregnane X receptor pathway. However data on the effects of antitubercular drugs on the levels of vitamin D is lacking. Therefore a pilot study protocol has been developed in collaboration with National Institute of Nutrition (NIN), Hyderabad.

Stored serum samples from a previous study (Adult PK study) were used for vitamin D estimation. The study population comprised of adult TB patients treated with category I or category II RNTCP regimens from 10 TB units in Chennai Corporation, Chennai, Tamil Nadu State, India. Patients with both pulmonary and extra pulmonary TB were included. The serum samples collected at initiation of the treatment (after 2 weeks of ATT) and at end of ATT were paired. The vitamin D estimation was undertaken in 131 paired samples at the National Institute of Nutrition, Hyderabad using HPLC and the data has been collected.

The levels of other micronutrients such iron, zinc and selenium will be estimated using atomic absorption spectrophotometry. Further the compilation and analysis of data will be undertaken.

<u>BC- 4:</u> Evaluation and characterization of potential therapeutic leads form AYUSH system of medicine for adjuvant therapy during anti tuberculosis treatment.

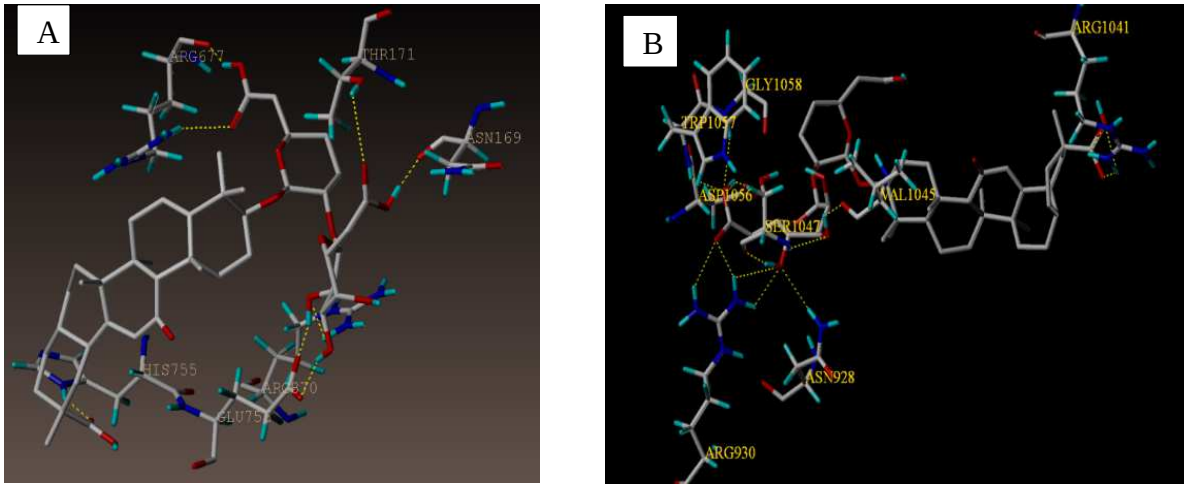
Principal Investigator	:	N Saravanan
Source of Funding	:	To be applied
Study period	:	2019-2022

During TB infection and treatment the pathological and metabolic events such as poor nutrient intake and malnutrition, altered immunity due to micro and macronutrient deficiency, altered inflammatory homeostasis in the lung and adverse drug reaction due to ATT occur in the host that may result in the progression of latent infection to active disease. In people with active disease who are undergoing ATT the aforementioned metabolic abnormalities may reflect in poor adherence to treatment, poor treatment outcome, acquired drug resistance and mortality in few instances. Hence the exploration of antimycobacterial compounds form natural sources should be in the interest of (i) increasing the nutritional status of the individual during infection and treatment, (ii) promoting the immunity of the host to achieve optimal clearance of *Mtb*, (iii) to aid pulmonary health in order to withstand the necrotic effects of *Mtb* infection and to (iv) preserve liver function during

treatment to avoid ATT induced adverse effects and consequent default cases an addition to their antimycobacterial potential. Therefore an the interdisciplinary group is formed to identify potential natural compounds, validate and characterize their medicinal properties using *in silico* and *in vitro* approaches under one umbrella followed by preclinical studies.

We have conducted virtual screening for potential antimycobacterial compounds from fifteen select phytochemicals for their efficiency of binding with the active targets (AT) of first line (INH, RIF, PZA and EMB) and second line (Fluoroquinolones, Bedaquilline and Capreomycin) antitubercular drugs (ATT). The selected proteins for the docking studies are (i) RNA Polymerase subunit C (PDB Id: 5ZX3) AT of RIF, (ii) Enoyl-[acyl-carrier-protein] reductase (PDB Id: 5VRL) AT of INH, (iii) Ribosomal protein S1(PDB Id: 4NNI) AT of PZA, (iv) Arabinosyl transferase (PDB Id: 3PTY) AT of EMB, (v) DNA gyrase subunit A (PDB Id: 4G3N) Active target of Fluoroquinolone, (vi) 2'-O-methyltransferase TlyA (PDB Id: 5KYG) AT of Caperomycin, (vii) F-Adenosine Triphosphate (ATP) synthase epsilon chain (PDB Id: 5YIO) AT of Bedaquiline. The results showed that seven novel phytochemicals namely Gardenin-A, Swertiamarin, Glycyrrhizin, Alizarin, Mangiferin, Laccaic acid and Aloe emodin are binding efficiently with AT of ATT drugs as evidenced by the number of hydrogen bond formation and C-score. These compounds will be further characterized for their other beneficial effects before initiating *in vitro* and *in vivo* studies.

<u>Fig.8.</u> Representative docking images of Glycyrrhizin with the (A) Enoyl-[acyl-carrier-protein] reductase and (B) Arabinosyl transferase.



<u>BC- 5:</u> Point of care estimation of Vitamin D and C-Reactive Protein for tuberculosis screening in household contacts of active pulmonary tuberculosis patients in Chennai, India: A pilot study

Principal Investigator	:	Dr. N Saravanan
Source of Funding	:	ICMR (Intramural)
Study Period	:	2020-2021

The launch of 'DOTS' (directly observed treatment, short-course) strategy by World Health Organization (WHO) has significantly decreased tuberculosis (TB) incidence worldwide. The deaths due TB have decreased by 47% overall between 1990 and 2014. Despite this achievement TB continues to be a major public health problem globally; based on the latest report, 9.6 million people developed new active TB and 1.5 million people died due to TB. The disease burden is highest in Asia and Africa, where India and China together contribute for almost 40% of the world's TB cases. The problems associated with missed or delayed diagnosis of active TB with poor access to high quality care lead to a higher risk of death, suffering, longer duration of infectiousness, increased transmission and unexpected economic burdens. Reports suggest that about one third of all incident cases of active TB were not diagnosed properly which eventually reflect in the abovementioned problems. In order to achieve maximum possible elimination of TB, WHO stressed the importance of 'systematic screening' of high-risk populations such as household contacts (HHC) of index cases to detect TB early and thereby accomplish decreased risk of (a) transmission (b) adverse social and economic consequences, and (c) adverse treatment outcomes.

Screening HHC of pulmonary TB patients for active TB is all the more important due to the following reasons,

(i) Among household contacts the prevalence of TB may reach ≥2.5%

(ii) Helpful in taking preventive measures such as isoniazid prophylactic therapy (IPT)

(iii) Essential to follow up the (i) contacts of multi-drug-resistant or extensively drug-resistant TB or (ii) people with HIV, diabetes whose risk for rapid progression to active TB is very high.

Being a promising approach contact investigation is part of the *Stop TB strategy* for many years and it is essential to have active screening strategies among HHC which could effectively decrease the costs of implementing intensified case finding we have proposed a pilot study to determine the diagnostic accuracy (sensitivity and specificity) and the predictive value (negative and positive predictive value) of point-of-care (POC) estimation of C - reactive protein and Vitamin D for active TB in household contacts of PTB patients. All approvals are obtained the study will be initiated

DEPARTMENT OF HIV

STUDIES COMPLETED:

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

Principal Investigator	:	Dr. Padmapriyadarsini; Dr. Hanna LE
Source of funding	:	DBT/NIH
Study Period	:	2013-2020

Background

The C-TRIUMPh study is a clinical study aimed at investigating host and microbial factors associated with TB treatment outcomes in Indian adults and children (active TB cohort), host and microbial factors associated with progression from latent infection to active TB disease in adults and children (Household Contact cohort), and host and microbial factors associated with TB transmission (Household Contact and Control Cohorts). The study also involves storage of different types of biological samples for experimental research.

Progress update

Follow up was completed for the remaining 73 Cohort A participants and 4 Cohort B participants. Details of samples stored from these individuals is provided in Table 18.

Table 18: Samples stored for the study during the period April 2019 to Mar 2020

Cohort	PBMC	Plasma	QGIT Supernatant	Whole blood in PAXgene tube	Whole blood for DNA
A	146	584	0	146	0
B	0	0	48	0	0
Total	146	584	48	146	0

Conclusion: The main study has been completed. Sub-studies using stored samples are being initiated.

HIV - 14: Study on HIV-1 Drug resistance patterns in ART experienced children with virological failure

Principal Investigator	:	Dr. Padmapriyadarsini; Dr. Hanna LE; Mr. K. Ramesh; Mr. Manohar Nesakumar
Source of funding	:	Intramural
Study Period	:	2017-2019

Background

There is very limited data on human immunodeficiency virus drug resistance (HIVDR) in treatment experienced paediatric populations in India. This study aimed to assess the prevalence and patterns among this population.

Aim/Objectives

To examine the type and frequency of occurrence of HIV drug resistance mutations seen in children experiencing virological failure at the end of 12 months of ART.

Methods

A cross-sectional retrospective analysis was undertaken in paired baseline and month 12 samples of children newly initiated on ART. Population-based sanger sequencing (PBSS) was attempted on samples which had viral load >1000 copies/ml at month 12 and their corresponding baseline samples. The obtained sequences of the protease and reverse transcriptase genes were assembled and edited to create a consensus sequence using Seqscape (Applied biosystems, USA) software version 3.0. Drug resistance mutations in the sequences were identified using the IAS 2017 HIV Mutation list and Stanford University HIV drug resistance database.

Results

Genotypic HIV-1 drug resistance results were obtained for 77 paired samples. At baseline, 6 children harbored NNRTI drug resistance mutations, two had NRTI drug resistance. One participant had dual class resistance mutations. At the time of virological failure, we observed a high burden of drug resistance; 63/77 (82%) children had at least one drug resistance mutation. Resistance to both NRTIs and NNRTIs was most common, with 49 (64%) participants harboring resistance to both drug classes. Two participants harbored resistance to Protease Inhibitors, NRTIs and NNRTIs, 14 (18%) participants harbored resistance to NNRTIs alone. No resistance mutations were observed in 14 (18%) participants, indicating that factors other than drug resistance contributed to virologic treatment failure in this pediatric population.

Participants harbouring HIV-1 drug resistance exhibited a broad range of mutations. M184V was the most commonly observed NRTI resistance mutation, found in 94% (46/49) of participants with NRTI resistance. The next most common mutations were associated with resistance to ZDV, 3TC, FTC, ABC and TDF, such as D67N, K65R, L74V/I, K219Q/E mutations, which were observed in 10%, 10%, 8% and 8% of NRTI resistant cases. Y181C, which confers resistance to all NNRTIs, and K103N which confers high-level resistance to both EFV and NVP, were observed in 45% (21/47) and 66% (31/47) of NNRTI-resistant participants, respectively. G190A and K101E, both of which confer resistance to EFV and NVP, were observed in 32% (15/47) and 8% (4/47) of NNRTI-resistant individuals, respectively. The two participants with Protease Inhibitor resistance mutations harbored the V32I and I54V mutations.

Conclusion

Our study reveals a substantial burden of HIV-1 drug resistance among the children experiencing virological failure to the first line regimen, which will greatly affect the treatment outcome. Implementation of timely drug resistance testing and access to newer antiretrovirals are needed to improve outcomes.

HIVL-17: Evaluation of mucosal immune responses to HIV infection in sero-discordant couples

Principal Investigator	:	Dr. Luke Elizabeth Hanna
Source of funding	:	DHR/HRD
Study Period	:	2017-2020

Background

Effective vaccine design relies on accurate knowledge of immune protection associated with a pathogen, so as to enable the induction of the most relevant and effective type of immune responses against it. Our understanding on the nature of the protective immune responses against HIV infection is still incomplete. This understanding will open up avenues for the design of an effective vaccine that can protect against HIV infection.

Aim

The aim of this study was to characterize the innate and adaptive immune responses that constitute "protective" immunity against HIV infection in the female genital tract (FGT) of exposed seronegative (ESN) individuals, as the FGT is often the first site of exposure to the virus.

Methods

The study population comprised or 37 HIV uninfected female partners of HIV-infected men (ESN women) and 35 age-matched, healthy, HIV-unexposed, uninfected women. The mucosal specimens obtained from these individuals included cervicovaginal lavage (CVL) and cytobrush specimens. The mucosal specimens were analysed for soluble immune mediators and secretory immunoglobulins (sIg) using commercial ELISA and cytometric bead array, subsets of natural killer (NK) cells, T follicular cytotoxic cells (Tfc), T follicular helper cells (Tfh) and T regulatory cells using flow cytometry, as well as the nature and composition of the cervicovaginal microbiome using next generation sequencing.

Results

The study identified significantly elevated levels of immune cell mediators, sIgs, and increased frequency of activated NK cells, Tfc and Tfh cells in ESN females as compared to HIV unexposed uninfected women. Further, the ESN females had decreased levels of immune activation markers and a higher abundance of *L. iners* and microbial dysbiosis.

Conclusion

The data demonstrate that a combination of soluble factors, mucosal NK and T follicular cytotoxic and helper cells in the FGT and a highly diverse cervicovaginal microbiome might play an important role in protecting against HIV infection.

HIVL-18: Differences in neutrophil response during the course of anti-tuberculosis treatment - a pilot study

Principal Investigator	:	Dr. Nancy Hilda;
Source of funding	:	ICMR Post-doctoral Fellowship
Study Period	:	2017-2020

Background
The pathogenesis of tuberculosis is the product of interaction between bacterial virulence and host resistance, which are two distinct and independent variables. A well-defined explanation of how our immune system aids in the elimination of bacilli and/ or how the bacterium evades the immune system of the host is the demanding need of the hour. In a previous study we showed that infected neutrophils undergo rapid cell death and *Mycobacterium tuberculosis* (MTB) strains modulate neutrophil functions. It is well known that neutrophils not only kill the pathogen but also modulate other immune cells, thereby forming a link between the early innate and adaptive immune response during microbial challenge with MTB.

Aim
This aim of the study was to evaluate alterations in the effector function of neutrophils and the immune modulations that occur during the course of anti-TB treatment.

Methods
Blood samples were obtained from 15 TB patients and an equal number of healthy controls and used for the various immunological studies. Sample was collected from TB patients at 3 time points (pre-treatment, 2 months post treatment initiation and at the end of treatment) and from the healthy controls at one time point only. Neutrophils were isolated from whole blood using dextran sedimentation process and further purified using MACS. The purified cells were cultured along with TLR ligands like LPS, FSL-1 and PAM3CSK4 overnight at 37^0C. The culture supernatants were analysed for a panel of cytokines using commercially available ELISA kits and the cells were used to examine the surface expression of TLR2, TLR4, CD64, CD16, PD1 and CD11b were analysed using flow cytometry.

Results
At the time of TB diagnosis, levels of IL-1beta, IL-8, TNF-alpha, MCP-1 and MIP-1 alpha were significantly elevated in the TB patients. Levels of IL- 1 beta , IL-8 and MIP-1 alpha declined following 2 months of anti-TB treatment. There was a further decline in the level of these cytokines at the end of treatment (sixth month). On the other hand, levels of TNF-alpha and MCP-1 were not significantly different at the different time points, suggesting that different cytokines follow different strategies for tackling infection. The pattern recognition receptors, TLR2 and TLR4, also showed increased expression in TB patients, indicating their role in infection. TLR2 expression was found to vary at different time points during the course of infection and treatment, providing evidence for immune modulation according to the requirement.

Conclusion
Elevated levels of cytokines and increased expression of TLRs in TB patients as compared to healthy volunteers provides evidence of the role played by the innate immune cells in counteracting TB infection. Interestingly, the observation of higher levels of cytokines and increased TLR expression in TB patients even at the end of treatment time point indicates that normalization of the perturbed immune responses take much longer than microbiological sterilization and clinical cure.

HIVL-19: RePORT (Regional Prospective observational Research in TB) India Common Protocol

Principal Investigator	:	Dr. Padmapriyadarsini
Co-Investigator	:	Dr. Luke Elizabeth Hanna
Source of funding	:	DBT/NIH
Study Period	:	2017-2020

Background

The RePORT India Consortium consists of five distinct TB CRUs spread throughout India working in collaboration to address a wide array of scientific themes related to tuberculosis. All the members of the Consortium came forward to implement a Common Protocol for collection of clinical, sociobehavioural and demographic data besides employing common protocols and SOPs for processing and storage of biological samples from the study subjects for future research. The common protocol is supported by a Central Biorepository located at NIRT and a central data management center (DMC) located at New Delhi.

Progress update

Provided below is a list of samples processed and stored for the Common Protocol at NIRT and samples collected at the linked CRUs and stored in the Central Biorepository at NIRT during the reporting period (Tables 19 & 20).

Table 19: Common Protocol samples collected during the period April 2019 to March 2020

Cohort	PBMC	PLASMA	QGIT (PLASMA)	PAXGENE (WHOLE BLOOD)	DNA (WHOLE BLOOD)
A	72	280	0	35	105
B	57	200	300	25	75
Total	129	480	300	60	180

Table 20: RePORT INDIA Common Protocol samples stored in the Central Biorepository during the period April 2019 to March 2020

SITE NAME/ SITE ID	PBMC	PLASMA	QGIT (PLASMA)	PAXGENE (WHOLE BLOOD)	DNA (WHOLE BLOOD)	URINE	SPUTUM	SALIVA	MTB ISOLATES
MVDRC(103)	30	262	0	37	111	144	52	60	80
JIPMER(102)	167	560	243	72	319	321	0	80	76
BJMC(106)	126	402	3403	144	485	889	1350	324	580
BMMRC(107)	141	486	2400	36	152	307	0	161	0
NIRT (105)	129	480	300	60	180	0	42	180	51
CMC(101)	24	80	227	0	0	80	0	80	148

Significance

NIRT has the pride of establishing and hosting an Indian owned and managed TB biorepository containing well-characterised specimens that can be usefully employed for the purpose of developing reliable TB biomarkers and diagnostics that will provide added impetus to TB prevention efforts in terms of diagnostics and vaccine development and treatment.

HIVL-23: Characterization of the immunological profile of HIV-2 Infected individuals

Principal Investigator	:	Dr. Hanna LE
Source of funding	:	Intramural
Study Period	:	2017-2019

Background

It is well known that the clinical phenotype of HIV-2 infection is distinct from that of HIV-1 infection. HIV-2 infection is majorly characterized by delayed progression to symptomatic disease and AIDS, even in the absence of ART. The distinctive nature of HIV-2 infection and disease has been particularly correlated with the protective nature of the immune response generated in response to HIV-2 infection. While intense research has been focused on understanding the nature of the immune cells involved in adaptive as well as innate immunity in HIV-1 infection, immunological studies on HIV-2 infection have been very limited.

Aim

The present analysis was carried out to understand the nature of the immune response elicited in response to HIV-2 infection, through phenotypic evaluation of T cell, B cell and NK cell subsets and compare and contrast it with the nature of the immune response seen in HIV-1 infection.

Methods

The study included 20 HIV-2 infected ART-naive individuals, 10 HIV-1 infected therapy-naïve individuals and 10 HIV negative healthy controls. The HIV-1 and HIV-2 infected individuals were matched for CD4 count. Blood samples were collected from all participants and peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation. Viable recovery of the cells was assessed using trypan blue dye exclusion method. T cell, B cell and NK cell populations were analyzed using multicolour flowcytometry. In addition, T follicular helper cell (Tfh) and regulatory T cell (Treg) subsets were also analysed. FACS data was analyzed using FlowJo and GraphPad Prism software (version6.0). Statistical significance between two groups was determined using the Mann-Whitney test (non-parametric).

Results

We found a higher frequency of memory follicular-homing CD4+ T cells as well as memory follicular T cells in HIV-infected persons as compared to healthy volunteers. Between the two HIV groups, those with HIV-1 infection had higher number of these cells as compared to those with HIV-2 infection. It was interesting to note that HIV-1 infected individuals also had a significantly higher proportion of central memory Tfh cells as compared to HIV-2 infected persons, while HIV-2 infected individuals had a higher proportion of effector memory Tfh cells as compared to those with HIV-1 infection. Similarly, we observed a significantly larger population of Tregs in HIV-2 infected individuals as compared to the uninfected and HIV-1 infected persons ($p<0.05$). While the proportion of resting Tregs was found to be similar in all the 3 groups, we found a significant increase in the proportion of activated Tregs in HIV-infected individuals as compared to the uninfected controls. Among the two HIV groups, HIV-1 infected individuals had significantly more activated Tregs than those with HIV-2 infection

(p<0.0001. We further analyzed the relative proportion of NK cell subsets and found significantly higher proportion of $CD3^{neg}CD56^{dim}CD16^{pos}$ cells in HIV-1 infected individuals as compared to HIV-2 infected persons (p<0.0001), in whom the frequency of these cells was similar to that seen in healthy controls. On the other hand, CD3neg CD56neg CD16pos cells were significantly expanded in those with HIV-2 infection and not in those with HIV-1 infection.

Conclusion

We conclude that both HIV-1 and HIV-2 infections induce phenotypic perturbations in immune cells, but to different extents. The immunological profile in HIV-2 infection is associated with better immune control than in HIV-1 infected individuals, thus proving that HIV-1 and HIV-2 antigens provoke different immune responses in the host, and that the response to HIV-2 antigens is more protective in nature.

HIVL-24: Inflammation and aging in HIV-infected individuals on long-term antiretroviral treatment

Principal Investigator	:	Dr. Luke Elizabeth Hanna
Source of funding	:	Intramural
Study Period	:	2016 – 2020

Background

Although ART prevents AIDS-related complications by suppressing HIV replication and preventing opportunistic infections on the one hand, on the other hand it contributes to non-AIDS defining co-morbidities in people aging with HIV. Patients on prolonged treatment with highly active antiretroviral therapy (HAART) frequently experience long-term side effects of the disease and/or treatment that mimic the natural aging processes. They suffer from a host of HIV-associated, non-AIDS conditions like cardiovascular disease, a number of infectious and non-infectious cancers, osteoporosis, liver disease, renal disease and neurocognitive decline, which are all associated with advancing age and chronic inflammation. Earlier studies have reported the presence of metabolic abnormalities in untreated as well as treated individuals. Factors like host immune response, opportunistic infections, ART drugs, and HIV itself are believed to contribute to this metabolic derangement. Till date there is no comprehensive, unbiased study on the plasma metabolic profile of people living with HIV (PLHIV) from India, a country that employs a standardized public health approach for treating HIV infected persons,

Aim

In a previous study we demonstrated increased risk of immune activation and inflammaging in HIV infected individuals in spite of successful antiretroviral therapy (ART). In this part of the study we analysed the metabolomic changes occurring in person infected with HIV and the associated risk of accelerated aging.

Method

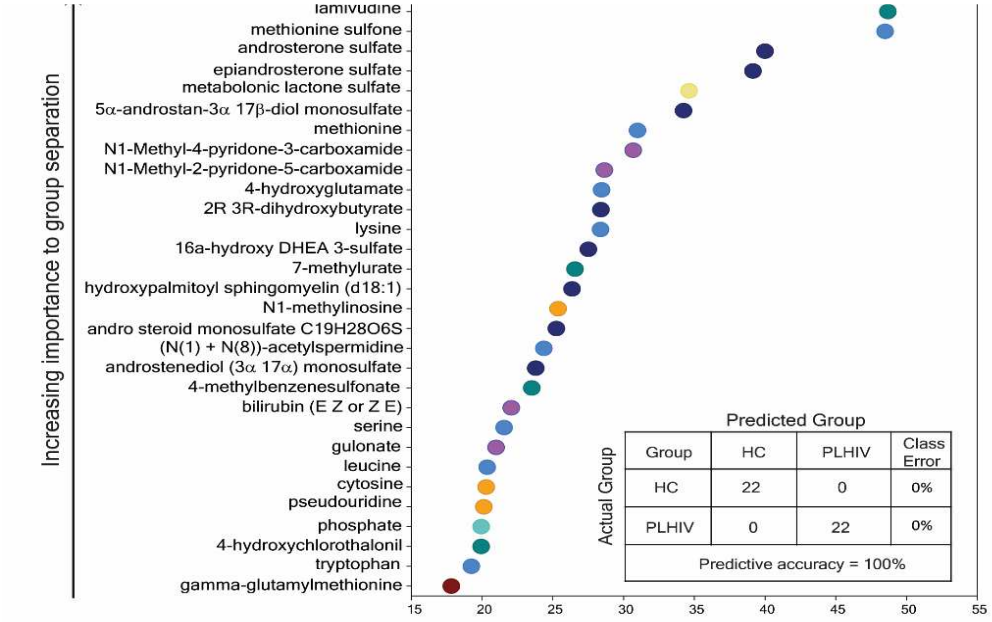
The study included 22 HIV-1 positive individuals on first line ART for more than five years (PLHIV herein) with suppressed viremia, and an equal number of age, gender and lifestyle-matched HIV-1 negative healthy individuals without any chronic illnesses (HC herein). Untargeted metabolite profiling was carried out by Metabolon Inc. (Durham, NC, USA)

using Ultra-High-Performance Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (UHPLC/MS/MS). Supervised analysis with Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA) and unsupervised analysis with Principal Component Analysis (PCA) were used to compare between the two groups.

Results

The study identified 250 significantly different metabolites [p<0.05, false discovery rate (FDR:q) <0.10] between the PLHIV and HC groups. Among them, 156 metabolites were found to be significantly lower and 94 were found to be significantly higher in PLHIV compared to HC. Random Forest (RF) analysis was performed to identify potential biomarkers among the significantly altered biochemicals, The biochemical importance plots showing the top 30 metabolites that strongly contribute to the separation between the two groups is shown in Fig 10. Among the top 30 metabolites, the most differentiating biochemicals in HC vs. PLHIV were involved in amino acid metabolism (8/30) and lipid metabolism (8/30). Two biochemicals that stood out as particularly interesting were methionine sulfone and metabolonic lactone sulfate.

<u>Fig 9:</u> Random Forest (RF) and clustering analysis



The RF analysis of named biochemicals resulted in predictive accuracies of 100% for HC vs. PLHIV. The biochemical importance plots display top 30 metabolites which most strongly contribute to the groups' separation for HC vs. PLHIV based on amino acid metabolism, lipid metabolism, energy metabolism, co-factors and vitamins, peptides and xenobiotic as indicated in different colors in the legend.

Conclusion

The study identified metabolic-abnormalities in several biological pathways involved in amino acid metabolism, energy metabolism, urea and TCA cycle, with changes in ceramides, phospholipids, and other complex lipids in PLHIV in spite of them being on suppressive cART for several years. The predicted effects of

the deranged metabolites on disease and bio-functions were related to risk of immunological, inflammatory, and neurological disorders in PLHIV. In the RF analysis, the top two metabolites identified were methionine sulfone and metabolonic lactone sulfate. Higher levels of methionine sulfone and ceramides are indicators of increased oxidative stress, a phenomenon that has been reported in earlier studies. Thus, the study reveals that even after long-term suppressive therapy, persistent chronic oxidative stress exists in PLHIV. If not controlled, oxidative stress could contribute to the induction of age-related chronic and degenerative diseases like cardiovascular disease (CVD), neurological disorders including depression and memory loss, rheumatoid arthritis, kidney disease, etc. and accelerate aging and mortality in PLHIV.

HIVL-25: Molecular characterization of full length HIV Type 2 envelope gene sequences from South India

Principal Investigator	:	Dr. Luke Elizabeth Hanna
Source of funding	:	Intramural
Study Period	:	2019-2020

Background

Exploring the characteristics of the HIV-2 envelope gene in individuals harbouring broadly neutralizing antibodies against the virus could throw light on the mechanisms of broadly neutralizing antibody production and provide useful clues for vaccine/immunogen design. In a previous study from our department, we screened plasma of 37 HIV-2 infected individuals for their neutralization potential and reported different levels of neutralization against HIV-1 and 2 viruses. One unique sample was found to exhibit potent neutralizing activity against both HIV-1 and 2 viruses whereas other samples exhibited different levels of neutralization against HIV-2 viruses alone.

Objectives

The goal of the present study was to amplify, sequence and characterize the HIV-2 envelope gene from the plasma of these participants.

Methods

Viral RNA was extracted from the plasma of 20 HIV-2 infected individuals and the full length envelope gene was PCR amplified using a nested approach. Amplification was successful in 14 samples and sequencing was performed. The envelope gene sequences were analysed using *in silico* tools.

Results

HIV subtyping and molecular phylogenetic analysis revealed that the individuals were infected with HIV-2 subtype A. Comparison of the deduced amino acid sequences with that of previously reported Indian strains showed these isolates were closely related and the overall genetic variation in V3 region between the strains was very low. Genotypic tropism prediction revealed that all the isolates were CCR5 tropic. We employed phylogenetically corrected methods to identify signature sites in the env sequences associated with the strongest sera neutralization activity and compared the patterns with that seen in the remaining Envs. Overall,

the signature analyses identified eight aminoacid signatures in env, of which seven were in V1, one was in the V3 region.

Conclusion
In this study, we retrospectively generated and analysed Env sequences of HIV-2 individuals with diverse levels of neutralization activity. These sequences revealed a number of Env signatures that coincided with the neutralization activity.

HIVL-26: Analysis of physicochemical properties and patterns in the variable loop regions (V1-V5) determining HIV-1 Coreceptor usage

Principal Investigator	:	Dr. Luke Elizabeth Hanna
Source of funding	:	Intramural
Study Period	:	2018-2020

Background
HIV-1 co receptor tropism typing (CRT) is a prerequisite for prescribing a co receptor antagonist. CRT by computational genotypic prediction offers a more feasible alternative due to greater accessibility, lower cost, and faster turnaround time. The third hypervariable region (V3 loop) in the gp120 protein has been recognised as the major determinant for co-receptor tropism of the isolates. However, the regions of the HIV-1 gp120 outside of the V3 loop have also been linked to co-receptor tropism. A reliable prediction algorithm would be desirable for two reasons. First, a sequence based method of phenotype prediction as an alternative to the more difficult experimental determination of phenotypes would be beneficial in the clinical setting. Second, the large number of existing HIV-1 sequences available in public databases could be classified accurately with an algorithm that can differentiate clearly between the X4, R5 and R5X4 genotypes, based on signatures within and outside the V3 loop of HIV-1 gp120.

Objectives
The goal of this study was to extract the sequence characteristics of all the five variable loops of the HIV-1 envelope protein and analyse them for physicochemical properties of the amino acids that constitute them, including hydrophobicity, bulkiness and polarity (referred in this study as position-dependent features (PDFs)) and other sequence-based position-independent features (PIDFs) such as net charge, length of the variable loop region and the number of potential N-linked glycosylation sites (PNGS).

Methods
A total of 3902 full length HIV-1 gp120 nucleotide sequences belonging to the three tropic groups of viruses, namely R5 (n=3308), X4 (n=215) and R5X4 (n=379), were obtained from the Los Alamos Database. We selected one representative sequence per subject in order to avoid biasing the results by the resampling of highly related sequences. In total, our final dataset consisted of 135 R5, 117 X4 and 109 R5X4 sequences obtained from the Los Alamos HIV database and 15 experimentally phenotyped sequences from our laboratory for the analysis. PLS Enhanced Discriminant Analysis (DA) was employed to identify discriminating features between the three viral groups. Variable screening (VS), two variable screening (TVS) and two sample logo (TSL) analyses

approach were employed for the identification of PDFs and PIDFs.

Results

The study identified a set of nine PDFs (eight in V1 region and one in V2 region) and two PIDFs (both in the V1 region) that aid in the discrimination between R5X4 viruses and R5 viruses. Similarly, our study identified a set of two PDFs (one in the V2 and one in the V3 region) that associated with better discrimination between the R5X4 viruses and X4 viruses with an area under the curve score (AUC) of 79% when a combination of the two variables were employed. In addition, we also identified 6 positions in the variable regions (four in the V1 region and two in the V3 region) that were found to be significantly different between the viral groups, and can add significantly to the accuracy of prediction when used along with V1 length and V3 charge.

Conclusion

In summary, our findings results suggest that coupling genotypic algorithms for tropism determination with the set of identified PDFs and PIDFs in the V1-V3 regions could improve the accuracy of prediction and may be used in combination with other methods as a supportive tool for prediction of co-receptor usage.

<u>HIVL – 27:</u> Integrative bioinformatics analysis of microarray data for identifying the differentially expressed genes in Active tuberculosis (ATB) compared to Latent tuberculosis (LTB) infection

Principal Investigator	:	Dr. N. Sudhakar / Dr. Tripathy SP; Dr. Hanna LE
Source of funding	:	Intramural
Study Period	:	May 2019-March 2020

Background

Tuberculosis (TB) is a communicable disease, transmitted almost exclusively through aerosols carrying bacteria belonging to the *Mycobacterium tuberculosis* (Mtb) complex. TB remains a major health problem worldwide and is the leading cause of mortality from a single infectious agent. In 2017, there was an estimated 10.0 million new cases and 1.60 million deaths (WHO) due to TB. The vast majority of Mtb infections result in a clinically asymptomatic contained state, known as latent tuberculosis infection (LTBI). About one quarter of the world's population is estimated to be latently infected with Mtb.

Aim

To analyse publicly available microarray data to identify differentially expressed genes and micro RNAs (miRNAs) in individuals with active tuberculosis (ATB) versus those with Latent tuberculosis (LTB) using an integrative bioinformatics approach.

Method

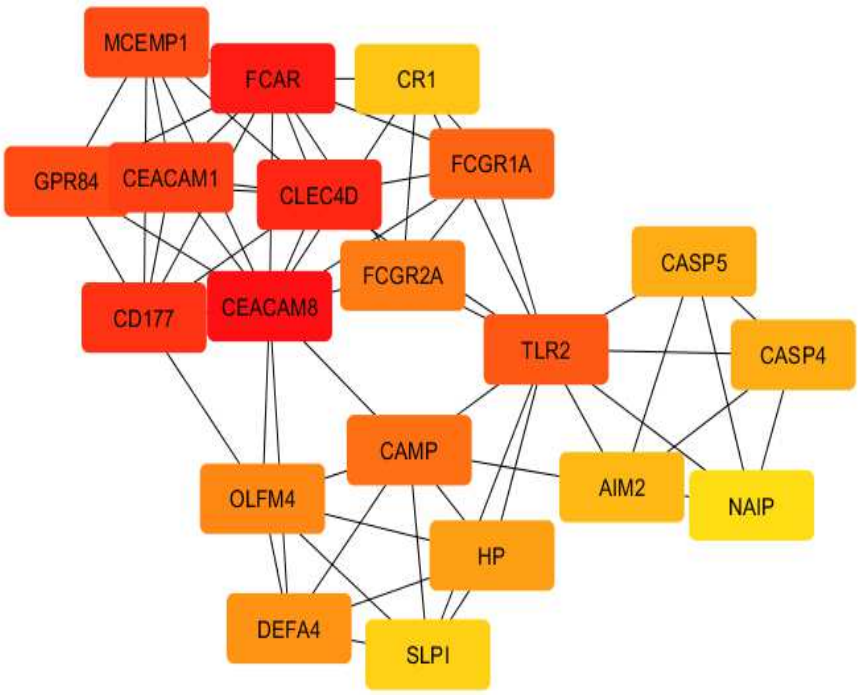
We re-analyzed the microarray dataset GSE37250 extracted from Gene Expression Omnibus (GEO) that contains raw data of the microarray profile of whole blood samples obtained from those with ATB and LTB from Malawi and South Africa. Differential gene expression in the

ATB vs LTB groups was identified using BRB Array Tool (https://brb.nci.nih.gov/BRB-ArrayTools/). Functional annotation and pathway analysis were performed using the DAVID bioinformatics tool (https://david.ncifcrf.gov/tools.jsp) and Reactome pathway analysis (https://reactome.org/). Enriched Gene Ontology (GO), KEGG pathway and Reactome pathway terms of up and downregulated genes were analysed for p values < 0.05 and enrichment scores ≥3. Protein-Protein Interaction (PPI) network was constructed using STRING database (https://string-db.org/). The PPI network was visualized in cytoscape 3.7.0 and the closely associated genes were identified using MCODE and CytoHubba plugin.

Results

The analysis identified 1117 DEGs (Differentially Expressed Genes) in the Malawi cohort (ATB = 51 samples, LTB = 35 samples) and 1486 DEGs in the South African cohort (ATB= 46 samples, LTB = 48 samples). Five hundred and thirty one genes were common among the two cohorts. Out of these, 376 genes were upregulated and 155 genes were downregulated. Gene Ontology (GO) terms of upregulated genes included innate immune response, antibacterial humoral response, defense response to bacteria, platelet degranulation and inflammatory response, and they were enriched with p value <0.05. The important KEGG pathway terms such as tuberculosis, complement and coagulation cascades, and phagosome were enriched significantly. Significantly overrepresented reactome pathways were related to neutrophil degranulation, immune system and interferon signalling. The GO, KEGG pathway and Reactome pathway results of downregulated genes were mainly related to wnt signalling, inflammatory response, cytokine signalling and B-cell receptor signalling. The resulted PPI network was significantly enriched with p value <1.0E-16, where 76 nodes connected with 151 edges. The following 19 nodes FCAR, MCEMP1, CEACAM1, CEACAM8, CLEC4D, GPR84, TLR2, CASP4, CAMP, HP, NAIP, AIM2, SLPI, CR1, DEFA4, CASP5, FCGR2A, FCGR1A and OLFM4 were identified as densely connected regions and also as important hub proteins **(Fig. 10)**.

<u>Fig.10:</u> Protein-protein interaction network displaying the interaction of upregulated genes from ATB vs LTB



Cytohubba plugin (MCC Method) analysis explored the most important hub nodes.

Conclusion

The study identified differentially expressed host response genes associated with ATB using bioinformatics analysis. The DEG need to be validated in ATB and LTB samples for potential diagnosis of TB and differentiation of ATB from LTBI and other pulmonary diseases.

STUDIES IN PROGRESS:

HIVL-20: NIRT PBMC Cryopreservation EQA Program

Principal Investigator	:	Dr. Hanna LE
Source of funding	:	CRDF Global
Study Period	:	2016-2020

Background

The use of cryopreserved peripheral blood mononuclear cells (PBMC) for immunological assays has increased greatly in the recent years. But the utility of cryopreserved cells depend largely on their viable recovery. Hence it is critical to ensure that good quality PBMC are stored in the Biorepository for future research. However, until NIRT embarked on this endeavour, there were no external quality assurance programs or providers for cryopreservation of PBMC in India. With the support of the RePORT India Consortium, the NIRT PBMC Cryopreservation program was established in 2016.

Progress update

Since its inception in 2016, the NIRT PBMC Cryopreservation PT program has been successfully conducting 4 quarterly surveys each year. During the reporting year, 4 quarterly surveys were rolled out and completed successfully. The NIRT PBMC EQA team assesses the viability and recovery of the PBMC processed at each of the participating labs and provide a score based on the results obtained. The team also helps the sites with trouble shooting as well as refresher trainings as and when required. Table 21 provides the details of the number of samples received and scored for each of the participating labs under this program during the reporting period

Table 21: PBMC EQAS Performed during the year April 2019 to Mar 2020

SITE NAME	SITE ID	NO. OF PBMC SAMPLES RECEIVED	NO. OF PBMC SAMPLES THAWED FOR SCORING
CMC(VELLORE)	101	16	9
JIPMER(PONDICHERRY)	102	16	11
MVDRC(CHENNAI)	103	16	9
NIRT(CHENNAI)	105	16	9
BJGMC(PUNE)	106	10	10
BMMRC(HYDERABAD)	107	24	11
	TOTAL	98	**59**

HIVL-28: Development and validation of a diagnostic kit for early detection of Human Papilloma Virus (HPV) infection in cervical cancer

Principal Investigator	:	Dr. N. Sudhakar
Source of funding	:	DST - Scheme for Young Scientists and Technologists (SYST) under SEED funding
Study Period	:	2019-2021

Background

Cervical cancer is caused by Human Papilloma Virus (HPV) infection. There are more than 100 HPV genotypes present and they are classified into high-risk and low-risk types. Currently available methods for early detection of HPV involve conventional PCR and Real-time PCR based techniques, which require sophisticated equipment and laboratory expertise, making it difficult to implement in low resource settings. This project necessitates the development of a detection kit for HPV testing using Loop-Mediated Isothermal Amplification (LAMP) assay that uses isothermal temperature for amplification of target gene for use in low resource settings. The LAMP assay is a simple, rapid, specific and cost-effective nucleic acid amplification method and is characterized by the use of 6 different primers specifically designed to recognize 8 distinct regions on the target gene (L2 region of HPV). Hence, specificity of the technique is extremely high.

Specific aims

i) To develop and validate an in house LAMP assay in HPV positive cervical cancer cell lines (SiHa, CasKi) and a HPV negative cervical cancer cell line (C33A).

ii) To collect cervical cancer tissue samples (n =100) from patients with cervical intraepithelial neoplasia (CIN) - CINI, CINII and CINIII and use them to compare the sensitivity of detection of HPV infection in these samples by the LAMP technique and PCR method.

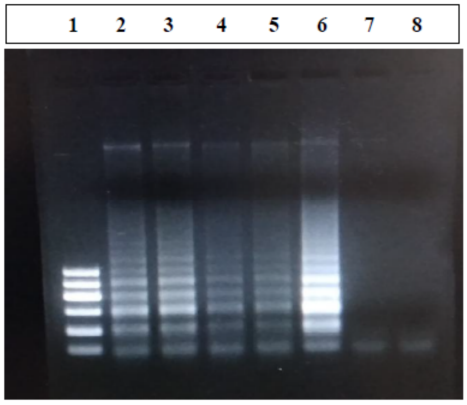
Methods

LAMP primers targeting the L1 region of the HPV genome were designed using Primer Explorer software https://primerexplorer.jp/e/. The designed primers were validated in genomic DNA isolated from HPV positive cervical cancer cell lines SiHa and Caski and a HPV negative cervical cancer cell line C33A. Tissue samples were collected from cervical cancer patients at the Institute of Obstetrics & Gynecology (IOG), Egmore, after obtaining ethics approval. We have collected cancer tissue biopsies and vaginal swabs from 52 women with cervical cancer. Genomic DNA was isolated from the tissue samples using the QIAamp DNA mini kit and the DNA was quantitated using NanoDrop spectrophotometer. HPV testing was done by PCR using primers specific for HPV-L1 region (GP5+ GP6+ primers) as well as the LAMP assay on all the 40 tissue samples.

Results

The results of the ongoing study and a representative picture of amplified products are shown in Fig. 11.

Fig. 11: LAMP Assay based HPV testing in cervical cancer samples.



LAMP Assay results showing amplification of HPV in cervical cancer samples. Lane 1 is 100bp marker, Lane 2 – Lane 5 representing cervical cancer samples showing amplification in LAMP assay, Lane 6 - positive control (SiHa cell line DNA), Lane 7 & Lane 8 - negative controls (Lane7 - C33A cell line DNA (HPV negative), Lane 8 - No template control.

Current status
The results of HPV testing using LAMP assay will be compared with standard PCR based method. The project is ongoing.

HIVL-29: Impact of malnutrition on immune responses to tuberculosis in Indian Children

Principal Investigator	:	Dr. Aishwarya Venkataraman / Dr. Syed Hissar
Source of funding	:	DBT
Study Period	:	2018-2022

Background
Globally, malnutrition is a one of the most common causes of death in children less than 5 years of age. Acute malnutrition affects 52 million children globally, of whom nearly 2/3rd (33 million) have moderate acute malnutrition (MAM). Malnourished children have increased susceptibility to infections like Tuberculosis (TB), probably due immunodeficiency caused by undernutrition but the immune dysfunction of malnutrition is poorly characterised. Hence there is an urgent need to better understand the immunology of malnutrition.

Tuberculosis and malnutrition are overlapping and interacting public health problems in India. The prevalence of MAM among children under 5 years of age in India is 20%, and India has the highest TB burden in world, accounting for about 25% of global cases annually. Although the WHO states that malnutrition is a contributing factor for childhood tuberculosis, there are limited studies to explain the mechanisms underlying this association. Therefore, collaborative studies are urgently needed, specifically on how nutritional status affects the risk and progression of tuberculosis and whether nutritional

intervention improves clinical outcomes or prevents TB disease.

Aims & Objectives

The goal of this study is to assess immune responses to *Mycobacterium tuberculosis* (Mtb) in children with MAM and compare them with that seen in well-nourished children and to evaluate the impact of a nutrition intervention on these immune responses.

The specific aims of the study are as follows:

1. To characterize innate and T-cell immune responses to Mtb in moderately malnourished and well-nourished children with TB disease.
2. To characterize innate and T-cell immune responses to Mtb in moderately malnourished and well-nourished children with latent TB infection (LTBI).
3. To assess the impact of a nutrition intervention on immune responses to Mtb in children with malnutrition.

Methods

Four groups of HIV-uninfected children will be recruited for the study, following caregivers' informed consent and assent of children >7 years of age. Innate and adaptive immune responses to Mtb will be evaluated in the 4 study groups: 1) MAM children with TB disease; 2) Well-nourished children with TB disease; 3) MAM children with LTBI; 4) Well-nourished children with LTBI. We have chosen a range of assays to compare innate, adaptive and functional immune responses to TB between groups. Whether nutritional supplementation improves immune function in MAM children remains uncertain. We will follow children in all four groups during 6 months of TB therapy / chemoprophylaxis, with 12 weeks of concomitant ready-to-use supplementary food given to MAM children, to evaluate longitudinal changes in innate and adaptive immune function, monocyte, lymphocyte ratio and mycobacterial growth inhibition.

Current status

Recruitment of children was due to start from 1st May 2020; however due to the current COVID pandemic and lockdown the study was temporarily put on hold. Recruitment to the study will be initiated from September 2020.

HIVL-30: National HIV Cohort Program – Cohorts of HIV Resistance and Progression in Indian Children and Adults (CoHRPICA)

Principal Investigator	:	Dr. Hanna LE/ Dr. Padmapriyadarsini; Dr. Bella Devaleenal
Source of funding	:	DBT/ICMR/IAVI
Study Period	:	2018-2022

Background

India stands at the crossroads in the response to its HIV-epidemic. To continue the progress and accelerate the pace towards elimination of the epidemic, India has set-out ambitious goals/targets and endorsed various global policies. However, achieving the 'last mile' in our response to the HIV epidemic in India will require population-based studies for informing better disease management strategies. These would provide context-specific evidence towards understanding the

disease and its outcomes, and enable design, development and effective implementation of interventions/products/solutions besides providing answers to various epidemiological, socio-behavioral, clinical and basic science questions related to HIV risks, transmission, pathogenesis, disease progression and resistance (including immunological, virological and genetic characteristics).

Aims

- To establish well-characterized cohorts of HIV-uninfected individuals at high-risk (including Exposed-seronegative) and HIV-infected individuals (including Early HIV-infection, HIV-infected adults – with and without comorbidities, and HIV-infected children).
- To establish a state-of-the-art biorepository of biological specimens collected from the above cohorts and other prospective and retrospective studies in India.
- To develop a national HIV/AIDS database (with clinical-laboratory-socio-demographic-and research data) to enable a singular digital platform for epidemiological analyses, generation of new research questions and conduct of advanced immuno-biological analyses.

Methods

This is a multicentric study that includes several ICMR as well as non-ICMR institutes in various parts of the country. The study will enrol high risk HIV-uninfected individuals from among key populations (MSM/TG, PWID and FSW - 350 in each group), as well as HIV-infected adults (250 individuals) with and without comorbidities like tuberculosis, diabetes and cardiovascular disease and HIV-infected children (100 individuals). NIRT will contribute to 350 MSMs and 100 HIV infected adults with and without comorbidities. It is hoped that the study will yield about 25-30 individuals with early HIV infection from the key populations across the various sites during the course of the study. All recruited participants will be followed up for a minimum period of 3 years and longitudinal blood samples will be collected at defined time points during the follow up period for routine investigations as well as for storage in the Central Biorepository that will be housed at ICMR-NARI for future research on correlates of protection/resistance to HIV infection, early events in establishment of HIV infection and HIV disease progression.

Current status

A total of 40 MSMs have been screened, of which 9 have been enrolled and are being followed up regularly at 3 monthly intervals. Screening and recruitment into the HIV-infected cohort has been initiated in August 2020 after obtaining NACO approval. The study is ongoing.

HIVL-31: Identification of Biomarkers that can predict progression from Latent Tuberculosis Infection to Active Tuberculosis Disease

Principal Investigator	:	Dr Luke Elizabeth Hanna/ Dr. Padmapriyadarsini
Source of funding	:	Intramural
Study Period	:	2020-2021

Background
The positive predictive value of the currently available tests for detecting latent tuberculosis infection like the tuberculin skin test (TST) and the interferon gamma release assay (IGRA) are very low and identifying latently infected individuals with the highest likelihood of progression to active disease continues to be a major challenge. The proposed study will use QuantiFERON supernatant to identify TB-specific biomarkers that can improve the diagnostic accuracy of the existing tests for detection of latent TB infection, and help in the identification of individuals with the highest short term risk of developing active TB disease. So far, there has been no exploration of biomarkers in QFT-GIT supernatants for TB disease progression.

Objectives
To identify biomarkers that can accurately predict progression from latent TB infection to active TB disease through:

- Multiplexed cytokine analysis of non-induced (Nil) and induced (TB Ag-stimulated) plasma (quantiFERON supernatant) of TB progressors and non-progressors.
- MicroRNA profiling of non-induced and induced plasma of TB progressors and non-progressors
- Proteomic profiling of the non-induced and induced plasma of TB progressors and non-progressors using a high throughput platform

Methods
Multiplexed cytokine analysis will be performed using Luminex platform. miRNA expression will be profiled using Next Generation Sequencing proteomic analysis will be performed using a high throughput protein profiling platform.

Results
The study is about to be initiated. All regulatory approvals have been obtained.

Significance
The identification of soluble prognostic biosignatures will aid in the development of simple assays with higher diagnostic accuracy to help in timely intervention and proper control of tuberculosis.

HIVL-32: Impact of HIV infection and antiretroviral therapy on premature onset of aging-associated diseases

Principal Investigator	:	Dr. A. Nusrath Unissa / Dr. Luke Elizabeth Hanna;
Source of funding	:	Intramural
Study Period	:	2019-2022

Background
To the best of our knowledge, there is scarcely any study from India that has investigated immunological and biological factors responsible for premature aging and co-morbid conditions that eventually lead to the high mortality rates in HIV individuals in spite of them being on suppressive life-long antiretroviral treatment. Recent studies carried out in our laboratory have identified signature

proteomic and metabolite profiles that are associated with several metabolic conditions in HIV-infected individuals. In the light of the above, the present study will help us to understand the impact of HIV infection and antiretroviral therapy on some of the important co-morbidities associated with aging such as cardiovascular diseases, diabetes, liver and kidney diseases and aid in the identification of important biomarkers that can predict the risk of these diseases in HIV-infected persons.

Objectives

To assess levels of the inflammatory, immuno-senescence and cellular aging markers in HIV infected individuals and to correlate them with the risk for development of important co-morbidities associated with aging such as cardiovascular diseases, diabetes, liver and kidney diseases.

Methods

This will be an experimental study comprising of two groups of individuals: 150 HIV positive individuals on long term antiretroviral therapy (≥5 years) with and without comorbidities, and 50 HIV-negative healthy controls matching for age, sex and lifestyle.

Progress update

Regulatory approvals have been obtained. Standardization of flow cytometric analysis and estimation of telomere length have been accomplished. Recruitment of participants into the study will be initiated shortly.

Significance

The study will help uncover some of the mechanisms and processes that contribute to the enhanced risk of premature onset of age-associated diseases and early mortality in HIV infected individuals. This knowledge will help in the design of methods to prevent the increased risk of morbidity and mortality in these individuals.

HIVL-33: Molecular dynamics simulation and structural analysis of HIV-1 Antisense Protein (ASP)

Principal Investigator	:	Dr. Luke Elizabeth Hanna
Source of funding	:	Intramural
Study Period	:	2019-2020

Background

The existence of an antisense protein (ASP) in the HIV-1 genome initiating from the TAR region was first demonstrated in 2006. But its structure and function are still not clearly understood, although there is some suggestion that it might contribute to the infectivity of the virus. In this study, we aim to simulate a stable structure for HIV-1 anti-sense protein and investigate the nature of its function.

Objective

Structural characterization of HIV-1 Antisense Protein using MD-simulation.

Methods

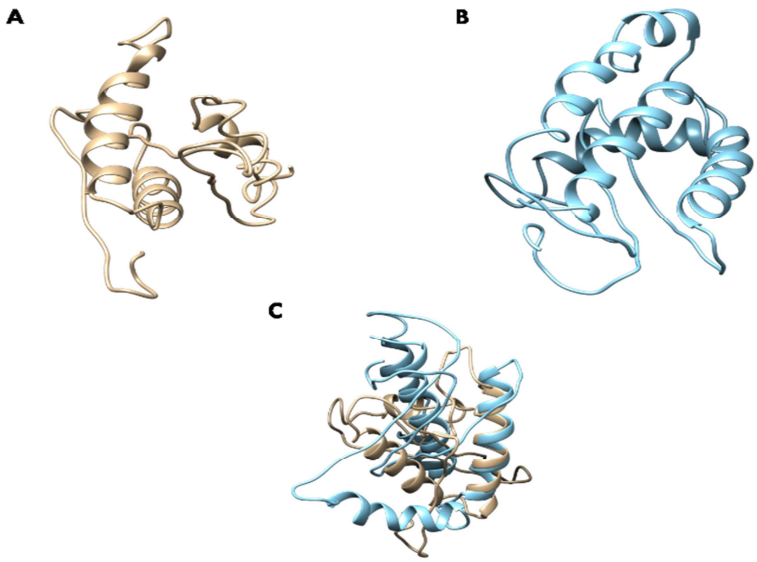
- Orf retrieval of the HIV-1 antisense protein using in-house perl program
- Building of a consensus sequence and sequence analysis
- Determination of secondary structures for the protein
- 3D structure prediction by threading

- 3D structure validation using ModRefiner and RAMPAGE
- ab initio 3D structure prediction and analysis
- MD simulation using Gromacs

Results

- ORFs for all 42 study sequences were retrieved using in-house perl program.
- A sequence comprising of 167 amino acid residues was obtained as the single representative consensus sequence for the ASP gene.
- Membrane topology of the consensus sequence was predicted using different online tools. All the servers showed that the protein is partially bound with the membrane such that a part of it is exposed to cytoplasm as well as to the extracellular domain.
- A 22 residue Env bound lipoprotein Signal Peptide was also predicted which might possibly have a role in the ASP folding.
- Tertiary structures were predicted using I-Tasser, Phyre, Quark and Robetta softwares. Figure 1 is the tertiary structure for the consensus sequence predicted by threading approach using I-TASSER and Phyre2.0 along with the superimposed structure of the models. In ab initio approach, the models derived from QUARK and ROBETTA had 2 beta strands each as shown in Fig. 12.

Fig. 12: Structure prediction by threading



HIV 1 Antisense Protein Structures predicted for asp_consensus_145aa by Phyre2.0 [color: cyan] and I-TASSER [color: gold] servers. Threaded structural output from Phyre2.0 (A) and I-TASSER (B), wherein only the best model is depicted here. (C) Superimposed structure showing unmatched region of I-TASSER and Phyre2.0.

Conclusion: The study is ongoing and the 3-D structure validation by simulation using Gromacs is in progress.

DEPARTMENT OF IMMUNOLOGY

STUDIES COMPLETED:

I – 14: CYP2R1 gene polymorphisms in pulmonary tuberculosis

Principal Investigators : Dr.M.Harishankar; Dr.B.Ramalingam
Source of Funding : Intramural, NIRT.
Study Period : 2018-20

Aim: To find out the association of Cyp2r1 rs10741657(G/A) and rs2060793(G/A) gene variants with susceptibility/protection to pulmonary tuberculosis in healthy controls (HCs) and pulmonary tuberculosis (PTB) patients and its influence on 25(OH)D levels.

Methodology: Genomic DNA was isolated from 104 HCs and 105 PTB patients by simple salting out procedure and genotyped by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) method. 25(OH)D levels estimated by ELISA method.

Table 22:

SNP	Allele	Allele frequencies		Genotype	Genotype frequencies		Odds ratio (95%CI)	p-value
		HCs	PTB		HCs n=104	PTB n=105		
rs10741657	G	0.59	0.71	GG	0.30(31)	0.51(54)	1	**0.0017**
	A	0.41	0.29	**AG**	0.58(60)	0.40(42)	**0.37(0.20-0.70)**	
				AA	0.12(13)	0.09(09)		
	Allele	**HCs**	**PTB**		**HCs n=72**	**PTB n=78**		
rs2060793	G	0.58	0.65	GG	0.31(22)	0.46(36)	**0.47(0.24-0.94)**	**0.031**
	A	0.42	0.35	**GA**	0.54(39)	0.39(30)		
				AA	0.15(11)	0.15(12)		

Results and salient findings

- In rs10741657, under dominant model (GG vs AG+AA), "AG" and "AA" genotypes as well as in rs2060793 under over dominant model (GA vs GG+AA), "GA" genotype significantly associated with protection to pulmonary tuberculosis.
- Based on sex, rs10741657 "AG" is significantly associated with protection and "GG" significantly associated with susceptibility to TB in males.
- A sufficient vitamin D level was found with rs10741657 "AA" and "AG" genotypes and "GG" genotype associated with 81.8% of vitamin D deficiency in PTB individuals.

Concluding remarks

rs10741657 "AG" and "AA" genotypes are associated with TB protection and with higher 25(OH)D levels. rs10741657 "GG" genotype individuals may be recommended for higher vitamin D supplementation for better outcome from the disease. Further studies with large sample size are needed to confirm this study finding

STUDIES IN PROGRESS

I-7: Early bactericidal activity of anti-TB drugs

Principal Investigator	:	Dr. K. R. Uma Devi
Source of funding	:	CRDF (CDC)
Study Period	:	2017-2019

Background and Rationale: Several MDR-TB treatment patients have DST results showing resistance to one drug within a class of drugs but susceptibility to other drugs in the same class. Experts agree there are no solid clinical data for evidence-based treatment decisions in these situations.

General approach: We propose a novel approach to developing clinically relevant evidence in a short time period using a variation on the method of EBA studies. In the novel approach, patients with RIF or INH resistance according to the rapid molecular test results, while completing the pre-treatment evaluation, phenotypic DST for the above drugs will be set up right away using the direct method in the MGIT 960 automated system. Hence results would be available within 1-2 weeks. Time is critical at this point in the process because CFU/ml will start decreasing as soon as treatment starts. The study subject would be treated with one drug for 6 days, the drug to which the patient's isolate was susceptible *in vitro*. The drug's effect in vivo will be measured by serial quantitative cultures. After the 6 days are over, the patient would be treated according to national guidelines. DST will be repeated on day 6 and 2 months later to ensure there was no acquired resistance.

Primary objective:
(i) To determine the bactericidal activity of RBT in patients whose baseline DST results demonstrate susceptibility to RBT and resistance to RIF
(ii) To determine the bactericidal activity of high-dose INH in patients whose baseline DST results demonstrate susceptibility to high concentrations of INH and resistance to low concentrations of INH

Sample size required:
15 - 20 patients for each of the five drug resistance pattern.

Methods: Potential subjects are screened with an approved rapid, molecular test to confirm the species as *M. tuberculosis* and detect mutations associated with RMP resistance and possibly INH resistance.
Among those found to have RMP or INH resistance, DST is carried out in liquid media by the direct method, which yields results in 7-14 days. Drugs to be tested include at least RMP, rifabutin, and 3 concentrations of INH. Quantitative bacteriology is carried out on days 0, 2, and 4, using a 16-hour overnight sputum specimen. In addition, rapid direct method DST using MGIT 960 was performed for initial screening. Standard phenotypic DST using the indirect method for the drug used during the period of monotherapy is repeated on specimens collected in the morning after the last day of monotherapy and again 2 months later for all patients who remain culture positive at those points in time.

Table 23: Status update of the participants recruited in the study

	High Dose INH arm	RBT arm
	MGIT results of INH-R@0.1 mcg & INH-S@0.4 or 2.0 mcg	MGIT results of RIF-R & RBT-S
No. of patients with DST results of interest	196	5
No. of patients who met baseline clinical eligibility	17	1
No. of patient consented to study	17	1
No. of patients started on monotherapy	17	1
No. of patients for whom 5 pooled sputum collections have been completed	17	1
Completed monotherapy	17	1
Completed 1 & 2 month follow-up DST	17	1

I-8: Whole Genome Sequencing and Transcriptome analysis of *Mycobacterium tuberculosis* clinical isolates from Bovine and Human Origin

Principal Investigator : Dr. Ahmed Kabir Refaya
Mentor : Dr. P. Kannan
Source of funding : Extramural – DST- SERB (N-PDF)
Study Period : 2017 – 2020

Background:

Bovine tuberculosis (TB) is one of the important areas of concern because of its serious impact it causes on economic losses and public health. Reverse zoonosis due to *M. tuberculosis* in bovine is increasingly being reported around the world. This situation warrants an urgent need of understanding the reason behind *M. tuberculosis* transmission from human to cattle. RNA sequencing (RNA-Seq) is a recently developed transcriptome profiling technique that revolutionizes the study of the transcriptome. Peripheral blood acts as a good study model and represents a reservoir of immune cells trafficking to and from the sites of active disease and lymphoid organs. In this study, we propose to characterize the host transcriptome response generated by human and cattle PBMCs upon infection.

Objectives:

- To assess the host transcriptome response generated by cattle and human *M. tuberculosis* clinical isolates to peripheral blood mononuclear cells (PBMCs) of cattle and human respectively.
- To validate the top 10 differentially expressed genes using quantitative real-time PCR (qPCR) in PBMCs

Methodology:

- **PBMC isolation and infection:**

PBMC was isolated from the blood collected from healthy volunteers and healthy cattle (n = 3 each) by density

gradient centrifugation method using Ficoll Histopaque. Approximately, 1 × 106 cells per ml were seeded into two 24- well plates (for each species) for culture in 5% CO2 incubator at 37 °C. PBMC were infected with *M. tuberculosis* strains each from bovine and human origin along with the standard laboratory strain (H37Rv)these strains at a multiplicity of infection of 1 for 4 hours in 37C followed by treatment with 6ug/ml of streptomycin to kill all extracellular bacilli. After 24-hours post-infection, the PBMCs were collected for RNA extraction.

- **RNA extraction , mRNA purification, Library Preparation and RNA sequencing:**

Infected and uninfected control PBMCs were lysed using RLT buffer from RNeasy Minikit (Qiagen) and RNA extracted as per the manufacturer's protocol quantified by NanoDrop™ 1000 spectrophotometer. mRNA was isolated using magnetic NEBNext Oligo beads and purified using magnetic mRNA isolation kit (NEB) and was further transcribed to cDNA using NEB NEBNext® Ultra RNA Library Prep Kit for Illumina® (E7530 and these libraries were sequenced on Illumina HiSeq 2000 platform according to the manufacturer's instructions.

- **Data analysis:**

Sequence reads were trimmed by removing adapter sequences using fastp program (v0.19.4) and the high quality reads were mapped to reference genome of homo Sapiens (GRCh37.p13) using HISAT 2 with a maximum of 2 mismatches. Prior to mapping of the genes the filtered reads were aligned to *M. tuberculosis* H37Rv chromosome (accession No.NC_000962.3) to check for any contamination. Subread software was used to analyze the gene expression levels using the features count mode. Differentially expressed genes (DEGs) in PBMCs infected with different bacterial strains were identified using DESeq2. Enrichment of Gene Ontology (GO) terms among the differentially expressed genes was analyzed using topGO, an R-bioconductor package.

Results:

Summary statistics of RNA seq data:

Each RNA-seq library generated a mean of 22 million reads. Deconvolutions and filtering of sequence reads to remove adapter-dimer sequences yielded a mean of 21.8 million per individual RNA-seq library. 19.7 million reads (92.35%) aligned well with *H.sapiens* reference genome (GRCh37.p13) and a mean of 0.8 million reads (3.6%) did not map to any genome location. None of the reads aligned to the *M.tuberculosis* genome ensuring no contaminations.

Gene expression and Differential gene expression among different groups

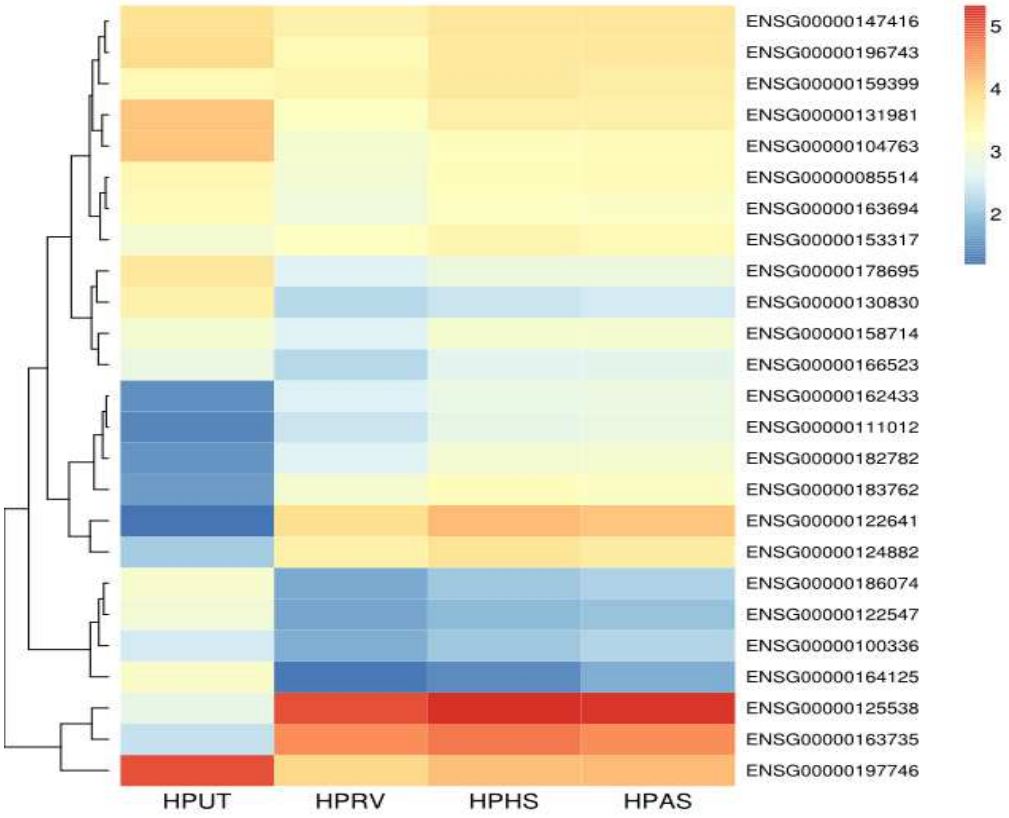
Gene expression level is measured by transcript abundance by counting the reads that map to genes or exons. A total of 33300 genes were expressed in all groups among which 23216 genes were common in all. Among the 23216 genes, a total of 3758 were differentially expressed significantly ($p<0.05$) in all three groups compared with uninfected control. 1980 genes were down-regulated and 1778 genes were up-regulated. 980 genes were commonly up-regulated in all three groups. 1093 genes were commonly down-regulated in all three groups. Heat map was generated for top 25 genes that were selected based on high significance ($p<0.001$) and those

which expressed fivefold higher or lower in all three treated groups compared with untreated (Fig. 13). From these top 25 genes top 10 genes (5 each from upregulated and down regulated genes) were selected for validation purpose in healthy volunteers and TB patients (Table 24).

Table 24: List of genes selected for validation in Healthy volunteers and TB patients

Gene ID	Regulation	Gene Name	Description
ENSG00000165457	Down	FOLR2	Folate receptor beta
ENSG00000124491	Down	F13A1	Coagulation factor XIII A chain
ENSG00000085265	Down	FCN1	Ficolin 1
ENSG00000180767	Down	CHST13	Carbohydrate sulfotransferase 13
ENSG00000011201	Down	ANOS1	Anosmin 1
ENSG00000108342	UP	CSF3	Colony stimulating factor 3
ENSG00000196611	UP	MMP1	Matrix metallopeptidase 1
ENSG00000166396	UP	SERPINB7	Serpin family B member 7
ENSG00000172551	UP	MUCL1	Mucin like 1
ENSG00000169908	UP	TM4SF1	Transmembrane 4 L six family member 1

Fig. 13: Heat map of top 25 genes



The study is ongoing.

I-9: Molecular Analysis of Monocyte Subsets from Humans Infected with *Mycobacterium tuberculosis*

Principal Investigator	:	Dr. Ramalingam B.
Source of Funding	:	DBT Ramalingaswami Fellowship
Study Period	:	2015-2020

Background: The molecular mechanisms for mononuclear phagocyte mediated protective immunity against *Mycobacterium tuberculosis* infection have not yet been completely deciphered. Reports with modulated frequencies in the subsets of mononuclear cells influenced us to study their phenotypic differences and to elucidate their functional role against tuberculosis infection.

Study Objectives

1. To phenotypically characterize and analyze the different monocyte subsets from whole blood of the tuberculosis patients, by immunophenotyping based on cell surface marker expression and comparison to normal healthy subjects.
2. To study the transcriptome profiles of monocytes within the study subjects and to validate the differentially expressed genes by quantitative real time PCR.
3. To identify the most promising candidate biomarker genes and pathway networks by comparing the transcriptomic profile of the monocyte subsets from active TB patients to the healthy subjects.

Experimental Approach

Blood samples from 4 groups (healthy controls (HC), latent TB infection (LTB), pulmonary tuberculosis (PTB) and drug resistant tuberculosis (DR-TB)) were collected and performed immunophenotyping of monocytes and dendritic cells using flow cytometry and extra cellular cytokine analysis.

Results: Our study findings identified, elevated frequencies of monocytes, ML ratio and intermediate monocytes and diminished frequencies of plasmocytoid DCs and cross-presenting myeloid DCs in PTB and DR-TB groups (Fig.14-17). Further, miRNA profiling and next generation sequencing studies have been performed within the groups, after subjecting them to FACS sorting for monocyte and outsourced for transcriptomics. Results generated from these studies will aid our research to focus towards enriching the transcriptomic knowledge for identification of differentially expressed genes together with their networks and pathways.

Fig.14: **Frequency distribution of monocytes (A) and monocyte to lymphocyte ratio (ML ratio) (B) among four groups.**

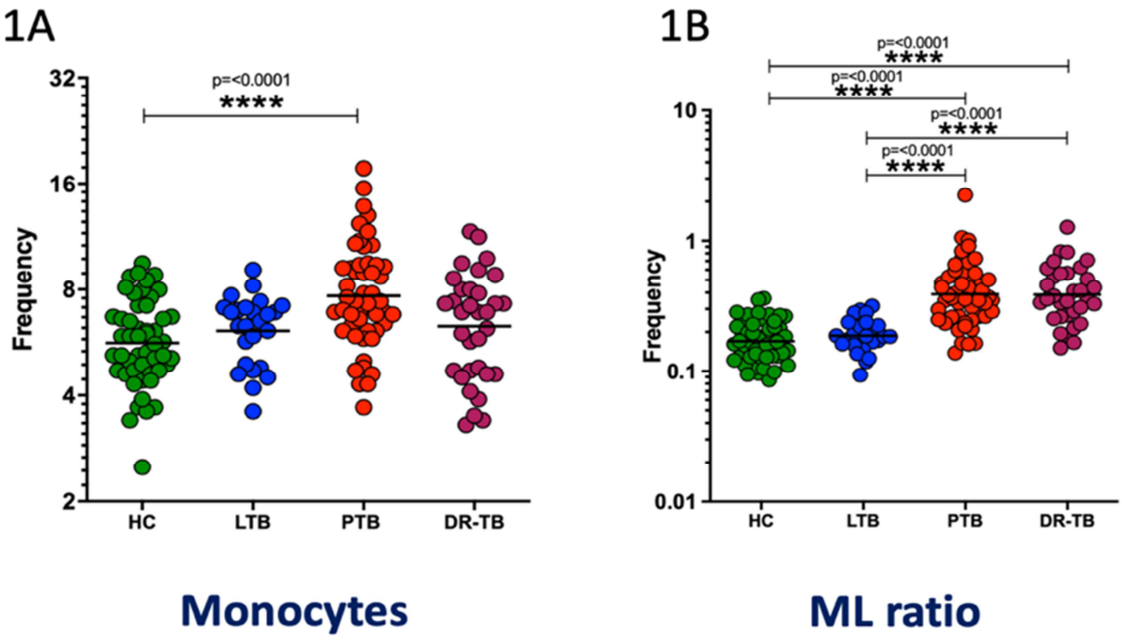


Fig. 15 & 16: **Frequency distribution of monocytes and dendritic cell subsets, Monocytes: Classical monocytes (2A), Intermediate monocytes (2B) and Non-classical monocytes (2C): DCs: Cross-presenting myeloid DCs (3A) and Plasmocytoid DCs (3B) among four groups.**

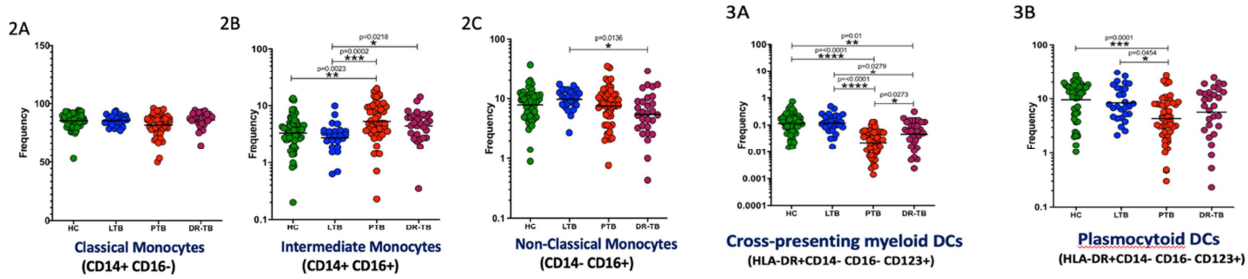
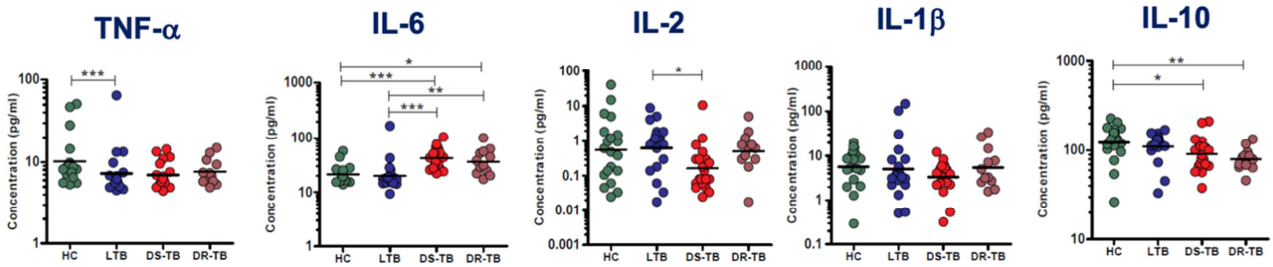


Fig. 17: **Comparison of mean concentration of circulating cytokines, TNF-α (A), IL-6 (B), IL-2 (C), IL-1β (D) and IL-10 (E) among four groups.**



The study is in progress.

I-12: Protecting and improving public health globally: Building laboratory, surveillance and workforce capacity to detect, respond to and prevent DR-TB in India

Principal Investigator	:	Dr K R Uma Devi
Source of funding	:	CDC (GHSA), Atlanta.
Study period	:	2015-2020

Background:
Current methods for detection of TB drug resistance are limited. Early detection of drug resistance is crucial for patients' treatment and to prevent DR-TB transmission. NGS capacity is the key to understand the molecular epidemiology of DR-TB, develop new molecular methods for detection of TB and drug resistance and assist in TB outbreak investigations.

Aims and objectives:
The proposed activities of this project is to build capacity to prevent, detect, respond to, and control the growing problem of DR-TB in India and prevent antimicrobial resistance, and strengthen surveillance systems, national laboratory systems, and workforce development.

Progress of activities performed at NIRT:

- Established the whole genome sequencing facility and capabilities for *M. tuberculosis* for the detection of DR-TB mutations by computational analysis at NIRT.
- The IQA and EQA validation done for NGS sequencing.
- Prospective drug resistant strains collected from various IRLs as identified by CTD for a national representative of DR-TB strains. Phenotype by MGIT DST for a panel of 14 first-line and second-line drugs was performed and the genotypic detection by NGS was compared for each isolates.
- Sensititre MYCOTB MIC plate based assay is being optimized.
- The prospective strain collection is ongoing.
- The discrepant isolates by genotype and phenotype will be tested in Sensititre MYCOTB MIC plate for further confirmation and validation.

Table 25: Nationwide TB samples collection and work progress in the study

CDC-GHSA study	Culture positives (Nos)	NGS done (Nos)	MGIT DST done (Nos)
Prospective isolates	2100	1388	1600
Retrospective isolates	166	166	166

The study is in progress.

I-15: Functional characterization of Rv0148 oxidoreductase of *Mycobacterium tuberculosis*

Principal Investigator	:	Dr. P. Kannan
Source of Funding	:	DST Inspire Fellowship
Study Period	:	2016-2021

Background:
M. tuberculosis resides in the host macrophages during infection and adapts to resilient stresses generated by the host immune system. In response, *M. tuberculosis* codes for short-chain dehydrogenases/ reductases (SDRs). These SDRs are nicotinamide adenine dinucleotide (NAD) reliant oxidoreductases involved in cell homeostasis. The precise function of oxidoreductases in *M. tuberculosis* was unclear.

Objective:
To evaluate the functional importance of oxidoreductase family gene Rv 0148 in *M. tuberculosis.*

Methods:
To assess the virulence of gene knock out mutant we conducted animal experiments using guinea pigs.The Duncan-Hartley strain guinea pigs in the weight of 250 to 350 gm were maintained in National JALMA Institute of Leprosy and Other Mycobacterial

Diseases, Agra, India in a biosafety level III facility. To evaluate the effect of Δ0148 deletion on the pathogenesis and *in-vivo* survival of H_{37}Rv. Guinea pigs (n=5) were infected with 80 to 100 bacilli of H_{37}Rv, Δ0148 and CΔ0148 strains through aerosol route. Animals were euthanized using Thiopentone sodium (100 mg/kg body weight) injection at 5^{th} and 10^{th} week post infection. After dissecting liver, lungs and spleen were observed for pathological damage graded based on Mitchison scoring system. Portion of left caudal lung lobe and spleen caudal segment from the infected animals were removed aseptically and homogenized in 5 ml saline by using Teflon glass homogenizer.

Results:
The animals were sacrificed and observed that the lesions were less in mutant group animals compared to H37Rv group. Further, homogenates need to be processed to evaluate the in-vivo survival of mutant in guinea pigs.

Conclusion:
An initial animal experiment result indicates Rv 0148 play a role in virulence of *M.tuberculosis.*

I – 16: *CYP27b1* gene polymorphisms in pulmonary tuberculosis

Principal Investigators	:	Dr.M.Harishankar; Dr.B.Ramalingam
Source of Funding	:	Intramural, NIRT.
Study Period	:	2020-21

Background: *Cyp27b1* gene encodes 1α-hydroxylase enzyme which synthesize active form of vitamin D3. Polymorphisms in this gene associated with vitamin D deficiency and TB outcome.

Aim

To find out the association of Cyp27b1 rs118204011(C/T) and rs11820 4012(A/G) polymorphisms with susceptibility/protection to pulmonary tuberculosis in healthy controls (HCs) and pulmonary tuberculosis (PTB) patients and its influence on 25(OH)D levels.

Methodology: Totally 100 HCs and PTB patients will be genotyped by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) method. 25(OH)D levels is estimated by ELISA method.

Statistical Analysis: Genotypic associations, p-values with OR adjusted for gender and age will be calculated by logistic regression under codominant, dominant, recessive and overdominant models using the online SNPstats program . The best fitting model of association will be determined using the Akaike information criterion (AIC) and Bayesian information criterion (BIC) provided by the software. The 25(OH)D levels between different genotypes in same group will be analyzed by paired "t" test and different group by independent "t" test. A p-value ≤0.05 will be considered statistically significant.

Table 26: Number of subjects studied and genotype details using PCR-RFLP method

Cyp27b1 SNPs	So far studied		PCR size	Restriction enzyme	Genotypes	Restricted fragment length in base pair(bp)
	HCs	PTB				
rs118204011(C/T)	73	76	279	*BsmI*	CC-single band CT-3 bands TT-2 bands	279bp 279+202+79bp 202+77bp
rs118204012(A/G)	43	46	248	*MluCI*	AA-2 bands AG-3 bands GG-single band	206+42bp 248+206+42bp 248bp

The study is in progress.

I -17: Identification of the latent tuberculosis specific marker by the immunoproteomic analysis of the cell wall and membrane proteins of *M. tuberculosis*.

Principal Investigator : Dr. K.R. Uma Devi.
Source of Funding : DST-SERB
Study period : 2018-2021

Background with rationale: The proteins of the cell wall and cell membrane of mycobacterium are unique and many of them play a crucial role in the pathogenesis of tuberculosis. Therefore, immunological characterisations of these mycobacterium surface associated proteins will help in understanding the pathogenesis of tuberculosis. However, the hydrophobic nature of mycobacterial cell wall and membrane proteins makes it technically challenging in terms of solubilisation and separation of these proteins. In

this study, we plan to perform a novel two dimensional separation approaches for separation of these hydrophobic proteins. The separated protein fractions will be subjected to immunological characterisation *in vitro* using biological samples. We anticipate that this new approach, will facilitate the identification of novel biomarkers for diagnosis of Latent tuberculosis infections.

Aims and objectives of the study:
The objective of the study is to identify latent tuberculosis specific markers by comparing immune responses against cell wall and membrane proteins of *M. tuberculosis* between latent and active tuberculosis participants.

To achieve that aim, the following experiments will be carried out,

1. To fractionate mycobacterial cell wall and membrane proteins using two dimensional separation approach

2. To study the cellular immune response (IFN-γ TNF-α and VEGF response) against isolated fractions by whole blood culture method

3. To study the antibody (IgG) response of the separated fractions by using sera of the study participants

4. To perform proteomic characterization of immunologically important fractions by mass spectrometry.

Methodology:
Hydrophobic membrane proteins of *M. tuberculosis* to be initially separated by reverse phase HPLC (RP-HPLC) method. The RP-HPLC separated fractions will be subjected to a second dimensional separation based on molecular weight by SDS-PAGE. The individual antigens and the less complex mixtures so obtained will be subjected to immunological characterization by cellular assays using whole blood and their antibody response will also be assessed.

Results:
During this year, large scale culturing (10 litres) of the S7 mycobacterial strain (clinical strain), sonication of the isolated bacterial pellet (in batches) and further clean-up was carried out using standard protocols. We were able to achieve10 mg of the whole cell lysate protein per 100 ml of the culture. In total, 1 gram of whole cell lysate has been obtained so far. Bacterial lysate preparation of the remaining pellet is underway. The study is ongoing.

I-18: ATTENUATED MYCOBACTERIA BASED VACCINE WITH A NOVEL STRATEGY FOR T CELL PRIMING

Principal Investigator	:	Dr. K.R. Uma Devi.
Ph.D student	:	Ms. J.S. V. Soundarya
Study period	:	2019-2024

Background with rationale: Currently available BCG vaccine has shown to have varying efficacy in adult population in different parts of the

world**.** Therefore in the current scenario, development of new vaccine candidates is a priority. rBCG co-expressing Ag85A-ESAT6 fusion protein of *M.tb* elicited more long lasting and stronger Th 1 type cellular responses in BALB/c mice. In the present study, additional modifications will be carried out in the $mc^2$6206 (attenuated M.tb) and BCG to provide enhanced immune response by a two prong approach. First approach is to add an additional deletion of ChoD or Tgs4 to $mc^2$6206 and BCG and second is to provide a targeted delivery of T cell specific mycobacterial antigens to the dendritic cells using these knock out strains.

Specific aims of the study:

1. Construction of rAMtb (Recombinant attenuated mycobacterium tuberculosis) and rABCG (Recombinant attenuated BCG) by deletions in either of two genes, Rv3409c (*ChoD)* or Rv3088 *(Tgs4*) in attenuated *M.tb* (mc^2 6206) and in the identical homologues in BCG (*Mb3443c and Mb3115)* expressing CFP10 and/or ESAT 6.
2. Construction of fused CFP10 and/or ESAT6 to Dec205 scFv, for secretion **(Antigen 85** signal sequence) from the mycobacterium for enhanced TB-specific T cells.
3. To test the efficacy of the constructed rAMtb or rABCG for their immunogenicity and to compare their efficacy with that of BCG.

Methodology:

Construction of fused CFP10 and/or ESAT6 to Dec205 scFv:

1. Designing of inserts - Dec 205 ScFv-ESAT6-CFP10-V5 (Insert1) for pBRL34 vector and Ag85p-19kDss-Dec 205 ScFv-ESAT6-CFP10-V5 (Insert2) for pMV306/206 vectors
2. Cloning and amplification of the fusion protein constructs into mycobacterial shuttle vectors pBRL34, pMV206, pMV306 has been achieved in *E.coli.*
3. Selection of positive clones through restriction digestion of the obtained plasmid from the recombinants.

Results:

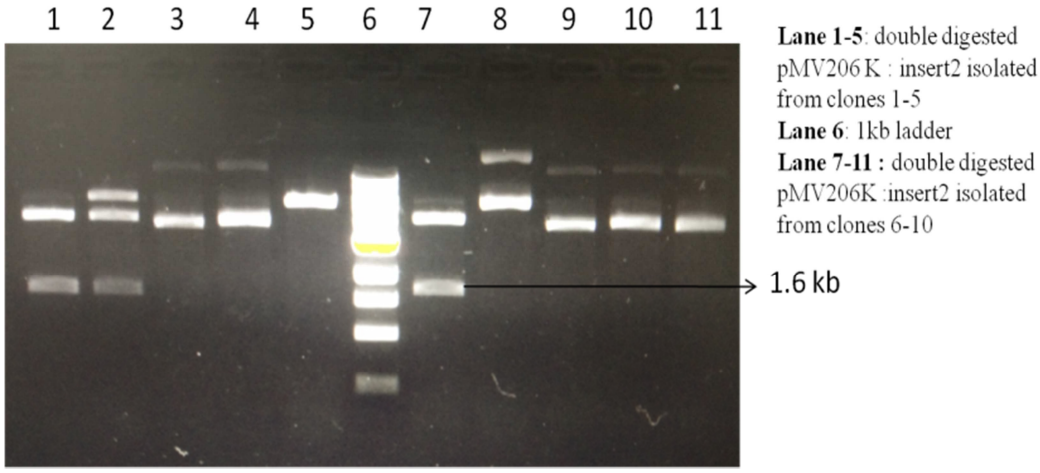
Construction of insert 2 has been achieved. The study is ongoing.

<u>Fig.18:</u> Representative culture plates – a) DH5α pMV206K:Insert2 colonies and b) Ligation control (SAP treated pMV206K)

(a)

(b)

Fig. 19: screening of pMV206K:Insert2 plasmids (double digested with HpaI and EcoRV).



I-19: Identification of tuberculosis specific biomarkers in children by the proteomic analysis of urine

Principal Investigator	:	Dr. D.Anbarasu.
Source of Funding	:	DHR-ICMR
Study period	:	2019-2021

Background: Newer diagnostic methods are urgently needed for the diagnosis of the childhood tuberculosis. Identification of the childhood tuberculosis specific biomarkers will be helpful in the development of the newer diagnostic method in diagnosing childhood tuberculosis. MS-based biomarker identification in urine of childhood tuberculosis participants is so far not carried out. So, in the present study we are studying urine using MS-based proteomic approach to identify the biomarker specific for childhood tuberculosis.

Aim:
To identify the childhood tuberculosis specific biomarkers by urine proteomic analysis.

Objectives:

i. To identify disease-specific biomarker for childhood tuberculosis in urine.
ii. To understand the disease-specific modification of the identified biomarker proteins in urine by using high-resolution mass spectrometry analysis

Methodology:
Study Groups:
Group A: Children with confirmed TB: (N=62)
Group B: Children having respiratory infection other than TB: (N=62)
Group C: Healthy children: (N=62)

Urine Proteomic Analysis:

The abundant proteins in the urine will be removed by using the centrifugal concentrators which having 50Kda molecular weight cutoff membranes (Merck Millipore, USA) to remove the high abundant proteins as elute. Both the eluted and concentrated urine samples are again concentrated using 3000da cutoff membrane centrifugal concentrators. This will retain only the protein molecules in the urine sample.

Both the highly abundant and low abundant urine proteins (400µg of urine proteins) are subjected to SDS-PAGE analysis. The separated proteins on the gel will be cut into 10 equal parts. Each gel lane will be subjected to in-gel digestion.

Results:

The recruitment of study subjects was initiated in October 2019. So far, we have successfully recruited 20 children with respiratory infection other than TB (Group B) and 5 children with confirmed TB.

The study is ongoing.

I-20: Gene knockout characterization of Rv2159, a alkyl hydroperoxidase of *Mycobacterium tuberculosis*

Principal Investigator	:	Dr. P. Kannan
Source of Funding	:	DST Inspire Fellowship
Study Period	:	2017-2021

Background:
Macrophages during *M. tuberculosis* infections produce reactive oxygen species (ROS) and reactive nitrogen species (RNS)**.** The oxidative stress and the resulting super oxides during *M.tuberculosis* infections are converted into various oxidants like HClO, H_2O_2 & $ONOO^-$ which damage the bacterial cells**.** *OxyR* and *soxR* are two prokaryotic regulators against peroxides and superoxides. Due to the evolutionary inactiactivation of *oxyR* in *M. tuberculosis*, peroxidase stress is managed by alkylhydroperoxidase reductase (AhpC)**.** The AhpC may assist *M. tuberculosis* in resisting against oxidative damage in absence of the catalase**.** Hence, studies like gene knockout will help in understanding the role of AhpC in *M. tuberculosis*. In this study, we have chosen AhpC family member Rv2159 of *M. tuberculosis*

Objective: To understand the functional role of AhpC family genes in *M. tuberculosis*

Methods: The gene knockout mutant of Rv2159 in *M. tuberculosis* was constructed by phage-facilitated allelic exchange. The mutants subjects to various stresses and macrophage infection assays.

Results:
Gene disruption shows increase in the growth of the knockout strain and displayed distinct difference in the *in-vitro* growth.THP-1 infection of mutant showed reduced *in-vivo* survival. Enhanced expression of IL-1β, G-CSF, IP-10, MCP-1(MCAF), and MCP-1a cytokines were observed in Δ2159 compared to H_{37}Rv. The exposure of mutant to oxidative stress using 2& 4mM concentration of H2O2 displayed the mutant was sensitive to H2O2. Investigated whether Copper, Zinc and SDS stresses disturb the metabolism of Δ2159, results suggested that mutant was sensitive to stress compared to H_{37}Rv and CΔ2159. To evaluate the drug resistance MGIT was performed, mutant displayed it has no role in drug resistance.

Conclusion:
The current study has brought to light the possibility to predict the function of AhpC genes involved in oxidative stress. The *in-vitro* and *in-vivo* experiments displayed the prominent role of Rv2159 in survival of *M. tuberculosis*. The study is ongoing

I-21: Study on Mutations Associated with Pyrazinamide Resistance in *Mycobacterium tuberculosis*

Principal Investigator	:	Dr.P.Kannan
Source of Funding	:	ICMR
Study period	:	2019 – 2022

Background:
Pyrazinamide (PZA) is an important first line drug in TB therapy. It is active against semi dormant *Mycobacterium tuberculosis* which is not killed by other TB drugs. Because of its indispensable sterilizing activity, all new TB drug candidates in clinical trials are used together with PZA. Although PZA has been continuously used to treat TB, the WHO does not include PZA in the group of antimycobacterial drugs to be routinely tested for resistance because PZA drug susceptibility testing (DST) is notoriously difficult and often inaccurate. Resistance to PZA will severely affect the treatment outcome in TB cases. In India no major studies have been conducted to understand PZA resistance among various clinical strains from presumptive drug resistant patients. This study will provide a unique opportunity to understand the genotype mutations and phenotypic correlation with PZA resistance in clinical strains.

Objectives:
The objective of this study is to understand the Pyrazinamide resistance in *Mycobacterium tuberculosis* strain isolated from presumptive drug resistant patients from Chennai.

Methods
Isolate selection:
All clinical strains isolated from presumptive drug resistant stored cultures from three districts of Greater Chennai were included in the study. Approximately 400 strains collected during 2017-2018 will be used for this study. All isolates will be processed immediately and subjected to phenotypic DST, PZase activity, and targeted gene sequencing. The aliquots of the bacterial cultures will be stored as glycerol stocks −80 °C.

Results:
Total numbers of 196 clinical isolates were collected among which 148 were MGIT positive for *M.tuberculosis* (Table 27) and remaining stored isolates either not grown or contaminated. MGIT positive strains were screened for PZA susceptibility testing. MGIT DST have completed for 143 isolates.

Table 27: Screening of first line drug resistance along with PZA resistance

Total no of isolates	INH Resistant	INH+RIF Resistant	INH+PZA Resistant	RIF+PZA Resistant	INH+RIF+PZA Resistant	PZA alone Resistant
143	38	8	14	01	07	15

148 MGIT positive strains were used for further studies. Resistance for Isoniazid (INH), Rifampicin (RIF) and Pyrazinamide (PZA) were screened. Among 143 isolates, 38 isolates were *INH resistant*, 8 isolates were *INH+RIF Resistant*, 14 were

INH+PZA resistant, 7 isolates were *INH+RIF+PZA resistant*, 1 isolate showed *RIF+PZA resistant* and 15 isolates were found to be *PZA alone resistant*.

The study is on-going

I-22: Studies on epigenome wide alterations in alveolar macrophages during *Mycobacterium tuberculosis* infections in guinea pig pulmonary tuberculosis model

Principal Investigator	:	Dr. P. Kannan
Source of funding	:	Extramural – DST- SERB
Study Period	:	2017 – 2021

Background:
The short or long term changes of host cell epigenome elicited by pathogens may play a role in disease initiation and progression. An increasing number of bacterial pathogens may also elicit chromatin modifications in various host species. In the present study we focus on *M. tuberculosis* induced epigenetic dysregulation in guinea pig which encode proteins or possess cell constituents that may interact with the epigenetic machinery of host cells. These interactions frequently result in a reprogramming of the host cell epigenome and gene expression pattern and associate with pathological changes, these epigenetic changes can be beneficial or disastrous to pathogens.. There has been huge advances in sequencing technology which can lead to a better understanding of the roles of epigenetics in the tuberculosis. Bisulfite sequencing remains the most sensitive and specific method for analyzing DNA methylation.

Objectives:
To understand epigenetic alterations in pulmonary macrophages during *M. tuberculosis* infections in guineapigs.

Methodology:
For the infection study, Hartley guinea pigs were brought to BSL III animal facility in JALMA Institute of Leprosy and other mycobacterial diseases at Agra. H37Rv was infected via aerosol route using nebulizer with 50-100 bacilli per animal. Following 9 weeks of infection, the guinea pigs (6 control and 6 treated) were euthanized (Fig.20) and pulmonary macrophages were collected. Genomic DNA was extracted from macrophages for whole genome bisulphate sequencing and chip sequencing.

Libraries of genomic DNA (gDNA) were prepared for whole-genome bisulfite sequencing (WGBS), a sequencing-based methylation analysis application. Genomic DNA was treated with bisulfite reagent, prior to library preparation for sequencing, The libraries were prepared for subsequent cluster generation starting from sample DNA through adaptor ligation, library purification, and quantification. Base quality distribution. Alignment files (sorted bam files) and alignment statistics, chromosome wise read alignment distribution, distribution of the methylated sites in the genic/intergenic regions were analysed.

Results:
Differentially methylated regions were identified and sorted as hyper methylated and hypo methylated regions. Among the differentially methylated Genes, Genes involved in epigenome modifications were selected and used for further analysis. (Table 28)

Fig 20: Comparison between Control and *M.tb* infected animal: A-lung lobes; B-spleen

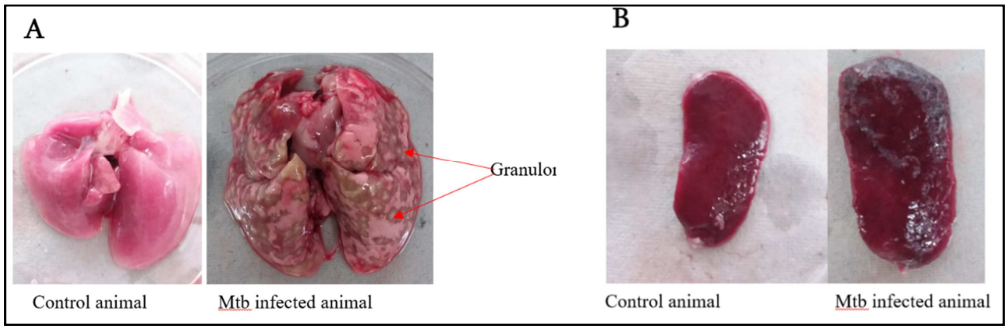


Table 28: Genes involved in epigenome modifications:

Gene ID	Entry name	Gene Name	Protein name
ENSCPOG00000001094	H0UVF4	NT5E	5'-nucleotidase ecto
ENSCPOG00000012562	H0VLQ3	HLA-DOA	Major histocompatibility complex, class II, DO alpha
ENSCPOG00000033895	A0A286XUP8	HLA-DOB	Major histocompatibility complex, class II, DO beta
ENSCPOG00000004277	H0V2S8	BTNL2	Butyrophilin like 2
ENSCPOG00000033475	A0A286XZI1	ZNF131	Zinc finger protein 131
ENSCPOG00000036046	A0A286Y312	SPRED1	Sprouty related EVH1 domain containing 1
ENSCPOG00000015287	H0VT03	CCBE1	Collagen and calcium binding EGF domains 1
ENSCPOG00000001394	H0UW38	RAB3C	RAB3C, member RAS oncogene family
ENSCPOG00000013970	H0VPZ2	SNCA	Alpha-synuclein
ENSCPOG00000039252	A0A286XSI1	PKNOX1	PBX/knotted 1 homeobox 1
ENSCPOG00000001151	H0UVK1	FAF1	Fas associated factor 1
ENSCPOG00000002148	H0UXV1	CCNK	Cyclin K
ENSCPOG00000001655	H0UWP9	GPC3	Glypican 3
ENSCPOG00000019915	H0VYM7	PKN1	Protein kinase N1

ENSCPOG00000003397	H0V0S6	SCMH1	Scm polycomb group protein homolog 1
ENSCPOG00000033310	A0A286XV43	ZNF746	Zinc finger protein 746
ENSCPOG00000015410	H0VTA6	Rarb	Uncharacterized protein
ENSCPOG00000034846	A0A286XLG5	RAB4A	RAB4A, member RAS oncogene family
ENSCPOG00000012350	H0VL87	POLR1A	DNA-directed RNA polymerase subunit
ENSCPOG00000031544	A0A286Y4F6	PCDH10	Protocadherin 10
ENSCPOG00000039715	A0A286XLI5	PAG1	Phosphoprotein membrane anchor with glycosphingolipid microdomains 1
ENSCPOG00000000591	H0UU94	Notch4	Uncharacterized protein
ENSCPOG00000004692	H0V3Q6	MSL3	MSL complex subunit 3
ENSCPOG00000032303	A0A286XCE0	ETV3	ETS variant 3
ENSCPOG00000024581	H0W1T3	BCL2	BCL2 apoptosis regulato
ENSCPOG00000012507	H0VLL1	ZNF704	Zinc finger protein 704
ENSCPOG00000015489	H0VTH2	IL16	Pro-interleukin-16 [Cleaved into: Interleukin-16 (IL-16) (Lymphocyte chemoattractant factor) (LCF)]
ENSCPOG00000034489	A0A286XHH6	CDKAL1	CDK5 regulatory subunit associated protein 1 like 1
ENSCPOG00000001954	H0UXE5	WWOX	WW domain containing oxidoreductase
ENSCPOG00000011312	H0VIW5	TRIM24	Tripartite motif containing 24
ENSCPOG00000009000	H0VDM2	CHD1L	Chromodomain helicase DNA binding protein 1 like

DEPARTMENT OF STATISTICS

STUDIES COMPLETED:

S-5 : Models Formulation using Cox Regression: A Rational Investigation on variable selection for Time-to-Event Randomized Clinical Trial Data on Tuberculosis

Principal Investigator	:	Dr. C. Ponnuraja
Source of Funding	:	ICMR
Study Period	:	2019-2021

Abstract: Handling of RCT data is unique and challenging for statisticians because it has both time-to-event outcomes and censored observations where the event was not observed during follow-up. Survival analysis of such data using Cox proportional hazard model will end up in some covariates contributing to the time-to-event outcome. But the possible limitation of this model is that it might not select some covariates which are statistically insignificant in unadjusted and adjusted regression approaches, but they could still practically contribute to the time-to-event outcome. Instead, when various permutations and combinations of all the covariates are formed as candidate models prior to the conventional method, this limitation can be minimized. This alternative approach will not only give additional useful information but also will help in better interpretation of the time-to-event outcome.

To address this issue we undertook survival analysis of the data obtained from a RCT conducted at NIRT, with 1236 culture positive PTB patients treated with three different ATT regimens. The main objective was to formulate various candidate model selections using Cox (PH) that yield clinically potential and interpretable estimates of the effect of exposure factors independently through Akaike Information Criterion (AIC) aspects. Additionally, it compared models with different forms of variable selection in Cox (PH) models. Also, it identified covariates that are influencing the time-to-sputum conversion as the event of interest. Furthermore, it examined the performance of univariate models on the prediction that how it could be relevant to the response. Predominantly this technique was applied in the development of a prediction model for the risk of delayed sputum conversion during TB treatment.

COX Proportional hazards model:

As in conventional regression, survival regression models allow for the quantification of the effect on survival of a set of predictors, the interaction of two predictors, or the effect of a new predictor above and beyond other covariates. For the past several decades the Cox proportional hazards model has been used comprehensively to examine the covariate effects on the hazard function for the time-to-event data. The proportional hazards model is appropriate when there is a permanent difference between the groups in the longer term in the context of the follow-up period. Cox proportional hazards models are derived from the underlying baseline hazard functions of the patient population and an arbitrary number of dichotomized covariates. Also, it does not assume an underlying probability distribution but it assumes that the hazards of the

patient groups are constant over time. That is the main reason it is being called “proportional hazards model”.

The hazard function plays a very important role in survival analysis. The Cox proportional hazards model investigates the relationship between predictors and the time-to-event through the hazard function. It assumes that the predictors have a multiplicative effect on the hazard and that this effect is constant over time. Cox model is the most popular mathematical modelling approach for estimating survival curve when considering several explanatory variables simultaneously. It is also called a semi-parametric model. The Cox PH model is usually written in terms of the hazard model The Cox PH model is given below as described by Cox (1972). That is

$$h(t, X) = h_0(t)e^{\sum_{i=1}^{p} \beta_i X_i}$$

where $h_0(t)$ is baseline hazard and β_i is parameter vector and X_i are independent variables. The Cox model formula says that the hazard at time t is the product of two quantities. The first of these, $h_0(t)$, is called the baseline hazard function. The second quantity is the exponential expression e to the linear sum of $\beta_i X_i$, where the sum is over the p explanatory X variables. This model gives an expression for the hazard at time t for an individual with a given specification of a set of explanatory variables denoted by X. That is, X represents a collection of predictor variables that is being modeled to predict an individual’s hazard. An important feature of this formula, which concerns the proportional hazards (PH) assumption, is that the baseline hazard is a function of t, but does not involve the X’s. Cox models have achieved great popularity, because they do not require the investigator to assume a particular survival distribution for the data. Instead, these models use a hazard function. In estimating the baseline hazard function, a Cox model uses the so-called Aalen-Breslow estimator, which is a generalization of the non-parametric Nelson-Aalen estimator of the cumulative hazard function (Kalbfleisch, JD.; Prentice, RL. The statistical analysis of failure time data. 2nd ed. Hoboken: John Wiley and Sons; 2002.). An additional justification on why it is called as “semiparametric model” is because of the lack of a parametric form of the survival distribution and this is the only model that consists of two components together; they are the baseline hazard as a nonparametric component and the exponential part as a parametric component.

Illustrative example

One of the Randomized Clinical Trial (RCT) enrolled about 1236 pulmonary tuberculosis patients into three different treatments including a control treatment. The total duration of the treatment period is six months. The study was conducted at the National Institute for Research in Tuberculosis (Indian Council of Medical Research), Chennai India. The event of interest is sputum culture conversion (positive into negative) during the treatment period and sputum test was carried out every month during the treatment period. The statistical package was used for all type of statistical analyses by R version.3.5.2.

The covariates considered for the analysis were,

1. Age (in years)
2. Sex (Male-1 and Female-0)
3. Treatment group (1- Control, 2-Trial Regimen-I, 3-Trial Regimen-II)
4. Weight at baseline (in Kg)

5. Pre-treatment sensitivity (Drug Susceptibility Test-DST)(Res. to any Drug-1 and Sens to all Drug-0)

Time and status were also involved here. The event was coded as 1 and censoring was coded as 0.

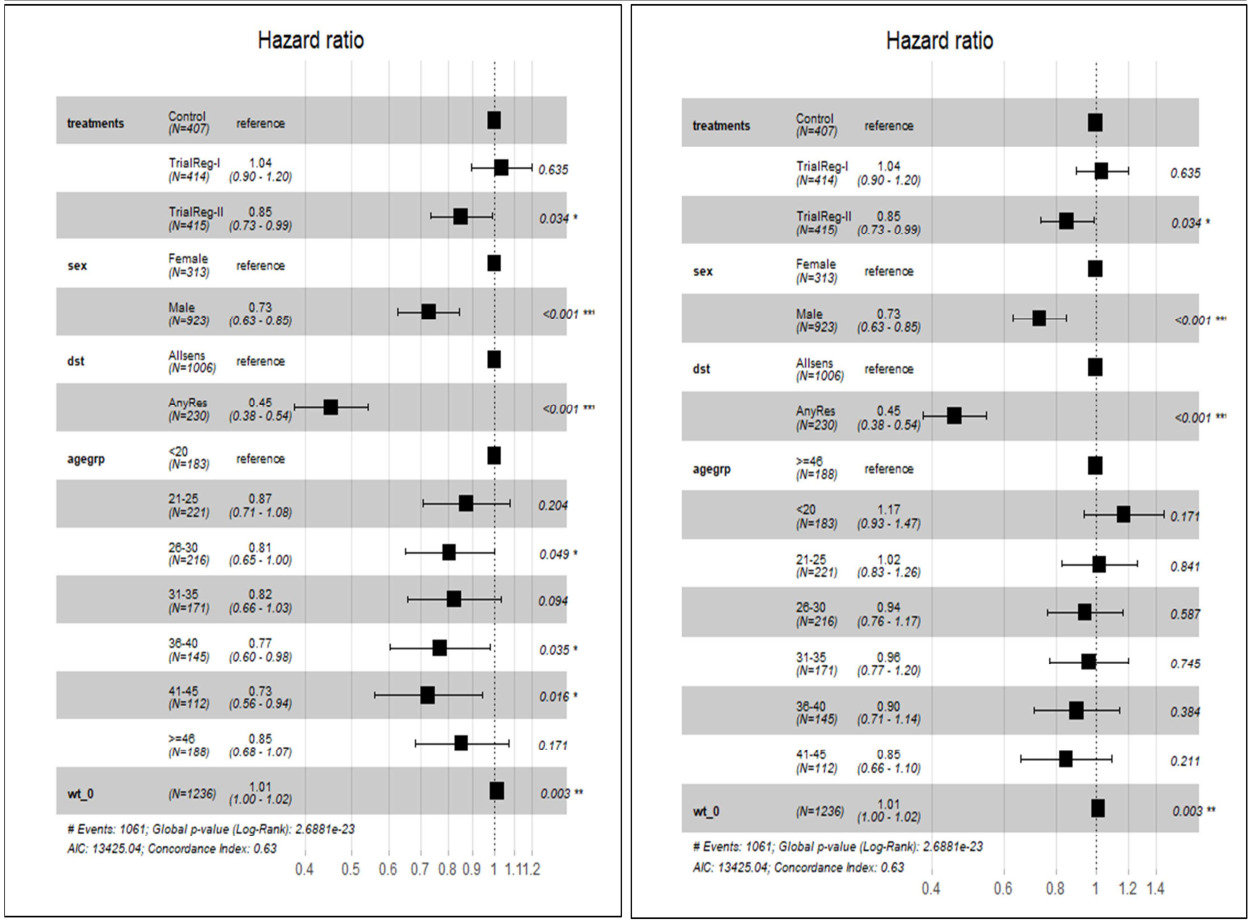
Table 29: CoxPH Model unadjusted

CoxPH Model unadjusted					
	coef	exp(coef)	se(coef)	z	p
Control(Ref)					
Trial Regimen-I	0.1626	1.1766	0.0762	2.1350	0.0327
Trial Regimen-II	0.1976	1.2185	0.0758	2.6080	0.0091
Female(Ref)					
sexMale	-0.3365	0.7142	0.0750	-4.4860	0.0000
Sensitive to all Drug(Ref)					
Res. at least to any one drug	-0.7902	0.4537	0.0904	-8.7400	< 2e-16
Age	-0.0037	0.9963	0.0028	-1.2990	0.1939
wt	0.0135	1.0136	0.0045	2.9730	0.0030

Every hazard ratio (HR) represents a relative risk of conversion and that compares one occurrence of a binary feature to the other occurrence. For treatments, an HR of 1.04 for trial regimen-I communicates that patients who received trial regimen-I had a reasonably quicker sputum conversion compared to patients who received control treatment (which served as a reference to calculate the hazard ratio). As shown by the forest plot, the respective 95% confidence interval is 0.90 - 1.20 and the result was non-significant. Nevertheless, trial regimen -II has an HR 0.85 and it conveys significantly slower conversion than compared to patients who received control treatment. In gender, when compared to female patients, the conversion scenario was significantly slower among male patients (HR-0.73(0.63-0.85)). From the DST point of view, patients from the “Sensitivity to All Drug” group served as reference and the group of patients in “Resistant to any drug” have slower conversion rate(HR-0.45(0.38-0.54)) which was also showing a significant difference. As per the age, it was classified into seven groups and the group of patients “<20years” served as the reference. As age progresses the sputum conversion rate was marginally decreasing when compared to the reference group. These differences are acknowledged with statistically significant. Another important factor, weight at baseline influenced the sputum conversion as outcome and the HR is 1.02(1.00-1.02)

Fig 21:

Forest Plot1. Adjusted Hazard Ratio with p-value



On the other hand, when the higher age group (>=46 years) was treated as the reference, the HR's of 1.17(0.93-1.47) and 1.02(0.83-1.26) for age groups "<20 years" and "21-25 years" respectively. It is also conveyed that these two groups were having higher conversion rate than compared to all other groups like age groups of "26-30", "31-35", "36-40" and "41-45" and their HR's of 0.94(0.76-1.17), 0.96(0.77-1.20), 0.90(0.71-1.14) and 0.85(0.66-1.10) respectively.

Using this model, we can perceive that the treatment group, gender, and age group variables significantly influence the patients' sputum culture conversion in this study. This is quite different from what it was saying with the Kaplan-Meier estimator and the log-rank test. Whereas the former estimates the survival probability, the latter calculates the risk of time to sputum conversion and respective hazard ratios. This analysis shows that the results that those methods yield could differ in terms of significance.

Table 30:

	B	SE	p-value.	HR	95% CI for HR	
					Lower	Upper
Control(Ref)						
Trial Regimen-I	.022	.075	.765	1.023	.884	1.183
Trial Regimen-II	-.125	.076	.100	.882	.760	1.024
Female(Ref)						
Male	-.243	.077	.001	.784	.675	.911
SENS(Ref)						
Res.to any drug	-.633	.091	.000	.531	.445	.634
wt_at baseline	.011	.005	.022	1.011	1.002	1.020
Age <21 (Ref)						
Age 21-25	-.113	.107	.289	.893	.725	1.101
Age 26-30	-.183	.109	.094	.833	.672	1.032
Age 31-35	-.166	.116	.153	.847	.675	1.063
Age 36-40	-.207	.124	.095	.813	.637	1.037
Age 41-45	-.265	.133	.047	.768	.591	.996
Age >=46	-.129	.115	.261	.879	.703	1.101
Control(Ref)						
Trial Regimen-I	.022	.075	.765	1.023	.884	1.183
Trial Regimen-II	-.125	.076	.100	.882	.760	1.024
Female(Ref)						
Male	-.243	.077	.001	.784	.675	.911
SENS(Ref)						
Res.to any drug	-.633	.091	.000	.531	.445	.634
wt_at baseline	.011	.005	.022	1.011	1.002	1.020
Age >=46 (Ref)						
Age (<21)	.129	.115	.261	1.137	.909	1.423
Age 21-25	.016	.108	.885	1.016	.822	1.255
Age 26-30	-.054	.108	.617	.947	.766	1.172
Age 31-35	-.037	.114	.745	.964	.771	1.205
Age 36-40	-.079	.121	.517	.924	.729	1.172
Age 41-45	-.136	.131	.298	.873	.676	1.128

The possible candidate models are being engaged for the time-to-sputum conversion of tuberculosis data. The ultimate candidate regression model is identified by the way of covariates with p-value > 0.05 one at a time until all regression coefficients are significantly different from the chosen alpha level of 0.05. Since the statistical testing at each step of the stepwise variable selection procedure is conditioning on the other covariates in the regression model, the concern of the multiple testing problems is demonstrated in the given table 30.

Table 31: Candidate model selection with five predictors which are likely to influencing time to sputum conversion in tuberculosis

Model num.	num.predictors	Models with predictor(s)	AIC
29	4	Treatment+sex+wt+DST	13419.89
31	5	Treatment+age+sex+wt+DST	13420.24
25	3	sex+wt+DST	13424.97
30	4	age+sex+wt+DST	13425.07
20	3	Treatment+sex+DST	13426.48
27	4	Treatment+age+sex+DST	13426.51
23	3	age+sex+DST	13430.25
14	2	sex+DST	13430.46
28	4	Treatment+age+wt+DST	13437.43
18	3	Treatment+age+DST	13438.36
21	3	Treatment+wt+DST	13441.54
24	3	age+wt+DST	13441.74
12	2	age+DST	13442.14
9	2	Treatment+DST	13442.16
15	2	wt+DST	13446.29
5	1	DST	13446.41
26	4	Treatment+age+sex+wt	13508.90
19	3	Treatment+sex+wt	13509.24
22	3	age+sex+wt	13515.09
13	2	sex+wt	13515.65
16	3	Treatment+age+sex	13517.61
7	2	Treatment+sex	13518.14
10	2	age+sex	13522.74
3	1	sex	13523.48
17	3	Treatment+age+wt	13524.57
6	2	Treatment+age	13527.14
8	2	Treatment+wt	13529.50
11	2	age+wt	13530.36
1	1	Treatment	13531.42
2	1	age	13532.37
4	1	wt	13535.67

Discussion:

The proportional hazards model has become the model of choice in the analysis of time to event data in clinical trials. In this research work our efforts to formulate models with Cox regression in parallel with conventional unadjusted and adjusted regression procedures using RCT TB data we found that top two formulated models not only recapitulated the variables derived with conventional procedures, but also the missed ones. Candidate model selection with five predictors which are likely to influence time to sputum conversion in tuberculosis have been identified with different models and its deviance criteria. The lowest deviance is the better the model.

STUDIES IN PROGRESS:

S- 6: Development of a Database of Clinical Study X-rays at NIRT, Chennai

Principal Investigator	:	Dr. C. Ponnuraja
Source of Funding	:	ICMR
Study Period	:	March 2020 – April 2021

Background: The NIRT has been conducting clinical trials to define better treatment for various forms of tuberculosis (TB) during the last seven decades. This involves laborious manual documentation of X-rays and follow-up for patients up to five years. In order to make the database of X-rays as an e-storage and an efficient electronic image capture (EIC) through a clinical trial management system (CTMS) is essential. The radiograph film digitization is a process to have electronic records in permanent archives.

Objectives: To convert the existing manual X-rays into DICOM images as an EDC system and to provide for architecting a solution to enable the interfacing of different applications to provide a complete EDC

Status: Study in progress

DEPARTMENT OF EPIDEMIOLOGY

STUDIES COMPLETED:

E-3: Tuberculosis among homeless persons, Chennai city

Principal Investigator	:	Dr. C K Dolla
Source of funding	:	ICMR, Extramural
Study period	:	2017-2020

Background

Homeless persons are at most risk of contracting the Tuberculosis disease, which is five times more than the general population. The study was carried out to estimate the prevalence of (Pulmonary Tuberculosis) PTB in pavement dwellers, shelter and roaming homeless people in Chennai city of south Indian state, Tamil Nadu. Chennai has large proportion of homeless population when compared to other cities.

Objectives:

(i) To estimate the prevalence of TB (active disease and latent infection) among homeless people of different zones in Chennai city;
(ii) To identify the distribution of risk factors associated with TB among homeless people

Methods

Study area: All zones of Chennai city
Sample size: 5000 homeless persons
The NGOs working with homeless persons were contacted and list of settlements of homeless in 38 (Pavement) areas and in 13 shelters was collected. Homeless settlements were identified through volunteers of these organizations. Homeless persons aged 15 years and above were screened for signs and symptoms of TB and chest X-ray posterior-anterior view was taken. Two medical officers read the chest X-ray and in cases of any disagreement, it was read by a third medical officer. Two sputum samples (one spot, one on next day early morning) were collected from persons with the following sign and symptoms: history of cough for more than two weeks, recent weight loss, haemoptysis, chest pain, fever and radiological abnormalities suggestive of TB. Auramine O phenol stain for AFB smear was prepared for fluorescent microscopy and solid culture by LJ medium was done. Study participants diagnosed with TB were referred to the nearest RNTCP centres for TB treatment.

Findings

A total of 4832 participants were surveyed. About 57% of the participants were females. The prevalence/1000 population (95% CI) of PTB was 3 (0 - 6), 15 (6 - 23),8 (2 - 14),13 (6 - 21),10 (5 - 15) among the age groups 15 to 25 yrs, 26 to 35 yrs, 35 to 45 yrs ,46 to 55 yrs and above 55yrs respectively. Smoking was prevalent in 34% of males, and 0.3 % of females. Alcohol usage was found in 40% of males compared to 0.4% in females. About 239 (4.9%) people had chest X-ray abnormality suggestive of TB. The prevalence /1000 population (95% CI) of TB among chest symptomatic was 47 (26 - 67) and among the asymptomatic it was 6(4-9).

STUDIES IN PROGRESS:

E-4: National survey for state-wise prevalence of microbiologically confirmed pulmonary tuberculosis in India

Principal Investigator	:	Dr Sriram Selvaraju
Source of funding	:	ICMR
Study period	:	2018-2021

Background

Estimated prevalence of microbiologically confirmed PTB in 9 sites in India among individuals aged ≥15 years varied between 170 to 528 averaging at 350 per lakh population. Noticeably, TB nationwide survey was never repeated after 1956. It is important to conduct a nationwide TB prevalence survey to closely monitor the progress towards TB control with the aim to 'End TB' as per Sustainable Development Goals (SDGs).

Objectives:

To estimate the point prevalence of microbiologically confirmed pulmonary TB among persons aged ≥15 years in India at National level and individually for 20 states / state groups.

Methods

Study Design: Cross sectional survey among all individuals aged ≥15 years.

Sampling Strategy: Cluster sampling design.

Sample size: For estimation of 20 State/UT groups we need a total national sample size of 5, 00,000. Cluster size (m) = 800; Number of clusters = 625.

Current Status: There are 23 teams operational in various parts of the state with a Central Project Management Unit at NIRT, Chennai. We have completed 115 clusters throughout the country and we have to postpone 7 clusters in between the operations due to COVID-19 Lockdown. Data in 20 clusters are collected by paper and in the remaining 102 by software. In the software based data collection, we have enumerated 116530 and. Of the eligible 80171 participants, we interviewed 73,733. We have taken chest x-ray for 72467 participants and excluded 508 pregnant women. The paper based data collection data entry is ongoing. There were 10151 eligible for first sputum collection and 9356 were processed for CBNAAT. There were 176 positive by CBNAAT. There were 8540 second sputum samples received in the IRLs and 6218 have been processed. There were 40 samples positive by smear on the 2nd sputum and 122 by culture. Of the 207 eligible for 3rd sputum, 191 samples were received at IRL and 124 were processed. There were 51 positive by CBNAAT and 19 by smear and 11 by culture in the 3rd sputum specimen. We have established the IGRA lab at NIRT, Thiruvallur for doing the TB Infection survey as a part of the National TB Prevalence Survey. We have started the operations of the IGRA lab and started the first IGRA cluster from Delhi. We have processed 566 samples for first IGRA cluster. Data collection would be initiated in the month of September 2020.

E-5: Sentinel surveillance for measuring the tuberculosis burden and trends in high risk group for tuberculosis

Principal Investigator	:	Dr Shrinivasa B M
Source of funding	:	Global Fund
Study period	:	August 2019- March 2021

Background

Early detection of TB is essential to further improve health outcomes for people with TB and to reduce TB transmission more effectively. Systematic screening in high risk groups is a potential strategy to complement the efforts of patient-initiated pathway of TB diagnosis, also called as “passive case finding”. Some risk groups pose a greater risk for developing tuberculosis. We therefore evaluate the effectiveness of new methodology of conducting sentinel surveillance for selected high risk groups for TB in India

Objectives:

Primary objective:

To estimate the burden of TB in high risk groups vulnerable population using an appropriate algorithm.

Secondary objectives:

1. To establish a national hospital based surveillance to examine trends and pattern of TB attributable to selected high risk groups/clinically vulnerable population attending tertiary care hospitals in India.
2. To establish a TB laboratory surveillance for the selected clinically vulnerable population attending tertiary care hospitals in India.
3. To estimate the prevalence of TB in selected clinically vulnerable population attending tertiary care hospitals in India.

Methods

Study Design: Multi-centric study involving four sites across Tamil Nadu, Uttar Pradesh Himachal Pradesh and Andhra Pradesh coordinated by National Institute for Research in Tuberculosis (ICMR-NIRT), Chennai.

Four identified high risk groups namely, Health Care workers, antenatal and postnatal women, people on immune suppressive treatment and patients with chronic kidney diseases are being screened for TB (CBNAT / X-Ray /Smear) from their regular outpatient department of the selected 4 tertiary care centres.

Phase 1: Retrospective secondary data collection from registers (data collection completed)

Phase 2 : Prospective cross sectional survey .

Current Status: Prospective data collection started for phase 2. Hitherto, a total of 2149 patients were screened. Out of these, 33 were newly diagnosed as TB. Of these 33 patients, 17 patients were on immunosuppressive therapy, 15 were chronic kidney patients and, one patient was a healthcare worker. Data collection will be completed by December 2020.

DEPARTMENT OF HEALTH ECONOMICS

STUDIES IN PROGRESS:

HE-1: Establishment of Regional Resource Centre for Health Technology Assessment in India (HTA-In)

Principal Investigator	:	Dr. M Muniyandi
Source of funding	:	DHR, MoHFW, New Delhi
Study period	:	2018-2021

Background: Ministry of Health and Family Welfare (MoHFW), Department of Health Research (DHR) had set up a system for the evaluation of appropriateness and cost effectiveness of the available and new health technologies in India as part of the research governance mandate of the DHR. The purpose of HTAIn is to design and institutionalise HTA that embodies modern best international practice which features transparent, inclusive, fair and evidence based decisions. These HTA evidences would serve as an important tool in prioritising national health spending on various health technologies such as devices, medicines, vaccines, procedures and systems developed to solve a health problem and improve quality of life. In this context HTAIn would make recommendations to the Government of India after suitable HTA of medical technologies, interventions and procedures for introduction / procurement in India.

Objectives

- To inform Government health department officials about undertaking public health programs (e.g. immunization, screening, and environmental protection programs).
- To inform research agencies about evidence gaps and unmet health needs.
- To inform hospitals, health care networks, purchasing organizations, other health care organizations, and help in decisions regarding technology acquisition and management.
- To inform clinicians and patients about the appropriate use of health care interventions for a particular patient's clinical needs and circumstances.

ICMR-NIRT Activities

- The resource centre at ICMR-NIRT will provide technical support for HTA.
- To provide necessary input and technical support to DHR for developing a policy perspective for HTA for use in public health programs in the country.
- To promote introduction and assessment of new and existing health technologies in the system and will provide support for adoption of health technologies.
- This resource centre will undertake HTA in terms of medical effectiveness, cost effectiveness, appropriateness, efficacy, safety, psychological, social, ethical, organizational and economic aspects.
- To build capacity in the country towards Health Technology Assessment.
- To support evaluation of health technologies for industry through DHR for products that may enter public health domain.

Summary and Progress: For this project we have been working closely with Government of Tamil Nadu and Government of India. Based on their demands and priorities we received different topics for Health Technology Assessment. So far we developed three proposals (1) Health Technology Assessment for screening of Type 2 Diabetes & Hypertension in India; (2) Health Technology Assessment for screening of Hepatitis B and C at Primary Health centers in Tamil Nadu; and (3) Health Technology Assessment for implementation of blood counters for diagnosis of dengue at primary health care settings in Tamil Nadu state. We received fund for the proposal on HTA for screening of Type 2 diabetes & hypertension in India and initiated. Study on HTA for screening of Hepatitis B and C at Primary Health centres in Tamil Nadu was presented in the Technical Appraisal Committee (TAC) meeting in DHR, MoHFW, New Delhi. We also conducted a systematic review and meta-analysis workshop for capacity building. The study is in progress.

HE-2: The Evaluation of a standard treatment regimen of anti-tuberculosis drugs for patients with MDR-TB Stage II (STREAM II) – Health Economics Component

Principal Investigator	:	Dr. M Muniyandi
Source of funding	:	Liverpool School of Tropical Medicine, UK
Study period	:	2018-2022

Background: Despite the widespread availability of an efficacious and affordable regimen and strategy for managing drug susceptible TB, the emergence of multidrug resistant TB (MDR-TB) remains a major challenge for global TB control efforts. The STREAM II trial is assessing whether the proportion of patients with a favourable efficacy outcome of new MDR-TB regimen is superior to that of a shortened regimen. The health economics component of the STREAM II trial in India aims to assess the impact of four alternative MDR-TB regimens.

Objectives

- To assess the costs (direct and indirect) imposed on patients enrolled in the study by MDR-TB treatment regimen.
- To assess changes to employment, socioeconomic status and financial well-being on patients enrolled in the study by MDR-TB treatment regimen.
- To assess health-related quality of life during treatment, and its relation to regimen and adverse events.
- To assess the health system resources required to provide MDR-TB treatment and associated patient care by MDR-TB treatment regimen.

Methodology

Perspective: A societal perspective will be taken, so that health systems and patient costs are included.

Intervention: Patients randomised to the control arm will receive usual clinical care based on the application of the 'WHO guidelines for management of MDR-TB' under RNTCP. Patients allocated to the study regimen will be treated at facilities that will have staff trained on the shorter standardised treatment regimen of MDR-TB. An economic

evaluation comparing two arms study regimen and control regimen will be conducted.
Study population: The participants of this study will be patients with MDR-TB and caregivers.
Data collection: Data related to health system and patient costs will be collected. For the health system, we need to estimate costs based on the quantities of resources consumed in the system for delivering different treatment regimens and the prices of those resources. For patients, we need to understand the economic benefits, if any, of one regimen over the other through estimating the costs patients incur throughout the treatment period and how such expense affects lives and livelihoods of patients of different socio-economic status. An additional analysis of health-related Quality of life (HRQoL) will also be conducted; data for this will be collected using the EQ5D-5L tool at the same time as the health system and patient cost data.
Data Analysis: Various costs will be analysed using excel and an operational model. The evaluation will include using patient pathway models to understand the impact on costs and quality of life.
Outcome Measures: The main health economics-related outcomes will be mean incremental costs incurred by patients, incremental cost to the health system of the study regimen compared with the control regimen (savings will be recorded as negative incremental costs). Additional health economics outcomes will be mean costs incurred by patients analysed by treatment outcome, patient costs by category (direct medical costs, transport, food and accommodation costs, and cost of guardians/accompanying persons and lost time), health systems cost by category (training, monitoring, service delivery and drugs) and costs related to adverse events.

Summary and Progress: So far we have collected information from a total of 49 patients enrolled in the main STEAM trail. Basic information on socio-economic status completed for all patients. Data collection on costs information regarding treatment and follow-up is in progress. Data collection on health status and quality of life of patients during treatment and follow-up is also in progress.

<u>HE-3:</u> Health Technology Assessment for screening of Type 2 Diabetes & Hypertension in India

Principal Investigator	:	Dr. M Muniyandi
Source of funding	:	DHR, MoHFW, New Delhi
Study period	:	2018-2019

Background: Increasing prevalence of diabetes and its related complications may contribute to increase in healthcare costs. Estimates indicate that a diabetic person utilizes twice as much resource than a non-diabetic person. Hypertension contributes to approximately 1.6 million deaths annually in India. The direct costs for treatment and complications and indirect costs arising from productivity losses due to diabetes and hypertension are huge. Moreover, coexistence of hypertension and diabetes also has economic implications. It was reported that on an average a diabetic patient with hypertension spent 1.4 times more than a diabetic patient without

hypertension. Government of India has already planned for carrying out screening of diabetes and hypertension and developed framework. However there are concerns on issues like which are the appropriate screening strategies for these diseases in developing countries. With this background we undertook an economic evaluation study with a holistic view of the screening program for diabetes and hypertension relevant to Indian context. We evaluated and identified an optimal screening strategy for diabetes and hypertension for India outlining preferred approach to screening, who should be screened with which tests for screening and confirmation and how often it should be done.

Objectives

- To assess the Quality Adjusted Life Years (QALYs) gained as a result of various screening strategies for Type 2 diabetes and hypertension in India.
- To ascertain the incremental cost per QALY gained with screening strategies for Type 2 diabetes and hypertension compared to no screening in India.
- To ascertain incremental cost per QALY gained with screening strategies for Type 2 diabetes and hypertension at 1 versus 3 versus 5 yearly frequency.
- To ascertain incremental cost per QALY gained with screening strategies at age groups of 18-30 years, 30-65 years, 25-65years and 40 to 65 years.

Methodology: The present study was undertaken as a model-based cost effectiveness evaluation of various screening strategies for diabetes and hypertension. The intervention involved screening and detecting individuals through risk score, biochemical tests like random blood glucose, fasting blood glucose and HbA1c for diabetes and sphygmomanometer for hypertension. The comparator was no screening scenario with conventionally diagnosed individuals with diabetes and hypertension. The cost-effectiveness analysis involved information on epidemiological demographic parameters related to diabetes and hypertension; cost parameters in intervention and comparator scenario; and health outcomes like life years and quality adjusted life years. Epidemiological and demographic parameters were collected through systematic review. Cost parameters from both patient and health system was collected through primary data using interview schedule. Health outcomes are assessed through EQ5DL quality of life measurement scale. A decision model in terms of combination of decision tree and Markov model to compute incremental cost-effectiveness ratios (ICERs) for different screening strategies for diabetes and hypertension was developed. This was a collaborative study with PGIMER, Chandigarh. NIRTs role in this study was to provide health system cost from Tamil Nadu and involve in analysis.

Salient finding: Our findings highlighted that, in the absence of screening, there are 9267, 28,206, 2982, 3030 and 1239 cases of stroke, myocardial infarction, end stage renal disease (ESRD), amputation and blindness due to diabetes and hypertension per 100000 population respectively. With the implementation of annual population based screening with random blood glucose test

followed by fasting glucose test (as compared to no screening), there is a reduction in 23%, 13%, 27%, 40% and 35% cases of stroke, myocardial infarction, end stage renal disease, amputation and blindness per 100000 population respectively. In the scenario of no screening, for a cohort of 100000 population, the lifetime treatment cost of complicated cases comprised of around 96.5% (Rs 7794 million) of the total cost, followed by cost of treating uncomplicated cases (3.37%; Rs 271 million). In the case of annual screening, treatment cost of uncomplicated cases constitutes the major component (64.5%; Rs 10929 million), followed by the cost of treating complicated cases (35%; INR 5980 million). The cost of implementing screening comprised of 0.5% (Rs 65 million) of the total cost. Implementation of annual population-based screening with random blood glucose test followed by fasting glucose test (as compared to no screening), lead to gain in 6387 life years, 19,656 quality adjusted life years and reduction in 1259 deaths (due to diabetes and hypertension) per 100000 population respectively. Only screening with once in a lifetime at 30 years of age is cost effective. Any increase in frequency of screening to every 5 year or 3 year or annually is not cost effective at the current level of health care utilization pattern for diabetes and hypertension. However, if the share of treatment for uncomplicated diabetes and hypertension at the proposed health and wellness centres (HWCs) rises, population-based screening for diabetes and hypertension starts to become cost effective. Once the HWCs treat at least 50% of the total uncomplicated cases of diabetes and hypertension, annual population-based screening starts to become cost effective. In addition, a feasibility and landscape analysis was undertaken to explore the challenges and opportunities with regard to population-based screening for diabetes and hypertension in India.

Conclusion: This study explored potential health system challenges and opportunities that need to be considered for Population Based Screening (PBS) from the health system perspective. There are potential challenges existing in various aspects of PBS were also recognized in improving coverage rates for screening, subsequent referral for confirmatory testing and put on treatment, focussing on follow up of those who started on treatment and how to achieve control for the disease conditions. Interventions that focus on the primary prevention like lifestyle management, health education and counselling should be the mainstay focus to ensure healthy lives. These findings will be useful to policy makers and health planners to improve the diabetes and hypertension in India.

HE-4: Social network analysis as a tool to improve active case finding at community level in Chennai, South India

Principal Investigator	:	Dr. N Karikalan
Source of funding	:	UNOPS
Study period	:	2017-2020

Background: Early case detection and treatment is important for controlling TB. Delayed TB diagnosis can increase the transmission of infection, hasten disease progression and increase the risk of mortality among populations. Active case finding (ACF) is being prioritized as a

strategy under the RNTCP in India to address the delay in diagnosis. ACF is resource intensive and holds considerable challenges since TB is an air borne disease and the susceptible individuals or subgroups cannot be singled out easily. Alternate and novel strategies (e.g., Social Network Analysis) for implementing ACF at the community level are needed. Social network analysis (SNA) is the study of social structure which connects individuals. Social network has three sub components, which are: i) The network relationships; ii) Network structures and iii) The network functions The characteristics of social network members with whom any TB patient interact greatly influence the TB transmission within the network. Social networks and the geographical places of networking contextualize the TB transmission in any community. There is a need to systematically understand the social network of TB patients which could provide insights on underlying social structure and the key social network members who influence the TB transmission within the network members and the key geographical locations which facilitate the transmission. In this study, we aim to test the feasibility of SNA as a strategy to explore TB transmission patterns which otherwise could have missed from being detected by routine contact investigation.

Objectives

- To identify key social network members facilitating TB transmission in North Chennai for complementing active case finding efforts
- To identify places of social congregation of TB cases and their social network contacts which facilitate TB transmission in North Chennai for complementing active case finding efforts.
- To identify TB case and contact dyads and other social network relationships patterns in program settings of North Chennai

Methodology: This is a mixed method exploratory study involving both quantitative and qualitative study methods. The study period is 24 months and is being implemented since November 2017. In phase one, TB patient's routine demographic, diagnostic and treatment related information from treatment and lab registers records were obtained from RNTCP TUs/DMCs of Chennai corporations in the North Zone since 2013. Analysis of this secondary data set was used to assess the geographic distribution of TB patients in specific streets and locations in the study TU. In the next phase, qualitative ego centric social network information was collected using in-depth qualitative interviews (IDI). Further quantitative ego centric social network survey was implemented with a target sample size of 300 newly diagnosed pulmonary TB patients in North Chennai TUs (Puliynathope TU, Thanthai Periyar TU, Vyasarpadi TU). This involved social network survey of index TB cases and their social network contacts. The pattern of socialization and the places of social aggregation of TB patients and their primary contacts were identified through questionnaire. The TB status of social network contacts of index cases was collected and further verified. We identified the TB suspects and patients who are among the primary and secondary social contacts of TB patients.

Results: A total of 300 Index TB patients (Ego) were interviewed. A total of 2544 primary network

members (Alters) were identified using name generator method in study TUs. Almost one thirds of social network contacts (non-household) of the index cases had TB retrospectively or in the present. Especially the strong prediction of TB among neighbourhood network-contacts is strengthened by the evidence that they have lived in close geographical proximity to the network- index patients. Close geographical proximity and consistent social relationship thus could hold significant potential in driving disease transmission outside households. Our social network survey method complimented by targeted geo-mapping of individual residences might be more useful than routine contact tracing, community spatial mapping, post-hoc network analysis and costly genome sequencing methods. The higher likelihood of alcoholic network-contacts to have TB underscores the contextualizing role of alcohol intake in TB endemic settings. This study also identified key hotspots and places of TB clustering in the study setting where case finding activities could be prioritized screening camps. Geographic proximity to hot sports and health facility was also found to have impact on the diagnostic and treatment aspects of index cases.

Conclusion: While we adhered to standard ego-centric personal network survey approach based only on reports and references of network index patient, alternatively screening of all identified social network contacts could have yielded more TB cases prospectively and help strengthen the NTEP program.

HE-5: Health Technology Assessment for screening of Hepatitis B and C at Primary Health centers in Tamil Nadu

Principal Investigator	:	Dr. M Muniyandi
Source of funding	:	DHR, MoHFW, New Delhi
Study period	:	2019-2020

Background: Hepatitis B viral infection (HBV) affects nearly 50 million people in India with an average prevalence of 4%. Hepatitis C virus infection (HCV) prevalence in the general population is estimated to be between 0.09-1.5% and it is reported that there are 6-12 million people with HCV in India. Prevalence of HBV and HCV was higher than average in key population like persons with sexual risk behavior, blood donors, individuals with STD, people living with HIV and men having sex with men (MSM), chronic kidney disease, on dialysis, thalassemia, haemophilia, leukaemias, those receiving immunosuppressives and cancer chemotherapy.

Methods: Decision tree cum Markov model was developed to estimate the cost effectiveness of strategies to screen and treat HBV and HCV or prevent HBV in population with various prevalence. The cost effective analysis was performed for the following strategies: (Strategy-1) screen for HBV infection and provide early treatment for positives and provide vaccination for negatives (proposed) (Strategy-2) screen for HCV infection and provide early treatment (proposed). The outcomes of the proposed strategy were expressed in incremental cost effectiveness ratios (ICERs) per quality adjusted life years (QALY) and life years (LY) gained and death averted

as compared to current strategy. Discount rate of 3% was applied for cost and QALY.

Findings: The strategy-1 had ICER of ₹ -1, 80,749 per QALY gained and strategy-2 had ICER of ₹ -1, 14,571 per QALY gained as compared with current strategy. Strategy-1 resulted in 505 discounted QALYs gained and Strategy-2 resulted 38 discounted QALYs gained. In terms of death averted 293 and 4 from strategy-1 and strategy-2 respectively. The other finding in the present study was shown in the OWSA, the quality of life score holds much influence on the ICER for HBV and HCV intervention. The proposed intervention will incur an additional budget of ₹ 142 crores for HBV and ₹ 57 crores for HCV implementation. It will vary depending on the proportion required for intensive care treatment for liver disorders. It was also estimated that the proposed intervention reduced out of pocket expenditure significantly to the patient.

Conclusion: The current results confirmed that the proposed interventions were dominant compared with current practice. It also indicates that the proposed intervention is worthwhile as result showed the screening key population for HBV and HCV at PHC level was more cost saving with negative ICER value per QALY gained.

<u>HE-6:</u> Measuring socio-economic risk-benefits and health related quality of life changes associated with tuberculosis disease disclosure

Principal Investigator	:	Dr. N Karikalan
Source of funding	:	IMPRESS Scheme of Indian Council of Social Science Research, New Delhi
Study period	:	2019-2021

Background: Tuberculosis (TB) patients often hide their disease from family, friends, neighbors, community and at workplace due to the fear of stigma and discrimination resulting from disclosure of the disease. TB remains one of the highly stigmatized diseases in India till date, and patients suffer both perceived and enacted stigma throughout their disease periods and even after completion of treatment. Disclosure has been extensively studied in the HIV disease context which is a highly stigmatized disease like TB. With regard to TB, only a few studies have assessed the disclosure status of TB patients and have assessed their experience after disclosing their disease.

Objective: To estimate the proportion of TB patients who disclose their disease status at different time points from diagnosis till the completion of treatment and to discern the disclosure patterns of TB patients.

Methodology: It is a prospective observational study involving quantitative methods. The study will be conducted in RNTCP treatment units in Greater Chennai and adjoining urban areas. Newly diagnosed pulmonary and extra-pulmonary TB patients registered for treatment under National TB Elimination Programme (NTEP). Patients who are lost to follow up will also be included in our study population. The study will be multi-

centric with the time frame of 24 months. Each patient will be followed for a period of six months or till treatment extension period.

Summary and Progress: The study was initiated in December 2019 after the recruitment of two Research Assistants and two days training has been provided for them in the month of January 2020. The study team was oriented towards the study proposal, objectives, research plan and the study tools. The study field work started in the last week of January 2020, and so far the study team had completed 80 baseline interviews among TB patients eligible for our study. Data was collected in the hard copy questionnaire and was then entered in the MS-Excel software. A template for data entry in excel has been created by the study team. So far twelve TB treatment units have been covered for conducting eighty baseline interviews with the TB patients newly registered. The follow up interviews for the baseline completed patients have been fixed for the mid line interview and will be conducted as per the time line for each patient.

ELECTRONIC DATA PROCESSING DIVISION

Electronic Data Processing Unit

The Electronic Data Processing division plays a key role in also setting up, maintaining and facilitating all IT related infrastructure and electronic facilities within NIRT. It also manages the data for all the epidemiological surveys and some operational research studies.

Maintenance of IT equipments:

The EDP division maintains an internal network of over 100 desktop workstations, network based equipment, operating systems and servers. 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.

The division helps in facilitating the 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 the staff under the NIH-ICER project. The ICER IT Support Team has been providing the 24/7 support and monitor to the data centre, Network Service, Application Service, Internet Service for Infrastructure support for ICMR-NIRT.

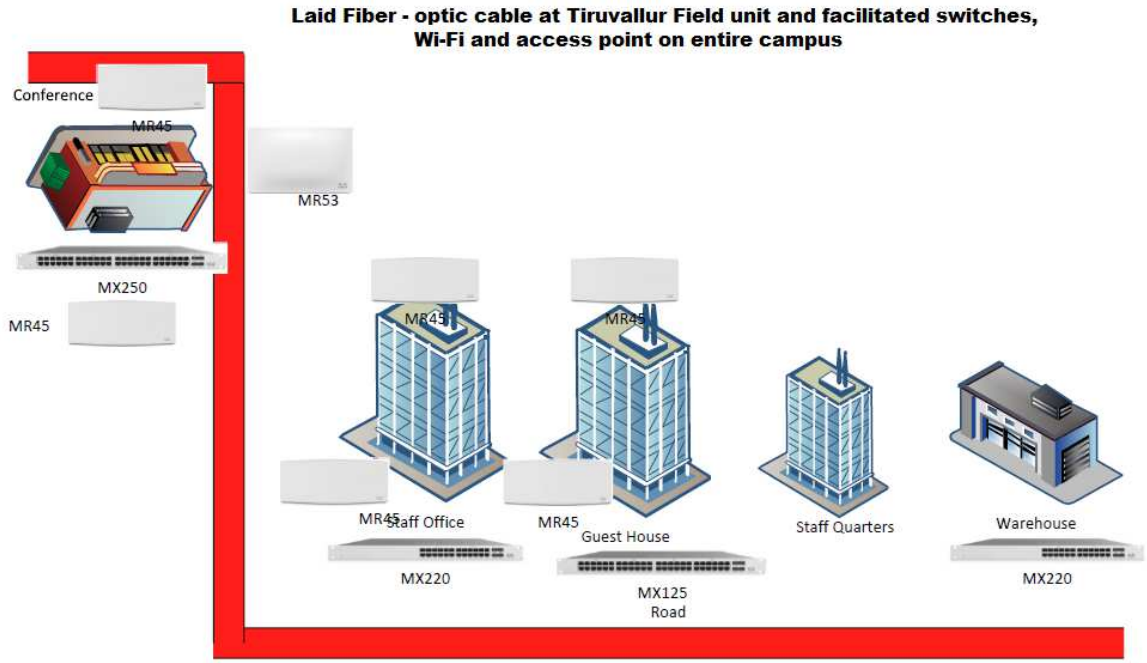
Highlights:

- User Account with new email access – 20 NIRT users, 75+ User Account extended, 80 Project Users
- Radio Frequency Facility enabled through National Knowledge Network Project to Tiruvallur & Madurai site funded by ICER Project.
- Bio-thermic Phase III completed in Main Lab Building, ICER, and Clinic Facility to monitor Freezers, Incubators, humidity, nitrogen Gas(-180) and network requirements completed.
- Bio-Thermic Facility for NIRT TB Vaccine Trial in Chennai & Tiruvallur
- New Core Switch installed in Data Centre and enabled 1Gig Speed Data transfer in Internal Network.
- Video Conference Facility has been built in Director's Office & Main Lab Building.
- New Network Connection in Canteen, Main Lab & Clinic Buildings
- Network Restructure with numbering in Main Lab Building Ground Floor Facility
- Basic Network Infrastructure with Fibre uplink for Tiruvallur Site created.
- Migrated the following servers to NIRT Domain from old domain.
    - Application Servers
    - Federated and Access Management Server
- New servers have been installed for National TB Prevalence Study (NTBPS) and Tamil Nadu TB Prevalence Study (TNTBPS) Research studies which are all in production.
    - Temperature Monitoring Server
    - Physical Application, Database & Image openMIND Server for National TB Prevalence Study
    - Storage Upgrade for Physical Servers for National TB Prevalence Study
    - TNTBPS server ready for production
- Project planning has been initiated for as proposal level for funding to upgrade the Data Centre with additional PAC unit and network

cabling, adding more power requirement for the Data Centre from 63AMPS to 250AMPS, adding additional Server Racks to Data Centre to equip more servers and additional storage facility

- Project planning has been initiated as proposal level for funding for converting one of the conference room to Tele-Learning room to user for the online training and conference.
- Warranty support for desktops and network printer to NIRT by ICER IT Support Team
- Video conference facility support to NIRT by ICER IT Support Team
- Continued structured and Managed Network Support to NIRT Chennai, Madurai & Tiruvallur site by ICER IT Support Team.
- External Service are provided to NIRT Staff while travelling,
  - ✓ https://mail.nirt.res.in
  - ✓ https://passwordselfservice.nirt.res.in
  - ✓ https://workingwith.nirt.res.in
  - ✓ https://federation.icerindia.org/nih-library
  - ✓ https://eduroam.nirt.res.in
  - ✓ https://prevalence.nirt.res.in
  - ✓ https://rcap.nirt.res.in
  - ✓ https://mcap.nirt.res.in
  - ✓ https://tempatures.nirt.res.in
- NIRT was the first institute in ICMR to be registered with Access Management Facility (AMF). The INFLIBNET Centre, as one of its core mandates provides access to scholarly e-resource to universities and research institutes in India under the e-ShodhSindhu. The INFED is being set-up as a centralized agency to coordinate with member institutions for the implementation of user authentication and access control mechanism distributed across participating institutions using standardized rules and metadata for exchange of attributes.

Fig.22:



Data entry/verification work:
The quantum of data records of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2019 to March, 2020 is shown (Table 32)

Table 32:

	No. of records entered and verified
Using epidata 3.1	
Clinical studies	1039
School Endline Survey	576
School study Endline Survey - Ambassadors	185
House hold Contact Card	171
PAMPER Study Double Entry	647
Using REDCap	
Patients' perception on Quality of Care in TB care settings in Chennai	555
Universal Drug susceptibility testing Facility survey data	45
House hold contact study 12th month follow up	1434
Using Excel	
IE-ART Secondary Data	4466
NTPBS	2109
Total	11227

REDCap

REDCap is a secure web application for building and managing online surveys and databases. It is specifically geared to support online or offline data capture for research studies and operations.

NIRT has obtained license under the REDCap consortium and has become functional from August, 2018.

REDCap Users:

Currently, the NIRT REDCap has 203 active users.

REDCap Projects:

Currently there are 35 projects under the NIRT REDCap. Of these 35 projects, 17 are in production mode, 9 under development and 9 projects are archived. Mobile apps are used for data collection by 8 projects.

Data Mart for NIRT:

A data warehouse solution called the NIRT DataMart is in progress in collaboration with OCICB/NIAID, National Institute of Health, US to convert all old data sets of EPID, ICER & Clinical studies. The role of EDP in this collaborative work is to prepare the raw data sets and also the ETL (Extraction, Transformation and Loading) programming based on CDISC specifications.

Supportive role to NIRT administration's eGovernance:

The division delivers a supportive role to the administration towards its electronic based administrative services like eProcurement and eMarketing, which are internet based avenues provided by the Central Government.

eOffice is a digital workplace solution, provided by the National Informatics Center, to achieve a simplified, responsive, effective and transparent working of all government offices. EDP facilitates the effective utilization of this digital solution at NIRT. In the past year, EDP customized the Leave Management module within eOffice for NIRT and now all the permanent staff apply their leave through this eLeave management setup.

INTERNATIONAL CENTRE FOR EXCELLENCE IN RESEARCH

STUDIES COMPLETED:

ICER-1: Effect of helminth infection on antigen-specific immune responses in latent tuberculosis in South India.

1. Immunology of TB and its co-morbidities: Helminth Coinfection Alters Monocyte Activation, Polarization, and Function in Latent Mycobacterium tuberculosis Infection.

Principal Investigators	:	Dr. Subash Babu
Source of funding	:	ICER and DST-SERB
Study Period	:	2013-2020

Background: Helminths are considered to be a risk factor for the development of active TB in individuals with latent TB infection (LTBI). This is due to the ability of helminth infection to modulate both innate and adaptive immune responses in LTBI, with major effects on T and B cells. With regards to helminth–TB coinfection, very few studies have examined the role of helminth infections in modulating monocyte function in the context of M. tuberculosis coinfection. Helminth infections are known to induce arginase-1 in macrophages, which promotes lung inflammation and disease severity in TB, and to dampen phagolysosomal maturation in macrophages (14). Helminth infections can modulate TLR expression and function on monocytes and induce alternative activation of macrophages in the setting of *M. tuberculosis* coinfection. However, very little is known is about the effect of helminth infections and subsequent anthelmintic treatment on monocyte function, in particular activation (as determined by expression of CD154, CD80 and CD86), M2 polarization (as determined by CD206 and CD163 expression), and function (as determined by phagocytosis, respiratory burst response, and cytokine response) in LTBI.

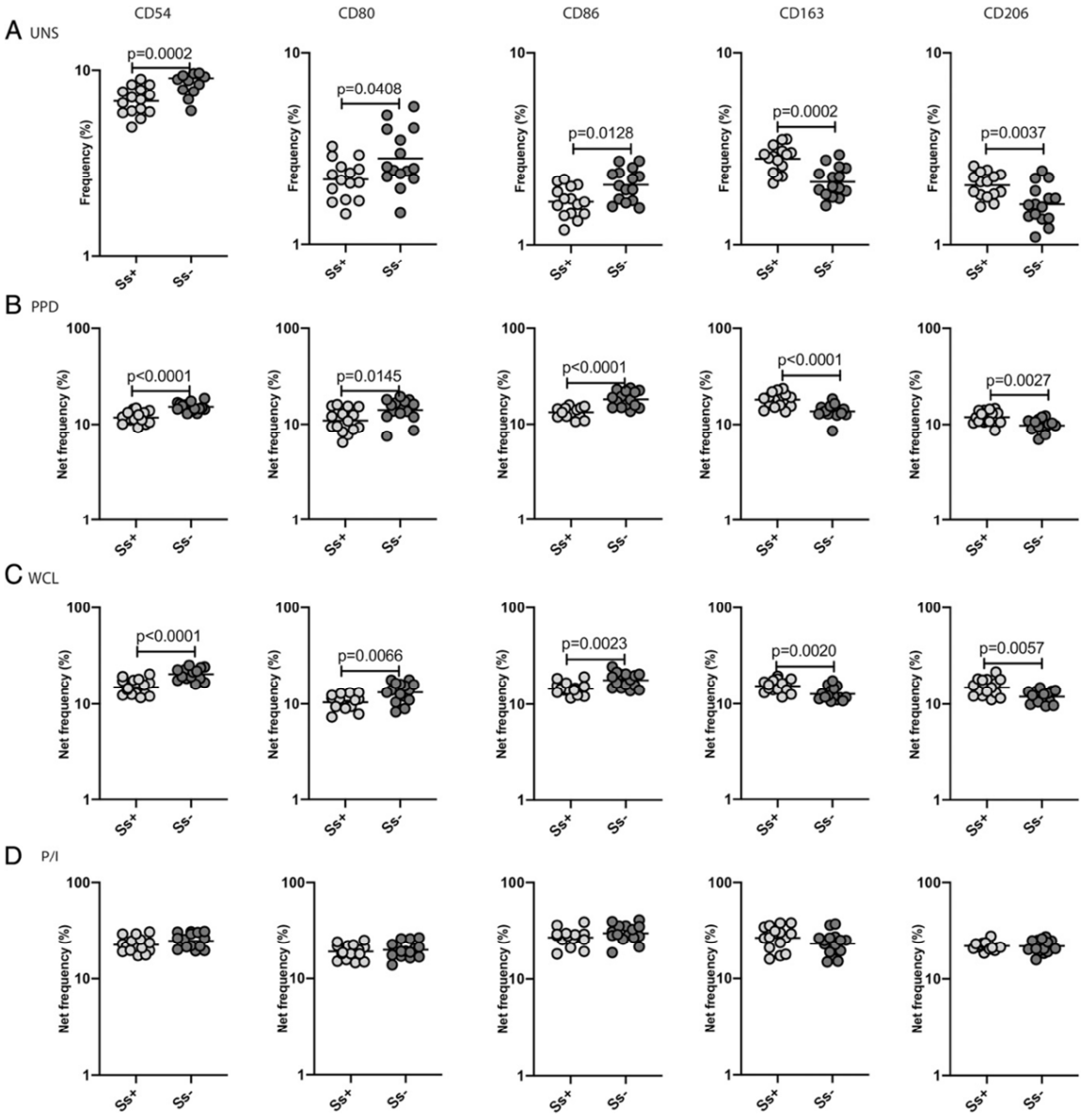
Aims/ Methodology: we sought to examine the activation, polarization, and function of monocytes in a helminth–LTBI coinfection. We examined the activation, polarization, and function of human monocytes isolated from individuals with LTBI with or without coincident *Strongyloides stercoralis* infection (*S. stercoralis*–positive and *S. stercoralis*–negative respectively). We measured the frequencies of monocytes expressing CD54, CD80, CD86, CD206, CD163 at baseline (absence of stimulation) and in response to mycobacterial-Ag stimulation than monocytes from *S. stercoralis*–negative individuals. We also measured the phagocytic activity and respiratory burst activity following mycobacterial-Ag or LPS stimulation and following definitive anthelmintic therapy.

Results: Our data reveal that the presence of S. stercoralis infection is associated with lower frequencies of monocytes expressing CD54, CD80, CD86 at baseline (absence of stimulation) and in response to mycobacterial-Ag stimulation than monocytes from *S. stercoralis*–negative individuals. In contrast, *S. stercoralis* infection was associated with higher frequencies of M2-like monocytes, as determined by expression of CD206 and CD163. Monocytes from *S. stercoralis*–positive individuals had a reduced capacity to phagocytose or exhibit respiratory burst activity following mycobacterial-

Ag or LPS stimulation and were less capable of expression of IL-1b, TNF-α, IL-6, and IL-12 at baseline and/or following Ag stimulation compared with those without *S. stercoralis* infection. In addition, definitive treatment of S. stercoralis infection resulted in a significant reversal of the altered monocyte function 6 month after anthelmintic therapy. Finally, T cells from *S. stercoralis*–positive individuals exhibited significantly lower activation at baseline or following mycobacterial-Ag stimulation.

Conclusion: Our data highlight the induction of dampened monocyte activation, enhanced M2 polarization, and impaired monocyte function in helminth–LTBI coinfection. Our data also reveal a different mechanism by which helminth infection modulates immune function in LTBI.

Fig. 23. LTBI/*S. stercoralis* coinfection is associated with decreased monocyte activation and increased M2 polarization.



Baseline and Ag-stimulated frequencies of monocytes expressing activation and M2 markers were determined by flow cytometry in S. stercoralis–positive (Ss+; light gray) and *S. stercoralis*–negative (Ss-; dark gray) individuals. The (A) baseline (unstimulated) as well as (B) PPD-, (C) WCL-, and (D) P/I-stimulated frequencies of monocytes expressing activation markers (CD54, CD80, CD86) and M2 markers (CD163 and CD206) are shown. Net frequencies were calculated by subtracting baseline frequencies from the Ag-induced frequencies for each individual.

2. Immunology of TB and its co-morbidities: Helminth infection modulates systemic proinflammatory cytokines and chemokines implicated in type 2 diabetes mellitus pathogenesis.

Principal Investigator	:	Dr. Subash Babu
Source of funding	:	ICER
Study period	:	2018-2020

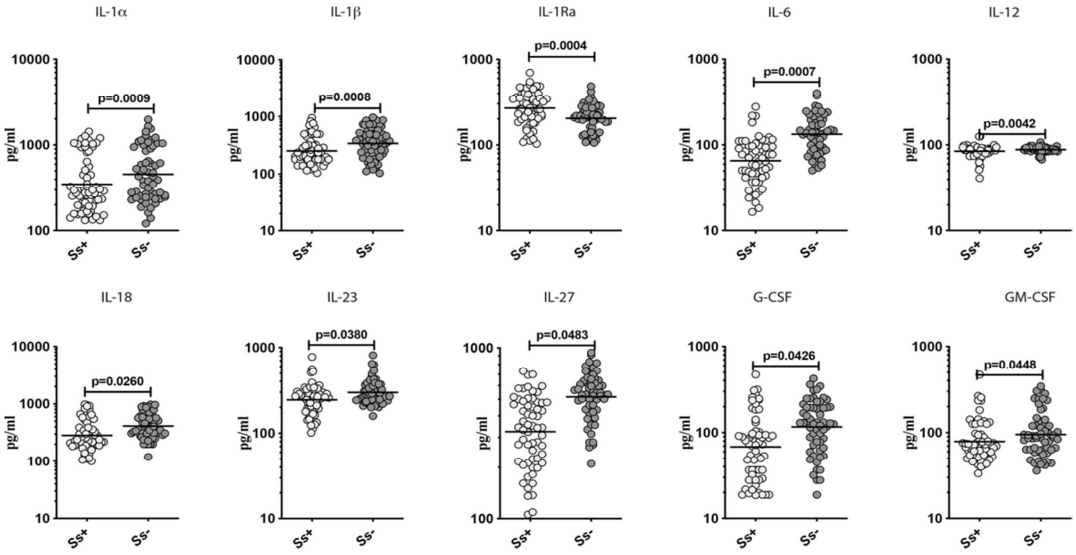
Background: The prevalence of helminth infections exhibits an inverse association with the prevalence of Type 2 diabetes mellitus (T2DM), and helminths are postulated to mediate a protective effect against T2DM. However, the biological mechanism behind this effect is not known.

Aims/ Methodology: We postulated that helminth infections act by modulating the pro-inflammatory cytokine and chemokine milieu that is characteristic of T2DM. To examine the association of cytokines and chemokines in helminth-diabetes co-morbidity, we measured the plasma levels of a panel of pro-inflammatory cytokines and chemokines in individuals with *Strongyloides stercoralis infection* (Ss+) and T2DM at the time of Ss diagnosis and then 6 months after definitive anthelmintic treatment along with uninfected control individuals with T2DM alone (Ss-).

Results: Ss+ individuals exhibited significantly diminished levels of the pro-inflammatory cytokines– IL-1α, IL-1β, IL-6, IL-12, IL-18, IL-23, IL-27, G-CSF and GM-CSF and chemokines– CCL1, CCL2, CCL3, CCL11, CXCL1, CXCL2, CXCL8, CXCL9, CXCL10 and CXCL11. In contrast, Ss+ individuals exhibited significantly elevated levels of IL-1Ra. Anthelmintic treatment resulted in increased levels of all of the cytokines and chemokines.

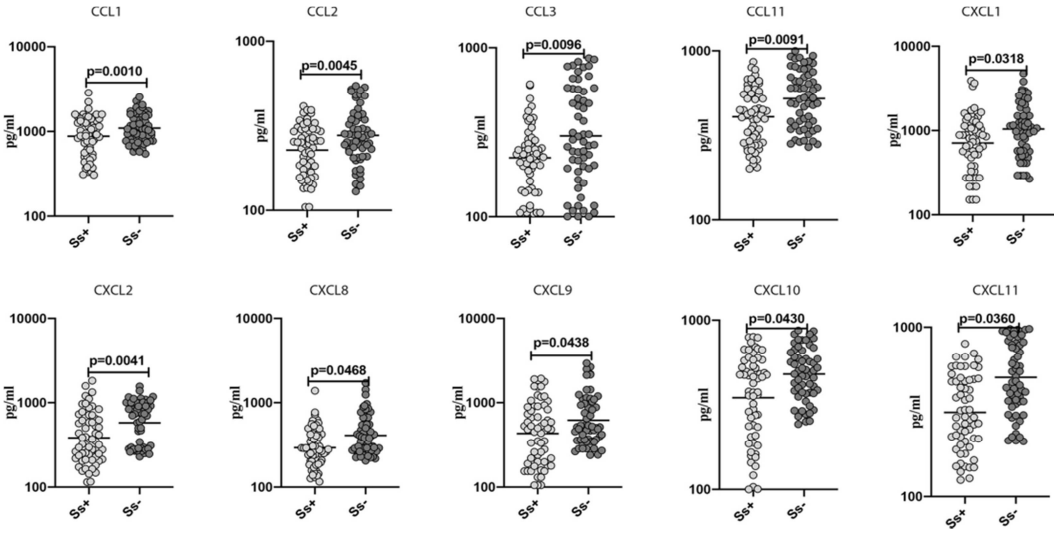
Conclusions: Thus, helminth infections alleviate and anthelmintic therapy partially restores the plasma cytokine and chemokine levels in helminth-diabetes co-morbidity. Our data therefore offer a plausible biological mechanism for the protective effect of helminth infections against T2DM.

<u>Fig.24:</u> Diminished plasma levels of pro-inflammatory cytokines in Ss+ individuals with T2DM.



Plasma levels of IL1-α, IL1-β, IL-1Ra, IL-6, IL-12, IL-18, IL-23, IL-27, G-CSF and GM-CSF cytokines were measured in Ss+ and Ss- individuals. Each dot is an individual subject with the bar representing the geometric mean (GM). p values were calculated using the Mann-Whitney U tests followed by Holm's correction for multiple comparisons.

<u>Fig.25:</u> Diminished plasma levels of pro-inflammatory chemokines in Ss+ individuals with T2DM.



Plasma levels of CCL1, CCL2, CCL3, CCL11, CXCL1, CXL2, CXCL8, CXCL9, CXCL10 and CXCL11 chemokines were measured in *Ss*+ and *Ss*- individuals. Each dot is an individual subject with the bar representing the geometric mean (GM). p values were calculated using the Mann-Whitney U tests followed by Holm's correction for multiple comparisons.

ICER -4: Characterization of immune responses in TB lymphadenitis

Principal Investigators	:	Dr. Subash Babu; Dr. D. Baskaran
Source of funding	:	ICER
Study Period	:	2015-2020

1. Immunology of extra-pulmonary TB: Diminished type 1 and type 17 cytokine expressing - Natural killer cell frequencies in tuberculous lymphadenitis

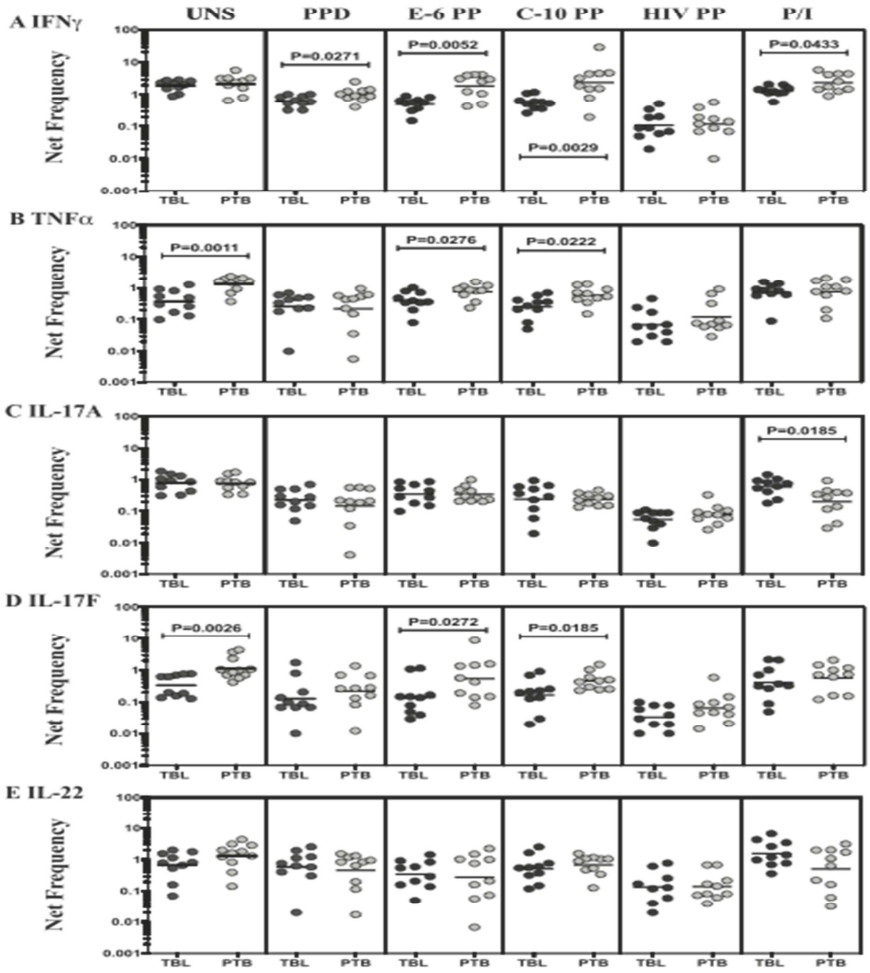
Background: Tuberculous lymphadenitis (TBL) is associated with the expansion of CD4+ and CD8+ T cells expressing Type 1 and Type 17 cytokines in the peripheral blood. However, the expression pattern of cytokine producing natural killer (NK) cells in both the peripheral blood and affected lymph nodes i.e. site of infection in TBL have not been examined.

Aims/Methodology: We have analyzed the baseline and mycobacterial antigen specific NK cell cytokine frequencies in whole blood of TBL and pulmonary tuberculosis (PTB) individuals. We have also examined the NK cell frequencies before and after treatment completion and in peripheral blood versus affected lymph nodes (LN) of TBL individuals.

Results: TBL is characterized by diminished frequencies of NK cells expressing Type 1 (IFNγ, TNFα), Type 17 (IL-17F) cytokines compared to PTB individuals upon antigen-specific stimulation. In contrast, TBL individuals did not exhibit any significant differences in the frequencies of NK cells expressing Type 1 and Type 17 cytokines upon completion of anti-tuberculosis treatment. LN of TBL is associated with altered frequencies of NK cells expressing Type 17 (increased IL-17F and decreased IL-22) cytokines when compared to peripheral blood.

Conclusion*:* we conclude that TBL individuals are characterized by diminished frequencies of NK cells expressing Type 1/Type 17 cytokines.

<u>Fig. 26.</u> Diminished antigen-specific frequencies of NK cells expressing Type 1 (IFNγ, TNFα) and Type 17 (IL-17F) cytokines in TBL.



Whole blood of TBL (n = 10) and PTB (n = 10) individuals were cultured with medium alone or mycobacterial or control antigens for 18 h. By using the harvested cells both baseline and antigen-stimulated cytokine frequencies were analyzed using multi-color flow cytometry. (A–E) Baseline (left panels), (A–E) PPD, ESAT-6 PP and CFP-10 PP (middle three panels), (A–E) HIV Gag PP (second last panels) and (A–E) PMA/I (last right panels) antigen stimulated frequencies of NK cells expressing respective Type 1 (IFNγ, TNFα and IL-2), and Type 17 (IL-17 A, IL-17F, IL-22) cytokines (each black colored round circle represents single TBL individuals, each grey colored round circle represents single PTB individuals). The bar represents the geometric mean values and P values were calculated using the Mann-Whitney U test. The net frequencies were calculated by subtracting the baseline from antigen-stimulated values for each individual. P value < 0.05 is considered as statistically significant.

2. Immunology of extra-pulmonary TB: Low body mass index has minimal impact on plasma levels of cytokines and T chemokines in tuberculous lymphadenitis

Principal Investigators	:	Dr. Subash Babu; Dr. D. Baskaran (email: sbabu@nirt.res.in; baskar.d@nirt.res.in)
Source of funding	:	ICER
Study Period	:	2015-2020

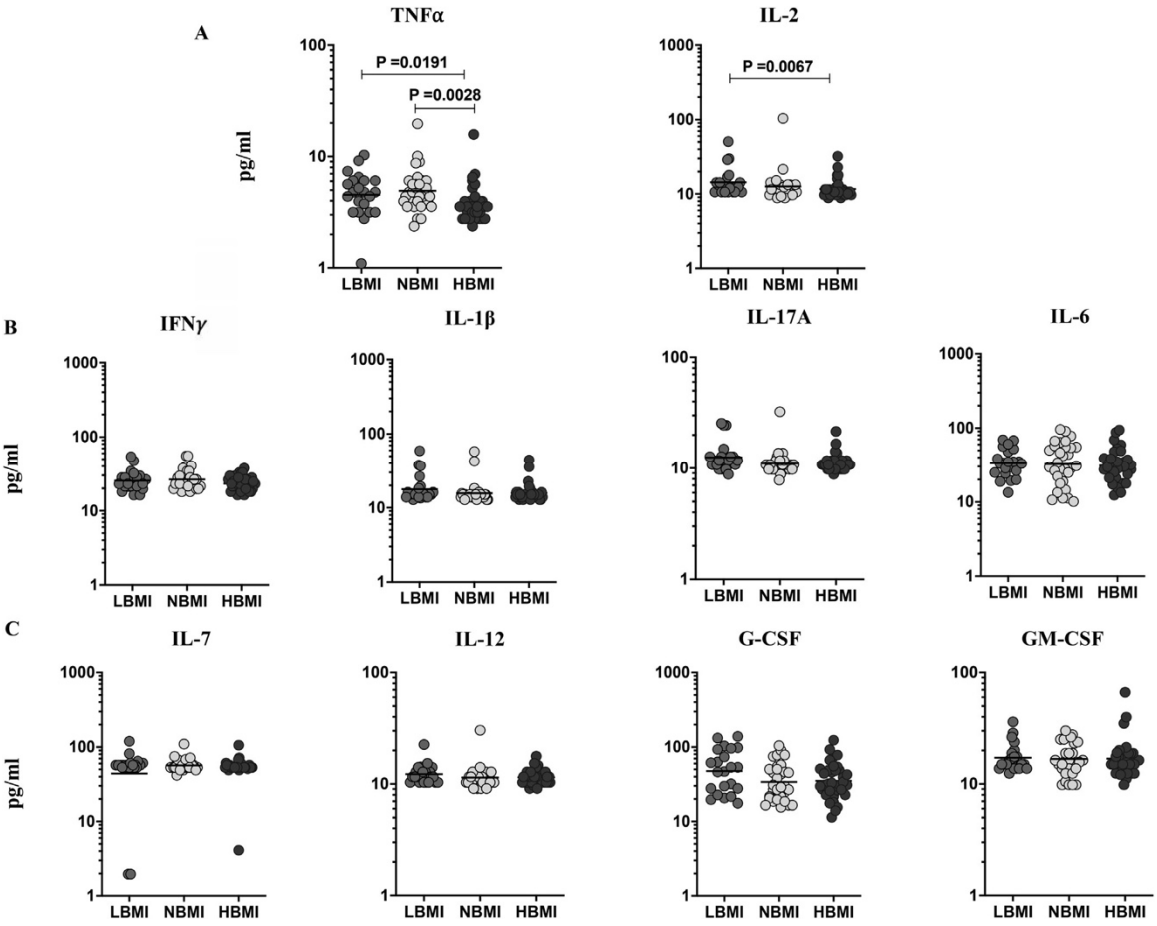
Background*:* Malnutrition, due to low body mass index (LBMI), is considered to be one of the key risk factors for tuberculosis (TB) development. The link between pro and anti-inflammatory cytokines and BMI has been studied in active pulmonary TB. However, the association of BMI with cytokines and chemokines in TB lymphadenitis (TBL) has not been examined.

Aims/Methodology*:* We have examined the plasma levels of a panel of pro- and anti-inflammatory cytokines and chemokines (CC and CXC) in TBL individuals coexistent with different BMI (LBMI, NBMI, HBMI) status.

Results: LBMI with TBL disease is associated with enhanced systemic levels of type 1 (tumor necrosis factor alpha [TNFα], interleukin-2 [IL-2]) and type 2 (IL-4, IL-13) cytokines in comparison with NBMI and/or HBMI. However, other pro-inflammatory (IFNγ, IL-1β, IL-17A, IL-6, IL-7, IL-12, G-CSF, and GM-CSF) and anti-inflammatory (IL-5 and IL-10) cytokines were not significantly different among the TBL individuals with different BMI status. Likewise, no significant differences were observed in the CC (CCL-1, CCL-2/MCP-1, CCL3/MIP1α, CCL4/MIP-1β, CCL11/eotaxin) and CXC (CXCL-1/GRO-α,CXCL2/GRO-β, CXCL9/MIG, CXCL10/IP-10, CXCL11/ITAC 1) chemokine profile among the TBL individuals with different BMI.

Conclusion*:* Our data implies that TBL individuals with LBMI are characterized by minimal effects on plasma cytokines and chemokines in TBL.

<u>Fig.27:</u> Elevated plasma levels of Type 1 cytokines associated with TBL individuals with LBMI.



(A) The plasma levels of Type 1 (TNFα, IL-2) cytokines were analysed by multiplex assay in LBMI (n = 22), NBMI (n = 31) and HBMI (n = 35) coexistent with TBL individuals. (B) The plasma levels of other pro-inflammatory (IFNγ, IL- 1β, IL-17A, IL-6), (C) (IL-7, IL-12, G-CSF and GM-CSF) cytokines were measured by multiplex assay in LBMI, NBMI and HBMI coexistent with TBL individuals. Each circle represents a single individual and the bars represent the geometric means. P values were calculated using the Kruskal-Wallis test with Dunn's multiple comparisons. *P < 0.05, **P < 0.01, ***P < 0.001 and ***P < 0.0001.

3. Immunology of extra-pulmonary TB: Altered systemic levels of acute phase proteins in tuberculous lymphadenitis and modulation after treatment

Principal Investigators	:	Dr. Subash Babu; Dr. D. Baskaran
Source of funding	:	ICER
Study Period	:	2015-2020

Background: Pulmonary tuberculosis (PTB) is characterized by elevated levels of acute phase proteins (APPs), but their association with tuberculous lymphadenitis (TBL) is poorly studied.

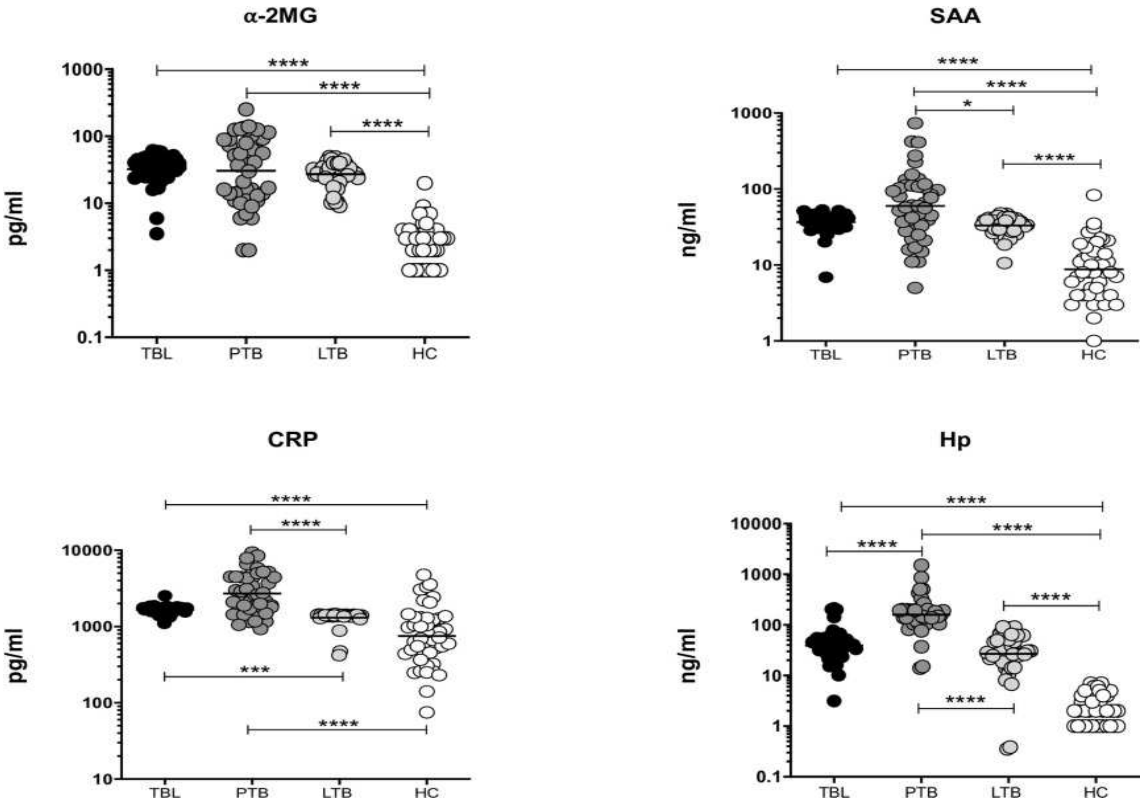
Aims/Methodology*:* We examined the systemic levels of APPs (alpha-2-macroglobulin [α-2MG], serum amyloid A [SAA], C-reactive protein [CRP] and haptoglobin [Hp]) in TBL, PTB, latent

tuberculosis (LTB) and healthy controls (HC) at baseline and in TBL after the completion of anti-tuberculosis treatment (ATT). We have also examined the association of these proteins with lymph node (LN) size, culture grade and multiple versus single LN involvement.

Results: TBL individuals exhibited increased systemic levels of α-2MG, SAA, CRP and Hp in comparison to HCs and increased CRP levels in comparison to LTB individuals. TBL individuals also exhibited decreased systemic levels of Hp compared to PTB individuals. APPs were not significantly associated with LN size, LN involvement and culture grade, indicating a lack of association with disease severity. Following ATT, post-treatment levels of α-2MG, CRP and Hp were significantly diminished compared to pre-treatment levels.

Conclusion: TBL disease is characterized by altered levels of APPs at baseline and modulated following treatment, indicating the presence of systemic inflammation.

Fig.28: TBL is characterised by altered plasma levels of acute phase proteins.



The plasma levels of acute phase proteins (α-2MG, SAA, CRP and Hp) were measured in TBL (n = 44), PTB (n = 44), LTB (n = 44) and HC (n = 44) individuals. We have shown our data as scatter plots with each circle representing a single individual and medians depicted with a bar. P values were measured using the Kruskal- Wallis test with Dunn's multiple comparisons.

STUDIES IN PROGRESS:

ICER-5: Effect of diabetes on the immune responses in tuberculosis

Principal Investigators	:	Dr.Subash Babu; Dr.Syed Hissar
Source of funding	:	DBT and NIAID
Study period	:	2014-2019

Immunology of diabetes-TB: Impact of Diabetes and Low Body Mass Index on Tuberculosis Treatment Outcomes

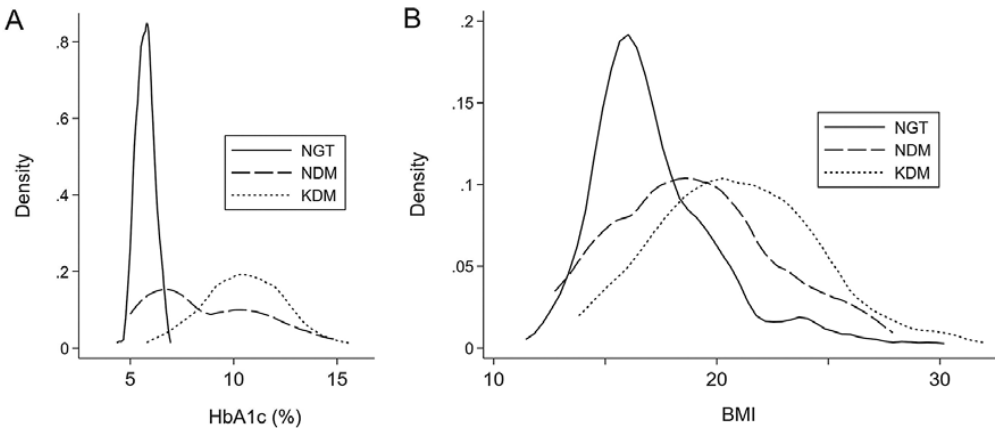
Background*:* Diabetes was identified as a tuberculosis (TB) risk factor mostly in retrospective studies with limited assessments of metabolic variables. The prospective effects of diabetes on TB severity study compared adults with pulmonary TB in Chennai, India, who were classified as having either diabetes or a normal glucose tolerance at enrollment.

Aim/ Methodology: The EDOTS cohort provided an opportunity to compare the relative impacts of a low BMI or DM on TB outcomes, and to determine whether the relationship between DM and TB outcomes differed by the level of BMI. Baseline TB severity, sputum conversion, and treatment outcomes (cure, failure, death, or loss to follow-up) were compared between groups with respect to glycemic status and body mass index (BMI).

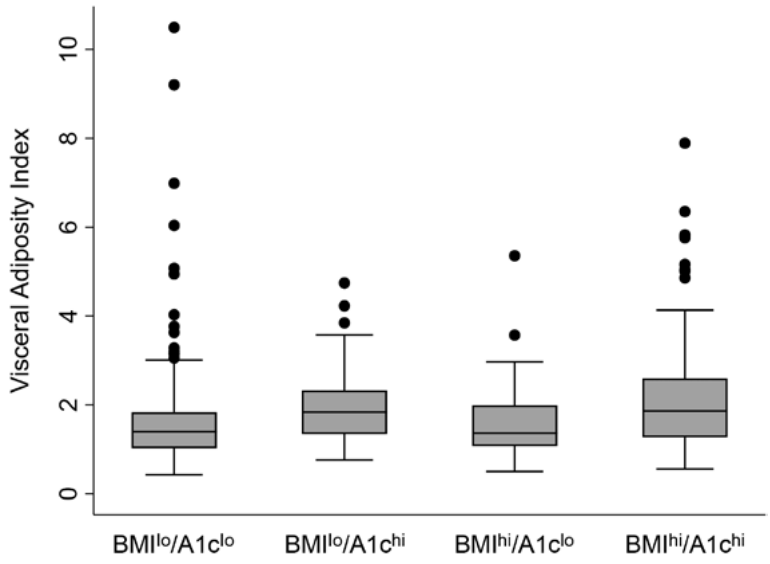
Results*:* The cohort of 389 participants included 256 with diabetes and 133 with a normal glucose tolerance. Low BMIs (<18.5 kg/ m2) were present in 99 (74.4%) of nondiabetic participants and 85 (33.2%) of those with diabetes. Among participants with normal or high BMIs, rates of cure, treatment failure, or death did not vary by glycemic status. Participants with low BMIs had the highest radiographic severity of disease, the longest time to sputum culture conversion, and the highest rates of treatment failure and death. Among participants with low BMIs, poorly controlled diabetes (glycohemoglobin [HbA1c] ≥8.0%) was unexpectedly associated with better TB treatment outcomes. A high visceral adiposity index was associated with adverse outcomes and despite an overall correlation with HbA1c, was elevated in some low-BMI individuals with normal glucose tolerance.

Conclusion*:* In this South Indian cohort, a low BMI was significantly associated with an increased risk for adverse TB treatment outcomes, while comorbid, poorly controlled diabetes lessened that risk. A high visceral adiposity index, either with or without dysglycemia, might reflect a novel TB susceptibility mechanism linked to adipose tissue dysfunction.

<u>Fig. 29:</u> Distribution of (A) HbA1c and (B) BMI, stratified by glycemic classification as NGT, KDM prior to incident TB, and NDM, based on enrollment screening oral glucose tolerance test.



<u>Fig. 30:</u> Visceral Adiposity Index (VAI), stratified by BMIs and HbA1c levels.



The VAI was calculated for the following 4 groups: BMI <18.5 kg/m2 and HbA1c <8.0% (BMIlo/A1clo; n = 138); BMI <18.5 kg/m2 with HbA1c ≥8.0% (BMIlo/A1chi; n = 44); BMI ≥18.5 kg/m2 and HbA1c <8.0% (BMIhi/A1clo; n = 50); and BMI ≥18.5 kg/m2 and HbA1c ≥8.0% (BMIhi/A1chi; n = 151). The horizontal lines indicate the medians, the boxes show the interquartile ranges, the whiskers show the highest and lowest values, and the dots represent outliers.

Abbreviations: BMI, body mass index; HbA1c, glycohemoglobin

ICER-6 : Impact of immune changes of pregnancy on tuberculosis

Principal Investigators	:	Dr.Subash Babu; Dr.Luke Elizebeth Hanna
Source of funding	:	NIH
Study period	:	2015-2020

Salient Findings: Immunological analysis underway.

ICER-7: Study to evaluate the effectiveness of BCG vaccine in reducing morbidity and mortality in elderly individuals in COVID-19 hotspots in India

Principal Investigators	:	Dr.Subash Babu; Dr.Padmapriyadarsini
Source of funding	:	ICMR
Study period	:	2020-2021

Salient Findings: Immunological analysis underway.

CONTRIBUTION TO THE NATIONAL PROGRAMMES

The Department of HIV at NIRT has been serving as one of the Regional Reference Laboratories to the National AIDS Control Organization's Early Infant Diagnosis Program and ART Programs. The summary of testing services provided for the National Programs is shown in Table 33.

Table 33: Summary of HIV-1 DNA PCR testing and viral load testing services provided for the NACO HIV Programme during the period Apr 2019-March 2020

	Testing Discipline									
	HIV-1 DNA PCR TESTING (NACO EID Programme)					HIV-1 Viral load testing services				
	Sample Details		Result Details			Sample Details		Result Details		
S.No.	Samples Received	Samples tested	Screening Detected	Confirmatory Detected	Not Detected	Samples Received	Samples tested	Target not Detected	1000 Copies/ml	>1000 Copies/ml
Total	2652	3088	98	59	2931	4165	4393	3466	441	286

PARTICIPATION IN EXTERNAL QUALITY ASSURANCE PROGRAMMES

The Department is enrolled in external quality assurance programmes offered by the best agencies for each of the diagnostic services it provides for NIRT's clinical trials and research studies. We have ensured successful ongoing participation and have achieved good scores that meet the QA body's criteria in all the testing disciplines. A summary of the EQA related activities for the current year is provided in Table 34.

Table 34: Summary of EQA related activities during the period Apr 2019-March 2020

Name of the Assay	EQA/Accreditation Body	Number of Panels tested	Results Received	Results	Remarks
HIV DNA PCR	Centres for Disease control	1	1	Certified (100%)	
HIV Viral load test	VQA RCPA	1 1	1 1	Certified (100%)	
Rapid HIV Testing (Round-I) Rapid HIV Testing(Round-II)	ICMR-NARI ICMR-NARI	1 1	1 1	Certified-100% Certified-100%	(Round-I) (Round-II)
Hematology/Peripheral Smear	ISHTM-AIIMS	3	3	Certified-100%	
CD4/CD8 testing	ICMR-NARI	1	1	Certified-100%	
HIV Drug resistance testing	VQA WHO Dry panel Assessment	1 1	1 1	Certified (100%)	

RNTCP ACTIVITIES IN NATIONAL REFERENCE LABORATORY, NIRT, CHENNAI (2019-20)

Contact Person	:	Dr. Rajesh Mondal
Source of Funding	:	Ministry of Health & Family Welfare, Central TB Division, New Delhi

ICMR-NIRT as one of the National Reference Laboratory (NRL) closely monitors five states (Andhra Pradesh, Gujarat, Kerala, Tamil Nadu, Telangana) and five Union territories (Andaman & Nicobar, Puducherry, Lakshadweep, Daman & Diu and Dadra & Nagar Haveli) for RNTCP activities in India. NRL Microbiologists visit each state at least once a year for 3 to 5 days for onsite evaluation (OSE) and monitoring EQA activities of smear microscopy, culture and DST by both phenotypic and genotypic methods as per the RNTCP protocol. During OSE visit, NRL microbiologist provide technical support for establishing quality assured smear microscopy, Culture & DST (C & DST) services, including facility design for the introduction of newer diagnostic tools (liquid culture and molecular tests) for the rapid diagnosis of MDR/XDR TB. NRL also undertakes yearly proficiency testing of IRLs/ C &DST labs as part of the certification process under RNTCP. Eight IRLs and 8 C & DST labs have been certified for diagnosis of DR-TB patients from the respective states. During 2019-2020, the Institute conducted 12th round of proficiency testing for 20 labs including three NRLs in India, with panel of 30 cultures for susceptibility testing for both first and second line anti-TB drugs. Retesting process has been completed for two C&DST laboratories in Medical College / NGO laboratory for certification by LPA.

As a NRL, NRL microbiologists has visited four states (Andhra Pradesh, Gujarat, Telangana and Kerala) and 5 UTs (Andaman & Nicobar, Dadar & Nager Haveli, Diu & Daman, Lakshadweep and Puducherry) for on site evaluation of sputum microscopy and 610 panel slides were used to assess 122 laboratory personnel for smear microscopy. Liquid culture DST training has been facilitated for 16 laboratory personals from Tamil Nadu and Kerala. 715 cultures received from Timor were processed for DST as a part of SNRL activity providing support on TB diagnostics to SEARO countries.

LIBRARY AND INFORMATION CENTRE

Library and Information Centre

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

The NIRT Library is being the heart of the Institute, supports its mission, to advance its research by providing quality resources on time through its digital platform. A hybrid library, moving towards an integrated electronic platform. The value of the information resources provides to scientists make them advance their research. NIRT Library has improved its core services this year.

SERVICES

Value-**A**dded **S**ervices (VAS)

- Access to electronic resources through **Digital Library** Portal *(since 2001)*
  - It has been updated with hyperlinks to
    - Institutional Repository
    - IRINS *(Indian Research Information Network System)*,
    - iThenticate *(plagiarism software)* Database
    - NIH Library
    - recent Open Access Resources
    - Science Citation Index
    - Web of Science *(ICMR e-Consortium Database)*
    - Journal Impact Factor-2018 list_by Clarivate Analytics *(a Web of Science Group)*
- **Automation**: Electronic Check-in and Check-out services since 2002
- **Website** Designing, Hosting and Maintenance
- NIRT Library took initiative for NIRT to become the part of **IRINS** (Indian Research Information Network System) database. This portal facilitates the NIRT Scientists to collect, curate and showcase their scholarly communication activities and provide an opportunity to create the scholarly network.

I. **S**elective **D**issemination of **I**nformation (**SDI**)

- e-Publications
- Digital Document Delivery Service *(DDDS)*
- Literature search
- Reference Assistance (Face-to-face, Telephone, E-Mail)
- Resource Sharing (ICMR; NIH; NML)

II. **C**urrent **A**wareness **S**ervice (**CAS**) – **Daily Service:**

- **Digital Information Alert Services** on
  - Press Clippings
  - New Article(s) Alert
    - Online First Article
    - Accepted Manuscript(s) online
    - In Press
    - High Impact Articles
  - Table of Contents
  - Weekly Updates
  - Monthly Updates
  - Information about Awards, Conferences, Seminars, Workshops, Webinars etc.

E-PUBLICATIONS

- **TB Alert** *(Monthly)*
- **HIV Monitor** *(Fortnightly)*
- **News Bulletin** *(Weekly)*

CLOUD COMPUTING

- ‘Automation’ migrated to Cloud Computing
- ‘Institutional Repository’ migrated to Cloud Computing

APPENDICES

SUMMARY SHEET OF PUBLICATIONS FROM NIRT (2019-2020)
(SORTED AS PER TOTAL IMPACT FACTOR ORDER)

Sl. No.	Publishing journals	No. of papers	Impact factor	Total Impact Factor
1	Adalya Journal	1	-	-
2	AIDS Research and Human Retroviruses	3	1.805	5.415
3	American Journal of Tropical Medicine and Hygiene	1	2.315	2.315
4	Annals of Indian Academy of Neurology	1	0.95	0.95
5	Biomedical Dermatology	1	1.70	1.70
6	BMC Infectious Diseases	2	2.565	5.13
7	BMJ Global Health	1	4.280	4.280
8	British Journal of Obstetrics and Gynaecology	1	5.193	5.193
9	Bulletin of Pure and Applied Sciences Section-E Math. & Stat.	1	0.011	0.011
10	Clinical Infectious Diseases	4	9.055	36.22
11	Clinical Pharmacokinetics	1	4.680	4.680
12	Current Science	1	0.756	0.756
13	Cytokine	3	3.078	9.234
14	Data in Brief	1	0.970	0.970
15	Elife	1	7.551	7.551

Sl. No.	Publishing journals	No. of papers	Impact factor	Total Impact Factor
16	European J of Medicinal Chemistry	1	4.8	4.8
17	Frontiers in Cellular and Infection Microbology	2	3.518	7.036
18	Frontiers in Immunology	4	4.716	18.864
19	Frontiers in Microbiology	1	4.259	4.259
20	Global Journal of Pure and Applied Mathematics	1	0.167	0.167
21	Indian Journal of Medical Microbiology	1	0.380	0.380
22	Indian Journal of Medical Research	1	1.251	1.251
23	Indian Journal of Pediatrics	1	1.136	1.136
24	Indian Journal of Public Health Research & Development	1	0.12	0.12
25	Indian Journal of Social Psychiatry	1	-	-
26	Indian Journal of Tuberculosis	5	0.294	1.47
27	Infection and Immunity	1	3.160	3.160
28	Infection Genetics and Evolution	1	2.611	2.611
29	Inflammation Research	1	3.061	3.061
30	Innate Immunity	1	2.298	2.298
31	Int Journal of Clinical Obstetrics and Gynaecology	2	-	-
32	International Journal of Current Research	3	-	-

Sl. No.	Publishing journals	No. of papers	Impact factor	Total Impact Factor
33	International Journal of Infectious Diseases	1		
34	International Journal of Mycobacteriology	1	-	-
35	Int J of Reproduction, Contraception, Obstetrics and Gynaecology	1	-	-
36	Int J Tuberculosis and Lung Disease	2	2.024	4.048
37	Journal of American Medical Association (JAMA) Network Open	1	5.032	5.032
38	Journal of Applied Science and Computations	1	5.8	5.8
39	Journal of Biomolecular Structure and Dynamics	1	3.220	3.220
40	Journal of Cellular Physiology	1	4.08	4.08
41	J Clinical TB and Other Mycobacterial Diseases	1	0.965	0.965
42	Journal of Complementary and Integrative Medicine	1	-	-
43	Journal of Ethnopharmacology	1	3.414	3.414
44	Journal of Global Antimicrobial Resistance	1	2.469	2.469
45	Journal of Immunology	2	4.718	9.436
46	Journal of Infectious Diseases	3	5.045	15.135
47	Metabolites	1	3.303	3.303
48	Memorias Do Instituto Oswaldo Cruz	1	2.368	2.368

Sl. No.	Publishing journals	No. of papers	Impact factor	Total Impact Factor
49	Microbiology Resource Announcements	1	-	-
50	Microbial Pathogenesis	1	2.581	2.581
51	National Medical Journal of India	1	0.644	0.644
52	New Journal of Chemistry	1	3.06	3.06
53	Open Forum Infectious Diseases	1	2.690	2.690
54	Ophthalmic Research	1	1.685	1.685
55	Pathogens and Disease	1	2.166	2.166
56	Pediatric Pulmonology	1	2.801	2.801
57	PLoS One	10	2.776	27.76
58	PLOS Neglected Trop Dis	2	4.487	8.974
59	Scientific Reports	5	4.011	20.055
60	The lancet Global Health	1	15.873	15.873
61	Transactions of Royal Soc. Tropical Med. & Hyg.	2	2.307	4.614
62	Tropical Medicine and International Health	2	2.308	4.616
63	Tuberculosis	2	2.790	5.58
64	Viruses	1	3.816	3.816
	Grand Total	104	173.113	301.203

LIST OF PUBLICATIONS

Publications in Journals :

i) International : 88

ii) National : 16

International:

2019:

1. Adams KN, Verma AK, Radha G, Harresh A, Dinesh Kumar S, Tripathy S, Uma Devi KR, Sherman DR, Urdahl KB, Lalitha R, Hernandez RE. Diverse clinical isolates of *Mycobacterium tuberculosis* develop macrophage-induced rifampin tolerance. Journal of Infectious Diseases, 2019;219(10):1554-1558.

2. Zayed H, Abdel Motal UM, Radha Ghnan A, Ponnuraja C, Doss CGP, Rizk N, Shebl FM. Retinopathy of Type 1 Diabetes in Arab Countries: Systematic Review and Meta-Analysis. Ophthalmic Research, 2019;61(3):125-136.

3. Gupte AN, Sriram S, Paradkar M, Danasekaran K, Shivakumar SVBY, Kannan T, Dolla C, Gomathy S, Pradhan N, Kohli R, John S, Raskar S, Jain D, Momin A, Balaji S, Gaikwad A, Lokhande R, Suryavanshi N, Gupte N, Salvi S, Murali L, Checkley W, Golub JE, Bollinger R, Padmapriyadarsini C, Mave V, Gupta A. Respiratory health status is associated with treatment outcomes in pulmonary tuberculosis. International Journal of Tuberculosis and Lung Disease, 2019;23(4):450-457.

4. Ayyanar S, Pattarayan D, Ramalingam B, Kar A, Mahapatra SK, Thimmulappa RK, Palanichamy R, Rajasekaran S. Tannic acid protects against experimental acute lung injury through downregulation of TLR4 and MAPK. Journal of Cellular Physiology, 2019;234(5):6463-6476.

5. Hassan S, Sudhakar V, Nancy Mary MB, Babu R, Doble M, Dadar M, Hanna LE. Computational approach identifies protein off-targets for Isoniazid-NAD adduct: hypothesizing a possible drug resistance mechanism in *Mycobacterium tuberculosis*. Journal of Biomolecular Structure and Dynamics, 2019;16:1-14.

6. Oliveira-de-Souza D, Vinhaes CL, Arriaga MB, Pavan Kumar N, Cubillos-Angulo JM, Shi R, Wei W, Yuan X, Zhang G, Cai Y, Barry CE III, Via LE, Sher A, Babu S, Mayer-Barber KD, Nakaya HI, Fukutani KF, Andrade BB. Molecular degree of perturbation of plasma inflammatory markers associated with tuberculosis reveals distinct disease profiles between Indian and Chinese populations. Science Reports, 2019;29;9(1):8002.

7. Gokul Raj K, Kadar M, Sridhar R, Baskaran D, Babu S. Enhanced mycobacterial antigen-induced pro-inflammatory cytokine production in lymph node tuberculosis. American Journal of Tropical Medicine and Hygiene, 2019;100(6):1401-1406.

8. Daskapan A, Idrus LR, Postma MJ, Wilffert B, Kosterink JGW, Stienstra Y, Touw DJ, Andersen AB, Bekker A, Denti P, Hemanth Kumar AK, Jeremiah K, Kwara A, McIlleron H, Meintjes G, van Oosterhout JJ, Ramachandran G, Rockwood N, Wilkinson RJ, van der Werf TS, Alffenaar JC. A Systematic Review on the Effect of HIV Infection on the Pharmacokinetics of First-Line Tuberculosis Drugs. Clinical Pharmacokinetics, 2019, 58(6):747–766.

9. Narendran G, Oliveira-de-Souza D, Vinhaes CL, Akrami K, Fukutani KF, Banu K, Padmapriyadarsini C, Ravichandran N, Sereti I, Swaminathan S, Andrade BB. Multifocal tuberculosis-associated immune reconstitution inflammatory syndrome - a case report of a complicated scenario. BMC Infectious Diseases, 2019;19(1):529.

10. Ramakrishnan K, Uma A, Senathipathy R, Vijayaraj S, Thirumalaikolundu-subramanian P, Rathinasabapati R, Mondal R. Pneumocystis carinii pneumonia in HIV infected patients from south India. International Journal of Current Research, 2019;11(6):4525-4527.

11. Ramakrishnan K, Uma A, Rathinasabapati R, Thiyagarajan V, Vijayaraj S, Thirumalaikolundu Subramanian P, Senathipathy R, Mondal R. Immunoglobuling (IGG) status in pulmonary and extra pulmonary tuberculosis. International Journal of Current Research, 2019;11(6):4560-4562.

12. Swathirajan CR, Nandagopal P, Vignesh R, Srikrishnan AK, Goyal R, Qureshi H, Saravanan S, Solomon SS, Hanna LE, Sivasankaran MP, Singla N, Mukherjee J, Chatrath S, Kopycinski J, Murugavel KG. Association of circulatory Tfh-like cells with neutralizing antibody responses among chronic HIV-1 subtype C infected long- term nonprogressors and progressors. Pathogens and Disease, 2019;77(4).

13. Lee DJ, Kumarasamy N, Resch SC, Gomathi NS, Mayer KH, Tripathy S, Paltiel AD, Freedberg KA, Reddy KP. Rapid, point-of-care diagnosis of tuberculosis with novel Truenat assay: Cost-effectiveness analysis for India's public sector. PLoS One, 2019;14(7):e0218890.

14. Mave V, Padmapriyadarsini C, Chavan A, Shivakumar SVBY, Kavitha D, Paradkar M, Kannan T, Kinikar A, Murali L, Gaikwad S, Hanna LE, Kulkarni V, Sathyamoorthy P, Suryavanshi N, Thomas B, Kohli R, Gomathi NS, Pradhan N, Bhanu B, Kagal A, Golub J, Gandhi N, Gupte A, Gupte N, Swaminathan S, Gupta A, for the CTRIUMPH RePORT India study team. Infection free "resisters" among household contacts of adult pulmonary tuberculosis. PLoS One, 2019;14(7):e0218034.

15. Munir A, Kumar N, Ramalingam SB, Tamilzhalagan S, Sivakumar S, Natarajan PL, Dina N, Padmapriyadarsini C, Natrajan M, Tripathy S, Uma Devi KR, Peacock SJ, Parkhill J, Blundell TL, Malhotra S. Identification and characterization of genetic determinants of isoniazid and rifampicin resistance

in *Mycobacterium tuberculosis* in southern India. Scientific Reports, 2019;9(1):10283:1-13.

16. Pavan Kumar N, Fukutani KF, Shruthi BS, Alves T, Silveira-Mattos PS, Rocha MS, West K, Natarajan M, Viswanathan V, Babu S, Andrade BB, Kornfeld H. Persistent inflammation during anti-tuberculosis treatment with diabetes comorbidity. eLife, 2019;8:e46477-1-19.

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Awards/Honours

1. **Bill and Melinda Gates Foundation Global Health Travel Award** to attend the Keystone Symposium held at Cape Town, South Africa from December 8 - 12, 2019. **Anuradha.R.**

2. **Bill and Melinda Gates Foundation Global Health Travel Award** to attend the Keystone Symposium held at Banff, Canada from Jan 17 - 21, 2019. **Anuradha.R.**

3. November 20th – 24th 2019 **Bill and Melinda Gates Foundation Travel** award to attend The American Society of Tropical Medicine and Hygiene, 68th Annual Meeting, Gaylord National Resort and Convention Center, National Harbor, Maryland USA (adjacent to Washington, DC). **Gokul Raj. K**

Participation in Conferences / Seminars / Workshops

INTERNATIONAL

1. Participation in the "5th Meeting of BRICS TB Research Network" at Beijing, China from 2nd to 3rd July, 2019 – **Dr. G. Narendran**

2. Participation in the "3HP Training of Trainers Workshop" at Johannesburg, South Africa from 10th to 12th July, 2018 – **Dr. Srikanth Tripathy**

3. Participation in the "21st Biennial International Conference of International consortium for Social Development" at Yogyakarta, Indonesia from 16th to 19th July, 2019 **- Dr. Beena Thomas**

4. Participation in the "9th International Conference on Social Work and in Health and Mental Health" at York, United Kingdom from 21st to 26th July, 2019 – **Dr. Beena Thomas**

5. Participation as a Technical Expert in the National Tuberculosis Reference Laboratory (NTRL) and National TB Program (NTP) for supervision and support of National Drug Resistance Survey (DRS)/NTRL at Dili, Timor Leste from 28th July to 9th August, 2019 – **Dr. S. Siva Kumar**

6. Participation in the "SAARC Training on Gene-Xpert Machine Operation and Maintenance for Laboratory Personnel" at Thimphu, Bhutan from 6th to 8th August, 2019 – **Dr. Rajesh Mondal**

7. Participation in the "WHO Meeting-Joint Monitoring Mission (JMM) of the National Tuberculosis Programme" at Yangon, Myanmar from 11th to 21st August, 2019 – **Dr. Rajesh Mondal, Dr. S. Siva Kumar**

8. Participation in the "12th International Workshop on Clinical Pharmacology of Tuberculosis Drugs" at London, UK from 8th to 11th September, 2019 – **Dr. AK. Hemanth Kumar**

9. Participation in the "STREAM Research Training Workshop" at London, UK from 11th to 13th December, 2019 – **Dr. G. Narendran**

10. Participation in the "7th BRICS TB Research Network Meeting" at Geneva, Switzerland from 5th to 6th March, 2020 – **Dr. C. Padmapriyadarsini**

NATIONAL

1. Participation in the stakeholders' meeting to identify & prioritise research questions for conducting systematic reviews in TB care & prevention at International Union against TB and Lung Diseases, South-East Asia Office, New Delhi on 29th – 30th April, 2019 **– Dr. M. Muniyandi**

2. Participation in the Meeting to discuss on cost effectiveness of MIP Vaccine for Leprosy at ICMR, New Delhi on 16th May 2019 **– Dr. M. Muniyandi**

3. Participation in the Expert Committee Meeting for Delamanid & Bedaquiline at ICMR, New Delhi on 3rd June, 2019 – **Dr. C. Padmapriyadarsini**

4. Participation in the meeting to discuss and finalize the framework on gender-responsive approaches to TB under RNTCP at CTD, New Delhi on 6th – 7th June, 2019 – **Dr. Beena Thomas**

5. Participation in the Training Programme on "Developing and sustaining India's capacity for Pre-Clinical drug discovery" at ICMR-NIN, Hyderabad on 7th July, 2019 – **Dr. Rajesh Mondal**

6. Participation in the Brainstorming Meeting on TB among Saharia at ICMR, New Delhi on 20th August, 2019 **– Dr. Beena Thomas, Dr. M. Muniyandi**

7. Participation as an Expert in the partnership meeting to discuss the Regional Road map for disabilities and assistive technologies and devices at ICMR, New Delhi on 4th – 5th August, 2019 **– Dr. M. Muniyandi**

8. Participation in the 25th Asia-Pacific Joint Regional Social Work Conference on Social Work partnerships towards an Equal Society at NIMHANS, Bengaluru on 18th – 20th September, 2019 – **Dr. Beena Thomas, Mr. P. Murugesan**

9. Invited to give presentation on "Added value of Next Generation Sequencing in clinical application for M.tb" at Bharathiyar University, Coimbatore on 19th – 20th September, 2019 – **Dr. K.R. Uma Devi**

10. Invited as a Guest Speaker to present on "Why Epidemiology is a corrective discipline of Public Health" at the National Conference no Pharmaco-epidemiology and Pharmaco-economics at JSS College of Pharmacy, Mysuru on 26th – 28th September, 2019 – **Dr. B.M. Shrinivasa**

11. Participation in the NTEG Meeting on Treatment of TB at NACO, New Delhi on 17th – 18th October, 2019 – **Dr. C. Padmapriyadarsini**

12. Co-Chair in TB Science 2019 Conference at Hyderabad on 29th – 30th October, 2019 – **Dr. C. Padmapriyadarsini**

13. Participation/Oral Presentation/Poster Presentation at the 50th Union World Conference on Lung Health at Hyderabad on 28th October – 2nd November, 2019 – **25 scientists and 10 research scholars/fellows from the Institute**

14. Participation in the WHO-GoI Joint Monitoring Mission for RNTCP, India at WHO-India Office, New Delhi on 17th – 20th November, 2019 – **Dr. C. Padmapriyadarsini**

15. Participation in the 8th National Conference of IOS: Scholarly Communication & Scientometrics at Institute of Scientometrics & ACTREC, Mumbai on 22nd – 23rd November, 2019 – **Dr. R. Rathinasabapati**

16. Invited as a Panellist for discussion on MDR-TB and presentation on "MDR-TB & Newer drugs" at NAPCON at Kochi on 24th November, 2019 - **Dr. G. Narendran**

17. Part of the Central Internal Evaluation Team of West Bengal RNTCP on 8th – 12th December, 2019 – **Dr. P.K. Bhavani**

18. Participation/Oral Presentation at the 37th Annual Conference of Indian Society for Medical Statistics at AIIMS, Patna on 5th – 7th December, 2019 – **Statisticians from the Institute**

19. Participation in the I Meeting of Task Force on LTBI Management in India and oral presentation on "Proposal for C-TB: Multicentric evaluation" at TB Association of India, New Delhi on 11th – 12th December, 2019 – **Dr. C. Padmapriyadarsini**

20. Oral Presentations at the International Conference on Recent Trend in Statistical Modeling and Methods at Annamalai University on 19th – 21st December, 2019 – **Dr. Ponnuraja, Mr. Padmanaban**

21. Participation/Oral Presentation/Poster Presentation at the 74th National Conference on Tuberculosis and Chest Diseases at Leela Palace, Chennai on 21st – 22nd December, 2019 – **Around 25 scientific and technical staff from the Institute**

22. Invited to give talk on "General awareness on Tuberculosis" at the National Seminar on "Exploring the scope of Plant Science" at Queen Mary's College, Chennai on 9th January, 2020 – **Dr. K.R. Uma Devi**

23. Oral Presentations at the International Conference on Recent Trend in Stochastic Modeling and its application at Manonmaniam Sundaranar University on 9th – 11th January, 2020–**Mr. Padmanaban, Dr. Srinivasan, Mr. Tamizhselvan**

24. Guest Lecture on "Molecular diagnosis of TB with Special reference to NGS" at 8th Annual Conference of Molecular Pathology Association of India at Jaipur on 10th – 12th January, 2020 – **Dr. S. Siva Kumar**

25. Guest Lecture at Bio Ethics and GCP in Health Research Sensitization Workshop at Thiruvarur Medical College Hospital, Thiruvarur on 24th January, 2020 – **Dr. Banu Rekha, Dr. P.K. Bhavani, Dr. C. Padmapriyadarsini**

26. Participation in the National Workshop on "Vaccine Research: Development to deployment" at ICMR, New Delhi on 4th – 5th February, 2020 – **Dr. K.R. Uma Devi**

27. Participation in the Seminar on Mathematical Modelling for Infectious Diseases at ICMR, New Delhi on 10th – 12th February, 2020 – **Dr. C. Ponnuraja, Mr. T. Kannan**

28. Guest Lecture at the National Conference on "Public Health challenges of tropical diseases-Moving towards universal access" at Central University of Tamil Nadu, Thiruvarur on 13th February, 2020 – **Dr. V.N. Azger Dusthackeer**

29. Participation in the Annual Meeting of WHO SEARO Advisory Committee on MDR-TB (rGLC) at NITRD, New Delhi on 12th – 13th February, 2020 – **Dr. C. Padmapriyadarsini**

30. Invited talk on "Human Papilloma Virus Infection and Cervical Cancer" at ISSRF 2020 "World Congress on Reproductive Health with Emphasis on Reproductive Cancers, Infertility & Assisted Reproduction" at Shri Mata Vaishno Devi University, Katra, Jammu & Kashmir on 13th – 17th February, 2020 – **Dr. N. Sudhakar**

Workshop(s)/Symposium/Other Events

Details available at NIRT Webpage: http://www.nirt.res.in/html/events.htm

Staff List as on 01/04/2020

Details available at NIRT Webpage: http://www.nirt.res.in/html/scientistPro.htm

Ph.D. Scholars

List of staff/students who have obtained their Ph.D. degree (Part Time) from the Bharathiar University

Sl. No.	Name of the candidate	Title of the Ph.D. thesis	Part-time / Full time	Supervisor/ Guide
1.	Ms. S. Valarmathi	Statistical Methods for Evaluating Treatment Outcomes in the Presence of Competing Risks Time to Event Clinical Trial Data	Part time	Dr. C. Ponnuraja
2	Ms. S. Santhi	Selection and Implementation of Sampling Procedures Indexed Through Acceptable Quality Level and Limiting Quality Level: A Zero Inflated Poisson Approach	Part time	Dr. C. Ponnuraja
3	Mr. C.L. Babu	Evaluating Clinical Trials Healthcare Data Using Clustering And Classification Procedures- A Data Mining Approach	Part time	Dr. C. Ponnuraja

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. Narayanaiah Cheedarla	Identification and characterization of neutralizing antibodies in clade 'C' HIV-1 infected individuals	Full time	Dr. Luke Elizabeth Hanna
2.	Mr. Ashok Kumar	Genetic identity and unique biological phenotype of early transmitted/founder viruses in recent HIV-1 infection	Full time	Dr. Luke Elizabeth Hanna

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

Sl.No.	Name of the candidate	Title of the Ph.D. thesis	Part / Full time	Supervisor/ Guide
1.	Ms. Vidya Vijayan	Characterizaiton of viral and host factor responsible for clinical differences seen in HIV-1 and 2 infection	Full time	Dr. Luke E. Hanna
2.	Mr.S. Sivasankaran	Evaluation of mucosal immune responses to HIV infection in discordant couples: Templates for a vaccine	Full time	Dr. Luke E. Hanna
3.	Ms. Hemalatha Babu	Inflammation and aging in long-term treated HIV-1 infected individuals	Full time	Dr. Luke E. Hanna

List of students who have registered (full-time) for their Ph.D. programme with The Tamil Nadu Dr. M.G.R. Medical University

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. thesis	Supervisor/Guide
1.	MARY REBECCA Y	ICMR SRF	Pharmacokinetic drug-drug interactions between first line anti-TB and anti-diabetic drugs	Dr.A.K.Hemanth 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.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
2.	Ms.Gunapati Bhargavi	INSPIRE Fellow	Functional characterization of oxidoreductases of *M. tb*	Dr.P. Kannan
3.	Mr. Kadar Moideen A.	ICER	Immune response to TB coincident with diabetes	Dr.B. Ramalingam
4	Ms. Pavithra S.	INSPIRE Fellow	Molecular analysis of monocyte subsets in humans infected with *M. tb*	Dr.B. Ramalingam
5.	Mr.S. Deepak	ICMR	Development of lactobacillus strains for the purpose of enhanced DNA stability, mucosal adhesion and delivery of therapeutic proteins	Dr. Luke E. Hanna
6	Mr. Anand B Sonawane	UGC	Molecular mechanisms of HIV pathogenesis in target cells	Dr. Luke E. Hanna
7	Ms.J.S.V. Soundarya	ICMR	Attenuated Mycobacteria based vaccine against tuberculosis with a novel strategy for T cell priming	Dr.K.R. Uma Devi
8	Mr.P.Venkatesan	Lady Tata	CRISPR mediated platform for diagnosis and rapid detection of drug resistance pattern in *M. tuberculosis*	Dr.K.R. Uma Devi
9	Mr. Krisna Moorthi P	ICMR	Immunomodulation of autophagy by vitamin D_3 in Macrophages infected with *M. tuberculosis*	Dr.K.R. Uma Devi
10	Ms.R. Ananthi	ICMR	Study on mutations associated with pyrazinamide resistance in *M. tuberculosis*	Dr.P. Kannan
11	Ms. Evangeline Ann Daniel	INSPIRE Fellow	Identification of biomarkers for predicting TB disease progression and treatment response	Dr. Luke E. Hanna

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. Kannan T	An evaluation of predictive statistical data mining techniques	Dr. K. Rajendran

OBITUARY

NIRT staff who passed away during the period 2019-2020

Mr. E. Raman, Senior Driver	-	08.04.2019
Mr. C.K. Valsarajan, Sr. Administrative Officer	-	04.05.2019
Mr. G. Subramanian, Lab Assistant	-	08.05.2019
Mr. M. Nagarajan, Sr. Technical Officer	-	06.06.2019
Mrs. Vijayal, Sr. Record Sorter	-	13.07.2019
Mr. V. Krishnamurthy, Sr. Technician –II	-	01.09.2019
Mrs. S. Lakshmi, Lab Assistant	-	11.11.2019
Dr. R. Parthasarathy, Deputy Director	-	02.12.2019
Mrs. R.K. Parasuraman, Assistant	-	24.02.2020

ICMR-National Institute for Research In Tuberculosis
No.1, Mayor Sathyamoorthy Road
Chetpet, Chennai-600031, India
Tel: +91 44 28369500; Fax: +91 44 28362525
www.nirt.res.in