

# ANNUAL REPORT 2016-17



**Indian Council of Medical Research  
New Delhi**

# Annual Report 2016-17



**Indian Council of Medical Research  
New Delhi**



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# DIRECTOR-GENERAL'S MESSAGE

It gives me immense pleasure to present the Annual Report of Indian Council of Medical Research (ICMR) for the year 2016-17. I would like to mention a few highlights. ICMR initiated a new flagship Programme “India TB Research Consortium” that aims to bring together all major national and international organizations to address overarching scientific questions to tackle TB in a mission mode. TruNAT Rif, an indigenous, cost effective, rapid molecular diagnostic kit for TB/MDR-TB has been developed in collaboration with ICMR, DBT and the industry. The feasibility study of TruNAT Rif at 100 microscopy centres in 50 districts across 10 states has been completed and should lead to scaled implementation across the country. In partnership with National Vector Borne Diseases Control Programme (NVBDCP), Drugs for Neglected Diseases Initiative (DNDi), and other international partners, we set up the Visceral Leishmaniasis consortium (VL), which aims to develop a forum for constructive discussion around the transmission dynamics of VL and re-energize efforts for research leading to its sustained elimination.



ICMR is exploring novel strategies for vector control and signed an MoU with Monash University, Australia for working on Wolbachia-based vector control strategy for Aedes mosquitoes. The Vector Control Research institute, National Institute of Malaria Research and NIRTH are all involved in testing new strategies for vector control. A Satellite Centre of NIIH, Mumbai started functioning at Chandarpur, Maharashtra to tackle sickle cell anemia and G6PD, benefitting more than 400 patients. Antenatal and neonatal screening programme would benefit the largely tribal population. Laboratories for prenatal diagnosis (PND) of Thalassaemia and Sickle cell anaemia were also set up at NIRTH, Jabalpur and RMRC, Dibrugarh.

In the area of Nutrition, ICMR's National Institute of Nutrition at Hyderabad developed a Mobile App on 'Dietary Guidelines for Indians based on the Recommended Dietary Allowances (RDAs) as well as a micronutrient mix for preschool children that is being considered by government for its flagship programmes. The new “Indian Food Composition Tables” comprising data of 586 varieties of Indian foods and their nutritive values was released during the year.

A new focus was on supporting the development, testing and validation of affordable indigenous technologies and 9 technologies developed by various ICMR Institutes were transferred to industry for commercialization. ICMR established collaboration with IIT, Kharagpur and through the Imprint programme funded 25 projects designing health technologies and finding better technological solutions for human health.

The other major significant activity of the year was the state level disease burden study (jointly by the Public Health Foundation of India (PHFI), ICMR & the Institute for Health Metrics and Evaluation (IHME) that will produce State-Level Disease Burden and Risk Factors estimates for the first time.

This will improve health programme budgeting and planning in the various states and help evidence informed policy making

Viral outbreaks and new emerging infections have always been a matter of serious concern. ICMR-NIV established surveillance at 25 sites in the country for Zika testing. Capacity building was done for 25 VRDL labs and 11 IDSP labs. Four cases of Zika virus were detected through ICMR's surveillance network (3 in Gujarat and 1 in Tamil Nadu) in 2017. Extensive investigations on AES in Gorakhpur showed that scrub typhus is the dominant cause of AES in that region. National Anti-Microbial Resistance Surveillance Network (AMRSN) expanded to 10 tertiary hospitals to enable compilation of National Data of AMR at different levels of Health Care.

ICMR-INDIAB, study is the first representative study providing authentic epidemiological data on diabetes, pre-diabetes, hypertension, dyslipidemia and obesity from the various States of India. The study has been completed in 14 states and one UT and the data obtained has been shared with the State health departments. Data provide a snap shot of the existing burden of pre diabetes, diabetes, hypertension and obesity in the country. National Cancer Registry Programme continued to provide data on cancer burden in the country through 30 Population Based Cancer Registries (PBCR) and 27 Hospital Based Cancer Registries (HBCR).

Partnership in Health Research with various international organizations/agencies was continued during the year. Total 7 exchange visits of Scientists were arranged for various collaborative programmes. 18 Scientists were selected for ICMR International Fellowship during 2016-17. ICMR also took initiative in newer areas of research including Evidence to Policy and Health System Research and Health Technology Assessment. ICMR scientists published a total of 720 research papers in various national and international journals and 12 patents were filed. ICMR funded a total of 1352 extramural research projects including fellowships in various areas of health research during the year to strengthen health research in the country.

ICMR also partnered in various implementation programs of the Government of India and provided expert advice whenever needed. ICMR is striving hard to provide research support to the diseases identified for elimination, as well as the newer programs of the ministry of health, like the national program for control of non communicable diseases and cancer. I am confident that in the coming years, ICMR will further broaden its mandate and attain new heights.



**(Soumya Swaminathan)**



# OVERVIEW

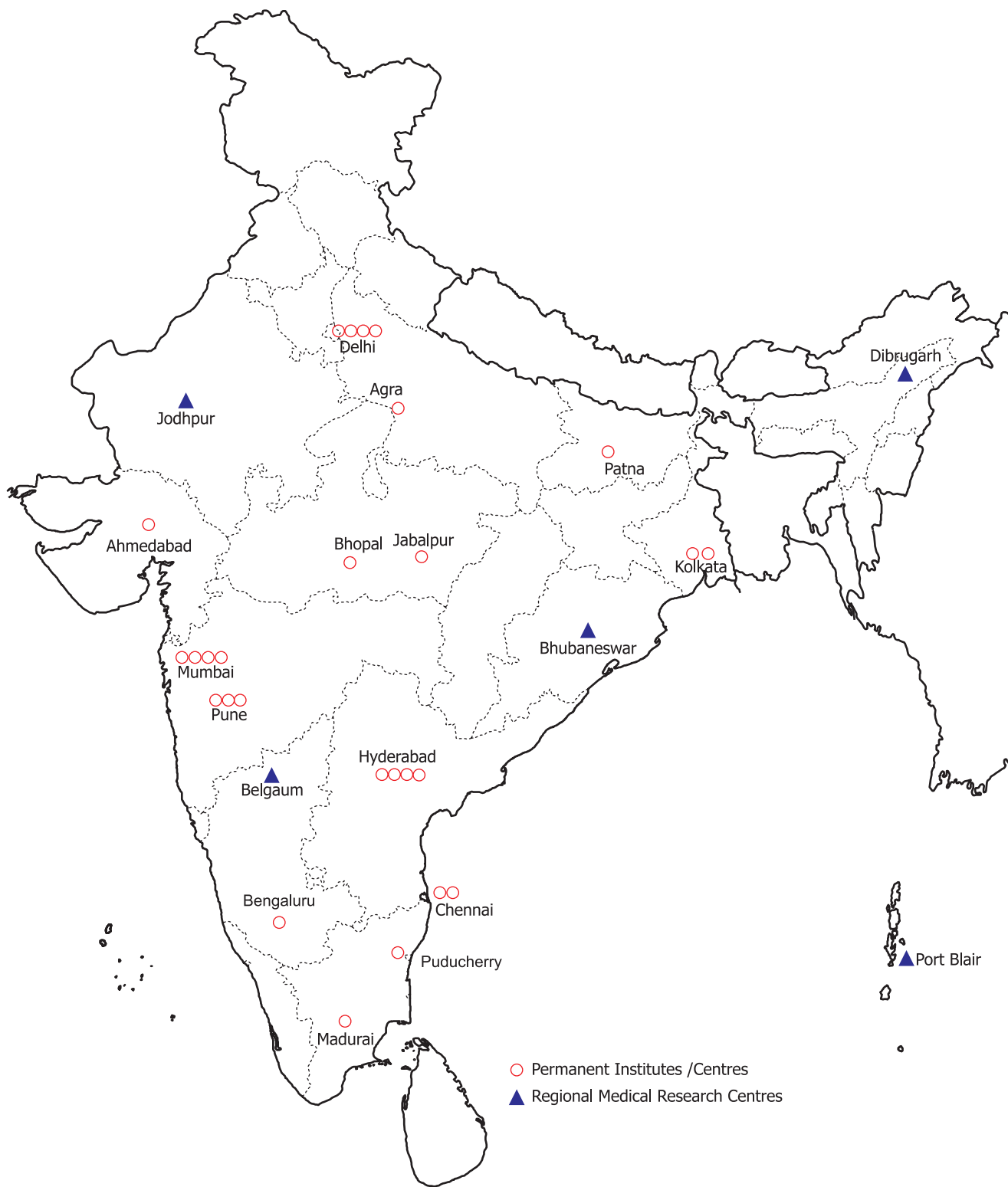
The Indian Council of Medical Research (ICMR), New Delhi, is over a century old autonomous organization, now working under the Department of Health Research (DHR), Ministry of Health and Family Welfare, Government of India. The ICMR has evolved over the years in line with changing health research needs of the country, effectively addressing the new challenges that have been emerging. The current strategy of ICMR is to have close interaction with national health systems, including disease control programmes, which are supported by the continued exploitation of scientific and technological advances from basic to applied research, biomedical to health sciences, and from laboratory to field research. The mission of ICMR is to promote better health in India through research. It provides stewardship, conducts and supports health research, generates knowledge and ensures its utilization, and develops resources for health research in areas of national public health importance.

ICMR promotes biomedical research in the country through intramural research (by its own Institutes/Centres) and extramural research (through grants-in-aid given to projects through a transparent rigorous review process mainly to non-ICMR Institutes). The extramural research is supported through (i) setting up Centres for Advanced Research in different research areas around existing expertise and infrastructure in selected departments of Medical Colleges, Universities and other non-ICMR Research Institutes; (ii) Task force studies which emphasise a time-bound, goal-oriented approach with clearly defined targets, specific time frames, standardized and uniform methodologies and often multicentric structure; (iii) long term projects targeting issues like vector control, nutrition, reproductive health, etc; (iv) Open-ended research on the basis of applications for grants-in-aid received from scientists from medical colleges and research institutes located in different parts of the country. The research activities carried out by the ICMR during 2016-2017 are highlighted in the following pages.

During this time period, LAMP assay was established using direct lysis of clinical samples for rapid and reliable diagnosis of kala-azar and PKDL. An automated tool, Target identification (TiD), was designed for drug target mining from whole proteome of bacteria within 2hrs. Peptide inhibitors against IL17 were designed by *In silico* mutagenesis, Protein-protein docking, molecular dynamics simulations.

There was establishment of Centre of excellence for Research, Diagnosis and Management of Primary Immunodeficiency disorders (PID), which will help in diagnosis, genetic counseling and management of these disorders. Successful establishment of Satellite centre was done for haemoglobinopathies at Chandrapur, benefitting more than 400 patients with establishment of antenatal and neonatal screening programme for sickle cell anaemia. There was establishment of Laboratory for prenatal diagnosis (PND) of  $\beta$ -Thalassemia and Sickle cell anaemia at NIRTH, Jabalpur and RMRC, Dibrugarh.

In NIRTH, Jabalpur, guidance documents for patients, ready reckoners for health workers, medical officers and reference manual for experts for prevention and management of ADRs to antitubercular



## ICMR INSTITUTIONAL NETWORK

drugs were prepared, released at the hands of Hon. Health Minister Dr. J.P Nadda on world TB day in March 2016 and put on central TB Division website. Acceptability and impact of patient guide is being evaluated. In Jan 2017, CDSCO ICMR handbook for applicants and reviewers of clinical trials prepared in collaboration with USFDA was released and workshop for subject experts held. Guidance document for clinical trials sites network has been prepared and will be used for tuberculosis network. Studies on ADR to antitubercular drugs in elderly, genetics of QTC prolongation, pharmacovigilance of bedaquiline, drugs for PKDL have been initiated. NIRT is conducting controlled clinical trials to establish policy for the treatment of pulmonary and extra-pulmonary TB. During this reporting period, one of the study provided the evidence for policy makers about the importance of daily regimen for HIV-TB dually infected individuals in comparison to intermittent regimen. NIRT initiated for the first time in India, a nationwide TB prevalence survey among tribal population. Together with this, a tribal intervention project for active case finding through mobile vans and community engagement was carried out in Gujarat.

A fast one-tube duplex RT-PCR assay was standardized for differential detection of dengue and chikungunya viral genome in patient's serum samples that could be used for regular laboratory screening. Kyasanur Forest disease (KFD) outbreak investigated in Sindhudurg, Maharashtra [March 2017], first time documented activity of KFD in Maharashtra. This warrants that besides Karnataka, KFD is spreading to other parts of the country. Studies at NIV, first time confirmed the existence of Zika virus in the country. ICMR provided diagnostic services for 29 viral diseases to several states in the country. A total of 36,913 samples were processed [referred as well as outbreak response]. A community-based cross-sectional sero-survey, conducted among 1085 individuals, in three age groups to determine scrub typhus endemicity in Gorakhpur district in lean period, revealed that IgG antibody prevalence was 50.8% and IgM antibody prevalence was 1.6%. Another survey carried out during peak season revealed that overall sero-incidence against *O. tsutsugamushi* was 21% among the respondents who were seronegative during the prior study.

ICMR conducted a National Rotavirus Surveillance Network study (NRSN) to examine long term trends and pattern of diarrhoea, attributable to rotavirus among children <5years of age, seen at in-patient facilities, from the different parts of India from 2012-16. Rotavirus was detected in 36.3% of children with acute gastroenteritis enrolled in surveillance. The highest positivity (43.1%) was observed among children between 12 and 23 months of age. Surveillance data from National Rotavirus Surveillance Network (NRSN) highlights the high rotavirus disease burden in India. With the Phased roll out of the rotavirus vaccine in the UIP, continued surveillance of rotavirus disease burden in the vaccine introducer states, is critical for the impact assessment of the rotavirus vaccine.

ICMR-NICPR has been instrumental in the formulation of "Operational framework document" for cancer screening and management, recently released by Ministry of Health and Family Welfare, Govt. of India, which has been designed to carry out population based screening in 100 districts of India using cost-effective screening tools. NICPR has also been designated as the training hub for implementing the guidelines. ICMR-NICPR was designated as the WHO FCTC Global Knowledge Hub on Smokeless Tobacco, which is the focal centre for all information on smokeless tobacco and is responsible for providing research and evidence (<http://untobaccocontrol.org/kh/smokeless-tobacco/>). 9 states of India have notified cancer as a notifiable disease. In 2016-17, Gujarat and Manipur also notified cancer. NCDIR continues to guide notification of cancer in states. There was setting up of Karnataka and Haryana state cancer registry to monitor cancer trends in response to the cancer notification.



Since January 2017, IJMR has also been made available live on IJMR App, both on Android and iPad devices. This user-friendly app has made IJMR readily available, thus helping in dissemination of information.

For facilitating online submission of International Collaborative projects for consideration of Health Ministry's Screening Committee (HMSC), a prototype for online submission of HMSC projects was prepared and the online submission of projects was opened on ICMR website in March, 2017.

In sync with GOI initiative of digitization, during the year, the ISRM division introduced modules for complete paper-less review of submitted concept proposals. The system processed 708 concept proposals. These proposals were completely reviewed online by 752 registered experts through the system and of these, 177 concept proposals were shortlisted for submission of detailed proposals. In addition to adhoc projects during current year, modules have been developed for online submission of proposals against specific 'Call for proposals' programmes. The module has been used for a). Call for Proposals for Participation in the Health Systems Research on Road Traffic Injuries (91 proposals), b). Call for proposals in the field of Ageing and Health (58 proposals), c). Call for Proposals on Indo-Norwegian Co-operation on Antimicrobial resistance (21 proposals). ICMR English bulletin (e - version) was initiated and quarterly issues for the year 2017 were planned. Modernization of ICMR website, based on GIGW guidelines has also been initiated. Focus was also to enhance the media coverage and expand the outreach of ICMR at various platforms, especially social media, to the community.

The following MoUs were signed during this period:

- A MOU has been signed between NIAID, NIH, USA and NIIH for "Understanding the Genomics of Primary Immunodeficiency Disorders (PID) using next generation sequencing (NGS)" under which DNA exome sequencing will be done in selected cases of PID free of charge from NIH.
- MoUs between ICMR and healthcare division of Emami Limited, Kolkata, were signed for the development of phytopharmaceutical product for the prevention and delay of type-2 diabetes.
- A Letter of Intent (LoI) between ICMR and National Institute of Infectious Diseases (NIID) of Japan was signed on 16th April 2016 at Japan.
- A MoU between Department of Health Research and the International Consortium on Anti-Virals (ICAV) of Canada was signed on 10th June, 2016 at New Delhi.

ICMR scientists published a total of 720 research papers in various National & International Journals.

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# COMMUNICABLE DISEASES

The research priorities of the ICMR have always coincided with health policy and priorities of the country. The research conducted by ICMR in the area of epidemiology and communicable diseases through its disease specific institutes, closely integrated with the stage Govt. priorities and national programme, has immensely contributed in various disease conditions. The country continues to face huge burden of infectious as well as Vector Borne Disease like, tuberculosis, diarrhoea, HIV/AIDS, malaria, dengue, chikungunya, respiratory infections etc. The management of these diseases is now becoming complicated due to emerging antimicrobial resistance and outbreaks of viral infections in various parts of the country, Zika virus being the latest example. Efforts were made by the ICMR to help the national programme by directing its research towards nationally relevant problems such that the research outcomes provide the technical advantage in framing the national programmes to control, prevent, eliminate and eradicate the spread of infectious diseases in the country. The highlights of the research studies carried out by ICMR institutes as well as through extramural mode in the field of Communicable diseases, is as follows.

## NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS, CHENNAI

National Institute for Research in Tuberculosis (NIRT) has a well-trained multidisciplinary team of Clinicians, Epidemiologists, Microbiologists, Immunologists, Biochemists, Statisticians, Social Scientists, Health Economist along with

Public Health and Paramedical Workers. NIRT is recognized as a WHO collaborating centre for TB research and training. The Institute has been recognized as an International Centre for Excellence in Research in basic medical sciences. Department of Bacteriology in NIRT has been recognized as a supra national reference laboratory for Mycobacteriology for South East Asian countries under WHO. The research contributions from NIRT has played a crucial role in the policy formulation for TB control in the country.

### MAJOR PROJECTS UNDERTAKEN

#### Clinical Research

- Cambridge Chennai Centre Partnership on Antimicrobial Resistance in TB: Focus on novel diagnostics and therapeutics.
- Two controlled clinical trials - moxifloxacin containing shorter regimens for drug sensitive TB and continuous vs intermittent regimens in HIV-TB co-infected patients.
- Two studies on the effectiveness of food supplementation on treatment outcomes, nutritional status in TB adults on retreatment regimen, and improving treatment adherence through evening DOTS.

#### Socio-Behavioral Research

- Investigating pre-treatment loss to follow-up (PTLFU) of smear-positive TB patients in the Revised National Tuberculosis Control Program (RNTCP) in Chennai city and Tiruvallur district, Tamil Nadu.

- Strengthening implementation and operational research under the Revised National TB control programme in India.
- An experimental study to enhance treatment adherence in TB patients who consume alcohol
- Estimate the burden of TB among the tribal population and develop an innovative health system model to strengthen TB control in the tribal areas.
- Targeted intervention to expand and strengthen TB control in tribal populations under the RNTCP, India (TIE-TB).
- Improving treatment adherence among TB patients through evening DOTS in Chennai, India.

#### Health Economics studies

- Identifying costs contributing to catastrophic expenditure amongst TB patients, registered under RNTCP in two metro cities in India.
- Comparative study of economic burden at the household level for TB patients, detected through active and passive case finding strategies in Tiruvallur district.

#### Bacteriology

- Operational feasibility and performance of TruNat MTB RiF assays in field settings under the Revised National Tuberculosis Control Program.
- Multi centric study to assess the operational characteristics of the Truenat MTB and RIF assays in intended settings of use.
- Microfluidics in development of rapid TB diagnosis.

#### Biochemistry & Clinical Pharmacology

- Pharmacokinetics of second-line anti-TB drugs in MDR-TB patients.
- Effects of co-administration of Unani Pharmacopoeial formulation (UPF; Qurs Tabasheer Sarthani (QTS) and Arq Hara Bhara (AHB) with Anti tubercular (CAT-I) drugs in adult Wistar albino rats.

- Pharmacokinetic drug-drug interactions between first line anti-TB and anti-diabetic drugs.

#### Immunology

- Protecting and improving public health globally: Building laboratory, surveillance and workforce capacity to detect, respond to and prevent drug resistance TB in India, Funded by CDC Early bactericidal activity of important anti TB drugs.
- Multicentric study from a country where intestinal tuberculosis as well as Johne's disease is endemic.
- Studies on epigenome wide alterations in alveolar macrophages during *Mycobacterium tuberculosis* infections in guinea pig pulmonary tuberculosis model.
- Whole Genome Sequencing and Transcriptome Analysis of *Mycobacterium tuberculosis* Clinical Isolates from Bovine and Human Origin.
- Evaluation of recombinant BCG strains, expressing major immunodominant antigens of *Mycobacterium tuberculosis* as vaccine candidate against tuberculosis.
- Construction of Rv 2159 and Rv 0418 gene knock out mutants of *Mycobacterium tuberculosis*.

#### HIV studies

- Role of Myeloid Derived Suppressor Cells in *Mycobacterium tuberculosis* reactivation in HIV-M.TB co-infected individuals.
- Identification of Biomarkers for tuberculosis diagnosis and treatment response.
- Molecular mechanisms of HIV pathogenesis in T- cells and endothelial cells.
- Role of microenvironment in the pathogenesis of HCV and HCV/HIV-associated hepatocellular carcinoma.
- Differences in neutrophil responses during the course of anti-tuberculosis treatment - a pilot study.

### Epidemiology

- Monitoring, learning and evaluation of the TB free Chennai initiative.

### Electronic Data Processing Division

- Retrospective observational time series study for fifteen years TB relational impact with climatic factors: Multi-centric study.
- Established server base real time data collection by Personal Digital Assistant (PDA), which reduced 80% of deferred patient treatment, after diagnosed through paper base data collection.

### International Centre for Excellence in Research

- Host response to Infection and treatment in lymphatic filarial disease and strongyloidiasis in India.
- Effect of helminth infection on antigen-specific immune responses in latent Tuberculosis.
- Effect of Diabetes on the immune responses to TB.
- Characterization of immune responses in TB lymphadenitis.

## NATIONAL JALMA INSTITUTE FOR LEPROSY AND OTHER MYCOBACTERIAL DISEASES, AGRA

### Targeted Intervention to Expand and Strengthen TB Control among the Tribal Population under RNTCP, India (TIE-TB Project)

A large and deprived tribal population in India estimated at an approximately 104 million (8.6% of the total population) with a huge burden of TB, requires services which are, truly & certainly, accessible and available. The extreme remoteness, intense deprivation from even a day's square meal and the harsh and isolated living environment, primarily contribute to high vulnerability of and poor access to healthcare by these populations. As such, provision of TB services to the tribal

population is not simply an issue of reducing the burden of TB in numbers but is a 'Standard of Care' issue.

The most significant aspect of the project is the deployment of the Mobile TB Diagnostic Van (MTDV), equipped with X-ray facilities and Sputum Microscopy facilities, which are offering diagnostic services for Tuberculosis at the doorstep of the patient's home, in difficult to reach areas of the tribal populations. This project has been initially undertaken in 5 States and 17 districts. 35 MTDVs, have been fabricated and equipped with sputum microscopy services and X-ray facilities and have been positioned in the 5 states of Madhya Pradesh, Gujarat, Chhattisgarh, Rajasthan and Jharkhand, in difficult to reach areas of the tribal belts. The vans have initiated services and according to a defined route plan, they are visiting the difficult to reach tribal areas and providing sputum services and also Chest X-ray services to presumptive TB patients.

The project is being implemented in 5 States and 17 districts covering a total population of approximately 17.65 million. This intervention is expected to improve the 'Standard of Care' among these extremely deprived populations. The efforts are expected to improve early seeking of care, reduction in out of pocket expenditure of individual patients and curbing of the individual patients from being directed to multiple providers for treatment, which results in huge economic burden to the patient and his family. The MTDVs have been operationalized at variable points of time and regular reporting of data is being initiated.

### Estimate the burden of TB among the tribal population and develop an innovative health system model to strengthen TB control in the tribal areas

The project will estimate the prevalence of TB in the tribal areas.

### Multicentre Phase II/III, Double-Blind, Randomized, Placebo Controlled Study to Evaluate the Efficacy and Safety of VPM 1002 in the Prevention of Tuberculosis Recurrence



### In Pulmonary Tuberculosis (TB) Patients After Successful TB Treatment In India

This is a regulatory clinical trial, with the objective of seeing the efficacy of VPM vaccine in reducing relapse rates in patients, who have completed treatment for Tuberculosis under RNTCP.

### Programmatic Implementation of *Mycobacterium indicus pranii* (MIP) vaccine as an immunoprophylactic under the National Leprosy Eradication Programme in high endemic settings.

The objective of the project is to measure the feasibility of introducing the *Mycobacterium indicus pranii* (MIP) vaccine as an immunoprophylactic under the National Leprosy Eradication Programme and also to estimate the impact of the intervention in reducing the burden of Leprosy in the community.

### Induction of Autophagy as a Strategy for Treatment of Tuberculosis

Assessment of amounts of particles inhaled by mice under different conditions of inhalation exposure is in progress. Preliminary results indicate that mice exposed for 30 or 60 seconds to about 425µg of aerosolised particles, inhale approximately 5% (18.59±0.97µg) of the amount available for inhalation during this period of exposure.

### Efficacy of α PD-1 in Immune Reconstitution and Adjuvant Immunotherapy against *M. tuberculosis*

We observed significant reduction in bacterial burden in both ATT and α PD-1 treated groups, relative to isotype control. Interestingly, spleen showed better log reduction (3 log) than that of lung (1.44 log), indicating possible impact of poly-functional T cells in containing bacillary dissemination. However, combination of chemotherapy (ATT) and immune rescue therapy (α PD1), demonstrated profoundly significant reduction in the CFU, both in lungs as well as spleen of the infected mice (p<0.001).

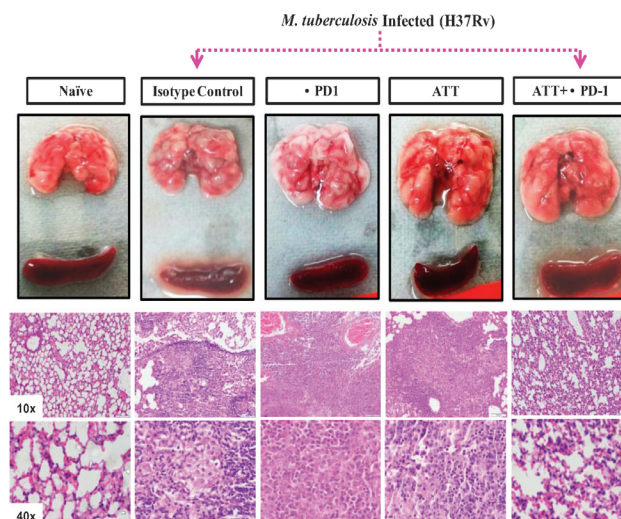


Fig. 1: *M. tuberculosis* Infected (H37Rv).

### Effect of hyperglycaemic conditions/diabetes on activation of latent tuberculosis infection

The mice model of latent Tuberculosis could not be established, as the CFU counts of lungs and spleen, indicative of latent Tuberculosis after BCG immunization with BCG, was not satisfactory as per referred literature. Subsequently, 8 weeks after infection with *Mycobacterium tuberculosis* H37Rv, the CFU counts did not indicate development. Continuing on the proposed line of work, streptozotocin was administered for induction of diabetes which was unsuccessful, as only 22 showed high blood glucose levels. Although negative results were obtained at each step of the proposed plan, the animals are still surviving and it is being planned to sacrifice them and harvest their lungs and spleen to check the CFU counts so as to rule out the confounding variable.

### Study of profile of deformity in new leprosy cases and to analyze predictive risk factors in the development and progression of the disability.

A total number of 40 patients were recruited in this study. Comparing observations of clinical charting and test methods for NFI with results of NCVs, showed impaired latency, amplitude and velocity in other peripheral nerves, besides in those clinically involved.

### Evaluation of *in-vitro* and *ex-vivo* antimycobacterial activity of selected plants traditionally used in tribal medicine against MDR *Mycobacterium tuberculosis* isolates and their active fractions investigation

Seven plants were identified for anti-TB activity against MDR- strains of *M. Tuberculosis*, using *in vitro* resazurin microtitre plate assay (REMA). The significant anti-TB activity was observed in plant extracts i.e., *Mesua ferrea* L. (Calophyllaceae), *Ochrocarpos longifolius* Benth. & Hook.f. ex T. Anderson (Clusiaceae) and *Holarrhena antidysenterica* (Roth) Wall. ex A.DC (Apocynaceae) with MICs 250 µg/ml to 31.25 µg/ml.

### Study of relative frequencies of CD39+CD73+ CD4, CD8 T regulatory cells, Th-17, Th-9, Th-22 cells in leprosy patients with reactions

We observed higher mean percentage of CD4+25+39+Foxp3+T reg cells in healthy individuals than patients when no stimulus was added. However, MLSA increased the percentage of these cells in leprosy patients without reactions. MLSA significantly increased the mean percentage of IFN $\gamma$  expressing CD4+ T-cells in leprosy patients without reaction and when compared with healthy individuals, significantly higher percentage of these cells was noted in these patients. Increase in mean percentage of IL-22 expressing CD4+T-cells in response to WCL was noted in leprosy patients with reactions than healthy individuals.

### Outreach Programme on Zoonotic Diseases

A total of 117 samples (53 milk samples from domestic livestock species and 64 sputum samples from suspected Tuberculosis patients) were collected and screened for the presence of *Mycobacterium bovis*. Of the 53 milk samples, 3 (5.66%) milk samples were found positive for the presence *M. bovis* using PCR. Of the 64 sputum samples, 42 (65.6%) samples were found positive for the presence of acid fast bacilli using ZN staining. All the isolates were identified as *M. Tuberculosis*.

### Identification of novel genetic mutations conferring of extensive drug resistance (XDR) in Indian isolates of *Mycobacterium Tuberculosis*

During the period, a total of 61 suspected multidrug resistant-Tuberculosis (MDR)-TB patients were included in this study. Of the 61 patients, 8 isolates of *M. Tuberculosis* were recovered. Of the 18 *M. Tuberculosis*, 4, 6 and 2 isolates were found as pan-susceptible (resistant to all first and second line anti-TB drugs), multi-drug resistant (MDR: resistance to both rifampicin and isoniazid with or without for other drugs) and extensively drug-resistant (XDR: resistance to at least isoniazid and rifampicin with additional resistance to any fluoroquinolone and at least one of three injectable anti-TB drugs), respectively.

### Whole proteome analysis of aminoglycosides resistant isolates of *Mycobacterium Tuberculosis*

We have analyzed the cytosolic proteome of amikacin (AK) and kanamycin (KM) resistant *Mycobacterium Tuberculosis* isolates by proteomic and bioinformatic approaches. Twenty protein spots were found over expressed in resistant isolates. Among these six proteins were with unknown functions or undefined role. String analysis also suggested that over expressed proteins along with their interactive partners might be involved in aminoglycosides resistance. Cumulative effect of these over expressed proteins could be involved in AK and KM resistance by mitigating the toxicity, repression of drug target and neutralizing effect.

### RESEARCH PROJECTS AT MRHRU, GHATAMPUR

#### Improving health and nutritional status of vulnerable segment of population by implementing multi-component health and nutrition education intervention as a sustainable model of intervention

The objective of the project is to estimate the present nutritional status of the vulnerable population, the children and the mothers, and assess improvement in nutritional status through extensive counseling

methods. The baseline survey has been completed and two rounds of counseling of selected population has been completed.

#### **Elucidating the strain differentiation and transmission dynamics of *M. leprae* through inter simple sequence repeats (ISSR-PCR) marker**

A total of 153 samples were collected from 71 villages of Ghtampur tehsil. A total of 57 (37.25%) patients were found to be positive for RLEP-PCR, while 36 (23.52%) were found for positive for AFB. It implied that RLEP-PCR is a sensitive tool for leprosy case detection rather AFB smear technique. A total of 82 (53.59%) patients were found to be positive for ISSR-PCR while 36 (23.52%) were found positive for AFB. It implied that ISSR-PCR is more sensitive in leprosy case detection.

#### **A study on endemicity of leprosy and utilization of health services in selected areas of Uttar Pradesh and Chhattisgarh**

A total of 164 slit skin smear, nasal swab samples and 161 blood serum samples for PGL-1 were collected. From healthy contacts, centre had collected 176 nasal swab and 124 blood serum samples. Thirty two patients were found positive for slit skin smear samples. PGL-1 ELISA study is under progress.

#### **Model Rural Health Research Unit, Haroli, Una, Himachal Pradesh**

The Department of Health Research, among its allocated 9 new functions to promote health research activities, besides the on-going work relating to the management and administration of ICMR, has initiated the scheme for 'Establishment of Model Rural Health Research Units (MRHRUs) in the States' during the 12th Plan period. Under this scheme, the state of Himachal Pradesh has been sanctioned one of the MRHRUs.

### **PUBLIC HEALTH**

#### **Line Probe Assay (LPA) - Strengthening/upgrading laboratories for the detection of MDR-TB cases in India, under the Revised National Tuberculosis Control Program (RNTCP)**

Annual proficiency testing & accreditation of different laboratories of Uttar Pradesh has been completed for LPA & LC-DST. During the period, we have monitored and accredited the laboratories of BHU-Banaras, IRL-Lucknow and IRL-Agra for LC-DST. A total of 293 sputum samples, suspected for TB patients were subjected to LPA for molecular DST testing. Of the 293 specimens, 51 (17.40%) and 164 (55.97 %) were found as MDR and pan-susceptible, respectively. A total no of 506 XDR presumptive cases were subjected to Liquid SL-DST. Of the 506 cases, 112 cases were found to be XDR, 121 were found to be Ofloxacin resistant, 13 were found to be Kanamycin resistant and 52 were found sensitive for both the drugs.

#### **Strengthening of Mycobacterial repository for translational studies (Phase II)**

Presently, we are collaborating with 62 Institute/ University in different parts of the country. Nearly 5750 well characterized isolates available in the repository are being maintained by regular passages and are available to support the investigators in the country.

#### **NRL (National Reference Laboratory) For Tuberculosis Activities**

NJIL & OMD, Agra is linked to supervise the activities in the sites of Uttarakhand (Number of districts-13; Number of DMCs-148; Number of CB-NAAT sites-9) and Uttar Pradesh (Number of districts-75; Number of DMCs-2021; Number of CB-NAAT sites-77). IRL, Agra and C&DST lab, JNMC, AMU, Aligarh are supported for the screening of XDR-Suspects by NRL-JALMA.

#### **Patient Care and Related Activities**

A total of 28734 patients, having symptoms, suggestive of leprosy and skin diseases attended the OPD in 2015-16. Out of the above, 3134 new leprosy cases were diagnosed and put on treatment. 18762 cases continued to be on treatment at NJIL & OMD. The surgical unit provides ulcer care to both out-door as well as; indoor patients. The radiography unit provides support to the patients for the radiological detection of Tuberculosis.



## NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES (NICED), KOLKATA

### RESEARCH AND SURVEILLANCE ON ENTERIC PATHOGENS

#### Drug resistance among enteric pathogens

Global emergence of fluoroquinolone (FQ) resistance in *Salmonella* Typhi (*S. Typhi*) has complicated the treatment of typhoid fever since the last decade. Widespread usage of FQs has major influences in reshaping the current *S. Typhi* population. Isolations of ciprofloxacin resistant and decreased ciprofloxacin susceptibility (DCS) *S. Typhi* isolates, varied from 16.7% to 25.8% and 69.3% to 82.1% over the last three years in ICMR-NICED, Kolkata respectively (Fig.) Overall, the non-susceptibility to ciprofloxacin was found to be about 98%. FQ resistance was mediated by mutations in quinolone resistance-determining regions (QRDRs) of DNA gyrase and topoisomerase IV genes. Gradual increase in genetic diversity of ciprofloxacin resistant *S. Typhi* isolates was also noticed by pulsed-field gel electrophoresis (PFGE) and majority of the isolates belonged to haplotype H58. Increased occurrence of FQ, especially ciprofloxacin resistance in *S. Typhi* Kolkata isolates, may indicate complete withdrawal of the drug from typhoid treatment to stop treatment failure cases of typhoid fever. Information on circulating pulsotypes of ciprofloxacin resistant *S. Typhi* with prevalent H58 haplotype is important in containment of this deadly organism.

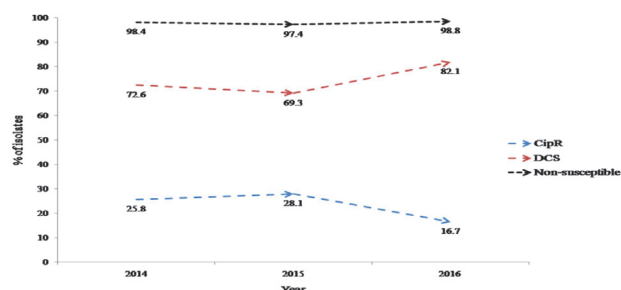


Fig. 2: Increased isolation of ciprofloxacin resistant and DCS *S. typhi* isolates in Kolkata over last three years (2014-2016).

*Campylobacter* species, particularly *Campylobacter jejuni*, have been documented as an important pathogen for causing acute bacterial gastroenteritis in humans and may require antibiotic therapy to reduce severity of the disease. High resistance towards fluoroquinolones had shifted the treatment of these infections towards macrolides. However, in India, an increasing occurrence of macrolide resistance in *Campylobacter* is being found mainly due to point mutation in V-region of 23S rRNA. To detect azithromycin resistance, ICMR-NICED has developed a PCR based assay, which was validated using disk diffusion method as the gold standard. The PCR based detection system showed 100% sensitivity and specificity and can serve as a simple and rapid method for detection of mutation conferring macrolide resistance with additional feature of identification of sensitive strains.

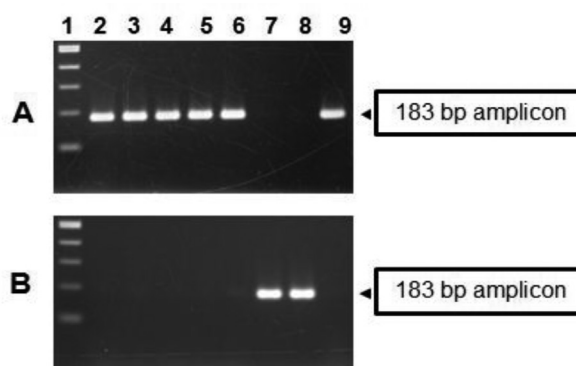


Fig. 3: PCR based assay to detect the azithromycin resistant strains using primers 23srRNA-Campy-1912F / 23srRNA-Campy-2075R [A] and sensitive strains using primers 23s-rRNA-Campy-1912F / 23s-rRNA-Campy-2074N-Rev [B] in representative *Campylobacter* strains of Kolkata. Lane 1 contains a 100-bp size ladder. Lanes 2 to 6 and 9- representative resistant strains. Lanes 7 and 8- sensitive strains.

On the other hand, acknowledging the huge clinical implications of the efficiency with which an antibiotic resistance gene is transferred from one bacterium to another, the mode of transmission of New Delhi-metallo- $\beta$ -lactamase-1 (NDM-1) was studied, both via mobile genetic elements and via outer membrane vesicles (OMVs). It revealed the first experimental evidence of intra and inter-species transfer of plasmid-harboring *bla*<sub>NDM-1</sub> gene in *A.*

*baumannii* via OMVs with high transformation frequency.

**Environmental dynamics of enteropathogenic bacteria**

Longitudinal surveillance on environmental dynamics of enteropathogenic bacterial species of the aquasphere in Eastern India (across Ganga Delta, West Bengal) and Western India (Gulf of Khambat, Gujarat) and their impact on the health of the coastal population and identification of bio-environmental markers has been a major working component. Impact of climate change on enteropathogens and risk assessment for prediction of outbreaks of waterborne diseases, are also being addressed. Interplay of certain specific environmental indices on the pathogenic conversion of the enteric bacterial species has been elucidated.



**Fig. 4:** Cross-sectional field activity in the Gangetic delta.

**Vaccine trials and efforts to develop vaccines against enteric pathogens**

As part of a multi-centric phase III trial, an oral live attenuated bovine-human reassortant pentavalent Rotavirus vaccine, developed by Serum Institute of India was tested for efficacy and safety among 700 healthy infants recruited by ICMR-NICED. Primary analysis of this three-dose vaccine revealed that the vaccine was safe and efficacious against severe Rotavirus acute gastroenteritis.



**Fig. 5.1:** Subject receiving intervention in study clinic.



**Fig. 5.2 :** Subject followed up in clinics.

Another trial studied the impact of IPV boost among 39 week old infants and compared with the boosting effect of OPV in age-sex matched infants. Analysis showed that IPV boost resulted into significantly higher seroconversion rates for Polio type 2 ( $p=0.03$ ) and Polio type 3 ( $p<0.01$ ) as compared to OPV boost. However, intestinal mucosal immunity at week 52 remained comparable between two groups. Results of this study can be considered as a part to support the final endgame strategy of global polio eradication initiative, which includes switching from trivalent oral poliovirus vaccines (tOPV) to bivalent oral polio vaccine (bOPV), and introduction of IPV.

Responding to the need of the hour, ICMR-NICED scientists have successfully explored the use of *Shigella* outer membrane vesicles (OMVs) as novel particles for development of next generation vaccine against *Shigella* species. The hexavalent

OMVs (from six major circulating *Shigella* strains) immunogen demonstrated about 80% protection against all the six strains. The study highlighted the possibility of using *tolA* mutant *Shigella* strain as a novel live attenuated *Shigella* vaccine. Further studies are planned towards elucidating the protective efficacy against homologous and heterologous strains as well as immunomodulatory signaling of innate and adaptive immunity. In March 2017, ICMR-NICED and MSD-Hilleman Lab signed a MoU for *Shigella* vaccine development.

The major emphasis of laboratory research in the Clinical Division of ICMR-NICED was on the identification of novel virulence factors of *Salmonellae* and the host immune responses, with an aim to develop novel antimicrobial agents and vaccines.

### Community-based interventions to reduce morbidities and mortalities from diarrhoea

A multi-component intervention was designed to improve diarrhoea related knowledge and practices of non-qualified practitioners' in order to provide appropriate clinical management of diarrhoea in urban slums of Kolkata. At one year of follow up, overall and cholera related knowledge showed sustained improvement in most of the domains. Moreover, exit interviews with the patients revealed that practitioners whose knowledge was improved, could satisfy their patients significantly more regarding treatment.

Recently, a cohort has been undertaken in a rural block of Hooghly district in West Bengal to identify the factors associated with failure of early initiation of breast feeding and/or early interruption of exclusive breast feeding.

### Surveillance of enteric diseases

The Clinical Division of ICMR-NICED carries out two major hospital-based surveillance studies. The diarrhoeal disease surveillance, conducted at the ID & BG Hospital, Beliaghata and Dr. B.C. Roy Children's Hospital, Kolkata (renamed as B C Roy Postgraduate Institute of Pediatric Sciences, Kolkata), recruits every 5<sup>th</sup> diarrhoea patient on two selected days in a week. Along

with socio-demographic, clinical and other relevant information, stool specimens are collected for elucidating major diarrheagenic pathogens and their resistance patterns. The findings are reported to local and State health authorities for formulating appropriate treatment guidelines. Another surveillance is being conducted at Dr. B.C. Roy Children's Hospital (renamed as B C Roy Postgraduate Institute of Pediatric Sciences, Kolkata) for enteric fever cases, detected through blood culture and Widal tests.

As part of the institutional diarrhoeal disease surveillance and National Rotavirus Surveillance Network, Rotavirus (RV) surveillance is conducted by ICMR-NICED to assess prevalence of Rotavirus infection among hospitalized children and to monitor circulating strains in the region. This surveillance is a part of the national network to provide baseline information as RV vaccine has been introduced in national immunization program in four states in India. Genetic characterization of positive strains revealed predominance of G1P[8] and G3P[8] genotypes during this period. Emergence and circulation of G3P[8] strains in Eastern India has been observed after more than a decade.

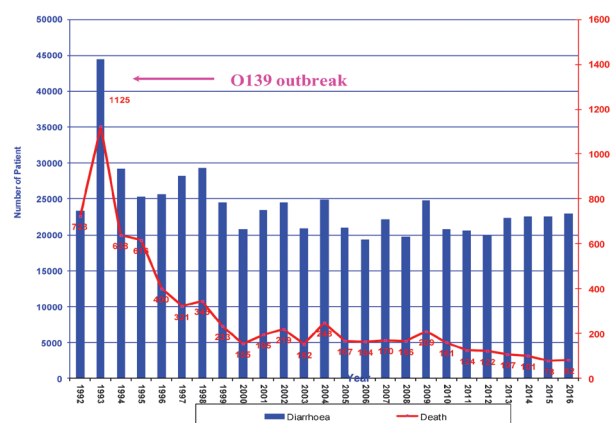


Fig. 6: Diarrhoea admitted cases and Deaths at ID Hospital.

The Parasitology Division has been entrusted with the monitoring of Soil Transmitted Helminths (STH) among school children aged 5-12 years and also for mapping of STH prevalence in North Eastern states of India. Depending upon the estimated burden, children may be included in regular deworming program.



Recently, one study has been initiated to improve the understanding about the role of climate variability on occurrence of diarrhoea diseases, adjusted for the effects of relevant non-climate factors and to determine the linkage between temporal changes in water quality and occurrence of diarrhoea diseases, including diarrhoea outbreaks. This study has been planned in the coastal Sundarbans area, having very high vulnerability from climate changes.

**SURVEILLANCE ON OTHER PATHOGENS**

Hospital based surveillance for respiratory virus infected cases in outpatient department and hospitalized cases has been undertaken to monitor seasonality, emergence of new subtypes and monitoring antiviral resistance among circulating influenza strains. The study is being done in collaboration with NIV, Pune and Centres for Disease Control and Prevention, Atlanta, USA. Human Metapneumovirus (19.7%), Adenovirus (19.1%) and Rhinovirus (18.3%) were found to be predominant among the ARI cases.

**SURVEILLANCE AND RESEARCH RELATED TO HIV/AIDS**

As the NACO-Regional Institute (East) for HIV surveillance, ICMR-NICED is actively engaged in providing technical and research inputs for prevention and control of HIV in the country with particular focus on East and North-Eastern states.



Fig. 7: HSS sample testing.

ICMR-NICED has been entrusted in supervision and monitoring, in addition to imparting training, for HIV sentinel surveillance (HSS) among antenatal

clinic (ANC) attendees and various high risk groups in the states/UTs of Andaman & Nicobar Islands, Chhattisgarh, Meghalaya, Nagaland, Sikkim and West Bengal.

Another important area of HIV-related work was exploration of emergence of HIV drug resistance (HIVDR) mutations, among antiretroviral therapy (ART) recipients. HIVDR limits the success of ART and therefore, needed to be monitored. The study revealed non-appearance of major or minor HIVDR among ART naïve individuals indicating absence of transmitted HIVDR in Kolkata, a metropolitan city in eastern India.



Fig. 8: Participants – EQAS Workshop.

One multi-site qualitative study has been planned with a focus to explore how people manage to stay HIV discordant in heterosexual marriage. This will entail understanding ‘beliefs’, ‘barriers’ and ‘facilitators’ pertaining to HIV-transmission within marriage. This study has the potential to suggest appropriate interventions in this context.

The National AIDS Control Organization has also entrusted ICMR-NICED as the lead institute to undertake an operational research for situation analysis of health facility preparedness for screening and treatment of syphilis among antenatal clinic attendees in India. This would be a multi-State operational research project (involving West Bengal, Haryana, Maharashtra, Tamil Nadu and Assam), with the objective of strengthening the elimination activities for congenital syphilis in India.

**OUTBREAK INVESTIGATIONS**

ICMR-NICED conducted several outbreak investigations during this period. On request from the State Health Authority, referred samples from various

State Govt. Hospitals were received and processed for diagnosis of dengue virus along with viral serotyping. Around 12,000 samples were screened and feedback results were sent to the concerned department with intimation to the State Health Authority. An outbreak of foodborne acute gastroenteritis in a rural area of South 24-Parganas district was successfully investigated by scientists from ICMR-NICED. The outbreak was found to be caused by *Shigella sonnei* and appropriate control measures were recommended for prevention and control.

### OTHER ACTIVITIES AND SERVICES

Under the SWACHH BHARAT campaign, the members of the 'Hygiene Committee of ICMR-NICED' have undertaken awareness activities in numerous schools and community settings in the city, focusing on various health issues. The campaign involved Voluntary Cleanliness Programme in the slum communities and a Tree Plantation Programme organized in collaboration with local municipal authorities.



Fig. 9.1: Tree Plantation program in urban slums of Kolkata.



Fig. 9.2: Voluntary cleaning activities involving local residents.

External Quality Assurance Scheme is one of the important tools to assess the performance of the laboratory and their ability to generate accurate results. National Reference Laboratory of ICMR-NICED is the proficiency testing provider for HIV antibody testing for the States Reference Labs (SRLs) of A&N, Assam, Jharkhand, Meghalaya, Mizoram and Orissa.

In consultation with the State Health Authorities, the Viral Research Diagnostic Laboratory (VRDL) of ICMR-NICED provided diagnostic services for a large number of suspected Dengue infections from various health facilities of West Bengal.

ICMR-NICED also provides testing services for samples of cases admitted with acute respiratory illness from different hospitals for detection of influenza A H1N1. Apart from West Bengal, samples from other states are also included for detection and further studies.

Vibrio Phage Reference Laboratory received strains of *Vibrio cholerae* isolated from six different states, including West Bengal, for phage typing study.

## ICMR VIRUS UNIT, KOLKATA

### VECTOR BORNE DISEASES

#### Seroprevalence of Dengue and Chikungunya virus infection during dengue outbreak in West Bengal

West Bengal suffered from vast dengue outbreaks all over the State in 2016. Massive dengue outbreaks were observed in few districts in South Bengal from middle of July till October, 2016. During the outbreak, ICMR virus Unit and VRDL-ICMR-NICED, screened dengue suspected blood samples for dengue NS1 and IgM by ELISA. 11,549 samples for NS1 and 4,545 samples for IgM were screened, and the positive cases were 3,272 (28.33%) and 1,392 (30.62%) respectively. Chikungunya IgM by ELISA was also tested for 2,171 samples with fever >5 days and 372 were seroreactive. 312 samples have also been tested for Chikungunya viral genome by conventional



one step RT-PCR and 36 were RNA positive. Dengue and chikungunya sero-nonreactive blood samples from 318 female patients, within age group 18-40 years, were also tested for Zika virus by conventional one step RT-PCR but none of them tested positive for viral RNA.

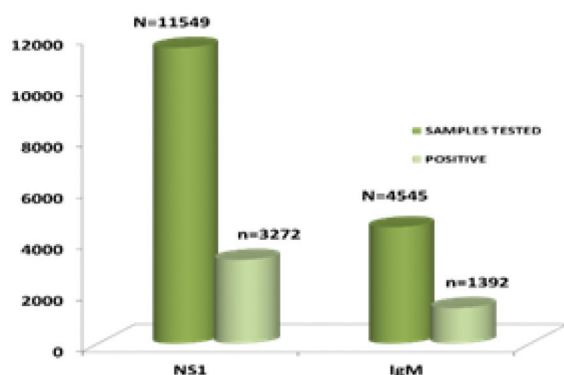


Fig. 10: Total sample tested for Dengue NS1 and IgM during 2016 dengue outbreak..

### Circulating Dengue Serotypes in West Bengal

All districts of West Bengal suffered from dengue outbreak in 2016. Centre processed 435 dengue NS1 seroreactive samples for dengue serotyping received from all over West Bengal and observed co-circulation of all the four dengue serotypes during the outbreak with different percentage. In this outbreak, Centre noticed that different dengue serotype was predominant in different areas of West Bengal, e.g., one district (Hooghly) predominantly suffered from DENV-2, whereas another district (North 24 Parganas), adjacent to Kolkata was affected by DENV-1, and few districts had co-circulation of all the four dengue serotypes. Overall, DENV-1 was the major serotype, followed by DENV-2 and DENV-4 during outbreak. Dengue serotype 3 was the least prevalent whereas, Centre found DENV-3 serotype was the major circulating strain in 2012 and 2015 dengue outbreak in Kolkata and its adjacent areas. Out of NS1 dengue seroreactive samples, 415 were dengue RNA positive by nested RT-PCR and 219 (52.77%) were DENV-1, followed by DENV-2 109 (26.26%), DENV-4 48 (11.57%) and DENV-3 was the least 39 (9.40%).

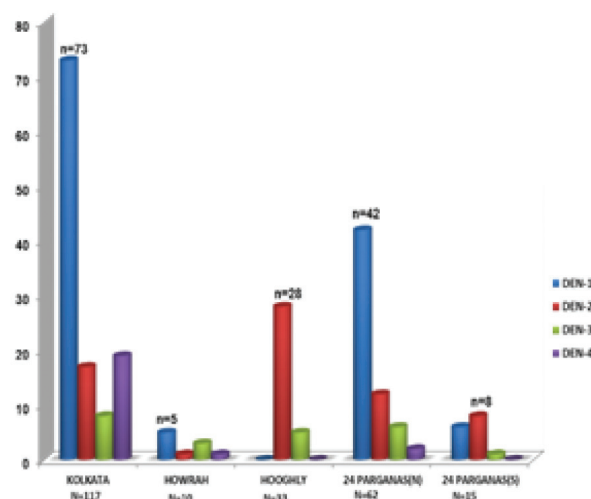


Fig. 11: Dengue serotypes in Kolkata and its adjoining districts in 2016 Dengue outbreak.

### VIRAL HEPATITIS

#### Genomic variations of hepatitis C virus among High Risk Group Population in Eastern Region

During this period, 203 HCV sero-reactive whole blood samples from different population groups were collected from Liver Clinic of different Medical Colleges of Kolkata. Demographic and clinical data were also collected and processed to re-confirm HCV sero-reactivity by ELISA. The detection of viremia was done by nested RT-PCR and quantitative real time PCR. HCV RNA positive samples were further processed for viral genotype analysis, based on core region of the HCV genome. HCV RNA positivity varied in different high risk population groups, e.g, RNA positivity in thalassemia patients and PWIDs was 65.38% and 90.90% respectively, whereas general population with chronic liver diseases (GP with CLD), it was 74.02%. HCV genotype distribution was also found to vary in different population groups, majority of thalassemia patients were infected with HCV genotype 3 (86.84%), approximately 57% of PWIDs and 70% of hemodialysis patients were infected with HCV genotype 1, whereas ~63% of the general population with chronic liver disease was infected with HCV genotype 3. A total of 50 HCV RNA positive individuals of different

genotypes were treated with Direct Acting Antivirals (DAAs), 5 of them showed relapse and belonged to genotype 3, the major genotype variant in India.

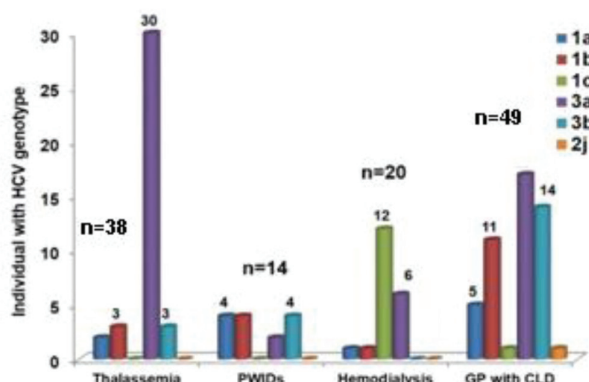


Fig. 12: HCV genotype distribution in different High Risk Group population.

### Studies on HCV within multitransfused β-Thalassaemia patients

During this period, 623 blood samples from multitransfused β-Thalassaemia individuals were collected from different blood Transfusion Centres and Day care, almost all over West Bengal and processed for HCV sero-reactivity by ELISA method. Overall, 133 out of 623 (21.35%) were HCV sero-reactive. In this study, it was found that the percentage of HCV seroreactivity varies from blood transfusion Centre to Centre. Samples were then tested for HCV RNA for viremia for active viral infection. 86 out of 133 (64.66%) seroreactive samples were HCV RNA positive by both qualitative and quantitative methods. Partial HCV core gene was amplified for viral genotyping and study observed that HCV genotype 3 was the major circulating strain within Thalassaemia patients (~86%), majority of them infected with HCV genotype 3a (~78%), followed by genotype 1 (~14%). Host single nucleotide polymorphism analysis of IL28B gene showed Thalassaemia patients carrying more unfavourable IL28B host genotype and HCV sero-clearance is associated with IL28B host genotype and prominent within low age group with favourable IL28B host genotype, as evident from serology and molecular HCV detection.

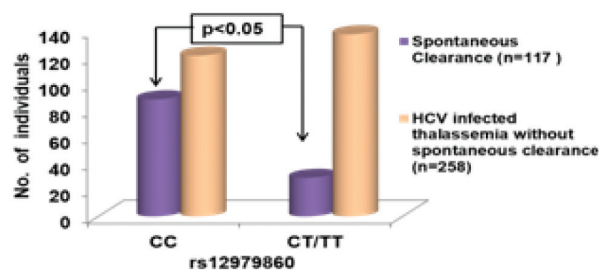


Fig. 13: Host IL28B(rs12979860) distribution among individuals with and without spontaneous clearance. Total sample tested for Dengue NS1 and IgM.

## HERPES VIRUSES

### Incidence of Human cytomegalovirus infection among immunocompromised patients in West Bengal, India

During this year, 309 immunocompromised patients were enrolled, of which 191 (61.8%) were seropositive for cytomegalovirus immunoglobulin G and 91 (29.4%) for immunoglobulin M, while cytomegalovirus DNA was detected in 112 (36.2%) patients. Seroprevalence in males was slightly higher than that in females. Multivariate logistic regression analysis revealed that age, house hold income and mode of HIV transmission were significant risk factors for HCMV infection.

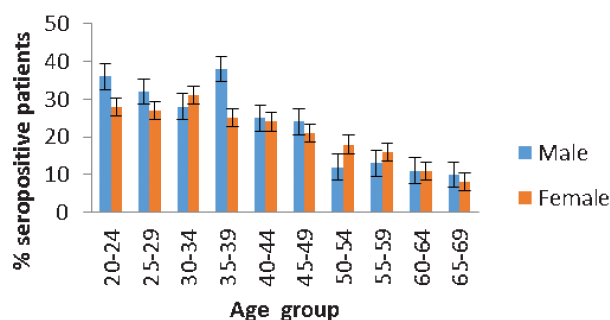


Fig. 14: HCMV seropositivity among AIDS patients of different age groups.

### Diagnosis, genotypic distribution and phylogenetic relationship between glycoprotein B (gB) gene in symptomatic congenital Cytomegalovirus (cCMV) infection

576 live-born infants from two different metropolis hospitals were tested within 2 weeks after birth. A total of 106 (18.4%) were tested to be positive

for CMV infection with or without symptoms. A total of 17 (2.95%) symptomatic newborns were affected more severely at birth with symptoms of CMV disease and the rest (n= 99) were considered as asymptomatic (18.4%). Among 17 symptomatic newborns, 5 (29.4%) of them were males and 12 (70.5%) were females. Genotyping based on the gB sequence was determined in samples from 17 symptomatic infants with congenital CMV infection. Single genotype was found in 14 (82.3%) samples. gB1 was the most frequent genotype observed in 4 specimens (23.5%). While, 17.6% of gB2 and gB3 genotype was observed (n=3 in each genotype). A single sample corresponded to gB4 (5.8%) and two for gB5 (11.7%). A mixed-genotype infection was detected in 3 (17.6%) cases. Among these mixed genotype infections, all were dual gB genotype infections and included gB1 and gB2 in 2 (11.7%), 5.8% (1/17) of each gB1-gB3 and gB2-gB5 genotypes.

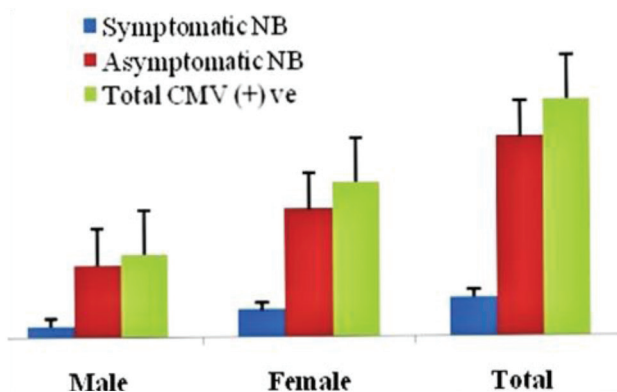


Fig. 15: CMV test symptoms.

## NATIONAL INSTITUTE OF MALARIA RESEARCH, NEW DELHI

### Presence of multiple knockdown resistance (*kdr*) mutations in *Aedes aegypti* with reporting of a new mutation F1534L associated with insecticide resistance

The control of several arboviral infections, mainly Dengue, Chikungunya and Zika virus solely relies on vector control measures in absence of specific drugs or vaccines against these infections. Emergence of pyrethroid resistance in vectors may pose a serious threat to the success of insecticide-based vector

control programme. One of the mechanisms of pyrethroid resistance in insects is alteration in the voltage gated sodium channel (target site of action for pyrethroids) due to point mutation referred to as knockdown resistance (*kdr*). NIMR reports the presence of a multiple knockdown resistance (*kdr*) present in an Indian population and discovery of a new mutation F1534L associated with DDT and pyrethroid resistance.

DNA sequencing of partial domain II and III of the VGSC was performed on *Ae. aegypti* collected from Bengaluru, India. It revealed the presence of mutations V1016G and S989P in domain II and two alternative *kdr* mutations F1534C and F1534L in domain III. The F1534L mutation is being recorded for the first time in *Ae. aegypti*, which is due to T>C substitution on the first position of the codon. Separate allele specific PCR assays (ASPCR) was developed for detection of *kdr* mutations V1016G and S989P. A PCR-RFLP based strategy was developed for genotyping of all the three mutations reported in domain III i.e., F1534L, F1534C and T1520I. V1016G and S989P were in complete linkage disequilibrium while they were having negative linkage disequilibrium with *kdr* alleles F1534C and F1534L. Genotyping of field populations collected in the year 2014 and 2015 revealed a low frequency of V1016G/S989P (13%), a high frequency of F1534C (52%), moderate frequency of 1534L (17%) and absence of T1520I. The new mutation F1534L showed significant protection against permethrin, deltamethrin and DDT, whereas F1534C showed protection against permethrin and DDT but not against deltamethrin.

The F1534L *kdr* mutation is being reported for the first time in *Ae. aegypti* that is associated with DDT and pyrethroid resistance. Presence of this mutation needs to be investigated in other parts of world.

### Vector Surveillance of Zika virus in selected high risk areas of India

The Project is a comprehensive project conducted at various sentinel sites of India. In Delhi, NIMR was identified as nodal Zika virus screening Institute. The project focused on screening of adult *Aedes aegypti*, collected from various localities of

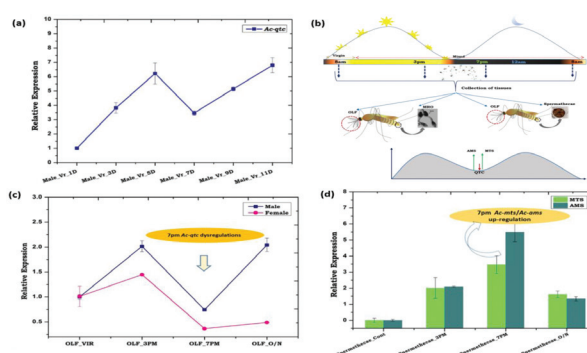


Delhi as well as from areas, where presence of Zika virus is suspected by various Government agencies. About 3104 adult *Ae. aegypti* were screened for detection of Zika virus in about 411 pools presently 157 localities of Delhi and other cities. Till date, none of these pools were found positive for Zika. In addition to this, 369 in 50 pools of *Ae. aegypti* from Ahmedabad, where clinical infection with Zika virus was reported, were also negative for Zika virus.

### Decoding the molecular secrets of mosquito private life

Seasonal elevation of vector population and acute transmission of many vector borne diseases has a critical impact that favours killings of millions of people globally. But how these factors influence the sex specific behavioral biology, especially feeding, mating and breeding of mosquitoes, are yet to be unravelled.

A study identified a unique transcript, encoding *Drosophila* homolog of *quick-to-court (qtc)* protein, from blood fed female *An.culicifacies* olfactory system. Through comprehensive molecular approach, we examined the sex-specific transcriptional regulation of this unique transcript *Ac-qtc*, and predicted its possible role with 'food choice' and/or 'mate choice' behavioural performance. An increased expression till 5<sup>th</sup> day post emergence and significant down regulation of *Ac-qtc* on 7<sup>th</sup> day in the virgin mosquito indicate that male mosquitoes sexually mature on 5<sup>th</sup> day for successful mating and reproduction (Figure 16a). A dual behavioral and molecular assay (Figure 16b) and experimental verification indicated that natural dysregulation of *Ac-qtc* in the late evening might promote the mating events for successful insemination (Figure 16-c). A blood meal response analysis indicated that *Ac-qtc* play significant role in host seeking and blood feeding behaviour in adult female mosquitoes (Figure 16d). Study data provides the first molecular evidence that *Ac-QTC* proteins may have a dual mode of action in the regulation of a cluster of mosquito olfactory genes that are linked to mating success and/or blood feeding in adult female mosquitoes.



**Fig. 16a, b, c, d:** Decoding the molecular secrets of mosquito reproductive cycle.

**Figure 16:** (a) Male achieve adulteration age on 5<sup>th</sup> day post emergence resulting auto dysregulation of *Ac-qtc* on 7<sup>th</sup> day in virgin mosquitoes, even in the absence of adult female mosquitoes; (b) Experimental hypothesis and schematic overview of the assay designed to correlate the function of *Ac-qtc* in the mating success of *A.culicifacies* mosquito; (c-d) Experimental demonstration of above hypothesis that late evening natural dysregulation of *Ac-qtc* manages successful insemination event in copulating couples:(c) sex-specific transcriptional profiling of *Ac-qtc* at different circadian time in the olfactory tissues; and (d) transcriptional response of sperme specific *Ac-mts* and *Ac-ams* genes in the spermathecae of mated mature adult female mosquito;

Study hypothesizes that further decoding the molecular complexity of the neuro-olfactory system may facilitate the identification of unique sets of genes managing sex specific 'mate choice' behaviour and thus, affect reproductive potential. A functional analysis of such molecular secretetes may be crucial to identify a suitable target for vector borne disease management.

### Efforts for Malaria Elimination in Punjab

In view of launch of malaria elimination in India, the state of Punjab has qualified for malaria elimination as per National Framework of Malaria Elimination, 2016-2030. The Department of Health and Family Welfare, Government of Punjab had collaborated with National Institute of Malaria Research (ICMR) in order to validate the data of malaria of the state and to provide technical expertise to



consolidate the efforts in elimination of malaria. A MOU was signed with the Government of Punjab and a field site unit was established at Dhakoli CHC, Zirakpur, District Mohali (Punjab) in the month of August 2016. With this joint venture, the goal of malaria elimination in the state is envisaged to be achieved. Preliminary information for baseline data collection from different districts of Punjab has been collected. Technical support and advisory to the state government has been envisaged under the joint collaboration so that the aim of malaria elimination in the state may be achieved within the time frame. NIMR will provide scientific support for vector control and treatment of malaria cases. With the launch of India's malaria elimination initiative, the states and UTs have a remarkable opportunity to get rid of this disease and contribute to better health and socio-economic development, especially among the country's most vulnerable populations. On the basis of surveillance data collected by the state government, five districts were reporting maximum number of malaria cases in the state. These districts are SAS Nagar, Patiala, Mansa, Bathinda and Ludhiana. Therefore, an action plan was prepared to first take up parasitological surveys in these five districts. Mosquito collections were carried out in all these five districts in the morning hours to know the anopheline fauna and vector density and their susceptibility to DDT, Malathion and Deltamethrin. *Anopheles culicifacies* was found 100% susceptible to malathion currently being used for Indoor Residual Spray (IRS) in Punjab. *Anopheles stephensi* was available in high density in Mansa, Bathinda and Ludhiana districts and showed 55-63% mortality against DDT, 90-95% mortality against Malathion and 97-100% mortality against deltamethrin. The density of primary malaria vector *An. culicifacies* was 11.8 in SAS Nagar, whereas it was low in other four districts. However, the density of *An. stephensi* was quite high in Mansa, Bathinda and Ludhiana districts in comparison to SAS Nagar and Patiala. The density of secondary vector *An. annularis* was high in SAS Nagar, Patiala and Mansa whereas, it was absent in Bathinda and very low in Ludhiana district. A

large labour force coming from malaria endemic states are working in Punjab in Agriculture, brick kilns and other small scale industries. Therefore, Malaria cases reported during NIMR surveys as well as from CHC/PHC were investigated to know whether the case is indigenous or imported. Out of 33 cases investigated through questionnaire based survey, 10 cases (30.3%) were found in migrant labourers with movement history. The 710 vector species (*An. culicifacies*, *An. stephensi* and *An. annularis*) collected during the surveys were assayed for detection of sporozoite and none was found positive.

#### **Inhibitor Identification of *P. falciparum* phosphoethanolamine methyltransferase for antimalarial drug discovery**

An essential enzyme phosphoethanolamine-methyltransferase (*Pfpmt*) of *Plasmodium falciparum* is characterized for rational drug designing. A sinex compound library was virtually screened and tested in *Plasmodium falciparum* culture (3D7) and  $IC_{50}$  was calculated. Amino acids sequence of *Pfpmt* gene in Indian isolate showed 100% identity with that of 3D7-*Pfpmt*. In addition, crystal structures of PvPMT and PkPMT are very similar with all atom RMSD = 0.452 Å. Compound library was built from Asinex compound library. Crystal structure of *Pfpmt* 3D7 (PDB Id: 3UJ9), Glide module of Schrodinger v9.6 was used for docking studies. In order to search novel inhibitors for this essential enzyme, Asinex compound library was subjected to computational ADMET analysis (DS3.5) and top 15 compounds obtained from Asinex were selected and procured based on Glide XP score. Further, five potent compounds were selected as it showed optimum drug-like properties and were non-carcinogenic, non-mutagenic, and non-toxic. In addition, these five hits showed good interaction with target protein and formed hydrogen bonds with crucial conserved amino acids for transmethylation as well as good inhibition activity ( $IC_{50} < 5\mu M$ ) in 3D7 culture. Top two compounds were selected as common inhibitors of *Pfpmt* and may be inhibiting *Pvpmt* and other plasmodium orthologs worldwide (Figure 17).

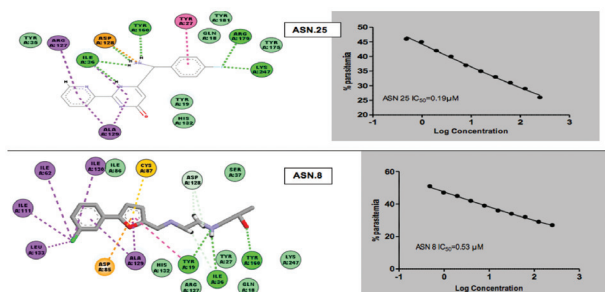


Fig. 17: Interaction and inhibition IC50 of two Pfprt inhibitors.

### Therapeutic efficacy of antimalarials in India

Nation-wide sentinel site system has been developed in the country, where therapeutic efficacy studies of recommended antimalarials have been conducted by NIMR in collaboration with National Vector Borne Disease Control Programme (NVBDCP).

Therapeutic efficacy of antimalarials against *Plasmodium falciparum* and *Plasmodium vivax* malaria were conducted at nine malaria endemic regions in the country (eight for *P. falciparum* and one for *P. vivax*), using standard protocols of World Health Organization. The conducted studies have shown that the efficacy of AS+SP at five sites ranged between 95.5–100% after 28 days of follow-up and the efficacy of AL against *P. falciparum* malaria in north-eastern region ranged between 98.6-100%. CQ remains 100% efficient against *P. vivax* at Kolkata. A random 20% samples were analyzed for single nucleotide polymorphisms in *dihydrofolate* reductase (*dhfr*) and *dihydropteroate synthase* (*dhps*) gene from AS+SP study sites, where majority of the samples (70.7%) showed double mutation in *dhfr* gene and 39% showed single mutant *dhps* gene, followed by wild types, the K76T mutation in chloroquine transporter (*Pfprt*) gene was observed in 58.5% samples. In northeastern region where AL is tested, majority of the samples showed double mutant *dhfr* and *dhps* gene, the K76T mutation was observed in 67.8% of samples and majority of samples showed wild type *P. falciparum* multidrug resistance 1 (*Pfmdr1*) gene. Till date, the generated data showed that the prescribed antimalarials, ACT (AS+SP & AL) against *P. falciparum* and chloroquine against *P. vivax* malaria patients by the National Program are effective and safe (Figure 18).

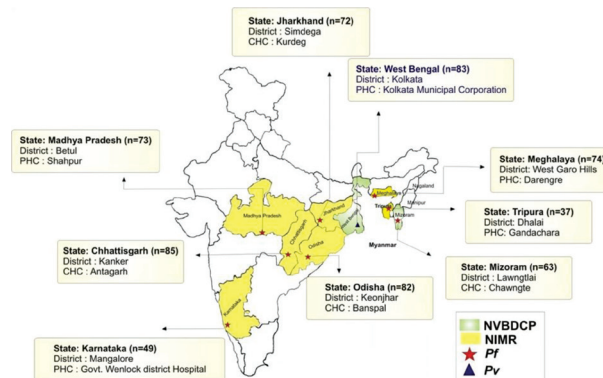


Fig. 18: Map showing study sites.

Mapping of Kelch 13 molecular marker in *P. falciparum* malaria patients across International Border states in North-eastern region of India.

The extent of mutations in Kelch13 gene, the recent molecular marker for artemisinin resistance was mapped for north-eastern region on a total of 388 *P. falciparum* infected patients collected from four study sites in the vicinity of international borders (Figure 19)). A total of twelve mutations (eight non synonymous and four synonymous) were observed in Kelch13 gene, however the mutation associated with artemisinin resistance were not observed in these sites near to international border.

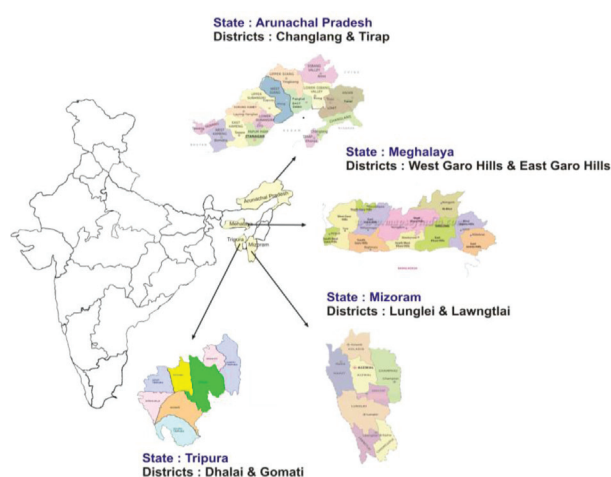


Fig. 19: The details of study sites for Kelch mapping.

### Comprehensive Case Management pilot programme in Odisha, India

Comprehensive Case Management Programme (CCMP) is being carried out by Government of Odisha, National Institute of Malaria Research and Medicines for Malaria Venture. It aims to assess the

impact of early diagnosis and treatment, supported by a strong surveillance system, on the incidence of malaria in different transmission settings in the state of Odisha.

CCMP approach is showing expected results in low endemic block. In other blocks, there has been a significant improvement in surveillance and early diagnosis and treatment which has permitted outbreak control and preventing complications.

More than 90% of malaria patients were followed up for complete treatment. Most cases are now diagnosed and treated at the ASHA level. The time from onset of fever to treatment has decreased with the larger proportion receiving treatment within 24 hours of onset of symptoms. In all intervention areas the number of cases detected has increased except in the low endemic block Bolangir which witnessed reduction in incidence. CCMP has led to a significant increase in access to diagnosis and treatment in all intervention areas. Increase in malaria cases due to improved surveillance followed by decline due to interventions has been shown in Figure 20.

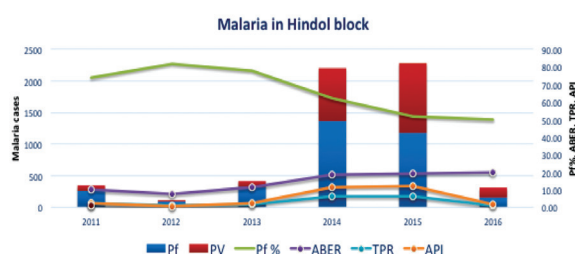


Fig. 20: Malaria in Hindol: Initial increase followed by decline.

### Clinical development of antimalarials

NIMR is actively involved in clinical development of antimalarials, and currently two clinical trials are ongoing.

**A Phase IIIb trial to assess the safety, tolerability and efficacy of dihydroartemisinin/piperazine (Eurartesim®) in Indian children and adolescent patients with acute uncomplicated *Plasmodium falciparum* malaria**

This is a multi-centre, phase IIIb, single arm trial to assess the safety, tolerability and efficacy of Eurartesimoral film coated tablet formulation (160/20 mg or 320/40 mg PQP/DHA) in children and adolescent patients with acute uncomplicated *Plasmodium falciparum* malaria.

**A multi-centre, open-label randomized trial to assess the efficacy, safety and tolerability of Triple Artemisinin-based Combination Therapies (TACTs) compared to Artemisinin-based Combination Therapies (ACTs) in uncomplicated falciparum malaria and to map the geographical spread of artemisinin and partner drug resistance**

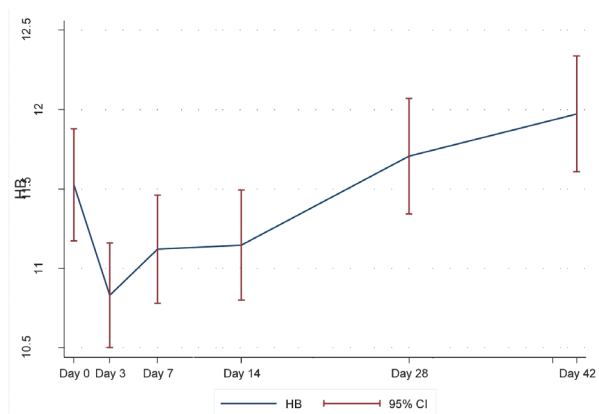
This is an open-label randomized trial comparing standard ACT treatment with matching triple artemisinin-based combination therapies (TACTs), evaluating efficacy in sites experiencing ACT failure and safety, tolerability and artemisinin and partner drug resistance in all sites.

In India, the trial is being carried out at three centres: Agartala Government Medical College; Ispat General Hospital Rourkela; and Medical College, Midnapur.

**Active pharmacovigilance for primaquine radical cure for the treatment of *Plasmodium vivax***

The project, being carried out in Odisha, tries to assess the drop of hemoglobin and recovery following 14-day primaquine treatment for *P. vivax* radical cure. Patients with confirmed vivax malaria, and having hemoglobin level of more than 7 g/dl were included in the study. The study enrolled 100 patients. Maximum fall in mean Hb was observed by day 3; while there was recovery by day 42 (Figure 21). No hemolytic symptoms were observed in enrolled patients. Preliminary analysis indicates that primaquine causes haemolytic anaemia in a fraction of patients. Further investigations are needed to understand the G6PD status of the subjects





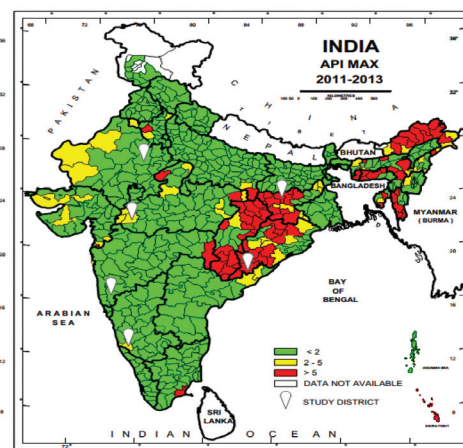
**Fig. 21:** Average change in hemoglobin level over time.

### Evaluation of Dengue Rapid Diagnostic Test Kits

Evaluation of nine brands of rapid diagnostic combi kits for diagnosis of dengue was carried out as a part of ICMR programme, in consultation with the National Vector Borne Disease Control Programme and the Central Drugs Standard Control Organization. Apart from National Institute of Malaria Research, National Institute of Virology, Pune also carried out the evaluation. Nine commercially available combo kits that can detect dengue NS1 antigen and IgM antibody, were evaluated. Overall sensitivity of the kits was greater for detection of NS1 antigen compared to detection of IgM antibodies. Thus, these kits can be useful for diagnosis of dengue in early stage.

### Estimation of malaria burden

A MoH&FW funded project on 'Estimation of malaria burden' was initiated in 2015 with collaboration with NVBDCP and NIMS to validate the reported incidence of malaria and mortality rates of malaria in 6 states of the country in 3 stratified zones, low, moderate and high incidence areas in one district in each state. Districts falling in each stratum were arranged in order of API and divided into two equal groups according to population and then arranged according to percentage of Pf cases in descending and ascending orders. Subsequently, one district from each group was selected using probability proportional to population size (PPS) sampling method from each group. In this manner two representative districts each from low, moderate and high burden districts were selected and overall, 6 study districts were selected in the country (Figure 22).



**Fig. 22:** Map of India showing geographical location of six study districts (white balloons) for capturing malaria morbidity and mortality. Firstly district level stratification of India was done on the basis of three Annual Parasite Incidence (API) classes, <2, 2-5 and >5 taking in to consideration APImax of malaria from 2011-2013 and then two study districts from each of the three strata were randomly selected as per PPS sampling method to conduct malaria burden estimation study.

Further, 3 Primary Health Centres (PHCs) were selected randomly from the list of all PHCs of each selected district, such that study population size within each selected PHC was about 70,000. In case, selected PHC was smaller (population less than 60,000), some population of the adjacent PHC or a Sub-Centre was included in the study area to obtain desired sample size. Similarly, the larger PHC (i.e. with Population > 80,000) was divided to select a contiguous segment of required population size. Overall, study population of surveillance area was 0.2 million/district. For death enumeration, an adjacent PHC of similar size and epidemiological features were selected, matching every surveillance PHC area.

The study concluded in mid 2016. It covered a sample of 2.4 million of population in both rural and urban areas within the chosen districts for morbidity and mortality estimation. Prepared by ICMR-NIMS, the study sampling frame covered wide spread areas, which were representatives of the diverse malaria situation found in the country. An active surveillance for detection of malaria cases in the study population was carried out by house to house visit every fortnight and testing of fever cases for malaria with the help of well trained 264 voluntary workers and supervisory staff. The number of malaria cases and deaths were compiled from both Private and Govt. Hospitals,

practitioners, pathology laboratories, Panchayats, Municipalities, crematorium and burial grounds. Verbal autopsy of death cases, reported from study area was performed using a pre-designed proforma and cause of death was confirmed by three expert who are independent medical professionals.

Based on the analysis of data, the estimated number of malaria cases in the country ranged from 3,792,018 to 3,958,137 during the study period of one year, with point estimate of 3,875,078 malaria cases in India (Table 1). Overall, the weighted estimate of Annual Falciparum Incidence (AFI) for the country was 2.20 (95% CI: 2.16-2.24) per thousand population. Based on weighted estimates of AFI and standard error of estimate, the estimated number of Pfmalaria cases in the country was worked out between 27,40,577 and 28,38,389 with point estimate of 27,89,483 P. falciparum cases (including mix infections). The overall point-estimates of deaths due to confirmed malaria were 19,067 with 95% confidence interval (CI) of 13,665–24,470 and the point estimate of deaths due to suspected malaria was 10,274 (95% CI= 7,694–12,853) with overall deaths of 29,340 (95% CI: 23,354-35,327), attributable to malaria in a population of 1.269 billion in India. The outcome will be useful for planning effective malaria elimination strategies by the NVBDCP and for designing appropriate National Malaria Control Policy.

**Table 1:** Salient findings and the gap between estimates number of malaria cases and deaths vs reported number of cases and deaths in India.

	Estimated (EMBI Study)	Reported (2016)
API	3.05 (2.99-3.12)	0.86
Cases	38,75,078 (37,92,018-39,58,137)	10,90,724
Deaths	29341 (23354-35327)	331
Confirmed	19067 (13665-24470)	NA
Suspected	10274 (7694-12853)	NA

### Cultivation of pre-erythrocytic stage of *Plasmodium vivax in-vitro*

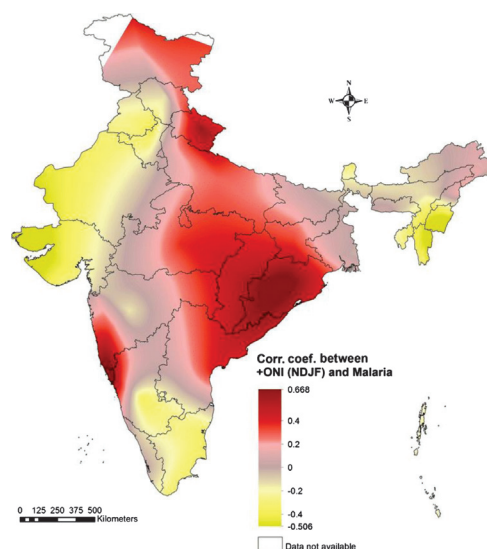
For the first time in India, *P.vivax* pre-erythrocytic schizonts (liver stage) were developed in hepatoma cell line using the facilities of Parasite bank in NIMR. Mosquitoes were fed on infected blood

through artificial membrane feeding apparatus and the fed mosquitoes were dissected on appropriate days for oocyst and sporozoites. These sporozoites from artificially fed mosquitoes were used for inoculating the hepatocytes/hepatoma cell line for the development of pre-erythrocytic stage parasites.

### Evaluation of El Niño Southern Oscillation as an early warning tool for malaria outbreaks in India

In order to find out the relationship between El Niño, rainfall and malaria incidence in different states of India, a study was undertaken at national level. Risks of malaria epidemics in relation to El Niño and Southern Oscillation (ENSO) events have been mapped and studied at global level. In India, where malaria is a major public health problem, no such effort has been undertaken that inter-relates El Niño, (ISMR) and malaria. In the present study, relationship between ENSO events, Indian Summer Monsoon Rainfall and malaria cases was determined with a point of view of using the tool for early warning of malaria outbreaks.

Correlation coefficients between ISMR, ‘+ winter ONI’ (of November, December, January and February months) and ‘malaria case index’ were calculated using annual state-level data of 22 years. The resultant correlations were further analysed on GIS platform to generate spatial correlation map (Figure 23).



**Fig. 23:** Relationship between El Niño and malaria in India.

The correlation between ‘+ winter ONI’ and ‘rainfall index’ showed that there is great disparity in effect of ENSO over ISMR distribution across the country. The resultant map showed that western part of India ( Gujarat, Rajasthan and parts of Karnataka) are likely to experience outbreaks of malaria if rainfall is good. Negative correlation with ONI (La Nina condition) shows heavy rainfall leading to outbreaks in the states of Rajasthan, Haryana, Gujarat, part of Tamil Nadu, Manipur, Mizoram and Sikkim indicating the likelihood of outbreaks. On the other hand, the states of Orissa, Chhattisgarh, Jharkhand, Bihar, Goa, eastern parts of Madhya Pradesh, part of Andhra Pradesh, Uttarakhand and Meghalaya may experience outbreaks, if rainfall is deficient.

With better resolution at district level and correlation with ONI of February onwards, forecast of malaria outbreaks can be made with better certainty so as to guide the national programme for planning intervention measures in view of ENSO events.

### WORKSHOP/TRAINING/EVENTS

1. Malaria Microscopy refresher training-I; 08 Nov. to 18 November,2016 (10 days).
2. Malaria Microscopy refresher training-II; 22 Nov. to 2 December,2016 (10 days).
3. WHO supported External Competency Assessment of Malaria Microscopists, 12 Dec to 16 Dec, 2016 (5 days).

## VECTOR CONTROL RESEARCH CENTRE, PUDUCHERRY

### LYMPHATIC FILARIASIS

#### Morbidity management and disability prevention programme (MMDP) for filarial lymphoedema

Filarial-Lymphoedema (LE) cases enduring acute dermato-lymphangio adenitis (ADLA) episodes were grouped based on the frequency of ADLA to select patients for administering antibiotic (penicillin or doxycycline) prophylaxis. The patients attending the filariasis clinic at VCRC are reviewed at least 3 times a year, are benefited

in terms of reduction in ADLA episodes and LE volume reduction. A Community based study carried out in 3 sets of patients (75, 74 & 63) in Villupuram District of Tamil Nadu has shown that regular practice of leg hygiene along with supportive treatment, reduces ADLA episodes significantly and also reduction in LE volume.

### Development and validation of sampling strategies for xenomonitoring as a post-MDA surveillance tool for lymphatic filariasis elimination programme

Application of a household-based sampling method developed at ICMR-VCRC for molecular xenomonitoring (MX) was investigated in one of the PHCs in Thanjavur district, Tamil Nadu, India. From the study, it was concluded that a reduced sample size of 300 pools by collecting 2 pools of 25 mosquitoes, each from 150 households could be used for surveillance. The strategy for MX, led to reproducible results and supported observed trends in LF infection in humans. qPCR assay results of low-cost VCRC-TE based DNA extraction method are comparable to that of commercially available qiagen method. MX has potential to be a cost-effective, non-invasive monitoring and evaluation tool with sensitivity even in low prevalence settings.

The strategy was successfully validated in one of the evaluation units (EU) in Cuddalore district Tamil Nadu. Validation in 3 districts with three different settings (TAS failed, TAS passed once, and twice) is in progress. The strategy is recommended as a standardized protocol for global LF elimination programmes.

### Adaptation, Validation and application of LYMFASIM model to predict risk of resurgence, following stopping MDA, based on transmission assessment survey

The stochastic micro-simulation model, LYMFASIM, for filariasis transmission and control was used to predict the impact of 3-drug regimen (Ivermectin, DEC & Albendazole) in comparison to the currently used 2-drug regimen for Mass Drug Administration for elimination of LF. The model



predictions showed that mass drug administration of 3 drug regimen with coverage of 70% could reduce the number of MDA by one or two rounds, compared to 3-4 rounds with 2 drug regimen.

**Prediction and evaluation of antigenic determinants of proteins of *Wuchereria bancrofti* for the development of an immunodiagnostic tool**

Cuticular collagen 2 (*col 2*) encoding genes of *Wuchereria bancrofti* are highly immunogenic and potential therapeutic candidates for blocking transmission. Four antigenic determinants of the cuticular collagen 2 protein of *W. bancrofti* were identified through bioinformatics tools, two of them showed immunogenicity against *W. bancrofti* infected sera. Purified polyclonal HRP-conjugated antipeptide antibody raised in rabbits were tested by indirect ELISA. Since the antibodies are polyclonal in nature, it was difficult to assess the non-specific interaction with negative controls. Hence, monoclonal antibody generation to get highly sensitive and specific antibodies and recombinant expression of the protein (CCEP) for positive control to validate the assay is in progress.

**Demonstration of strategies to enhance community compliance for MDA towards Lymphatic filariasis elimination in Palakkad district of Kerala state**

A three arm study was undertaken in three phases: 1) pre-intervention, 2) intervention and 3) post-intervention in 21 sites of Palakkad district under Mass Drug Administration for ELF. A community preparation intervention was developed and implemented by formation of small groups, strengthening of existing groups and networking between the groups and imparting intensive behaviour change communication, based on the information from the pre-intervention phase. After intervention, there was a significant improvement in the coverage and consumption of drugs in all the three arms. The relative change in consumption is significantly higher in the intervention arm (68.3%) compared to Arm II (52.6%), where strengthening of social mobilization with VCRC support was carried out and control arm (55.6%) reflected

the effectiveness of the intervention activities. Reduction in Mf prevalence was significant in intervention arm compared to non intervention arm (control arm).

**A community based study, to compare the safety, efficacy and acceptability of a triple drug regimen (Ivermectin, Diethylcarbamazine and Albendazole) with a two-drug regimen (Diethylcarbamazine and Albendazole) for lymphatic filariasis elimination programme**

A community based large scale efficacy, safety and acceptability study with 3-drug regimen is being carried out in comparison to the current 2-drug regimen, to find out alternate strategy to accelerate LF elimination in ‘hardcore’ districts. The safety data shows that 3-drug regimen pose no safety concern.

**Effectiveness and operational feasibility of mass DEC fortified salt as a supplementary intervention to mass drug administration towards elimination of the lone foci of diurnally sub-periodic *Wuchereria bancrofti* in Andaman & Nicobar Islands**

Under a collaborative study with RMRC, Port Blair, distribution of DEC fortified salt in Noncowry Islands of Andaman & Nicobar Islands to assess the operational feasibility, community acceptance, efficacy and effectiveness of DEC salt distribution as a supplementary strategy to MDA, has recorded coverage over 85%. Salt content analysis by HPLC showed that DEC concentration varied from 0.01 – 0.17% with a mean of 0.14% from 245 salt samples collected from kitchen. Salt samples tested at the household level, for DEC homogeneity indicates that the levels of DEC in the salt are in the therapeutic range. Mosquito samples collected from intervention (DEC salt) as well as control villages were assessed for filarial infection and the preliminary analysis showed 4.2%, irrespective of the intervention.

**MALARIA/SCRUB TYPHUS**

**Comparative assessment of the impact of combo vector control [long lasting insecticide treated**

### nets (LLIN) plus indoor residual spraying (IRS)] versus single measure (only LLIN or IRS) on malaria transmission in Koraput district of Odisha State

The study showed that the room spray (IRS) coverage and the use rate of LLIN could be enhanced to >75% through reinforced IEC activities and strengthening the advance information system. With the level of coverage, a significant reduction was observed in vector abundance and malaria incidence in the three arms (IRS alone, LLIN alone and combination of LLIN+IRS). However, the reduction was not significantly ( $p>0.05$ ) different between the arms, indicating that the combination had no added advantage.

### Prevalence and distribution of haemoglobinopathies (Sickle cell and Thalassemia) and G6PD deficiency in relation to malaria among tribal groups in Odisha State, India

Prevalence of G6PD deficiency among different tribes was studied in Odisha State. Out of 1,423 people from six tribes screened so far, 67 (4.7%) were found to have G6PD deficiency. Since, *Plasmodium vivax* is prevalent in tribal areas, the study results provide evidence that has implications on the treatment regimens to be used for the treatment of *vivax* patients in the area.

### Field evaluation of DawaPlus 3.0 and DawaPlus 4.0 long-lasting insecticidal nets from Tana Netting, UAE against natural populations of *Anopheles culicifacies* s.l. and or *Anopheles fluviatilis* s.l. in experimental huts in Odisha, east-central India

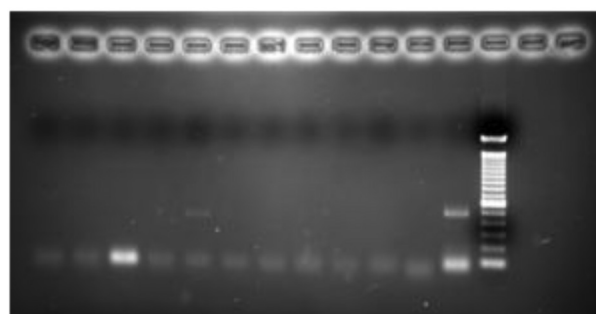
Two candidate LNs, DawaPlus 3.0 washed or unwashed and DawaPlus 4.0 washed or unwashed were evaluated in experimental huts in comparison to a positive control, DawaPlus 2.0 and a negative control (untreated polyester net), in terms of deterrent effect, blood feeding inhibition, induced exophily and killing effect for 42 nights during 7 weeks in one site in Malkangiri district of Odisha State, India, against the pyrethroids resistant

malaria vector, *An. culicifacies*. The performance of unwashed or washed (20 times) DawaPlus 3.0 in terms of deterrent effect, blood feeding inhibition, induced exophily and killing effect was significantly higher than the negative control (untreated net) and comparable with/marginally better than the positive control. Relative to the negative control, the overall performance in terms of deterrent effect, blood feeding inhibition, induced exophily and killing effect of unwashed or washed (20 times) DawaPlus 4.0 was significantly higher and with the positive control, the performance was comparable.

### Prevalence of scrub typhus vectors/rodent hosts and the pathogen, *Orientia tsutsugamushi*, in areas reporting human cases of AES in Gorakhpur district, Uttar Pradesh

Studies on prevalence of scrub typhus vectors/rodent hosts and the pathogen *Orientia tsutsugamushi* in areas reporting human cases of AES in Gorakhpur district, Uttar Pradesh, showed high abundance of mite vector species with an estimated *Leptotrombidium deliense* index of 13.8 per animal, well above the critical level (0.69 per animal), in the 13 villages surveyed. The study also demonstrated natural infection of *O. tsutsugamushi* in animal hosts and vector mites in the AES reporting villages of Gorakhpur district and confirmed transmission of scrub typhus (Fig. 24).

1 2 3 4 5 6 7 8 9 10 11 12 13



**Fig. 24:** Nested PCR amplification of 483 bp of *O. tsutsugamushi* in rodent blood samples collected from Gorakhpur, UP (Lane 1-4 & 6-10 negative samples, Lane 5: positive sample, Lane 11: negative control, Lane 12: positive control, Lane 13: 100bp ladder).

### DENGUE/KFD/JE/ZIKA

### Preliminary studies on Kyasanur Forest Disease virus in ticks and antibodies in rodents

### in potential risk areas of adjoining States to Karnataka

Preliminary studies on Kyasanur Forest Disease virus in ticks and rodents in potential risk areas of adjoining states to Karnataka showed high abundance of *Haemaphysalis* vectors in the villages in forest fringes of the six districts surveyed in Western Ghats of Tamil Nadu, Karnataka and Kerala. KFDV infection was detected by NIV in the tick vector in two of the six districts. The outcome of the study was useful in mapping KFD risk areas in the districts and strengthening the ongoing preventive measures such as vaccination, supply of insect repellents to the high risk groups, particularly, Cholanaikkan tribes living deep in the forest, Kattunaikkan tribes engaged in the fire line works and other forest staff/workers and intensive health education (Fig. 25).



**Fig. 25:** Tick collection by flag dragging at Nedumkayam reserve forests in Malappuram district of Kerala.

### Demonstration of mosquito vector control and prevention of dengue/chikungunya through partnership and community empowerment in selected rural areas of Puducherry

In view of developing a sustainable surveillance system for *Aedes* mosquito control, a collaborative approach involving school students has been designed with the Department of Education, Puducherry. Students were trained on assessing breeding sources in and around their houses using

a simple pictorial proforma in the local language. A training workshop was conducted jointly with state NVBDCP for students and teachers of selected schools on consolidation of data collected on mosquito vector breeding and for online transmission to NVBDCP for appropriate action.

### Monitoring JE vector abundance in an area with mixed vegetation and varied water bodies using RS and GIS

Moderate Resolution Imaging Spectroradiometer (MODIS) data was used for monitoring JE vector abundance in the areas with mixed vegetation and varied water bodies at Gorakhpur, as it provides substantially improved radiometric and geometric property of mixed vegetation types in an area within a season/annual cycle. The NDVI values derived from Moderate Resolution Imaging Spectroradiometer (MODIS) imageries could be used as ‘proxy’ for monitoring the vector abundance in mixed vegetation areas (like Gorakhpur).

### Vector surveillance for ZIKV in selected high risk areas of India

In view of the declaration of global public health emergency, ICMR-VCRC, CRME and NIMR are carrying out vector surveillance for ZIKV in Dengue hot-spot areas, in collaboration with respective state Health Departments. Altogether, 4240 mosquito samples have been collected and processed from Kerala state (4 Dists.) and Puducherry. None of these mosquito specimens were found infected with ZIKV, ruling out active transmission of ZIKV from the high risk villages/urban areas selected in the study. The studies are ongoing.

### MICROBIAL/CHEMICAL AGENTS FOR VECTOR/PARASITE CONTROL

#### Isolation and development of newer microbial agents for vector control

The metabolites of an indigenously isolated *B. amyloliquefaciens* (VCRC B483) were found to exhibit mosquitocidal, anti-bacterial and keratinase activity. Methanolic extract of the metabolites were indicative of isoforms of Bacillomycin, Surfactin and Fengycin. The metabolites exhibited antifungal



activity against phytopathogens, *Fusarium sp.* (VCRC F25) and *Curvularia sp.* (VCRC F26). Hence, these metabolites could be further explored for the development of vector control products and/or drug products.

DNA finger printing of *Bacillus thuringiensis* subsp. *israelensis* (VCRC B-17) strain, development of an improved production process/formulation and a real time PCR assay for quantification of delta endotoxin.

The process technology related to the mosquito larvicidal biopesticide, Bti (VCRC B17) has been licenced to firms and is expected to be used for mosquito vector control under national programme. Hence, DNA fingerprint of the strain becomes useful to monitor the use of the strain in the breeding habitats. Studies have shown that *rpoB* gene can be used as molecular/phylogenetic marker for the identification of *B. thuringiensis* subsp *israelensis*.

Attempts to develop a cheaper production media as well as a new formulation, has resulted in arriving at horsegram (*Macrotyloma uniflorum*) and broken Bengal gram (*Cicer arietinum*) based production media and formulating the cell mass, produced in the latter medium into tablets, using disintegrants, fillers, glidants and lubricants.

### TECHNOLOGY TRANSFER

A process for the preparation of mosquito larvicidal formulation from *Bacillus thuringiensis* var. *israelensis*—Technology licensed to 3 firms, Advance Crop Care Pvt Ltd, Indore; Tropical Biosciences Pvt Ltd, Chennai & Microplex Biotech & Agrochem Pvt. Ltd, Wardha during the year 2016.

### Development of new mosquito control agents based on anthranillic diamides targeting the insect ryanodine receptor

A total of ten molecules belonging to a new class of anthranillic diamide insecticides were synthesized, purified and tested against *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* and showed LC<sub>50</sub> values below 5 ppm against larvae

of all the three species. Two of them showed LC<sub>50</sub> values of 0.512 and 0.7 ppm against larvae of *Cx. quinquefasciatus*, 0.337 and 0.573 ppm against *An. stephensi* and 1.282 and 1.985 ppm against larvae of *Ae. aegypti*, respectively.

### NEW VECTOR CONTROL TOOLS

#### Evaluation of MAGNet, an alpha-cypermethrin long-lasting insecticidal mosquito net, against susceptible malaria vector populations in experimental huts in Odisha State, India

The performance of MAGNet after 20/25 washes was comparable to unwashed MAGNet as well as to the reference net, DuraNet. Compared to the untreated net, the performance of unwashed or washed MAGNet was significantly higher than the untreated net (negative control) and comparable to/ marginally better than the positive control (Duranet) in terms of mortality, deterrence effect, blood-feeding inhibition and induced exophily of the malaria vector, *An. fluviatilis* in experimental huts.

### FACILITY

#### BIOMEDICAL INFORMATICS CENTRE OF ICMR

#### ‘VectorInfo’ - a web repository of medically important Indian arthropods

Development of a Vector Informatics Database – ‘VectorInfo’ – a repository of data on medically Important Indian Arthropods is continued and development of a digital data entry system for Zika/ Dengue vector surveillance project is in progress.

### NATIONAL INSTITUTE OF VIROLOGY, PUNE

#### Influenza surveillance and diagnostic services

An upsurge of influenza activity was observed from January 2017 onwards. Clinical samples from suspected H1N1 patients were referred to NIV by different clinics/hospitals across Maharashtra for diagnosis of influenza A/H1N1pdm09. A total 790 clinical samples were tested by real time RT-PCR and shown 13% positivity (105/790) for A/

H1N1pdm09, 3% (24/790) for A/H3N2 and 1% (9/709) for influenza B viruses. HA gene sequences of influenza H1N1pdm09 isolates showed that 2016-17 strains were similar to vaccine component A/Michigan/45/2015 and similar to globally circulating strains.

Laboratory network has been established for epidemiological and virological surveillance for influenza and non-influenza respiratory viruses RSV, human metapneumovirus (hMPV), parainfluenza virus (PIV) 1-4, adenoviruses, rhinovirus in different geographical areas of India. A total of 320 clinical throat/nasal swab samples from ARI and 479 from SARI patients were obtained and tested in duplex real time PCR for respiratory viruses. Overall, of 799 samples, Influenza A/H1N1pdm09 was detected in 71 (8.8%), PIV in 44 (5.5%), Rhinovirus in 21 (2.6%), HMPV in 15 (1.8%), Influenza B in 10 (1.2%), Adenovirus in 7 (0.8%) and RSV in 4 (0.5%) samples, respectively.

Feasibility study to track community mortality due to Respiratory Syncytial Virus (RSV) in infants/children under 2 years of age, in a rural high-risk community in Melghat, Maharashtra has been initiated. A total 690 samples were tested for RSV by real time PCR, 99 and 2 were found positive for RSV A and RSV B respectively. Genetic analysis of G gene of RSV showed that ON1 genotype of RSV A was predominantly in circulation.

#### **Community-based surveillance of viral diseases/syndromes in Janata Vasahat in Pune city, Maharashtra**

The objective of the project in collaboration with B J Medical College, Pune was to monitor the attacks of acute febrile illness along with Influenza like illness (DLI) and acute diarrhoeal diseases (ADD) in the study area among children aged < 6 years for association and contribution of various viral and bacterial agents. In a population of 31489, during April-November 2016, 174 ILI episodes were recorded. One influenza type B case was confirmed from 33 ILI cases. Specimens were collected from 59/103 (57.3%) DLI cases. Viral etiology could be confirmed in 26 cases; dengue (13), Chikungunya (9) and co-infection of dengue and Chikungunya

(4). Dengue cases, reported during rainy season (June-October), were mainly adults. Incidence of DLI in August was 4 per 1000 persons per week. Surveillance of fever, influenza like illness and dengue like illness has helped to know the base line incidence of viral disease syndromes and seasonal variation of various viral bacterial diseases in community.

#### **Multi-centric hospital-based surveillance of acute encephalitis syndrome for viral etiology among children in selected districts of Maharashtra and Andhra Pradesh**

The aim of the project was to undertake surveillance of AES hospitalization among children <15 years for detection of viral etiologies to JE, Chandipura and enterovirus. JE IgM ELISA and Chandipura virus PCR testing by three site laboratories was highly concordant with NIV during quality assurance. A total of 1561 hospitalized children <15 years were screened during December 2014 to March 2017. There were 311 AES cases among 474 sampled patients. Case fatality ratio was 21%. Among 311 eligible AES cases, 145 consented for enrolment. Follow up of 74 recovered cases identified sequelae in 12 cases. Virus etiology was identified in 59/311 (18.97%) AES cases. JE was confirmed in 45 cases. Vaccination against JE was reported in only 2 of 45 JE cases. Chandipura virus was confirmed in 4 cases. Five cases were detected with Dengue and Chikungunya. Most of the AES cases occurred during July to October 2016. JE confirmation was also observed mostly in September 2016. Chandipura evidence was mostly in July 2016. JE continues as the leading cause of childhood AES in central India, in spite of vaccination. Surveillance needs to be strengthened for assessing actual disease burden. Vaccination coverage and effectiveness studies are needed for evaluating impact of vaccination so as to guide prevention and control strategies in future.

#### **Molecular characterization of dengue and Chikungunya virus circulating in India**

Dengue samples during 2016 from New Delhi revealed the circulation of DENV-2 and DENV-



4 while samples from Pune and Vellore showed circulation of all the four serotypes. Phylogenetic analysis of the viral genome sequences revealed that DENV-2 grouped into cosmopolitan genotype, DENV-3 belonged to G-III, and DENV-4 grouped into GI genotypes. In Pune, DENV-1 GIII was found to be circulating while in Tamil Nadu, circulation of two genotypes of DENV-1, GIII and GI was detected for the first time. DENV-1 GI was introduced from Singapore and associated with large outbreaks in Singapore and Sri Lanka. This study underscores the need for close molecular monitoring of DENV.

Phylogenetic analysis of Chikungunya virus based on E1/E2 gene sequences of the isolates from Pune, Nasik, Bangalore, New Delhi revealed the circulation of the Indian subcontinent sublineage strains, mainly possessing mutations E1: K211E and E2:V264A in the background of E1:A226. Selection pressure analysis revealed sites E1:211 and E2:264 showing evidence of positive selection. Some of the novel mutations noted in E1/E2 could be associated with epitopes or virulence determining domains, implying the role of viral factors among others contributing to the molecular epidemiology of the virus.

#### ***In-vitro* effect of human cathelicidin antimicrobial peptide LL-37 on dengue virus 2**

Vitamin D inducible human cathelicidin antimicrobial peptide LL-37 is known to have antiviral activity against many viruses. However, the effect of LL-37 on dengue virus (DENV-2) infection and replication is not known. Investigation of antiviral effects of LL-37 on DENV-2 revealed that pretreatment of the virus with 10-15  $\mu$ M LL-37 significantly reduced the viral infection and replication in Vero E6 cells. However, post infection, LL-37 had no effect on viral replication. *In-silico* analyses suggested that LL-37 inhibits DENV-2 infection by binding to the envelope (E) protein. Together, *in-vitro* and *in-silico* studies suggest that LL-37 inhibits virus entry by possibly binding to E protein. The results might have implications for prophylaxis against DENV infections and further *in-vivo* studies are needed.

#### **Study of Kyasanur Forest Disease (KFD) in patients and disease progression in monkey model**

Using KFD viremia and antibody persistence data obtained from 70 patients, an algorithm for efficient early diagnosis of KFDV infection was developed, which would help in, improved evaluation for the diagnostic period and early detection of KFDV infection. KFD virus was diagnosed till 18th POD and anti KFD IgM and IgG antibodies were detected till 121 days and 473 days post infection, respectively. These finding will help in providing timely diagnosis, thereby improving the management of KFD cases and adopting strategies for prevention and control of disease.

#### **Zika virus surveillance and vector competence studies**

Nation-wide surveillance for Zika virus (ZIKV) in ante-natal women and cases of acute febrile illness was initiated. As an apex laboratory for Zika diagnostics in the country, study confirmed 4 cases (3 from Ahmedabad, Gujarat, and one from Tamil Nadu) of Zika infection, during the period. RCVRDL and VRDL network together screened a total of 44357 samples from different states. Vector competence studies have shown that Indian strain of *Aedes aegypti* mosquitoes are susceptible to Zika virus (ZIKV), replicated to high titers and transmitted the virus horizontally to infant mice. Based on IFA and Real time RT-PCR data in co-infection experiments, it was concluded that ZIKV may have a relative advantage in replication dynamics over DENV and CHIKV. However, transovarial transmission could not be demonstrated.

#### **Risk factors and seroprevalence of CCHF infection among humans in rural population in Gujarat (NIV-ICAR, Gujarat)**

Many outbreaks and sporadic cases of CCHFV have been reported from different districts of Gujarat State since 2011. Sero-prevalence of CCHF in 33 districts of Gujarat was performed to study the epidemiology and risk factors. Initial findings

reveal, IgG antibody in only CCHF survived cases and close contact cases from CCHF affected areas. Highest risk in Gujarat is being posed by tick bites and human-to-human transmission.

#### **Enhancing Biorisk mitigation awareness in public health community and creating laboratory networks for enhanced diagnostic capabilities to deal with surveillance and outbreaks of Viral Hemorrhagic Fever and Respiratory Illness diseases (CDC-GHSA funded)**

During April 2016-March 2017, VHF network laboratories screened a total of 1542 samples of suspected VHF cases for Dengue, Chikungunya and Zika virus and highest prevalence was shown for Dengue and Chikungunya virus; no Zika virus was detected from these centres.

#### **Establishment of facility for production of standard virus positive controls for diagnostic PCRs and RT-PCRs tests for the important public health viral diseases**

Protocol for preparation of positive control and gamma irradiation for different viruses has been standardized and Gamma inactivated positive controls for Chikungunya, Japanese encephalitis, West Nile and Zika viruses were prepared.

#### **National Hospital Based Rota Virus Surveillance Network (NRSN), West Zone, India**

National hospital based rotavirus surveillance was conducted to estimate the rotavirus disease burden and distribution of rotavirus genotypes (G.P types) in four zones (South, North, East, West) in India. NIV, Pune a referral centre of west zone of India includes four peripheral centres (Pune, Ahmedabad, Mumbai, Surat) and one regional centre (Belgaum, Karad). During the study period, overall rotavirus positivity (2013-2016) in west zone was observed to be 33.3%. Highest rotavirus positivity was observed in Pune region (48.3%) and lowest (23%) in Ahmedabad region. In west zone, G1P[8] was the predominant rotavirus genotype followed by G2P[4], G9P[4], G3P[8], G1P[6] along with mixed infections. Also a changing trend in circulation of rotavirus strains was observed in certain sites.

#### **Longevity of immunological memory following Hepatitis E infection**

To understand whether hepatitis E virus (HEV) infection elicits long lasting immunity, a study was carried out in recovered individuals, 1-26 years post HEV infection. Results showed persistence of anti-HEV antibodies in 91% and detection of antibody secreting plasma cells (ASCs) in ~95%. This suggested that it is not only antibodies but also HEV-specific ASCs as a potential correlate of protection. This data could be useful in future HEV vaccine trials.

#### **Evaluation of role of miR122 in Hepatitis E**

Molecular mechanisms of liver pathology in Hepatitis E virus infection are not yet clear. Given the important role of miR-122 in liver pathobiology, centre has investigated possible role of miR-122 in HEV replication and pathogenesis. The study carried out in hepatoma cells indicated that miR-122 facilitates HEV replication possibly via direct interaction with its target site in HEV genome. This role of miR-122 presents novel opportunities for antiviral therapy and management of Hepatitis E.

#### **Measles virus genotypes circulating in India and contribution of rubella infection in suspected measles cases**

At National Reference Laboratory (NIV), the throat swabs/urine specimens (n=380) or PCR products (n=219) obtained from suspected measles cases (2011-2015) were referred for MeV genotyping. Indian MeV sequences (2011-15) were compared with 1996-2010 sequences. Circulation of measles genotypes B3 (n=3), D4 (n=49), and D8 (n=351) strains were observed in 19 states and 3 UTs. During this period, 64 MeV isolates were obtained from 253 clinical specimens. The genetic diversity within Indian B3, D4, and D8 genotypes was 0.3%, 1.1%, and 2.1%, respectively. The genetic divergence of Indian B3, D4, and D8 measles strains with the WHO reference sequences was 2.5%, 2.6%, and 1.8%, respectively. The data would be useful for the national immunization program. As a part of measles outbreak based surveillance, 4,592 serum samples were referred during 2010-

2015 from Karnataka (n=1,173), Kerala (n=559), and Maharashtra (n=2,860) to NIV, Pune and NIV Bangalore unit. Overall, 62.9% samples were confirmed for measles and 27.7% for rubella, highlighting the need for rubella surveillance in the country.

### Susceptibility of avian influenza viruses isolated from India to neuraminidase inhibitor antiviral drugs

*In vivo* antiviral assays were used to determine the susceptibility of HPAI H5N1 virus to oseltamivir carboxylate (OC). It was found that significant reduction in haemagglutination HA or 50% egg infectious dose (EID<sub>50</sub>) titres, is a more appropriate indicator of the susceptibility of HPAI H5N1 viruses to oseltamivir than 50% egg lethal dose (ELD<sub>50</sub>) and also the egg model is suitable to study the susceptibility of low as well as highly pathogenic avian influenza viruses.

### Role of complement during pandemic influenza A (H1N1) infection

A collaborative (NCCS-NIV) study on the role of complement in controlling the pandemic influenza A(H1N1)pdm09 virus infection, showed that deficiency of intact complement results in heightened vulnerability to the pandemic influenza A(H1N1)pdm09 virus infection in mice, leading to complete mortality, and that synergy between the classical and alternative pathways of complement is crucial for efficient protection.

### Entomological studies

*Vector competence of certain Culex and Aedes mosquitoes to Chittoor virus:* As part of characterization of Chittoor virus (CHITV), the Indian variant of Batai virus, studies to determine the vector competence of *Culex tritaeniorhynchus*, *Cx quinquefasciatus* and *Aedes aegypti*, were carried out. All the three species replicated the virus while vector competence could be demonstrated only by the two *Culex* species. Both the *Culex* species demonstrated experimental transmission of the virus to infant mice. Since Batai virus is known for genetic reassortments, active circulation

of the virus, availability of susceptible hosts and competent vector mosquitoes, pose a serious threat to public health.

*Mosquito population dynamics in Pune region:* An entomological study was undertaken to investigate the composition (diversity) and seasonal dynamics of mosquito population in Pashan area of Pune. A total of 21 species were observed in the region. Mosquito abundance was found correlated positively with maximum and minimum relative humidity and rainfall. Interesting finding was that the mosquito abundance is modulated by diurnal temperature range (DTR), as low DTR corresponds to high mosquito abundance.

### Detection of Chikungunya (CHIKV) antigen from infected mosquito in anti-capsid monoclonal antibody (MAb) based antigen capture ELISA

An antigen capture ELISA was developed using anti-capsid protein MAbs to CHIKV. The ELISA specifically detected  $5.5 \times 10^4$  PFU/ml of CHIKV from the tissue culture stock and no reaction was evident with Dengue virus. The ELISA also detects CHIKV from intra-thoracic inoculated mosquito harvested on post infection day 1-10. To mimic field conditions, combination of infected and uninfected pools of mosquitoes were also screened in this assay. The ELISA detected CHIKV from even a single infected mosquito. In both the test formats, the results co-related well with Real-Time PCR. This sensitive assay will be useful to undertake vector surveillance.

### CORE FACILITY ACTIVITIES

*Bioinformatics:* Phylogeography studies of genotypes D4 and D8 of measles virus (MeV), which are the major circulating genotypes in India was undertaken using nucleoprotein (N) gene sequences (n=756) representing 86 countries (1973-2016) to study the spatiotemporal transmission dynamics. D4 genotype transmissions were noted from India to Australia, USA and Western Europe in recent years. D8 genotype transmissions were noted from India to several countries upto 2011 while importations mainly from the region of



the North America into the country were noted in recent years upto 2016. Recent transmissions from both measles endemic countries and from the countries that have eliminated or are nearing elimination, brings out the importance of continued worldwide MeV surveillance activities for eradication of the disease. In another study, protein structure modeling and simulation studies helped in identifying co-evolved mutations (V344M, I354L) in the polymerase gene PB2 of the 2009 pandemic Influenza (H1N1) viruses. These mutations were found to be established in the PB2 gene over the period 2010-2013, and may have played a role in improving replication competence by mediating increased efficiency of host m7GTP cap binding.

*Electron Microscopy: Characterization of the effect of dengue virus NS1 on endothelial cell gene expression and cytoskeletal morphology-* Cultured endothelial cells were exposed to Dengue virus NS1 protein of all four serotypes *in-vitro* at normalized physiological concentrations. High resolution limited gene expression sets relevant to vascular inflammation and capillary physiology was studied along with dynamics of cytoskeletal morphology. The findings reveal highly conserved gene expression alterations among all four serotypes that affect capillary vasomotor tone, hemostasis and changes in kalikrien-bradykinin axis. Morphological changes in cytoskeleton were highly consistent with both infection and exogenous exposure that was seen as stress fiber formation and focal adhesion changes. Collectively, these findings indicate potentially novel and direct engagement of endothelial cells by direct exposure of NS1 protein relevant to vascular dysfunction of severe dengue disease.

## FIELD UNITS

### Kerala Unit

*Distribution of Kyasanur Forest Disease (KFD) virus in tick population in forest areas of Kerala:* Tick surveillance was conducted from Wayanad district in four forest ranges and Malappuram district in two forest divisions. Larval and Nymphal tick abundance was observed in small patches of forest fragmentation and open areas in teak plantation

along with ephemeral water bodies. Three pools of *Heamaphysalis spinigera* were positive for KFD virus.

*Impact of climate change on mosquito abundance in coastal wetlands of Alappuzha district Kerala:* Mosquito Larval survey was conducted in coastal brackish water and paddy field of Alappuzha district. Major Japanese encephalitis vectors were observed in brackish water and these mosquitoes were tested for the ability to survive in brackish water. Among the mosquitoes tested, *Culex tritaeniorhynchus* exhibited a better survival and biology up to 7.5 parts per thousand of salinity.

### NIV Bengaluru Unit

As a part of WHO-SEAR laboratory network BU confirmed and reported 2.7% (228/8377) of Sabin Like Polio viruses (serotype 1 & 3), 23.3% (1951/8377) of Non-Polio Entero viruses, 30.3% (143/471) of Measles and 28.4% (134/471) of Rubella virus, from the states of Karnataka, Kerala and Southern districts of Bihar. It is the first laboratory in Karnataka state to start case based study for Measles and Rubella virus. As State Sentinel Laboratory for NVBDCP for Bangalore urban and rural area, the unit investigated and reported 45.8% (1021/2229), 17% (379/2229) and 4.3% (6/138) of Dengue, Chikungunya and Japanese Encephalitis cases respectively. The unit reported 34.6% (9/26) of positive Rubella cases under the congenital Rubella syndrome project. As a part of Rubella sero surveillance project, BU confirmed 85.7% (90/105) positive IgG antibodies among pregnant women.

### Gorakhpur Unit

During 2016, laboratory diagnosis was provided to a total of 1965 clinically suspected AES cases, hospitalized in Baba Raghav Das Medical College, Gorakhpur. A total of 3402 clinical specimens (CSF and blood/serum) were investigated for JE infection and anti-JE IgM antibodies were detected in 173 cases (9.11%). In JE cases, 59 deaths (34.1%) were recorded with highest case fatality rate in the age group of 1-10 year children, accounting for 127 (73.41%) of JE cases with 42 (72%) deaths.



In 2016, overall mortality in the hospitalized AES cases was 27%, which is recorded to be 2% higher than the mortality documented during the year 2015. During 2017, a total of 55 JE cases (7.4%) were diagnosed among the 743 AES cases admitted till date (Aug 30, 2017) with 12 deaths (21.81% CFR). In view of improving the quality of storage, transport and lab testing protocols, AES cell was established during 2016 in the Pediatric department of BRDMC with the support of ICMR. In this planned study, clinical specimens collected from 350 cases were tested for anti-JE and anti-OTs IgM and Dengue virus NS1 antigen by standard ELISA assays. In addition, specimens were processed for detection of *Orientia tsutsugamushi* (OT) and other rickettsial genomes by PCR. Among the 300 cases, anti-JE IgM was diagnosed in 5.5%, anti-OT IgM in 60% and 6.6% specimens showed positivity for Dengue virus NS1 antigen ELISA. Among 330 cases with paired specimens (CSF and whole blood) OTs PCR testing resulted in 21.51% positivity and 4% cases tested positive for *Rickettsia*. Findings suggest JE infection in about 10% AES cases and infection with scrub typhus and members of spotted fever group. During 2017, a total 205/743 (27.6%) AES cases were tested positive for OT infection and OT genome was detected by PCR in >40% of IgM positive cases. The CFR in OT positive cases was documented to be 14.14% while in remaining undiagnosed AES cases CFR was 30%. Among the other AES associated etiologies, infections with Dengue (1%), Parvo P4 (6%), Human Herpesvirus 1 (3%), Human Enterovirus (5%) and *Rickettsia* of the Spotted Fever Group (3%) were detected. In combinations with the results obtained by ICMR on serosurvey in healthy population and demonstration of presence of OT genome in mites captured from field, these findings highlight association of OT and other rickettsia infection in cases occurring in the region.

### HUMAN RESOURCE DEVELOPMENT

- **VRDL Training programs conducted:** Resource Centre for Virus Research and Diagnostic Laboratories (RCVRDL) imparted training to 117 participants VRDLs network. RCVRDL provided standard training, protocols and non-commercial essential reagents. Institute also provided quality assurance to different VRDLs.
- **Other Nation Training Programs conducted:** The institute has trained laboratory staff/personnel from ICMR and other national laboratories in enhancing diagnostic capabilities to detect emerging/re-emerging threats like CCHF, Zika, Yellow fever and KFD etc.
- **WHO Training programs conducted:** NIV also provided training to personnel from government laboratories of WHO-SEAR countries (Bhutan, Nepal, Burma, Maldives etc.) under the HR development program.

### CERTIFICATE OF NABL ACCREDITATION

- Three laboratories at NIV; viz., Human Influenza, Avian Influenza and BSL-4 have fulfilled the requirements of lab accreditation policies and received ISO/IEC 17025:2005.

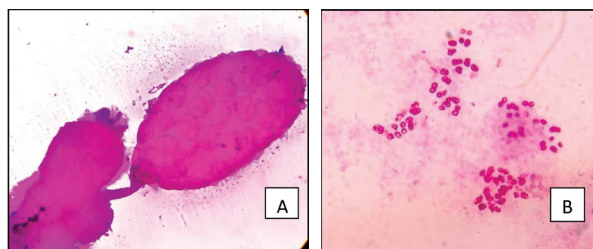
### ACADEMIC PROGRAM

- Eight (08) students were awarded Ph.D. Degree by Savitribai Phule Pune University.
- NIV conducts two-year post graduation course (M.Sc. Virology) affiliated to Savitribai Phule Pune University, Pune. The course has an intake of 20 students per year.

### CRME, MADURAI

#### Designing and development of novel equipment for generating germ free and gnotobiotic mosquitoes

The main objective was to design and develop a novel equipment for growing “germ free” and “gnotobiotic” mosquitoes to investigate the role of normal indigenous microbiota in the survival of vector mosquitoes. A simple, innovative and cost effective prototype was developed to understand the role of each microbial species which are responsible for the normal development of mosquitoes. When the mosquito was grown in the device, in the absence of any microbes, their growth potential was very much hindered.



**Fig. 26A:** Ovary of *Aedes* mosquito stained with Gimenez stain  
B. Stained *Wolbachia*.

### Screening of *Anopheles* vectors for *Wolbachia* infection

The main objectives were to screen for naturally available *Wolbachia* in *Anopheles stephensi* and other major malaria vectors and to investigate effect of *Wolbachia* infection on the biological characteristics. Gimenez staining method is under standardization for the observation of *Wolbachia* endobacteria from mosquito specimens.

### Vector Surveillance for ZIKV/JEV in selected high risk areas of India

An ICMR extramurally funded network project on Vector surveillance for Zika/JEV in selected high risk areas of India was undertaken for surveillance of Zika virus in vector mosquitoes in certain districts in Tamil Nadu. A total of 422 pools of *Aedes aegypti* and *Aedes albopictus* comprising 9020 adult vector mosquitoes collected from Madurai, Dindigul, Theni, Tirunelveli, Coimbatore, Ramanathapuram, Krishnagiri, The Nilgiris, Chennai and Kanyakumari districts in Tamil Nadu were found negative for Zika virus infection by RT-PCR.

### Designing and evaluation of a simple, eco-friendly and cost effective ovitrap for the control of Dengue/Chikungunya vectors

The prototype device has been successfully designed and the documents have been submitted to ICMR, New Delhi for processing for Patent. Laboratory evaluation is in progress.

### Patent submitted

1. Device for generating germfree and gnotobiotic mosquitoes.
2. Novel mosquito ovitrap.

## NATIONAL AIDS RESEARCH INSTITUTE, PUNE

### EPIDEMIOLOGY

#### Kaposi's Sarcoma-associated Herpes Virus (KSHV) or HHV-8 and HIV-1 in MSM population

With very few reports among the MSM population in India, a total of 51 samples have been tested for the presence of HHV-8 IgG antibody using HHV-8 IgG antibody ELISA kit (Sunlong Biotech, China). Ten out of 51 samples were positive for HHV-8 antibody, of which 4 samples were also reactive for HIV-1.

#### Viral Hepatitis among Men having Sex with Men (MSM) and Injecting Drug Users (IDUs) in Pune

Preliminary data from ongoing studies among MSM and IDU in Pune reveal a low prevalence of Hepatitis B in both groups (MSM-1.6% and IDU-2.4%). However a large proportion of IDU (28.6%) had ever been infected by Hepatitis C, HIV and Hepatitis B/C co-infections among IDU was 3.5%.

#### Prevalence and Incidence of Scrub Typhus infection during lean and epidemic period in selected blocks of Gorakhpur district in Uttar Pradesh, India

Repeat cross sectional sero-surveys conducted during lean (April- May 2016) and peak (September to December 2016) Acute Encephalitis Syndrome (AES) periods in Gorakhpur, India, confirmed the endemicity of scrub typhus infection and found significant increase in IgM positivity during peak period indicating active scrub typhus infection during AES epidemic could potentially be the cause for AES cases. Overall, 70% sero-positivity against *O. tsutsugamushi* was seen with 5% IgM positivity and 69% IgG positivity during peak AES period. IgM positivity was significantly higher (8% vs 3.9%,  $p=0.008$ , CI:4.8,11.2) while IgG positivity was significantly lower (59% vs 67%) among fever cases. The sero-prevalence during peak AES period was significantly more than in the

lean period of AES cases (70% vs 50.97%). IgM positivity in lean period was 1.6%.

Overall, sero-conversion against *O. tsutsugamushi* was 21% among the respondents who were sero-negative during the lean AES period. Factors independently associated with scrub typhus during lean AES period were female gender (AOR=1.66, CI (1.18,2.35),  $p=0.004$ ), no formal education (AOR=3.28, CI (2.17, 4.95),  $p<0.001$ ), not wearing footwear during outdoor activity (AOR=1.98, CI (1.12,3.49),  $p=0.018$ ), not taking bath after work (AOR=1.62, CI (1.09,2.41),  $p=0.018$ ) and being a rural resident (AOR=1.76, CI (1.21,2.55),  $p=0.003$ ).

### **Design and development of strategies to optimize data quality in large scale bio behavioural surveys among high risk population sub groups in India**

Computer Assisted Personal Interviewing (CAPI) and Integrated Information Management System (IIMS) technology was used for the first time in the large-scale national Integrated Biological and Behavioural Surveillance (IBBS) by NACO for data collection and management. Study evaluated the strengths and weaknesses of this technology in survey research. Despite challenges, the technology was huge success with respect to data coverage, response rates, cost effectiveness and acceptance by field staff and respondents. The real time availability of the data during and after the survey supported the survey monitoring activity and early release of reports. However, such techniques require more focus on competencies of human resource, training needs and concurrent evaluation system for optimal and effective use of technology.

### **Identification and Characterization of HIV-1 subtype for samples from Nepalese Population**

HIV-1 subtype analysis was carried out as requested by National Public Health Research laboratory (NPHL), Nepal, a low HIV prevalence country. Sequencing analysis of the env gene (560 bp), amplified from 124 plasma samples indicated that

119 sequences (96%) belonged to HIV-1 subtype C and subtype A (1.6%). However, presence of an CRF\_AE (1.6%) and a B/C recombinant (0.8%) was also observed.

## **HIV BIOLOGY**

### **HERC-5 polymorphism in HIV infected individuals**

HERC-5 is one of the novel host restriction factors which block the release of HIV particle during later stages of the HIV life cycle. Study reported that the known SNP (rs34457268) at exon-18 of HERC-5 gene is not associated with the HIV-1 susceptibility in our study population. SNPs detected at other intronic regions may have some role in the regulation of HERC-5 which needs further investigation with a larger group of individuals.

### **Antibody-Dependent Cellular Cytotoxicity (ADCC): a new frontier in vaccine research**

This collaborative project between India and Australia aimed to identify unique dominant ADCC epitopes of HIV-1 antigens from non-progressive HIV infection, for potential use for HIV vaccine design. Study identified a region from V3 loop of envelope protein and a region from Tat protein of HIV-1 as dominant ADCC epitopes from Indian LTNP and a Vpu protein region from Australian LTNP. It was observed that cART reduces the ADCC responses in both C subtype as well as B subtype infected individuals overall and marked reduction was observed in individuals with higher CD4 counts at baseline. The study also found that ADCC responses against pandemic H1N1 & H3N2 influenza strains in both HIV infected and healthy individuals were similar and showed cross reactivity. Among the HIV infected, these were marginally higher among LTNPs as compared to progressors in case of pH1N1.

### **Characteristics of cervicovaginal cellular effector mechanisms against HIV in Indian women**

The innate and adaptive effector immune cells at cervicovaginal surfaces of the HIV infected women were studied. HIV infected women showed a higher frequency of defective natural killer (NK) cells



and increased degranulation of NK and CD4+T cells; (surrogate markers of their activation and cytotoxicity), as compared to uninfected women. Degranulating immune cells were associated with increased cervical viral load. Overall, we report that increased degranulation of immune cells at the mucosal surfaces are detrimental and hypothesize that this may contribute to increased HIV transmission and disease progression.

### Polymorphism in CD16 receptor on the ADCC response to HIV-1 infection

CD16/ FC $\gamma$ RIIIa receptor is a critical component of the antibody dependent cellular cytotoxicity mounted against HIV by natural killer (NK) cells. The influence of polymorphism in CD16 receptor on the ADCC response to HIV-1 infection is not known. Study found a predominance of VF genotype frequency for FC $\gamma$ RIIIa receptor. ADCC response in HIV infected persons in the study showed a trend of association with the hierarchy of VV>VF>FF genotype of FC $\gamma$ RIIIa receptor.

### Genetic and neutralization properties of the envelope gene in HIV-1 and HIV-2 monotype and dual infections

Co-receptor tropism plays an important role in HIV pathogenesis. The co-receptor tropism of HIV strains isolated from mono (HIV-1/HIV-2) and dually (HIV-1 and HIV-2) infected individuals was studied. This study data suggests 50% existence of dual tropism in Indian HIV-1 and HIV-2 infection. Although study reported *in vitro* phenotypic co-receptor tropism for the first time, it is difficult to attribute these findings to the independent tropism of the two viruses, as it needs further genotypic confirmation.

### Impact of HTLV on HIV-1 replication

Study explored the effect of HTLV-II APH-2 and CREB cellular protein interaction on basal and / or Tat mediated transcription of HIV-1 in case of HIV-1/HTLV-II dual infection. The Luciferase expression assay carried out in 293T cell line exhibited a decrease in luciferase expression in

presence of Aph-2, which confirmed the negative effect of Aph-2 on Tat mediated transcription.

### PRODUCT DEVELOPMENT & TESTING

*Terminalia paniculata* and *Polygonum glabrum* extracts (NARI-NCL 051 and 052, 053 and 054) and their fractions, sub-fractions and pure molecules (n=66) were tested for anti-HIV1 activity using TZM-bl assay and for determining the mechanism of action using cell based (HIV-1 entry and fusion inhibition) and enzymatic assays (reverse transcriptase and protease inhibition).

It was observed that the *Terminalia paniculata* and *Polygonum glabrum* showed anti-HIV activity. Both these extracts inhibited HIV-1 entry, however, they could not inhibit HIV-1 fusion. The extracts of *Terminalia paniculata* were also found as potent protease and reverse transcriptase inhibitors. However, the activity of the extracts reduced upon fractionation. Comparatively, the extracts/fractions of *Terminalia paniculata* showed good anti-HIV1 activity.

### SEXUALLY TRANSMITTED INFECTIONS

#### Novel Mutations identified in drug resistant *Neisseria gonorrhoeae* strains from Delhi, -Pune, Mumbai and Hyderabad

Three novel mutations in *gyrA/ parC/ mtrR*, ten in *penA*, four in *ponA*, eight in *porB* and twenty in *rpsJ (tetM)* were reported to NCBI database. Double mutations in *gyrA* combined with single mutations in *parC* and *mtrR* gene in *N.gonorrhoeae* may have contributed to high level of ciprofloxacin resistance in India. Mutations in *porB* alone or combined with *penA* and *ponA* increased the level of penicillin resistance.

#### *Lactobacillus* flora candidates isolated from HIV-positive and HIV-negative women as potential probiotics

Seven *Lactobacillus* strains [*L. crispatus* (4/10), *L. gasseri* (2/9) and *L. jensenii* (1/6)] with optimal adherence and colonization properties have been identified as likely excellent candidates for development as a vaginal tract probiotics.



## ANTIRETROVIRAL THERAPY

### Case control study to understand determinants and biomarkers associated with Immuno-virologic discordance

A study conducted at 2 ART centres in Pune to understand determinants of Immuno-virologic discordance at 1 year after ART initiation and to assessment of the immune restoration in patients could identify low numbers of Virologic non-responders [VNR -CD4 rise more than 50 cells/mm<sup>3</sup> over baseline and viral load >1000 copies/ml] as compared to immunologic non-responders [INR-CD4 rise less than 50 cells/mm<sup>3</sup> over baseline and undetectable viral load].

INR patients showed improved cytolytic T cell activity against other viral infections like Influenza, EBV and CMV as well as improved NK cell functionality, similar to the matched responders (CD4 rise more than 100 cells/cumm over baseline and undetectable viral load and matched for age, sex, baseline CD4 count with INRs), and significantly higher than treatment failures. They showed significantly higher CD4 T cell activation than responder groups, which needs to be investigated further.

### Use of Herbal drugs by PLHIV

A cross-sectional survey among PLHIV in Pune found that overall 11 % of PLHIV used herbal drugs, of whom 55% used them for co-morbid conditions like hemorrhoids, gastritis, hair fall, weight loss, diabetes, joint pains and 45% for increasing immunity and “HIV cure”.

### Retention in free ART clinic

Retention in care is important for optimal clinical outcomes in HIV infected individuals. Ten year retention among HIV infected individuals initiated on ART in a programme clinic at Pune, India analyzed by changes in the national guidelines for ART initiation showed that the median CD4 counts at the time of registration (linkage) showed significant increase with the changes in the national guidelines from 200 cells/mm<sup>3</sup> to 350 cells/mm<sup>3</sup>. However, there was no significant difference in the loss to follow up (LTFU) and opted out

(disengaging from HIV care) patients, initiated on ART in 10 years. The survival probability increased significantly with the increase in CD4 cut offs for ART initiation. Efforts should be taken for preventing patients from disengaging from HIV care in the programme, by planning effective interventions.

### Renal impairment due to tenofovir among patients in NARI ARTC

The renal function of HIV infected individuals, initiated on Tenofovir Disoproxil Fumarate (TDF) based regimen between 2012-2016 and completed at least 12 months of follow up, was retrospectively analysed using Cockcroft-Gault formula at baseline and over time. A significant decline in creatinine clearance at all the visits from baseline was noted. The dose adjustment for TDF was required for 10 patients based on decline in creatinine clearance.

### Compartmental drug resistance in paired CSF and plasma samples

Paired CSF and plasma specimens from 2 cases with HIV associated neurological problems were tested for HIV drug resistance to NRTIs, NNRTIs and PIs. A 100% concordance in DR mutations in both cases indicated transmission of resistant HIV into the CSF.

### Validation of method to quantifying the plasma Tenofovir in HIV PrEP (Pre-Exposure Prophylaxis) trial samples

A method for estimation of tenofovir in plasma samples with a lower limit of detection 1ng/ml, lower limit of quantification: 5ng/ml with 2.5 fold linearity at 9 points ( $r^2$ : 0.9974) was validated at NARI. Testing of study samples have been initiated to assess adherence endpoints.

## BEHAVIOURAL STUDIES

### Primary Prevention of Intimate Partner Violence in India

One hundred recently-married women and men belonging to low socio economic strata in Pune were studied, to understand the determinants of Intimate Partner Violence. On multivariate

analysis, 'education of spouse', 'lack of satisfaction of the spouse's family with the *'maanpaan'* during marriage' and 'lack of conflict negotiation skills' were correlated with DV among newly-married women. Among men 'jealousy', 'time spent alone with partner each week after marriage', 'attainment of the husband ideal' were found to be significant correlates of DV perpetration among men. These data will help in development of an IPV prevention module.

### **Understanding needs and feasibility of online web platform to conduct sexual health survey among young college going adult population**

Formative research was carried out to understand the knowledge needs of college going young adult population with respect to sexual health and HIV/AIDS. Only 53 (70%) students had undergone some sex education in school/college. About 70% agreed that social media, networking sites and TV channels had an impact on sexual health. Students told that while they would prefer to get sex education from parents or siblings as they are reliable source of information, they actually got it from friends/internet. Reliable Web and mobile based applications with interactive sessions to address individual's needs were recommended and to be placed on college web sites.

### **Community Engagement for JE and AES prevention and control- Development of Abridged intervention module**

On the advise of JE expert group, an interim rapid assessment to study the impact of ongoing Community Engagement Intervention (CEI) for modification of treatment seeking behaviour and creating demand for JE vaccination and its acceptability at Bhathat block of Gorakhpur was undertaken. The findings of interim analysis which highlighted that exercises to bring community together to access Govt. facilities and story narration were critical. Based on this, an abridged module 'Healthy community - Community Responsibility', especially tailored to highlight the activities that are best accepted and most useful in the community, was developed and published in January 2017.

## **IMPLEMENTATION SCIENCE**

### **'Opted out' patients in ARTC: Unaddressed potential leak in the programme**

This retrospective study was conducted in the NARI ARTC. The patients who no longer wished to continue treatment with the programme because of their personal choices or reasons and stopped coming for treatment were labeled as 'opted out'. The predictors for 'opting out' of the ART treatment were analyzed by comparing them with HIV infected individuals who were regularly taking ART. The results showed that patients that were initiated on ART within one year and CD4 decline during follow up were significantly more likely to be opted out from the treatment. The programme has no clear operational guidelines for tracking opted out patients as compared to loss to follow up or transfer out patients. Addressing this important issue of 'opted out' to prevent the leakage in the cascade, will be useful in retention.

### **Community-based HIV testing**

In accordance with the UNAIDS 90-90-90 targets, India's NACP needs to focus on ensuring, that 90% of PLHIV are tested, diagnosed and linked to care. NARI undertook different field based studies to assess feasibility of community based HIV testing among different populations. Feasibility and efficacy of community based rapid HIV testing was tested in 10 different villages in 2 sub-centres (Wadgaon and Karanjagaon subcentres) in Maharashtra. Different strategies like house to house visits, targeting construction sites, hotels, companies and ware house have been used. Of 2151 HIV test seekers, 42% were female and 24% reported unsafe sexual behaviour. There was a low (0.7%) awareness about the nearest government HIV testing facility.

### **Hidden sex workers & willingness to testing**

A rapid field assessment among FSW in Kolhapur district, in 2016 found a rising predilection for home/secret based female sex work in both rural and urban areas and a high dependence on peers

for accessing HIV testing services among these sex workers (FSW). Reservations towards accessing testing services from well branded ICTC due to fear of stigmatization and desire to keep their activity hidden, were strongly expressed. FSWs who are extremely hidden and probably not linked to the program were found to be less likely to be receiving testing and care services but willing to utilize opportunities for community based confidential HIV screening.

### **SUPPORT ACTIVITIES FOR THE NATIONAL AIDS CONTROL PROGRAM**

NARI supported the 15<sup>th</sup> round of HIV Sentinel Surveillance among Antenatal Clinic attendees (ANC) at 203 sentinel sites. Implementation of Linked Confidential Testing was assessed.

Laboratory support for the National AIDS Control Programme: NARI as an Apex laboratory for HIV serology and CD4 count estimation, carried out External Quality Assessment (EQA), monitoring quality control for HIV diagnosis and CD4 estimation, training, and analysis of EQA data and Kit Quality Testing. Testing of DBS samples for 'Early Infant Diagnosis' for diagnosis of HIV exposed babies as well as viral load testing of potential ART failures was also done.

### **OTHER STUDIES AND ACTIVITIES**

#### **Building a Research Analytic Initiative at NARI-India (BRAIN-I)**

This two year initiative aims at developing a grant leading to a program to provide enhanced training in statistical applications, epidemiology, and analysis, tailored to the needs of the clinical and socio-behavioral scientists at NARI. A formal needs assessment including a "Watch and Learn" activity and assessment by self and supervisors helped to understand the training and knowledge gaps and inform the development of a structured participatory curriculum. Mapping of various MPH (and master's level research training) programs in the country has been completed.

### **Support to SAARC countries for HIV drug resistance genotyping**

ICMR-NARI's WHO accredited HIV drug resistance laboratory supported HIVDR genotyping for two international HIV pre-treatment and acquired drug resistance (ADR) surveys conducted by Nepal and Myanmar, and supported Sri Lanka's treatment program, testing over 900 Dried Blood Spot (DBS) specimens.

## **ENTEROVIRUS RESEARCH CENTRE, MUMBAI**

### **POLIOMYELITIS SURVEILLANCE**

Over the past several years, Enterovirus Research Centre (ERC), Mumbai, has contributed significantly to the understanding of epidemiology of poliomyelitis and developing polio vaccination strategies in India. ERC has been accredited as WHO Global Specialized Laboratory (GSL) for polio, participating in Acute Flaccid Paralysis (AFP) surveillance in India by virological investigation of fecal specimens from AFP cases. AFP surveillance plays a crucial role in monitoring cases of paralytic poliomyelitis and provides evidence of the elimination of indigenous wild polioviruses. No wild poliovirus has been detected since 2011 in India and was declared polio free in 2015 by WHO. In 2016, a total of 46562 cases of AFP were reported through AFP surveillance in India, out of which 10204 stool samples from 5165 AFP cases were reported from Maharashtra, Madhya Pradesh and Goa and tested at ERC for the presence of polioviruses and other Enteroviruses. Polioviruses were isolated from 172 (3.3%) AFP cases and Non-Polio Enteroviruses (NPEV) from 1250 (24.2%) AFP cases. In India, type 2 VDPV was detected from 1 AFP case from Bihar whereas remaining poliovirus isolates were Sabin-like. However, no VDPV circulation was observed in Indian population in 2016.

### **Environmental surveillance for detection of wild poliovirus**

In 2016, a total of 416 sewage samples (Mumbai: 156, Patna: 78 and Hyderabad: 182) were tested for



the presence of polioviruses. Six type 2 VDPVs were detected from sewage samples collected from various sites in India, out of which, 3 were isolated from Delhi, 1 from Kolkata, 2 from Hyderabad. Along with AFP surveillance, the environmental surveillance also confirmed the absence of wild poliovirus transmission in India during 2016.

### **Polio endgame strategy “Global switch from OPV to bOPV”**

In 2016, WHA endorsed the phased withdrawal of OPV and introduction of inactivated poliovirus vaccine (IPV) into childhood routine immunization schedules. Type 2 OPV has been withdrawn through a globally synchronized “switch” from trivalent OPV (all three types) to bivalent OPV (types 1 and 3). Due to the switch, the type 2 polioviruses isolated from AFP cases/environmental samples in India has gradually reduced to almost zero. Type 2 poliovirus was last isolated from an AFP case in May 2016.

The number of Sabin2 viruses isolated from sewage samples have dropped from 16 in April to 2 in December 2016, without any type 2 detection during the intermediate months (October and November). The 2 Sabin viruses were detected in December from sewage samples collected in Hyderabad during environmental surveillance, which was due to the use of tOPV in a private clinic. VDPV2 was last detected in sewage in June 2016.

### **CONTAINMENT OF SABIN2**

After the switch from tOPV to bOPV on 25<sup>th</sup> April 2016, Sabin2 has been under containment. Under Sabin2 containment, all the type 2 Sabin-like isolates received from National Polio Laboratories, suspected VAPP type 2 isolates, Sabin 2 NIBSC reference strains and VDPV2 isolates, undifferentiated NPEV isolates/stools from various projects including Rotavirus project and glycerol stocks of Sabin2 DNA clones, were discarded by autoclaving with proper validation.

### **MEASLES & RUBELLA SURVEILLANCE**

ERC, the Global Specialized Laboratory for polio laboratory network in SEAR has contributed

significantly in the polio eradication program in India. ERC with all necessary infrastructures was proposed by ICMR to participate in Measles elimination and Rubella control network. ERC staff received training for Measles and Rubella serological and molecular diagnosis at NIH, Thailand. ERC was included in WHO Measles and Rubella network from June 2016 after 100% proficiency scoring. Serum samples from outbreaks detected in various parts of Maharashtra i.e. Mumbai, Thane, Raigad, Palghar, Nashik and Goa from July 2016 were tested at ERC for Measles and Rubella by standard IgM detection method. A total of 326 sera were tested from July 2016 to March 2017 out of which 207 were found to be positive for Measles and 41 were positive for Rubella.

### **Cross - sectional serologic assessment of immunity to polioviruses in different risk states of India (India polio seroprevalence study 2016)**

Seroprevalence studies have been conducted in various parts of India especially in high risk areas of Uttar Pradesh and Bihar since 2007 to help the programme to monitor the sustainance of immunity levels of children in the country. A study was conducted to assess the seroprevalence to all three poliovirus serotypes among children aged 6–11 months living in three risk categories for polio after tOPV to bOPV switch and to compare seroprevalence among infants who have received/not received IPV.

A total of 1113 serum samples were collected from Bihar (366), Madhya Pradesh (377) and Chhattisgarh (370). It was found that all high risk states in India have >97% seroprevalence against poliovirus serotypes 1 & 2 and >92% for type 3. These results indicated that one full dose of IPV introduced in routine immunization has led to improved seropositivity against all the three poliovirus serotypes.

### **Molecular characterization of Non Polio Enteroviruses isolated from AFP cases from India (2014 and 2015)**

In recent years, significant interest has been developed in the circulation, detection and



identification of Non Polio Enteroviruses (NPEVs) isolated from Acute Flaccid Paralysis (AFP) cases. NPEVs isolated from AFP cases are not further characterized; therefore, their role in causing AFP remains unexplored. An ICMR project was undertaken in collaboration with National Polio Laboratories (WHO/NPSP) to determine the genetic diversity of NPEVs isolated from 4870 AFP cases identified from different parts of India during 2014 and 2015 by molecular typing, using partial VP1 gene sequencing. It was revealed that 87 serotypes (average) out of 108 known serotypes were identified each year from AFP cases. NPEVs belonging to species B EVs were most predominant (80.5%) as compared to species A (13.6%) and species C (5.8%) EVs isolated from AFP cases. The study suggested that molecular typing method is a useful tool for EV serotyping.

#### **Host virus interaction and the significance of apoptosis in infections by viruses causing Hand, Foot and Mouth disease (HFMD)**

CVA6 has been identified as an emerging pathogen for a series of HFMD outbreaks in Europe, North America and Asia. The study was carried out to investigate whether CVA6 Indian isolates can infect human neuronal cells in-vitro and induce apoptosis. Infection of CVA6 Indian isolates in human muscle and neuronal cells have shown that these viruses infect both the cells leading to apoptosis by both mitochondrial and TNF associated pathway. However, there are differences in release of pro-inflammatory cytokines between the cells. CVA6 infected neuronal cells up regulate TLR-3 and all pro-inflammatory cytokines such as IL-1 $\beta$ , TNF- $\alpha$  and IL-8 but not IL-6 whereas muscle cells induce TLR7, 9 and only IL-6, not other cytokines. More importantly CVA6 infected neuronal cells did not induce interferons as seen in case of infected muscle cells. These findings indicated that CVA6 has a tendency to infect neuronal cells, producing disease causing cytokines, which may lead to pathogenesis.

#### **Studies on poliovirus infections in children with immunodeficiency**

Immunodeficient children when exposed to poliovirus, may harbour poliovirus infection for several months or even longer, which is a major risk to community and the polio eradication programme. The project is being carried out in collaboration with NIIH and Wadia Children's Hospital, Mumbai, to screen the immunodeficient patients for enterovirus infection and study the long term excretors. From 151 diagnosed PID (Primary Immune-Deficient patients), stool Samples of 42 patients (4 SCIDs, 3 CVIDs, 7 XLAs and 28 other PIDs) with humoral, combined and other PIDs were assessed and followed up for enterovirus excretion. Stool specimens of 8 patients (19%) were tested positive for enteroviruses from which 5 (12 %) patients tested positive for Non-Polio Enteroviruses and 3 (7%) were tested positive for polioviruses. A 6 years old male child, a case of leaky SCID was excreting poliovirus since last two years and then rapidly stopped excretion. Genetic sequencing of virus isolates identified type 3 VDPV with up to 41 (4%) nucleotide changes at the age of 4 years which subsequently showed up to 93 (10%) nucleotide divergence at 6 years of age. The comparison of immunological parameters during excretion and after the child stopped poliovirus excretion, revealed rising cytotoxic T lymphocytes with exceptionally high NK cells. However, the B cell count of the child was still low with no change in immunoglobulin status. The result indicates that the T cells not the B cells, are important to stop virus replication. Further studies to analyze the immune response are being carried out.

#### **Cytokine responses to EV71 in cultured human cells**

Enterovirus71 (EV71) is the second most important Enterovirus of public health after poliovirus. Excessive pro-inflammatory cytokine responses such as IL-6, IL-1 $\beta$ , and TNF- $\alpha$  etc. contribute to the severity of EV71 infection. In this project,

the pattern of cytokine release by human cells infected with EV71 indigenous genotypes were analyzed in-vitro in order to gain information on clinical severity and pathogenesis of these strains. Genotype C1, C2 and G induced the release of all pro-inflammatory cytokines, the only difference observed was, Genotype G infected cells did not produce IFN- $\gamma$ . The results reciprocated the clinical status of the genotypes as they were isolated from cases of encephalitis, HFMD and AFP. Most importantly, from the two indigenous genotypes (D and G) EV71 genotype D infected cells did not produce any inflammatory cytokines. Based on our results the Genotype D could be considered as a candidate strain for vaccination studies and needs further characterization, which is being planned.

## RAJENDRA MEMORIAL RESEARCH INSTITUTE OF MEDICAL SCIENCES, PATNA

### SURVEILLANCE AND EPIDEMIOLOGY

**A study to explore the possible markers for conversion of asymptomatic into VL and its role in disease transmission – A multi-disciplinary approach**

The study was conducted in highly endemic PHCs of Muzaffarpur (Paroo and Sahibganj), Saran (Garkha), Nalanda (Islampur – *outbreak occurred in 2015*) and Vaishali (Patepur) districts of Bihar. Out of 4856 populations, 3920 was screened with rk-39, of which 124 (3.2%) persons were found rk-39 positive. Amongst the rk-39 positive cases, 76 (1.9%) had past history of VL and rest 48 (1.2%) were marked as asymptomatic cases. Besides, the ADA value was observed elevated ( $88 \pm 12.34$ ) in asymptomatic subjects as compared to endemic healthy control ( $62 \pm 8.12$ ). The Asymptomatic cases are under close observation.

**Private practitioners and Kala-azar case detection in a highly endemic district of Bihar: Actual role and potential**

It was observed that Private Practitioners (PPs) were more accessible and approachable to patients when compared to the Govt. facilities. They perceived

themselves to be important healthcare provider, and viewed themselves in different roles within the national program viz. provide right direction for diagnosis & treatment, refer patients at appropriate place for their ailments, guide patients in taking proper treatment, health motivation, save patients from specific disease, etc.

### DIAGNOSTICS

**Development of non-invasive loop-mediated isothermal amplification (LAMP) assay for diagnosis of Visceral leishmaniasis from oral fluid and urine samples**

This study was based on the diagnosis using Loop-mediated isothermal amplification (LAMP) assay of oral fluid and urine samples from confirmed VL cases, other disease patients such as Leprosy, Tuberculosis, Malaria and Typhoid as well as healthy controls and the DNA isolations of the clinical samples, where sensitivity and specificity was 88.46% and 100% respectively. The study is in progress to improvise sensitivity and specificity of this diagnostic tool on large number of oral fluid samples as well as in urine samples.

### TREATMENT

**Evaluation of efficacy and safety of Amphotericin B in two different doses in the treatment of Post Kala-Azar Dermal Leishmaniasis (PKDL)**

In an open label randomized trial, it was observed that Amphotericin B at 0.5mg/kg daily regimen is equally efficacious compared to 1mg/kg regimen, moreover; it reduces the frequency of infusion related side effects as well as nephrotoxicity. Therefore, this regimen can be considered for treatment of PKDL.

**A Prospective, Single Arm, Open label study to Assess Efficacy and Safety of a single dose (10mg/kg body weight) of AmBisome in children (aged 5 to 15 years) with Visceral Leishmaniasis (Kala azar)**

Post-treatment assessment, done at Day-30, revealed 100% initial cure, as all were clinically and

parasitologically cured with complete regression of spleen size and remarkable improvement of hematological parameters and body weight. During 6-months follow up period, 2 cases were relapsed at 4 and 6 months post-treatment. However, 3 patients did not turn up for six months follow up visit. Definite cure rate at 6-months evaluation was 95 (95%) [95% confidence interval 87.62-102.38]. These relapsed cases were successfully treated with Amphotericin B in the dose of 1mg/kg body weight for 15 infusions in 5% dextrose on alternate days.

## BASIC RESEARCH

### Screening cocktail *Leishmania* antigen (PDIS-70) along with immunomodulator for their role in immunity and protection in Visceral leishmaniasis

Mice immunized with *LdPDI*-DNA construct was found to be most immuno-reactive which was associated with substantial rise in Th1 and Th17+ CD4 cell response. Study conclude from the data that although *LdPDI* is essential for parasite survival but it has the vaccine potential since *LdPDI* immunized mice especially in DNA format evoked substantial protective response.

### Screening of Ornithine De Carboxylase inhibitor along with immunomodulator for their role in immunity and therapeutic implication in visceral leishmaniasis

Recombinant *LdODC* protein was observed having direct correlation with parasite growth and it significantly increased promastigotes and

axenic amastigotes growth after 96h of culture at 10 µg/ml.

### The role of Ornithine De Carboxylase of *L. donovani* in immunopathogenesis and to evaluate its vaccine potential against Visceral Leishmaniasis

A DNA based vaccine study was initiated by formulating ODC gene of *L. donovani* into construct of mammalian expression vector pcDNA3.1. The ODC construct so prepared was further used into downstream application at later stages in this immunization studies. Immunization with sub lethal dose was performed on week-1, 3<sup>rd</sup> and 5<sup>th</sup> and 10 days, thereafter the experimental groups were infected with  $3 \times 10^7$  metacyclic promastigotes. Immunological pattern for IL-2, IL-12, IL-10, IFN- $\gamma$  and TNF- $\alpha$  was evaluated, day-20 post inoculation of infective dose (Table 2). The trend observed so far has revealed that although considerable proportion of T cells proliferate (CFSC+ and IL-2 up regulation), protective immune responses are triggered and *Leishmania* proliferation in infected splenocyte inhibits against ODC during earlier stage of infection. The study is in progress.

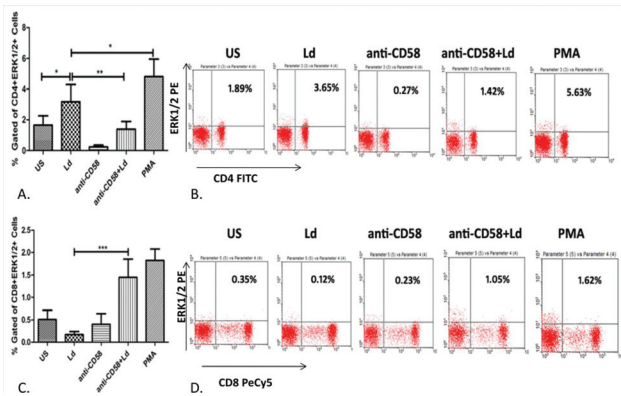
### ROLE OF LFA-3 IN VISCERAL LEISHMANIASIS

Relevance of LFA-3 in pathogenesis of Leishmaniasis is higher expression in time dependent manner, which correlated with the higher expression of Foxp3 and IL-10 in TLR2 and TLR4 dependent phosphorylation of ERK.

**Table 2:** Cytokine production in non-immunized and immunized host with DNA constructs Vector and SLA emulsified with Saline

Group of cytokines	Control	Non-immunized	ODC construct	SLA	Vector
INF- $\gamma$	33.03 $\pm$ 0.73	56.37 $\pm$ 3.23	76.33 $\pm$ 2.11	88.34 $\pm$ 2.75	65.02 $\pm$ 2.33
IL-12	121.55 $\pm$ 0.37	198.33 $\pm$ 5.11	238.11 $\pm$ 2.38	178.11 $\pm$ 3.22	239.77 $\pm$ 3.78
TNF- $\alpha$	65.33 $\pm$ 8.75	350.17 $\pm$ 5.66	412.33 $\pm$ 2.03	675.17 $\pm$ 1.73	340.11 $\pm$ 7.11
IL-10	17.55 $\pm$ 0.75	110.17 $\pm$ 3.31	128.75 $\pm$ 3.57	140.76 $\pm$ 7.98	122.04 $\pm$ 2.22
IL-2	3.73 $\pm$ 2.11	66.20 $\pm$ 0.73	112.55 $\pm$ 11.75	130.71 $\pm$ 0.31	70.75 $\pm$ 3.33





**Fig. 27:** Bar diagram showing effect of CD58 on ERK phosphorylation. (A) bar diagram showing % gated of CD4+ERK1/2+ cells (B) representative dot plots of figure A. (C) bar diagram showing % gated of CD8+ERK1/2+ cells (D) representative dot plots of figure C. Healthy PBMC were cultured in presence or absence of anti-CD58 and *Leishmania donovani* and % gated of CD4+ERK1/2+ and CD8+ERK1/2+ cells were observed by flow cytometry (n=5).

### Relevance of Natural Killer T cells (NKT cells) in Visceral Leishmaniasis

One of the *L. donovani* mediated pathogenesis mechanism was identified as the parasite induced MIP1 $\beta$  expression in phagocytes, which attracts CCR5+ positive CD4+NKT cells in infection site. The CD4+NKT cells were predominantly producing TGF- $\beta$  at infection site. It was also inferred that TGF- $\beta$  secreted from CD4+NKT cells prompts tyrosine kinase and caspase 3 mediated apoptosis in CD8+NKT cells.

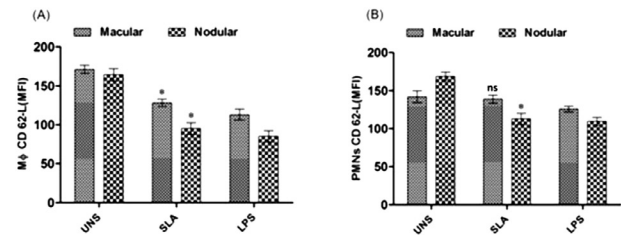
### Role of vector (Indian *Phlebotomus argentipes*) saliva proteins in Visceral Leishmaniasis

Construct of PagSP01, PagSP02 and PagSP07 was evaluated for immunoactivation and observed protective.

### Study on modulation of Innate Immune response and Immunopathogenicity in Indian PKDL patients

Innate immune response pattern during different clinical manifestations of PKDL patients was examined in skin biopsy samples. Macular patients, which usually are difficult to treat, were observed with gross impairment in the abilities of their PMNs for trans-endothelial cell migration (CD62-L low shedding, CD11b down regulation) at inflammatory site and this was further validated through an ERK-1

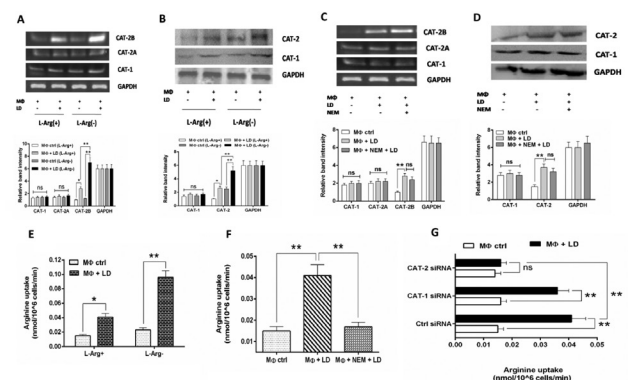
dependant more IL-10 production by such patients. The nodular patients of PKDL were observed with more improved trans-endothelial abilities of their innate cells and they were supported by production of higher IFN-g and TNF-alpha in p38 MAPK dependant manner.



**Fig. 28:** CD62-L on PMNs and M $\phi$ , represented as mean fluorescence intensity (MFI).

### Studies on the role of L-arginine in the immunomodulation of host response in Visceral Leishmaniasis

It was observed that intracellular survival of *L. donovani* depends on the availability of extracellular L-arginine mediated through CAT-2, which is the main isoform associated with its transport. Extracellular L-arginine availability was also found to regulate host arginase and iNOS balance in *Leishmania* infection. It was also observed that inhibition of L-arginine transport as well as arginase resulted in decreased polyamine production, limiting parasite survival inside macrophage. Besides, PI-3 kinase regulates arginase-iNOS expression and alters Th1/Th2 balance, thereby contributing to *Leishmania* survival inside macrophage. Thus, our findings provide evidence for targeting L-arginine metabolism as an adjunct therapy for the control of *Leishmania* infection.



**Fig. 29:** Effect of *Leishmania* infection on expression of CAT-1 and CAT-2 and L-arginine transport.



### Studies on Functions of Universal Minicircle Sequence Binding Protein (UMSBP) in *Leishmania donovani*

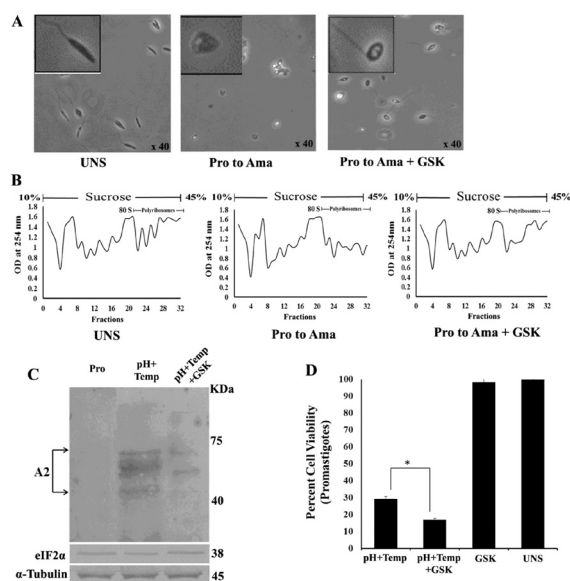
It was observed that kDNA binding activity of USBP is regulated by cellular redox signalling mechanism. Moreover, USBP regulates the expression level of MDR1 through kDNA loss, depletion of Cyt.b which leads to disruption of complex-III activity and reduction in ATP level which modulates the MDR1 mediating AmB efflux and thereby, regulates the drug resistance phenomena.

### Studies on the Inhibitor of Serine Proteases of S1A family (ISPs) in *Leishmania donovani*.

The experimental data suggests LdISP2 as an important molecule with significant inhibitory property over gut proteases and its down regulation severely affect gut protease activity inside sand fly midgut.

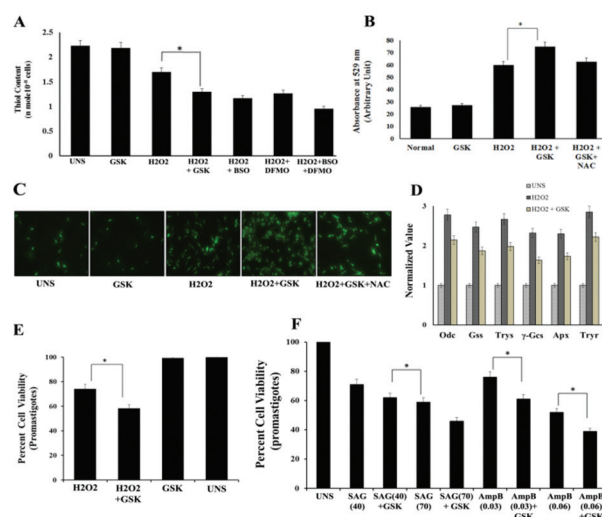
### Role of translation Initiation Factor 2-alpha (LdeIF2 $\alpha$ ) of *L. donovani* in parasite survival under stress condition

Inhibition of LdeIF2 $\alpha$  phosphorylation in presence of GSK2606414 hampers promastigote to amastigote differentiation and decreases parasite viability under exposure to combination of elevated temperature/acidic pH (Fig. 30). Further, inhibition of LdeIF2 $\alpha$



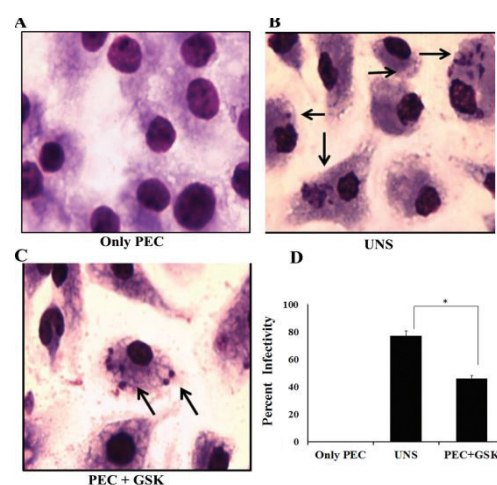
**Fig. 30:** Inhibition of LdeIF2 $\alpha$  phosphorylation in presence of GSK2606414.

phosphorylation under H<sub>2</sub>O<sub>2</sub> exposure reduces antioxidant levels and leads to accumulation of intracellular ROS which results in decreased parasite viability in an oxidative environment (Fig. 31).



**Fig. 31:** Inhibition of LdeIF2 $\alpha$  phosphorylation under H<sub>2</sub>O<sub>2</sub>.

Effect of inhibition of LdeIF2 $\alpha$  during course of infection was also studied. Infection rate of parasites pre-treated with or without GSK2606414 for 3 h on PECs isolated from mice showed 40% reduction in infection (P < 0.05) for GSK2606414 treated parasites compared to untreated parasites (Fig.32).



**Fig. 32:** Effect of inhibition of LdeIF2 $\alpha$ .

Effect of common anti-leishmanial drugs viz; Sodium Antimony Glucamate (SAG) and Amphotericin B (Amp B) on phosphorylation status of LdeIF2 $\alpha$  was studied. It was found that ROS generated by these drugs, also induces LdeIF2 $\alpha$  phosphorylation (Fig.33).

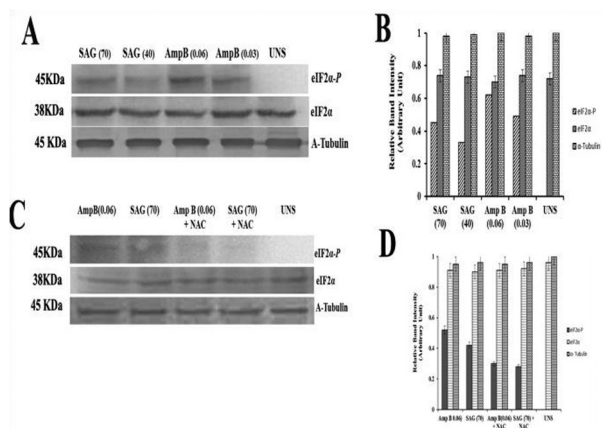


Fig. 33: Effect of common anti-leishmanial drugs.

Taken together, these findings indicate that stress induced phosphorylation of LdeIF2 $\alpha$  plays a crucial role in differentiation and parasite survival under stress and also a possibility of an involvement in protection against drug induced stress.

### Study on immunoregulatory role of PD-L1/PD-1 pathway in human visceral leishmaniasis

Experiment was performed for evaluating the frequency of PD-1 & its ligand expression (PD-L1) on immune cells (T cell sub-sets and APCs) respectively.

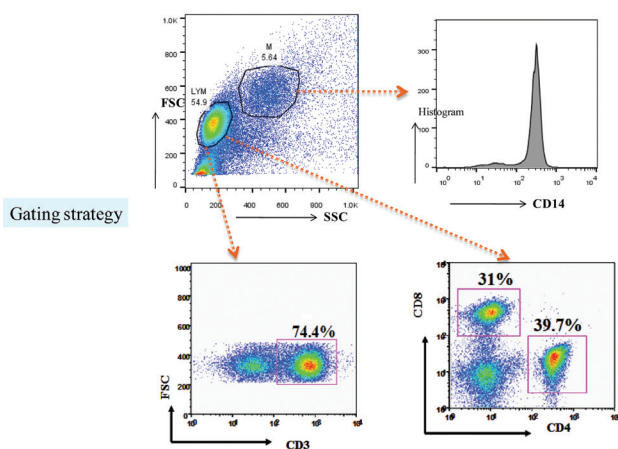


Fig. 34: Representative FACS plots showing the gating strategy of T cell sub-sets (CD3, CD4, & CD8) and monocytes (CD14) cells in VL patient.

The expression of PD-1 was more likely due to activation of T cell sub-sets and it may function as a cell death inducer on expressed cells.

### Ascorbate based regulation of intracellular redox metabolism in clinical isolates of AmB resistant *L.donovani*

Study has shown the central role of ROS in the mechanism of *Leishmania* drug resistance and differentiation using LdAPx gene knockout (KO) AmB resistant parasites, overexpressed (OE) LdAPx sensitive parasites, resistant parasites pretreated with inhibitor compared to wild type (WT) AmB sensitive parasites. Thus, it was inferred that this APx could be fundamental in a rational approach to the design and discovery of drugs against *Leishmania*.

### Pathophysiology of lipoproteins in *Leishmania donovani* infection

The serum lipid profile analysis of VL patients revealed reduced level of total cholesterol, HDL-c, LDL-c in patients having high parasitic (LD bodies) score. Data suggests that parasite burden affect the serum cholesterol and lipoprotein level in VL patient.

### Study of effect of Zinc trafficking in macrophage antimicrobial response in Visceral leishmaniasis

Level of serum zinc and zinc binding serum proteins, prooxidant & antioxidant levels and markers of inflammation were studied in VL patients and healthy control. Data suggests oxidative stress and increased inflammation are associated with low serum zinc level in VL patients.

### Understanding the role of LdIscS and LdIscU proteins of ISC machinery in mediating iron fluxes during transformation from promastigotes to amastigotes stage

Macrophage infection studies using LdIscS-overexpressing parasites showed significantly enhanced infectivity of LdIscS-OE parasites as compared to wild type parasites, confirming the role of LdIscS and ISC machinery in pathogenicity of *Leishmania* parasites.

## VECTOR BIOLOGY & CONTROL

### Study on the semiochemical mediated response in the oviposition behaviour of *P. argentipes*

Female *P. argentipes* preferred the substrate treated with Rabbit's larval rearing medium (RLM) for the oviposition purpose over the Cow's larval rearing

medium (CLM), CLM provides best survival to the developing stages of sand flies leading to the emergence of adults flies. The chemico-analysis revealed that Heptacosane [ $C_{27}H_{56}$ ], a Cuticular Hydrocarbon (CHC), as a common semichemical compound in both type of extracts and is responsible for attracting and stimulating oviposition responses among *P. argentipes*.

### Impact of DDT spray on Kala-azar in Bihar - A longitudinal study

*P. argentipes* population was susceptible to DDT in the non-endemic villages (except the Pipraradhe village of Muzaffarpur district). Conversely, vector population of three endemic sites (Azadnagar, Madhopur and Tarawa) had developed resistance against DDT. Mortality rate of vector collected from the resistant and tolerant reported villages ranges from 46.15% to 78.33% and 80.36 % to 89.29 %, respectively. Sand fly densities, pre-IRS (7-days before) and post-IRS at monthly interval till the next spray in all the study villages were monitored by CDC light traps. Longitudinal monitoring of *P. argentipes* density of these villages was performed at monthly interval

### Evaluation of active molecule having insecticidal effect to *Phlebotomus argentipes* (Diptera: Psychodidae) in the laboratory

Out of 97 plants collected from different endemic and non-endemic areas of Kala-azar, two indigenous plants (PK90 and PK48) were identified the extract of which showed insecticidal effect on sandfly.

### Integrated vector management for the VL control vis-à-vis case study - A pilot study

Bioavailability test of insecticide in intervention (BII) revealed resurgence of the sand fly population after some months. Probably, it may be due to reduction in the bioavailability of insecticidal content in both IRS as well as ITN. Data analysis for intervention-wise reduction is under process. The study is continued to assess mass acceptability of intervention(s) by the community.

### Study on protein variants from DDT resistant strain of *Phlebotomus argentipes* (Diptera: Psychodidae) from Bihar, India

The purification profile of GST protein from crude homogenate of sand flies through Glutathione-Agarose beads showed one specific band at 26 kDa along with one impurity at 64kDa, which may be an interacting protein with GST.

### Understanding behavioral profile of DDT resistant sand flies for exploring scope of IGR as an alternate technique for containing the population of *Phlebotomus argentipes*

Establishment of DDT resistant colony of *P. argentipes* has been accomplished. It was confirmed that the descendents emerged out in subsequent generations from highly resistant parent bear characteristic feature of DDT-resistance.

## BIOINFORMATICS

### Whole Transcriptome Analysis of VL and PKDL patients by Next Generation Sequencing of *Leishmania donovani*

Comprehensive transcriptome analysis showed that there are separate set of genes for regulation or switching of various genes of the parasite (during VL/PKDL) and host to up-regulate and down-regulate the genes that makes the parasite fittest for its survival. Some of the highly expressed human genes (up-regulated) are AJ271736.10 (IL9R gene; interleukin 9 receptor; repeat region), *Homo sapiens* mature T-cell proliferation 1(MTCP1), *Homo sapiens* membrane protein, palmitoylated 1, 55kDa (MPP1), transcript variant 4, *Homo sapiens* filamin A, alpha (FLNA).

### Whole Genome Sequencing of *Phlebotomus argentipes* by Next Generation Sequencing (de novo sequencing)

As per the pair end data and matepair data assembled, the size of the genome of *Phlebotomus argentipes* has been found to be 141MB approximately. The CDS (Coding sequences) of the genome were predicted from generated scaffolds using Augustus software. The assembly consists of 48,179 and 43,411 scaffolds for DDT-sensitive and DDT-resistant samples respectively. A total of 17,700 and 10,766 CDS were obtained for DDT sensitive and DDT resistant samples respectively using Augustus software.



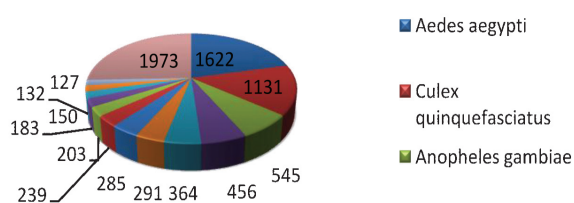


Fig. 35: Insect-gDNA top blast hit species distribution.

### Concept of fabrication of novel drug delivery technology and Nanomedicine to improve the current options of treatment or vaccination against leishmaniasis

It was observed for the first time that efficacy of GINPs encapsulated AmB and miltefosine increases two fold. *In vivo* studies also demonstrated the high cure rate which suggests the reliability of the Nano formulation.

### Mining the genome of *Leishmania donovani* for the development of novel vaccine candidate for the control of visceral leishmaniasis

It was revealed that cocktail of three *Leishmania* class-1 restricted synthetic peptides (Cocktail1: p1, p2, p4, p5; Cocktail2; p7 and p9; cocktail3: p1,p2,p4,p5 and p14) triggered a greater population of CD8<sup>+</sup> T Cells to produce IFN- $\gamma$  production as compared to soluble leishmania antigens (Positive control). Though similar pattern was not observed in vaccinated mice group, but an elevated level of protective cytokines (IL-2, IFN-  $\gamma$  and IL-12) was observed in vaccinated animal model.

### VIROLOGY

Stool samples routinely collected from different Hospitals (Child Care Centre & NMCH) in the year 2014-2016 were screened for the presence of Human Calicivirus (Norovirus, Sappovirus), astrovirus, adenovirus, and enterovirus. Out of total 1459 stool samples, 111 samples were found to be positive for adenovirus (7.61%), 36 samples were found to be positive for astrovirus (2.54%) and 8 samples were found to be positive for norovirus (0.54%). It was the first ever in Bihar to sequence and phylogenetic analysis of the gastroenteritis causing viruses. Mixed infection of astrovirus and adenovirus were also found. Astrovirus and adenovirus type 40 (clustering with M633 Japanese strain Dugan)

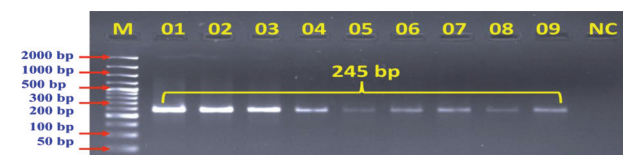
were also confirmed by sequencing and conduct phylogenetic analyses. RT-PCR and sequencing of amplicons of astrovirus from Patna, with specific primers targeted to the conserved domain of ORF1a (289 bp) of the astrovirus genome, showed maximum homology to the astrovirus strain (“5–158”) from Madagascar. Samples which weren’t positive for viral gastroenteritis were sent to institute’s bacteriology lab of RMRI for diagnosing bacterial and protozoan etiological agents.

After Rotavirus, Adenovirus and Astrovirus was found as the most frequent cause of viral gastroenteritis in both hospitalized and non-hospitalized children. Maximum positivity of the viruses was seen in children less than two years of age.

### TUBERCULOSIS

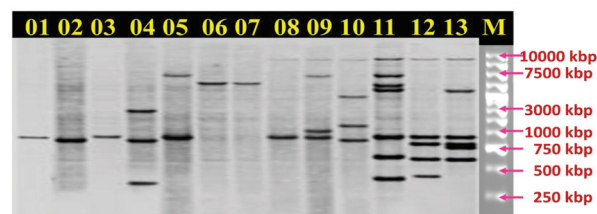
#### Genotyping of *Mycobacterium tuberculosis* isolates among the Tribal and Non-tribal Pulmonary TB patients of Bihar using IS6110 element RFLP analysis

A total of 102 sputum samples from tribal (n=48) and non-tribal (n=54) symptomatic Pulmonary TB patients having prior history of receiving anti-TB treatment were collected and diagnosed. Genomic DNA of *M. tuberculosis* was checked using 245 bp probe amplified by PCR using IS6110 primers (INS 1 & INS 2).



M: Mol.wt. marker, L1: H377V; L2-05: Clinical isolates from tribal population, L6-9: Clinical isolates from non-tribal population, NC: Negative control

Fig. 36: PCR Amplification of *M. tuberculosis* (H37Rv) and clinical isolates of *M.tuberculosis* using IS6110 primers (245bp).



L1-7: Tribal population, L8-13: Non-tribal population, M: 1 Kbp Mol. Wt. marker

Fig. 37: Genotyping of *M. tuberculosis* isolates using IS6110 DNA by Finger Printing method.

## SPECIAL ASSIGNMENT

As entrusted by DGHS, RMRI undertook holistic intervention approach in Vaishali district as model for Kala-azar elimination in close collaboration with Care India, MSF, DNDi, Bihar State Health Dept. and NVBDCP. Planning, execution and strict supervision & monitoring of the activities viz. IEC, training & re-orientation, active case detection, treatment with single dose AmBisome, IRS using DDT (1<sup>st</sup> round) & Synthetic pyrethroid (2<sup>nd</sup> round) etc were the key component. The concerted effort led to remarkable reduction in kala-azar incidence at block level in Vaishali district. As suggested by VBDSF, the model has been replicated in other districts as well.

## NIRTH, JABALPUR

### VECTOR AND VECTOR BORNE DISEASES

#### Studies on HRP2 and HRP3 expression in *Plasmodium falciparum* parasites from endemic States of India: A prospective evaluation

A study was undertaken to evaluate the histidine-rich protein 2 (*HRP2*) and *HRP3* gene variations / deletions in *P. falciparum* parasite. The samples were collected from malaria-endemic eight States of India (North East, Orissa, Madhya Pradesh, Chhattisgarh, Jharkhand, Maharashtra, Gujarat and Rajasthan). The commercially available malaria rapid diagnostic test's (RDT's) detects *P. falciparum* HRP2 and cross react with HRP3, a structural homologue. In the designed study, a total of 1392 *P. falciparum* positive samples collected from eight endemic states were subjected to PCR amplification and sequencing of *Pfhrp2* and *Pfhrp3* genes. The sequence analysis revealed a total of 16 and 11 distinct repeat motifs in *Pfhrp2* and for *Pfhrp3* respectively, including some newer repeat types. No correlation was found between variations in the size of *Pfhrp2* repeat types 2 and 7, nor between any combinations of repeat motifs, and performance of a commercial RDT at low parasite densities. The study revealed sequence diversity in *Pfhrp2* and *Pfhrp3* genes from Indian isolates of *P. falciparum* is meagre and not likely to negatively

influence performance of currently used HRP2 based RDTs.

#### Bionomics of malaria vectors and their sibling species, and to establish their role in malaria transmission in Chhattisgarh, India

The highly malarious districts, Bastar and Korla of Chhattisgarh state were selected in the study to determine the abundance, distribution, breeding, host preference, insecticide susceptibility status, sibling species composition and parasite prevalence in vector species.

A total of 17 different *Anopheles* species were collected. *Anopheles culicifacies* was the predominant vector species available throughout the year in the study area. The sibling species complex of *An. culicifacies* constituted species C, while in *An. fluviatilis* complex, species T was the most abundant one. Blood meal analysis of both *An. culicifacies* and *An. fluviatilis* revealed that they are primarily zoophilic. Breeding takes place mainly in stream, rocky pits and seepage water. Insecticide susceptibility tests showed that *An. culicifacies* is highly resistant to DDT and Malathion, showing variable resistance to Pyrethroids.

#### An assessment of intervention measures for prevention of malaria in pregnancy: A prospective longitudinal study in Central India

A prospective longitudinal study was undertaken in two CHCs (Ranapur and Meghnagar) of district Jhabua to understand the effect of intermittent preventive screening and treatment (IST) on malaria in pregnancy (MIP). The total of 610 pregnant women under the eligibility criteria were enrolled in the study. The prevalence rate among pregnant women was 2.8% (17/610) and the major species involved in the infection was *P. falciparum* 65% (11/17). Interestingly, 2% (14) pregnant women were found malaria positive during follow up. Regarding anaemia status during the time of delivery, 81% (295/365) women were having moderate and only one woman had severe anaemia, respectively. A total of 512 deliveries were recorded in the Delivery Unit (DUs). Out of

512, 71% (365/512) deliveries were attended in the hospital and rest 29% (147/512) were unattended. In the SMT group, a total of 519 pregnant women were enrolled in the study. Out of which 13% (68/519) of pregnant women were showing symptomatic signs (history of fever from past 14 days). Among 68, 7 pregnant women were (10%) positive for malaria and most of them were having *P. falciparum* infection, 86% (6/7). Moreover, 14% cases were positive for dual parasitic infections (*P. falciparum* and *P. vivax*). A total of 858 women were followed up successfully and out of which 6% (48/858) were symptomatic during follow up. Only one positive case was found infected with *P. vivax*. At the time of enrollment at homes, 89% (463/519) and at the time of delivery at DUs, 86% (181/210) women were having moderate anaemic condition. The present study demonstrated that ASHA, ANM, AWW and community health worker's visit during antenatal period to treat malaria in pregnant women may increase routine health care of pregnant women and reduce the adverse effect of malaria on pregnancy. The study would help in developing policies to reduce burden of malaria or its adverse effect on pregnancy.

#### State intervention model for malaria control in highly malarious district Balaghat in Madhya Pradesh by intensified blood surveys and drug administration

Mass screening for malaria was carried out in 102 villages (42,293 population covered) of Birsa community health centres (CHC) and 77 village (28209 population covered) of Baihar CHC by using rapid diagnostic test (RDT). The malaria prevalence rate was highest in Songudda PHC (42%, 24% and 19% in the first, second and third round, respectively) and lowest in the Mandai PHC. More than 90% cases were having *P. falciparum* infection. Moreover, Age- group-wise analysis revealed that malaria positivity was significantly higher among 1- 4 year old children than other age groups. The present study revealed that the malaria positivity significantly declined during survey period from 42% to 24% and to 19% in the first, second and third round, respectively.

#### Molecular epidemiological study of *Plasmodium falciparum* field isolates and the incidence of malaria in endemic regions of Central India

*Plasmodium falciparum* invade erythrocyte through alternate pathways, was well characterized in laboratory adapted clones but this phenomenon is not well studied among field isolates. So, the following selected invasion ligands of *P. falciparum* –*PfPRH4*, *Ripr*, *EBA-175* and *AARP* were analyzed by molecular methods in field isolates. A total of 25 *P. falciparum* positive samples were successfully cultured and used for the invasion assay. Out of which sample no. 23, 24, 18 and 8 were successfully amplified and analysed for *PfPRH4*, *Ripr*, *EBA-175* and *AARP* gene sequences. Overall, all the genes were found to be highly conserved among the different *P. falciparum* field isolates with very limited polymorphisms except *EBA-175* which was found polymorphic as compared to other genes.

#### Preparatory study for the control of malaria in the district Gadchiroli, Maharashtra.

A study was initiated with collaboration of Society for Education, Action and Research in Community Health (SEARCH) Gadchiroli, Maharashtra to study the vector composition and to evaluate susceptibility status of *An. culicifacies* against commonly used insecticides in public health. The predominant vector species in the study area belongs to anopheline genus viz. *An. culicifacies* and *An. subpictus*. However, *An. annularis* and *An. vagus* were also present in area. *An. culicifacies* is the known malaria vector, which is found resistant to DDT, Malathion, Cyflutrin, Lambda cyhalothrin and Deltamethrin while resistant status against permethrin is yet to be verified.

#### A field based study to assess burden of malaria in pregnancy (MIP) in two blocks of district Balaghat, Madhya Pradesh

The study was carried out in Baihar and Birsa blocks of district Balaghat, MP from the period of August 2014 to June 2017. A total of 1664 pregnant women (PW) were screened for malaria (581 primigravids, 564 secundigravids and 519 multigravids) using



RDTs and microscopy. The overall malaria prevalence was found to be 23% (26.5% among primigravids, 19.7% in secundigravids and 22.4% in multigravids; 22.8% in 2014-15, 21.8% in 2015-16 and 23.9% in 2016-17) at the time of enrolment. Women were treated following NVBDCP guidelines. Most of the infections were due to *P. falciparum* malaria (87.4%) and were asymptomatic (81.6%). The *P. falciparum* malaria prevalence was significantly higher among primigravids compared to secundigravids [OR 95%CI, 1.6 (1.2-2.2),  $p = 0.0029$ ] and multigravids [OR 95%CI, 1.4 (1.0-1.9)  $p = 0.017$ ]. Malaria prevalence among Baiga PW (42.1%) was significantly higher compared to Gonds (22.9%) [OR: 2.4; (1.7-3.4),  $p < 0.00001$ ] and non tribals PW (8.2%) [OR: 8.1; (3.8-19.2),  $p < 0.00001$ ]. Prevalence of anemia and severe anemia among pregnant women was 80% and 19% respectively. Birth outcomes of 300 malaria exposed PW revealed 18.5% lower birth weight, 5% still birth and 3% abortion. Further, 1.3% new born died shortly after the birth. Only 42.7% deliveries occurred at health centres.

#### Mid-term evaluation of mass drug administration (MDA) in the control of Lymphatic Filariasis

The study was carried out on the instruction of state vector borne disease control program of Madhya Pradesh to evaluate the compliance rate of mass drug administration in the elimination of lymphatic filariasis after 11<sup>th</sup> and 12<sup>th</sup> round of annual MDA. One urban ward and three villages in consultation with the program officer of each district were selected on the basis of low, medium and high compliance rate of MDA. Overall 16 sites (4 urban and 12 rural) were surveyed. In 11<sup>th</sup> and 12<sup>th</sup> round of MDA, survey was conducted in Damoh, Katni, Umaria and Chindwara districts covering 516 households with eligible population (excluding less than 2 year children, pregnant women and severely ill patients). Overall, 85.5% respondents and 77.7% of the eligible members of their households received drug in 11<sup>th</sup> round, whereas the corresponding proportion was 78.3% and 69.3% in the 12<sup>th</sup> round of MDA. Highest coverage was recorded in Chindwara district where 99.5% respondents received drug followed by

district Damoh (93%). However, population wise highest coverage was recorded in district Umaria (85.6%).

Consumption of drug was highest in district Chhindwara (95.9%) among respondents and 75.7% among eligible population in 11<sup>th</sup> round, while in 12<sup>th</sup> round highest consumption was in district Umaria. Consumption of drug was above the threshold level of 65% in 11<sup>th</sup> round in three districts whereas in 12<sup>th</sup> round only in Umaria district, it was equal to required rate essential for elimination. However, in Katni district, consumption rate was much below than the required rate of MDA. Coverage and compliance rate of MDA in urban areas in both the rounds of MDA was lower than the average compliance rate of MDA in the district

#### HEMOGLOBINOPATHY AND SICKLE CELL ANAEMIA

##### Establishment of prenatal diagnosis of $\beta$ -Thalassemia syndromes and sickle cell disorders in Madhya Pradesh, Assam and the Andaman and Nicobar Islands

Initiatives to establish a prenatal diagnosis facility at ICMR-NIRTH, Jabalpur in collaboration with NSCB Govt Medical College was accomplished under the multi-centric efforts. Two gynaecologists underwent training for chorionic villus sampling (CVS) procedures at AIIMS, New Delhi in April 2016. Subsequently, workshops on Hemoglobinopathies and prenatal diagnosis were also organized for sensitizing and training doctors and technical staff from Govt. Hospital in April, 2017. The registration and licence from state health authorities for initiating CVS sampling at the institute through the partner NSCB medical college, Jabalpur was obtained (No. CLN1608672). With the completion of hands training on CVS sampling by medical doctors and establishment of molecular lab by ICMR-NIRTH, prenatal testing for sickle cell and thalassemia syndromes is geared at NIRTH institute.

##### Micro mapping of G6PD deficiency among the tribals of India and its importance for anti-malarial therapy

Efforts were made to map the G6PD mutations among tribal populations of Madhya Pradesh under the multi-centric study on micro mapping of G6PD deficiency among the tribal populations of India. During the year 2016-17, one thousand six hundred seventy tribal individuals were screened. Eighty four G6PD deficient individuals were identified and these individuals and their family members were counseled on the management and prevention of hemolytic crisis due to anti-malarial and other oxidant drugs. New G6PD variant (551 C-T) in exon 6 was observed among the tribal groups of Madhya Pradesh and named G6PD Dindori. Further, district health authorities were also intimated about these affected individuals and necessary preventive care in case of crisis.

#### **Screening for sickle cell disorder among Ashram school students-MP State**

Under the joint initiative of ICMR and Ministry of tribal affairs, a total of 1589 tribal school children between the ages of 7-16 were screened for various hemoglobinopathies in particularly sickle cell anaemia. Out of which, 215 were found to be sickle cell trait and 11 were homozygous for sickle cell disease.

#### **Screening for G6PD deficiency among the tribal populations living in malaria endemic zones and its correlation with anti-malarial therapy**

Screening for G6PD deficiency among tribals living in highly malarious districts of Odisha has been initiated to identify and prevent incidence of haemolytic events among G6PD deficient individuals due to anti-malarial drugs. For this study, a field laboratory at Biswanathpur CHC, Kalahandi district has been established in June 2017. Since the initiation of the study, 507 fever cases have been investigated for various haemoglobin disorders. Among 507 patients, only 3 individuals were found to be G6PD deficient indicating a low prevalence of G6PD deficiency in the area.

#### **Morbidity profile of sickle cell disease (SCD) in central India**

ICMR-NIRTH has been operating a bi-weekly sickle cell clinic in the NSCB Govt. Medical

College, Jabalpur since 2000, where, more than 1000 sickle cell patients receive regular treatment. During the year 2016-17, sixty new patients were enrolled for treatment. Early detection and counseling provided at sickle cell clinic, resulted in symptomatic relief and reduced the painful crisis events in these SCD patients.

Newly diagnosed SCD patients were registered with ICMR-NIRTH sickle cell clinic operational at NSCB Govt. Medical College for management and care on OPD basis.

#### **Screening the resident tribe of Lahaul & Spiti, district (H.P) for sickle cell anaemia and other abnormal variants of Haemoglobin**

This is a new initiative at Keylong field station to screen tribal populations in Lahul and Spiti valley of Himachal Pradesh for hemoglobinopathies. In this field station, 1325 samples were screened for sickle cell anaemia and thalassemia. So far, no sickle hemoglobin carrier is detected among the screened population. The study is ongoing.

### ***Communicable Diseases***

#### **TUBERCULOSIS**

#### **Multi-centric cohort study of recurrence of tuberculosis among newly diagnosed sputum positive pulmonary tuberculosis patients treated under RNTCP**

In a prospective, multi-centric cohort study to estimate the recurrence of TB among newly diagnosed pulmonary TB patients, who have successfully completed treatment and to assess risk factors for unfavourable outcomes to treatment. New smear positive pulmonary TB patients treated under RNTCP were enrolled as study participants and were subjected to the structured interview, sputum examination for smear, culture and DST, blood tests for diabetes mellitus and HIV infections. The study highlights high recurrence of TB (24.8%) which is more than double the reported figure of 10-12% in different parts of the country by various workers. The majority of recurrences (82%) occurred in first six months. The study findings also highlight high proportion of unfavourable treatment

outcomes (deaths 10% and treatment failure 17%) compared to the national average of 4% and 2%, respectively.

### **Estimate the burden of TB among the tribal population and develop an innovative health system model to strengthen TB control in the tribal areas (Study site: Madhya Pradesh) - Multi-centric ICMR Task Force Study**

This cross sectional, multi-centric ICMR task force study was carried out to estimate the prevalence of TB and to find out health seeking behavior patterns to develop feasible interventions, improve case finding and compliance for TB amongst tribal groups in the country. The study was initiated in randomly selected 16 clusters of the state in 4 phases *viz.*, Phase I (Situational analysis), Phase II (Qualitative assessment), Phase III (Quantitative Assessment) and Phase IV (Intervention). Situational analysis along with the mapping (phase I) was completed in 30 villages (16 clusters +14 additional). Further, qualitative assessment (Phase II) and TB disease survey (Phase III) was also completed in all the 16 clusters. Moreover, thirty-one focus group discussions (FGDs) and interviews of 124 key informants (Medical officers, ANM, ASHA, STS/STLS, Sarpanch, Patient) have been completed. FGDs and interviews of key informants highlight their knowledge and perceptions about the TB disease, use of TB diagnostic and treatment facilities and the suggestions for improvement. In a Survey, total 14,409 population covered, 653 (4.5%) were found chest symptomatic. A total of 72 sputum positive cases (smear and/or culture) have been detected so far and are referred for treatment under RNTCP. The phase IV (intervention) activities are being undertaken in selected villages. The study is in progress.

### **Targeted intervention to expand and strengthen TB control in tribal populations under the revised National Tuberculosis Control Programme, India. (The TIE-TB Project)**

The Revised National Tuberculosis Control Programme (RNTCP) is covering the entire country for TB control. However, limited improvements

in certain aspects are impediment for effective implementation of the RNTCP services. For achieving universal access, the primary two aspects, 'the access to services' and 'the awareness among the community' were targeted. This project focuses on interventions of community engagement and targeted use of mobile vans equipped with digital X-ray and sputum microscopy services to improve the accessibility of TB care services and health seeking behaviour of the tribal populations. NIRTH is covering two states under the project *viz.*, Madhya Pradesh (four districts: Jhabua, Alirajpur, Barwani and Dindori) and Chhattisgarh (three districts: Sarguja, Surajpur and Balrampur). The baseline survey including collection of the relevant data has been completed and the intervention phase using mobile vans has been initiated.

### **Comparative study of line probe assay and Xpert MTB/RIF for detection of MDR**

The objective of the study is to compare the performance of Xpert MTB/RIF with Genotype® MTBDRplus for detection of Rifampicin resistant TB and to study the mutation patterns associated with *rpoB*, *katG* and *inhA* genes in this area. Out of 351 specimen tested by line probe assay (LPA), 108 were negative for *M. tuberculosis*. 209 (85.5%) specimens were sensitive for both rifampicin and isoniazid. However, 18 and 10 specimens were found multidrug and mono-resistant to either Rifampicin or isoniazid, respectively. The discordance between Xpert MTB/RIF and LPA were resolved by sequencing and it was found that it was consistent with the findings of Gene Xpert.

### **SCABIES**

#### **Intervention programme for the management of scabies in Baiga tribe of the Dindori district**

The study was conducted in 7 blocks including 201 villages. The blocks were divided randomly into two groups *i.e.* Group A (receiving Ivermectin and GB Lotion) and Group B (receiving GBH Lotion). The identified positive cases were treated with group A and B medications, respectively. Total 451 and 449 positive cases were detected in group A and B, respectively. Among Ashram schools of



all the blocks, a total of 1271 positive cases were identified. Detailed analysis is in progress.

### **National hospital based rotavirus surveillance network**

This is a multi-centric ICMR task force study on rota viruses. Being a regional laboratory, NIRTH had 2 peripheral sites; Netajii Subhash Chandra Bose Medical College (NSCB), Jabalpur and Kamla Nehru Gandhi Medical College (GMC), Bhopal. Total 261 stool samples were collected in year 2016, 144 samples at NCSB, Jabalpur and 117 at GMC, Bhopal from diarrhoeic children with respective parents/guardian's consent. Total fifty samples were positive for group A rotavirus by ELISA. The confirmed positive samples were used for *VP4* and *VP7* amplification by reported primers for P and G typing. *G1P8* was the commonest type identified followed by *G1G12P8* and *G9P4*.

Phylogenetic analysis of *VP6* gene showed that this area has *VP6I1* and *VP6I2* in circulation. *VP6I1* was earlier reported only from north eastern part of the country.

### **Enhancing biorisk mitigation awareness in public health community and creating laboratory networks for enhanced diagnostic capabilities to deal with surveillance and outbreaks of high-risk group viral pathogens causing viral hemorrhagic fevers**

This is ongoing project initiated and monitored by NIV, Pune since August 2016, to diagnose hospitalized febrile illness patients for viral hemorrhagic fevers such as Dengue, Chikungunya and Zika. In 2016, total of 459 Dengue and Chikungunya suspected hospitalized patients were tested. Out of which, 16 % and 6 % samples were found positive for Dengue and Chikungunya infections, respectively. Moreover, 2% samples were found positive for both Dengue and Chikungunya infections. However, team has not yet encountered a single case of Zika virus infections.

### **Establishment of grade II virology laboratory**

Presently, virology laboratory of NIRTH, Jabalpur provides diagnosis for more than twenty different

viruses. The samples are collected by NIRTH team and government health agencies of Madhya Pradesh. Moreover, along with viruses diagnosis, it is involved in outbreaks investigations too. In 2016-17, more than 2300 samples were tested by different serological and molecular diagnostic tests. The study revealed that Dengue, Hepatitis and Influenza are the important viral diseases. Besides, four Dengue outbreaks were investigated and Dengue virus 3 was noted as a dominant serotype circulating in the year 2016.

### **A pilot study on evaluation of rapid diagnostic test (RDT's) kit for dengue in tribal-rural areas of Madhya Pradesh under NIRTH, Jabalpur**

To compare the sensitivity and specificity of the RDT's with ELISA and molecular tests, a total of 164 samples were collected in 2016-17 from 19 districts of Madhya Pradesh. The RDT's stored at three different storage temperature (*i.e.* 4°C, 37°C and 45°C) are being evaluated. Further, the tests will be compared using ELISA and qRT-PCR as gold standard. The study is in progress.

### **Studies on prevalence and risk factors associated with hepatitis B infection in Lahaul and Spiti, Himachal Pradesh**

The ELISA test was standardized for screening of HBsAg and HCV Ab at Keylong field station. Since December 2016, two hundred and fifty samples were collected from patients with symptoms of hepatitis residing in Lahaul valley and were tested; 5.0 % (13 / 250) patients were found positive for HBsAg and one sample was HCV antibody positive. The study is in progress.

## **SOCIAL SCIENCE AND BIO-STATISTICS**

### **Knowledge, Attitudes, and Practices (KAP) survey at Gadchiroli**

Gadchiroli district is an insurgency prone area of Maharashtra and contributes around 46% malaria while its population is only 1% of the state of Maharashtra. The district is designated as tribal district. As part of designing a strategy for malaria control in the district, NIRTH in collaboration with SEARCH and NGO conducted a qualitative KAP

study on malaria. The study revealed the existence of misconception, poor/less use of preventive measures and anti-malarial services. Moreover, it indicated, a need to develop local sensitization programme for mass awareness, promotion of ITN/LLIN, planned the IRS and capacity building for ASHA to deliver prompt diagnosis and anti-malarial treatment.

### Reported RTI at Lahaul and Spiti district of Himachal Pradesh: A Pilot study

A pilot study was initiated to evaluate reported RTIs in Lahaul and Spiti district. The adverse reproductive outcome experienced by participants included induced abortion 9.7% (31/318), miscarriage 16.7% (53/318), still birth 2.5% (8/318) and premature birth 1.3% (4/318). Around 27% (112/418) were aware of symptoms of RTI and among them 21.4% (24/112) had reported to multiple symptoms of RTI during three months preceding the survey. The study is in progress.

### Extramural Research

#### Anti Microbial Resistance Surveillance Network and Stewardship Program

ICMR initiated National Anti Microbial Resistance Research and Surveillance Network (AMRSN) to enable compilation of National Data of AMR in 2013 in six Nodal centres (NCs) which are focusing on six pathogenic groups:

- (i) Diarrhoeagenic bacterial organisms: CMC, Vellore.
- (ii) Enteric fever pathogens: AIIMS, New Delhi.
- (iii) Enterobacteriaceae causing sepsis: PGIMER, Chandigarh.
- (iv) Gram negative Non-fermenters: CMC, Vellore.
- (v) Gram positives including MRSA : JIPMER, Pondicherry.
- (vi) Fungal infections: PGIMER, Chandigarh.

The following six hospitals/medical colleges were added as regional centres to the network in 2016-2017

1. Hinduja hospital. Mumbai
2. Apollo hospitals, Chennai

3. AFMC, Pune
4. Tata Medical Centre, Kolkata
5. Sir Ganga Ram Hospital, New Delhi
6. MGIMS, Wardha

### RESEARCH OUTCOME

The results of the surveillance network provide valuable and essential data which can form baseline for targeted antibiotic policy (speciality wise, syndrome wise and category wise) and implementation of antibiotic stewardship in a tertiary care medical facility.

The basis of targeted antibiotic policy and antibiotic stewardship guidelines is the prevalence of pathogens and the susceptibility profiles of the same. In contrast to western data, our isolates from serious hospital acquired infections show a clear dominance of gram negatives (90% of respiratory, 80% of body fluids, 75% CSF and pus and 50% of blood); in body fluids and respiratory specimens empiric therapy may be targeted to gram negatives only. The data emanating from this network since last quarter of 2014 has shown that *S. typhi* multidrug resistance (MDR) to ampicillin, chloramphenicol and trimethoprim –sulfamethoxazole is showing a downward trend and resistance to fluoroquinolones in *S. typhi* is increasingly reported. This finding is very promising as this provides an evidence to start using first generation simple cheap drugs. Among the Enterobacteriaceae species, *Klebsiella* and *E. coli* cause most of infections which are 100% sensitive to colistin followed by imipenem and meropenem but resistant to 3rd generation cephalosporins (80%). Most of hospital acquired infections are caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. All isolates of *Pseudomonas aeruginosa* were susceptible to colistin, followed by imipenem (85%), amikacin (80%), ciprofloxacin (80%), piperacillin-tazobactam (58%) and meropenem (50%) In *Acinetobacter baumannii* maximum susceptibility was to colistin (99%) followed by imipenem (53%) and meropenem (53%). Susceptibility to Amikacin has increased by 27% from 2014 to 2015. The network has 10 hospitals and will be expanded to total 20 hospitals and medical colleges across the country.

Antimicrobial Stewardship Program: In order to rationalize antimicrobial use in hospitals which are part of AMRSN, ICMR initiated AMSP activities in these hospitals. Treatment guidelines, infection control guidelines and AMSP Curriculum was prepared by ICMR, Chennai. Four workshops were conducted across the country to train teams from 32 hospitals across the country. More than 150 doctors, nurses and pharmacists from 30 hospitals were trained in AMSP.

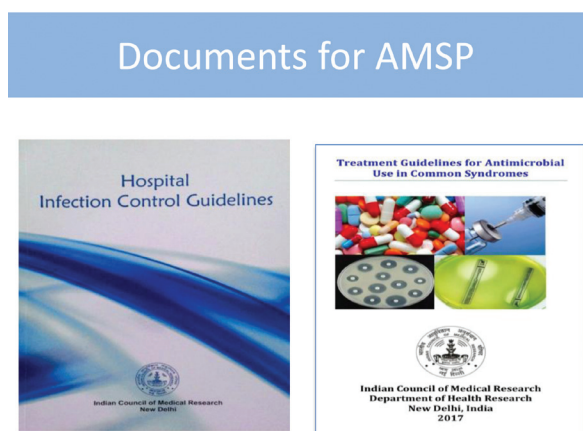


Fig. 38: Documents for AMSP.

## DIARRHOEAL DISEASES

- A multicentric study entitled, “Gastrointestinal Tract Pathogen Repository (GTPR)” was carried out by the National Institute of Cholera and Enteric Diseases, Kolkata. Gastrointestinal Tract Pathogens Repository at NICED has been working as valued source of archived entero pathogens. Working on different types of entero pathogens and limited molecular typing of the strains, required use of robust methodologies. All methodologies/activities were based on specific SOPs developed specifically for GTPR activities. All SOPs were made available in the GTPR laboratory. A total of 786 clinical *V. cholerae* strains were isolated, of which 690 for O1, 64 for O139 and 32 for non-O1, non-O139 are presented in the website. These strains were collected from clinical cases from different places of India including Kolkata. In addition, 15 strains of Diarrhoeagenic *E. coli* (enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and entero aggregative *E. coli*), and 12 strains of

*Salmonella typhi* have been archived. To comply with Global Action Plan (GAPIII) and guidelines from National Task Force (NTF) on the containment of wild Polio viruses, GTPR facility no longer holds any stool specimens as well as total nucleic acid extract. GTPR activities have now been taken up as a part of Institutional activity to increase archiving of more number of entero pathogens.

- ICMR conducted a “National Rotavirus Surveillance Network (NRSN)” study to examine long term trends and pattern of diarrhoea attributable to rotavirus among children <5years of age, seen at in-patient facilities from the different parts of India from 2012-16. The study was coordinated by National Institute of Epidemiology, Chennai. Rotavirus was detected in 36.3% (7783/21421) of children with acute gastroenteritis, enrolled in surveillance. Rotavirus infection was detected throughout the year in all sites. The highest positivity (43.1%; 2862/6644) was observed among children between 12 and 23 months of age. Among infants aged <3 months and <6 months, the proportion of rotavirus positivity was 17.0% (253/1488) and 24.7% (953/3866) respectively. Children aged 6-11 months had positivity of 40.7% (2743/6741). Rotavirus infections usually occurred more commonly during the cooler months of December - February (53.2%; 3302/6212), followed by September–November (36.2%; 1663/4600). Analysis of diarrhoeal disease severity showed that 39.4% (4998/12692) of children with severe to very severe diarrhea were rotavirus positive. Only 3.4% (734/21421) of enrolled children had a history of rotavirus vaccination, and among them 223 children (30.4%) were rotavirus positive. Analysis of overall distribution of various rotavirus genotypes showed the preponderance of G1P [8] strains (52.9%; 2139/4041) followed by G9P [4] strains (8.7%; 353/4041). Surveillance data from NRSN highlights the high rotavirus disease burden in India and emphasizes the need for continued monitoring



to reduce morbidity and mortality associated with rotavirus gastroenteritis in India. With the phased roll out of the rotavirus vaccine in the UIP, continued surveillance of rotavirus disease burden in the vaccine introducer states is critical to the impact assessment of the rotavirus vaccine.

### HIV/AIDS & STDS

- Under ICMR's Task Force study on development of a DNA subunit HIV vaccine, the bacterial and viral vaccines constructs have been revived and the cGMP batches are being produced. The animal toxicity studies are being carried out.

### LEPROSY

- In an effort to support the National Leprosy Eradication Programme, ICMR in collaboration with NJIL&OMD, Agra and Central Leprosy Division has introduced the MIP vaccine to the contacts of leprosy patient in project mode in 2 states in Gujarat and Bihar. The project was successfully launched in Gujarat in May 2017 and is ongoing. The MIP vaccination would also be compared with the rifampicin chemoprophylaxis and the combination of MIP vaccination and Rifampicin to see the impact in each group. The most effective intervention would then be taken for scale up in the entire country subsequently.
- NJIL&OMD, Agra has developed and validated the new RLEP-PCr for early detection of leprosy in field conditions. The test is currently being used at RLTRI, Raipur which is the Institute under the NLEP for leprosy detection. The ICMR has also helped build up a molecular laboratory at RLTRI, Raipur with help of NJIL&OMD, Agra for providing support in leprosy case detection to neighbouring Institutes.
- Nikusht: A real time leprosy monitoring software was developed by ICMR and has been launched successfully for use under

RNTCP. The training has been completed in 8 states and the states have started the use of the software. The training in other states would be completed soon.

### PROJECTS IN NORTH EAST REGION

The ICMR sanctions 10% of the budget allocation of ICMR to the North East Region (NER). In the Division of Epidemiology and Communicable Diseases (ECD) at ICMR, the focus of research in North East region is primarily to conduct translational research and to cater to the health problems of region for the benefit of public health. The projects from the NER in the areas of communicable diseases, after getting approved by the North East Project Review Committee (NE-PRC) are processed in the Division for funding by ICMR under the North East. The North East India shares international borders with neighbouring countries like Nepal, China, Myanmar and Bangladesh and being a gateway to South East Asia, attains a specific importance to various problems due to communicable diseases and cross border transmission. Among them are: vector borne diseases; viral diseases including dengue / DHF, JE, Chikungunya, AES, West Nile Virus, Measles, Hepatitis, HPV, HIV / STD; rickettsial diseases; acute diarrhoeal diseases; bacterial diseases, TB and some parasitic diseases (including neurocysticercosis, paragonimiasis etc.).

### ONGOING NORTH EAST STUDIES:

During 2016-17, 6 multi centric studies have been ongoing broadly in areas of Surveillance of Chikungunya virus activity; Diagnostic evaluation of radiologically confirmed neurocysticercosis patients; Efficacy & predation of indigenous larvivorous fishes against the malaria vector; Effectiveness of single dose against *Japanese encephalitis*; Drug resistance mechanisms in enterobactriacae causing neonatal sepsis; Epidemiological and virological survey of Nipah Virus. The brief details of progress of these projects are given below:

- Surveillance of Chikungunya virus activity in Assam and Meghalaya.

- Evaluation of loop mediated isothermal amplification and polymerase chain reaction in clinically suspected and radiologically confirmed neurocysticercosis patients in North East India.
- Efficacy & predation of certain indigenous larvivorous fishes against the vector(s) of malaria in and around coal mining areas of Garohills, Meghalaya.
- Effectiveness of single dose of live attenuated SA 14-14-2 vaccine against *Japanese encephalitis* in adults over a period of 3 years in two districts of Assam.
- Assessing drug resistance in enterobactiaceae causing neonatal sepsis in North East India: resistance mechanisms and transmission.
- Multi-site epidemiological and virological survey of Nipah virus: Special emphasis on North East Region of India.

## TUBERCULOSIS

- TIE-TB Project & IR/OR Project: A unique Tribal TB case finding initiative was started by ICMR and the Central TB Division. This innovative project aims to bridge the gap in last mile service delivery to underserved tribal populations. Mobile vans equipped with facilities to diagnose TB at the door-step of the community, will help find TB cases in early stages, thereby improving chances of cure and effectively cutting down the chain of transmission. 35 Mobile TB Diagnostic Vans equipped with X-Ray and Sputum Microscopy facilities are being deployed across 17 districts in the states of Chhattisgarh, Madhya Pradesh, Gujarat, Rajasthan and Jharkhand covering a population of about 176 lakhs. This will be a unique model, if successful can be implemented in the whole country.

## INDIA TB RESEARCH CONSORTIUM

ICMR established India TB research Consortium, a flagship initiative, that aims to bring together all major national and international stakeholders to

develop new tools (drug, diagnostics, and vaccines) for TB. A funding of 20CR was sanctioned by Tata Trusts and an interim Secretariat was established and activities were initiated. Landscape analysis documents were prepared and the leads were shortlisted as per their level of advancement in each thematic area and reviewed by the working groups and the international Scientific Advisory Group. The clinical trial projects for treatment of drug sensitive and drug resistant TB have been finalized. The prevention of disease trial for the vaccines (VPM1002, MIP and DAR 901) are being prepared. The proposals for Active case finding in congregate settings and Institutional settings have been finalized. The new cost effective models for Public private partnership for active notification and management were through call for proposals and would be reviewed.

- Indigenous molecular TB diagnostic: ICMR's initiative in collaboration with DBT and MOH&FW on promoting indigenous TB diagnostics undertook validation of a new cost effective molecular diagnostic TrueNat Rif for detection of MDR-TB. The Kits were found to be at par with the gene xpert an imported molecular test currently used under RNTCP. The new molecular test is cost effective and gives results faster than gene Xpert and can be used at District Microscopy centres. The test recently completed feasibility testing in 100 DMCs under RNTCP.
- In an effort to tackle the rising problem of crohn's disease, a multicentric project involving the scientist from veterinary and human side has been undertaken to see the association of MAP with the disease. The two groups are working closely in humans and animals to formulate the guidelines for detection of MAP in animals and humans.

## VECTOR BORNE DISEASES

### Malaria

- a. In view of launch of National Framework for Malaria Elimination by GOI in which malaria elimination is set to be targeted for

2030, studies demonstrating effective plan for eliminating malaria in a specified area would be encouraged and supported. A Comprehensive study is being planned in the state of Odisha, where ICMR's three institutes are working (NIMR, RMRC, Bhub. and VCRC) to bring 10 low endemic districts of Odisha to elimination level. Also, the high density districts will be targeted for reduction in API by 50%.

- b. Demonstration of malaria elimination by utilizing the strategy outlined in the National Framework for Malaria Elimination would also be attempted in Car Nicobar Island by RMRC, Port Blair.
- c. Vector Biology and bionomics studies to be supported to study impact of early biting, and outdoor transmission/ site of transmission to devise effective vector control strategies.
- d. Necessity for a third round of IRS in areas, where vectors are present during major part of the year should be determined through studies.
- e. Advantage of combo vector control strategies i.e., LLIN and IRS need to be assessed further in the light of preliminary results of a study by VCRC.
- f. The proposals to monitor resistance/ susceptibility to insecticide in malaria vectors in Uttar Pradesh, Madhya Pradesh, Jharkhand would be supported in 2016-17.

### Lymphatic Filariasis

- a. An open, 12-month study, to compare the safety, efficacy and acceptability of a triple drug regimen (Ivermectin, DEC & Alb) with a two-drug regimen (DEC & Alb) is being planned to be undertaken in Yadgir district of Karnataka in collaboration with NVBDCP. The study is being funded by Gates Foundation.
- b. Xenomonitoring is potential tool for enhanced TAS and mapping. Studies undertaking xenomonitoring through networking mode involving zonal entomological teams are proposed to be undertaken.

- c. Coverage and compliance gaps to be analyzed through operational research to bridge the gap and social scientist to be involved to improve the outcome of the studies.
- d. Studies assessing the status of unsurveyed areas using risk prediction maps and areas adjoining endemic areas.

### Lieshmaniasis

- a. RMRIMS, Patna has undertaken a demonstration project in Vaishali district towards achieving elimination of kalaazar in that district. Similar studies in other districts using the role model of Vaishali will be planned to be undertaken.
- b. Studies on asymptomatic cases, PKDL and HIV-VL co-infection are needed. Diagnostic and treatment guidelines have to be developed. Guidelines for Short course treatment for PKDL would be developed.
- c. Using IVM strategy involving environmental modification and chemical control methods towards achieving kalaazar control/ elimination.
- d. Studies to establish the role of the three recently identified sibling species of the *Ph. argentipes* complex by Zoological Survey of India South Zone of Chennai, TN, RMRIMS, Patna, Bihar and VCRC, Puducherry.
- e. Resistance in *Ph. argentipes* to DDT, malathion and pyrethroids to be monitored in Bihar, Jharkhand, West Bengal and Uttar Pradesh.

### Dengue/JE

- a. A study to examine the *Ae. albopictus* sub-group species in Kerala by NIMR Field Unit in Chennai and VCRC, Puducherry.
- b. Studies on biology and bionomics of JE vectors in Assam by RMRC, Dibrugarh and in Odisha by RMRC, Bhubaneswar.

Vector Control Research Centre, Puducherry is conducting out the a) Phase I laboratory studies



and b) Bio-ecological studies on *Aedes aegypti* population.

- ICMR in partnership with Ministry of Health and Family Welfare (MOH & FW), Directorate of National Vector Borne Disease Control Programme (NVBDCP), Drugs for Neglected Diseases Initiative (DNDi), London School of Hygiene and Tropical Medicine (LHSTM) and Bill & Mellinda Gates Foundation (BMGF) has set up the VL consortium, which aim to develop a forum for constructive discussion around the transmission dynamics of VL and brings together the scientific, logistic and practical expertise, and to define the gaps in our understanding that threaten sustained elimination to analyze the existing or new findings, develop protocols, methodologies and actions that can rapidly provide the missing information.

Two Assembly meetings have been held so far. 1<sup>st</sup> SPEAK Meeting on 3-5<sup>th</sup> November 2016 in New Delhi and 2<sup>nd</sup> SPEAK on 24-25<sup>th</sup> April, 2017 in Varanasi, UP.

### Projects in North East Region

- Prevalence of West Nile virus (WNV) and its coexistence with Japanese encephalitis virus (JEV) was recently concluded at RMRC, Dibrugarh. WNV cases were found to be 6-10% among the acute encephalitis (AES) cases. JE vaccination among the paediatric as well as adult population, cross-protection was studied, no cross protection was observed for (WNIRGT07), while 33.33% protection was elicited against the other circulating strain (NIRGC07). *Mansonia uniformis*, *Culex vishnui* and *Culex tritaeniorhynchus* were the predominant species 26.88%, 25.66% & 11.66% respectively. WNV RNA was detected in a human clinical sample and in 19 mosquito pools. Densities of preponderant WNV vectors were found to be strongly associated with different climatic variables. This is the first report of WNV vector incrimination in Eastern India.
- The epidemiology of scrub typhus (ST) and its vectors in selected areas of Assam and Arunachal Pradesh were studied. Demonstrating wide circulation, accounting for about 20% of the febrile cases with a significant aetiology of AES. Pathogenic Karp like strains were prevalent, beside scrub typhus, presence of other rickettsial diseases viz., spotted fever (SF) and typhus group rickettsiae (TGR) are also evident. Detection of dual infection of SFGR and TB in an AES presenting patient calls for concern among clinicians as well as health workers about the potentiality of rickettsial infections worsening the health system in TB endemic region like NER. Detection of *Rickettsia sengelensis* in the centre's flea collection is a maiden report from Southeast Asia.
- An exploratory study was taken up by Assam state AIDS control society in three districts of Assam (viz. Cachar, Karimganj and Nagaon) using a mixed-methods approach. The female sex workers in these districts probably were serving as the main reservoir for HIV where the infection was spread through unprotected sex. This highlighted the need of an intervention in this area, which might encompass appropriate behavioral as well women empowerment (especially economic empowerment) issues. The (outgoing) migrant labourers and truckers showed comparatively lower levels of awareness about HIV/AIDS and many of them also engaged in high-risk behaviours, indicating the requirement and scopes for future work with these groups too.
- The survival of *V. cholerae* during the interepidemic period, in the rectal swabs from diarrhoeal patient previously infected and environmental sample in tea garden in three districts of Upper Assam were evaluated. No *V. cholerae* could be isolated by culture nor amplified by PCR in the rectal swabs. *OmpW* gene specific for *V. cholerae* was successfully amplified by PCR in environmental samples but were non-culturable. The study concludes that environmental monitoring and

surveillance primarily based on detection of *V. cholerae* and virulence-associated factors in *V. cholerae* isolates from environmental sources by molecular and biological method may have role in prediction of outbreak.

- Prevalence of bacterial pathogens colonizing the genital tract of pregnant women and comparison of those causing early onset neonatal sepsis were studied. A total number of 668 pregnant women were studied. Colonization rate of Group B *Streptococci* (GBS), *Escherichia coli* and *Enterococcus* at 35-37 weeks was 15.1%, 7.8% and 33% respectively. Among those GBS colonized, 21.6% of them had a history of urinary tract infection, 20% of them had associated fever and 22.4% of them gave history of vaginal discharge. The study concluded that Group B streptococcus, *Escherichia coli* and *Enterococcus*, the pathogens known to cause neonatal sepsis in this region were found to colonise the genital tract of pregnant women with a prevalence rate of 15.1%, 7.8% and 33% respectively.
- Host innate immunity and hepatitis B persistence was studied. Found that the T-cell regulatory protein FOXP3 to be significantly upregulated in Chronic Hepatitis B with HBeAg positive status. As regulatory T-cells turns the immune response down, upregulation of FOXP3 may be playing a vital additional role in persistence of HBV infection. Documented the upregulation of the proto-oncogene 'FOS gene' in CHB with HBeAg positive status & HCC cases compared to controls. Four HBV genotype C, D, I and A have been found to be circulating. Genotype C was the most common genotype in Arunachal Pradesh, while Genotype D was the most common in Tripura and Assam. Probable escape mutant was detected in position G145A in three cases while probable drug resistance to lamivudine (V173A) was also detected. Mutation at position A128 V was common and was detected in 40% of the genotype D.

## TUBERCULOSIS

**TB Diagnostic:** Truenat which is an Indigenous molecular, POC, cost effective diagnostic test for TB/MDR-TB, will have a major public health impact as it can diagnose TB cases including DR cases at the most peripheral i.e District Microscopy centre and enabling the initiation of treatment on the same day of diagnosis thus preventing transmission of TB. Being cost effective will have additional advantage.

**Tribal TB project** on mobile diagnostic van intervention for control of TB in Tribals in 5 states is ongoing, will help in diagnosing TB cases in vulnerable population, thus enable the RNTCP to extend the intervention in other states as well.

## Viral Infections & Vaccine Preventable Diseases

- (i) Development of Multiplex Real Time RT-PCR for Dengue and Chikungunya viruses by NIV, Pune.
- (ii) Development of IgM ELISA kit for Measles by NIV, Pune.
- (iii) Setting up surveillance for diagnosis of Zika virus disease: ICMR's NIV, Pune has conducted three training programmes wherein they have trained a total of 25 laboratories (including IDSP labs) for Zika virus diagnosis. The trainings were done from 15<sup>th</sup> to 19<sup>th</sup> February 2016, 18<sup>th</sup> to 22<sup>nd</sup> October 2016 and 27<sup>th</sup> – 28<sup>th</sup> January 2017. All these labs are routinely testing for Zika virus in Dengue and Chikungunya negative patients with history of fever or travel. The results are being compiled at NIV, Pune on fortnightly basis. A total of four cases of Zika virus have been detected till now.
- (iv) Establishment of Congenital Rubella Syndrome surveillance in India at these sites: PGIMER, Chandigarh, AIIMS, Jodhpur, IGICH, Bengaluru, KEM, Pune, CMC Vellore. Primary Objective of the study is to establish a facility-based surveillance for CRS in selected medical colleges/hospitals in different parts of country to monitor the time trends of the disease. Continued surveillance in these

facilities for 5-7 years will generate data about the impact of rubella vaccination.

### TRIBAL HEALTH RESEARCH

- A multi-centric study on “Estimate the burden of TB among the tribal population and develop an innovative health system model to strengthen TB control in the tribal areas” is being carried out at National Institute of Research in Tuberculosis, Chennai which is the coordinating centre with 5 other study sites i.e, RMRC (Port Blair), PIMS (Puducherry), NIRTH (Jabalpur), RIMS (Ranchi), RMRC (Bhubaneswar) to estimate the prevalence of tuberculosis and the health seeking behaviour patterns among the tribal groups in various states of the country. This multi-centric study, using a mixed method approach in six states of India in phase I included Nicobar Island, Chhattisgarh, Madhya Pradesh, Jharkhand, Maharashtra and Odisha. The tribal districts which had >70% tribal population were randomly selected and each site was allotted clusters/villages using the PPS method. For these six sites the number of clusters was 48 with a population of approximately 800 individuals  $\geq 15$  yrs per cluster. The quantitative assessment involved interviews with all household members of  $\geq 15$  years age, identification of chest symptoms, those with a history of TB and collection of two sputum samples from them. The qualitative analysis involved in-depth interviews with MOs and other health care providers (HCP) and FGDs with tribal representatives, TB patients and their families to understand awareness of TB, health seeking behaviour patterns, gaps and barriers in utilization of TB services and challenges faced for better utilization of TB services. Among the 48 clusters, data have been received from 31 villages. Around 31339 individuals have been interviewed, of which 1148 (4%) were found chest symptomatic. Among this, 113 (9.8%) were new TB cases (361 per 100,000). Data from qualitative analysis reported challenges such as difficult geographical terrain, lack of transportation

and accessibility issues, weak health system which included non-availability of staff, poor attitude of HCPs, and dependence on traditional healers. The socio-cultural factors such as alcohol use, poor awareness on TB, financial constraints, and misconceptions on TB influenced their health seeking behaviour.

- ICMR, had established 10 THRU in two Phases; 6 THRU viz NIRTH (Jabalpur), RMRC (Bhubaneswar), RMRC (Port Blair), NIIH (Mumbai), NIN (Hyderabad) and RMRC (Dibrugarh) were funded in Phase I in 2013 and 5 THRU viz RMRC (Belagavi), DMRC (Jodhpur), VCRC (Puducherry), NIE (Chennai) and RMRIMS (Patna) were provided funding in Phase II in 2014 under the Tribal Health Research Forum (THRF). Also ICMR had established 4 field units (Rayagarch, Kalahandi, Keonjhar and Kandhamal) in tribal regions under RMRC, Bhubaneswar. Five staff positions have been provided to each of the 10 THRU to enable them to carry out specific research activities on identified diseases and health conditions.

**Overall mandate** of THRU are:

- a) Knowledge and data bank to local and State govt.
- b) Morbidity and mortality profile of tribes.
- c) Epidemiology of infectious diseases like malaria, diarrhea, and TB; determination of new control tools and changing patterns.
- d) Nutritional deficiency disorders and their control.
- e) Assessment and prevalence of NCD risk factors.
- f) Mapping of G 6 PD deficiency, sickle cell disorder and Thalassemia among ethnic groups.
- g) Studies on ethno medicine to empower Tribals.
- h) IEC intervention to improve health status.



All the above mentioned 10 THRU are functioning at the moment to provide cohesive information of the health problems of the indigenous community in the country and accordingly develop a successful model of an intervention plan.

- A multi-centric study on “Newbornscreening for sickle cell disease and providing comprehensive care in tribal population of Madhya Pradesh and Gujarat” was carried out under the coordination of NIIH, Mumbai with NIRTH (Jabalpur) and Valsad Raktdan Kendra (Valsad, Gujarat) as investigating centres. A total of 3477 newborn babies from Gujarat (n=2944) and Madhya Pradesh (n=533) were screened over 3 years using high-performance liquid chromatography (HPLC) and the diagnosis was confirmed by molecular analysis. A total of 87 SCD babies in the age group of 6 months to 6.6 years were followed-up clinically and haematologically once in every three months. In Gujarat, 2.5% sickle homozygous/sickle  $\beta$ -thalassemia and 22.0% sickle heterozygous babies were identified, while in Madhya Pradesh, 1.7% sickle

homozygous and 7.9% sickle heterozygous babies were identified. Pain, severe anemia requiring blood transfusions and fever with infections were the major complications. Among the SCD babies (87) followed-up, 13.8% of babies were presented with either severe or moderate clinical presentations, whereas 86.2% babies had a milder presentation. The presence of ameliorating factors, such as high Beta haemoglobin, Xmn-I polymorphism, and  $\alpha$ -thalassaemia did not have any impact on clinical severity. The parents of SCD babies were educated and counseled for home care. The distribution of mobile phones to these SCD families had a tremendous positive response and helped to reduce morbidity and mortality of these cases. Genetic counseling to the affected families has increased the awareness and acceptance for prenatal diagnosis.

## ZOONOSES

Around 20 Ad-hoc projects were completed during the period.

# REPRODUCTIVE HEALTH

**N**ational Institute for Research in Reproductive Health (NIRRH) and Genetic Research Centre, are situated in Mumbai, Maharashtra. These are dedicated towards addressing the various issues of reproductive health in accordance with the national needs and priorities. They are actively involved in development of clinical, operational guidelines and information, education and communication material on priority areas for use in the national programs for reproductive health.

## NATIONAL INSTITUTE FOR RESEARCH IN REPRODUCTIVE HEALTH & GENETIC RESEARCH CENTRE, MUMBAI

### INFERTILITY

#### *Female Infertility*

#### **A Genetic Analysis of Polycystic Ovary Syndrome (PCOS) with Special Emphasis on Genes involved in Insulin Resistance**

PCOS is a multifactorial endocrine disorder where multiple genes interact with each other and environmental factors. Several women are reported to present with increased prevalence of subclinical cardiovascular disease risk factors. In the reporting year, we have investigated the association of promoter polymorphisms of the *PON1* gene, which regulate the expression of paraoxonase 1 enzyme, with risk of PCOS development and its related traits. This enzyme impedes oxidative modification of LDL and favorably modulates insulin sensitivity. Of the six major polymorphisms, -907G/C polymorphism showed significant association with PCOS susceptibility in both additive and

recessive genetic models. Additionally, the -108C/T polymorphism showed association with decreased serum testosterone levels in women with PCOS. All polymorphisms were in strong linkage disequilibrium with each other and were observed to influence PON1 activity.

#### **PON1 Expression, Activity and its Relationship with Oocyte and Embryo Quality in Women with PCOS Undergoing Assisted Reproductive Technique**

Reactive oxygen species (ROS) are generated in the growing follicles due to high metabolic activity. Balance of pro- and anti-oxidants in the follicular fluid (FF) is critical to maintain oocyte quality. Paraoxonase 1 is instrumental in inhibiting the oxidation of low density lipoprotein (LDL), lipid and protein peroxidation, thereby preventing cellular damage as well as facilitating glucose uptake. Therefore, low PON1 expression and activity in FF may be associated with poor oocyte quality. The FF concentration of lipid peroxidation products, as well as activities of important antioxidant enzymes, paraoxonase, catalase and superoxide dismutase in FF, were found to be comparable in the controls and women with PCOS. Preliminary analysis indicates that granulosa cells show increased glucose uptake in the presence of PON1 enzyme; however, this needs to be investigated further.

#### **Deciphering the Putative Epigenetic Mechanisms Pertaining to Polycystic Ovary Syndrome**

Epigenetic alterations such as DNA methylation and histone modifications play a vital role in altering gene expression levels and chromatin assembly in response to environmental determinants in physiological and pathological conditions. Global

DNA methylation analysis using ELISA to measure 5'-methyl cytosine content and bisulfite-PCR of LINE1 elements is an effective predictor of the overall epigenetic state of different cell-types or tissues in a study cohort. The study investigated these changes in circulating leukocytes and ovarian granulosa cells of controls and women with PCOS. Global methylation levels in both these cell types remained unaltered between controls and PCOS, however a single hypomethylated CpG dinucleotide (CpG -4) in LINE1 elements was found to be strongly associated with PCOS and its hormonal traits measured in serum and follicular fluid. Tissue-specific comparison between leukocytes and granulosa cells showed that the latter are relatively hypomethylated, irrespective of normal or disease-states, indicating that such hypomethylation is required to drive the expression of tissue specific genes to govern complex and dynamic ovarian functions. Further, the comparison of gene expression levels of DNA methyltransferases (DNMTs) that are responsible for de novo and maintenance methylation showed that DNMT3A levels are altered in granulosa cells of PCOS women.

### Understanding Follicular Angiogenesis in Women with Polycystic Ovary Syndrome (PCOS)

The follicular growth in PCOS is arrested at preantral stage leading to anovulatory infertility in women. Studies have implicated altered angiogenesis in PCOS, which may be one of the factors responsible for follicle growth arrest and corpus luteum insufficiency. However, mechanism of dysregulated folliculogenesis and vascular formation in PCOS is not clearly known. In the current study, expression of angiogenic factors in follicular fluid and granulosa cells obtained from women with PCOS and healthy controls undergoing IVF, were studied. The levels of vascular endothelial growth factor A (VEGFA) in follicular fluid and serum of PCOS women were found to be higher than in non - PCOS women. In the transcriptome study of granulosa cells, several angiogenic factors such as VEGFA, fibronectin and insulin-like growth factor binding protein 4 were also dysregulated. This indicates that angiogenesis

in follicles of PCOS women may be altered and this may contribute to pathophysiology of PCOS.

### Implementation of Multidisciplinary Model of Care for Women with Polycystic Ovary Syndrome (PCOS): Developing a Cohort of Adolescent and Infertile Women for Research in PCOS at NIRRH.

A multidisciplinary model of care to address most concerns of women with PCOS has been established at the institute with a team of IVF specialist, endocrinologist, dermatologist, dietician and yoga expert. This is a unique platform wherein research and services meet and is first of its kind in the government research Institute in India. Cohorts of adolescent and Infertile women with PCOS will be studied and followed at the clinic. Physical, hormonal, biochemical, ultrasound and emotional health parameters of the women with PCOS will be studied over a period of time. The clinic was inaugurated by Honourable DG ICMR, Dr Soumya Swaminathan on 30th April 2016. More than 300 women with PCOS are currently being managed at the clinic.



Fig. 1. Inauguration of NIRRH Multidisciplinary PCOS Clinic.

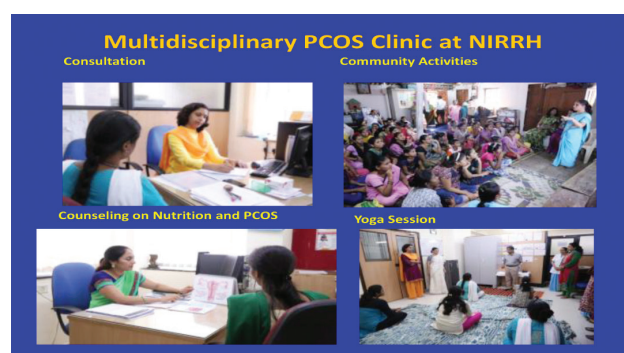


Fig. 2. Multidisciplinary PCOS Clinic at NIRRH.



### Autoimmune Markers for Early Diagnosis of Endometriosis

The mean serum levels of anti-endometrial antibodies of SLP2a, SLP2b, SLP2c, TMOD3a, TMOD3b, TMOD3c, TMOD3d, TPM3a, TPM3b, TPM3c and TPM3d) in early stages (Stage I-II) were significantly higher than that of advanced stages (Stage III-IV) of endometriosis ( $p < 0.05$ ). The sensitivity and diagnostic accuracy of all these biomarkers were higher in detection of Stage I-II endometriosis than in the detection of Stage III-IV endometriosis at an acceptable specificity.

### Role of Homeobox Gene HOXA10 in Endometrial Decidualization and in Pathogenesis of Endometriosis

The aim of this study is to understand the functions of HOXA10 gene in endometrial physiology and pathogenesis of endometriosis. Towards this, study team had generated transgenic mice that overexpress a shRNA against HOXA10. Presently, study team has identified about five founder lines which are being characterized for their copy number and transgene expression. In one of the founder lines, study team has observed that loss of HOXA10 causes endometrial hyperplasia and endometritis. It was observed that in mice with reduced HOXA10, there is increased proliferation of the epithelial cells and extensive splenomegaly. These observations underscore the importance of endometrial HOXA10 in both these disorders.

### Development of an Animal Model for Endometriosis and Identification of Novel Biomarkers

Study developed a mouse model of endometriosis and reported that lesions can be sustained in this model for a very long time. At the molecular level, study team showed lesions mimic several features of human endometriosis. Study used plasma from these animals and evaluated the proteome profiles using 2D gel electrophoresis, ITRAQ and label free LC MS/MS. Preliminary results showed altered levels of several proteins in the plasma of endometriosis mouse. One such protein was

fibrinogen, which was validated further, where study observed hypofibrinogenemia during early endometriosis.

### The Factors of Relevance in Endometrial Adhesiveness to Embryonic Cells

Optimal abundance of specific adhesion molecules on the surface of endometrial and embryonic cells is extremely critical for the initiation of pregnancy. While sizeable data are available on quantitative changes in specific Cell Adhesion Molecules (CAMs) at transcriptional and post-transcriptional levels in the endometrium before and during implantation; mechanisms involved in the distribution of these CAMs to the endometrial cell surface are yet to be decoded. Intracellular trafficking is one of the major pathways that govern the localization of specific adhesion molecules to the cell surface. Rab GTPases are the master regulators of the intracellular trafficking machinery. Rab11A, one of the Rab GTPases, is known to be involved in recycling and exocytosis. Studies conducted during the reporting year, demonstrated a decrease in the expression of Integrin  $\alpha V$  in the cell surface protein enriched fractions in Rab11A knockdown clones. A significant decrease in the surface localization of functional Integrin  $\alpha V\beta 3$  heterodimer in Rab11A knockdown cell clones was also observed. These observations indicate the role of Rab11A in the trafficking of integrin  $\alpha V\beta 3$  to the surface in endometrial epithelial cell line.

### Effect of Metformin on Hormonal, Metabolic and Endometrial Profiles in Obese Bonnet Monkeys (*Macaca Radiata*)

Metformin, an anti-diabetic agent, is also used in the treatment of reproductive disorders such as Polycystic Ovary Syndrome (PCOS). There exists data to suggest that metformin treatment leads to enhanced uterine vascularity and blood flow in PCOS; however it is not known whether metformin has any direct effect on endometrium. In the previous year, study reported significantly higher cell surface localization of integrin  $\alpha V\beta 3$ , an important marker of endometrial receptivity, in metformin treated Ishikawa cells. Studies conducted during the year, demonstrated higher

synthesis of integrin  $\alpha v$  and sirtuin 1 in Ishikawa cells, on treatment with metformin. In brief, these studies indicate that endometrium is another site of action for metformin.

## MALE INFERTILITY

### Understanding the Molecular Mechanisms of Gonadal Development in the Mouse

The aim of this study is to understand how the testis and ovaries are formed developmentally and what are the genetic players involved in this process. Lhx2 is a Lim homeobox protein that is essential for tissue specification. Centre's earlier studies had shown that Lhx2 is expressed in the mouse gonads, but its role is largely unknown. To understand the role of Lhx2, study performed RNAseq of RNA from developing gonads and identified large numbers of genes to be altered with several genes having sexually dimorphic expression. Study also identified genes whose expression was unique to either specific to XX or XY gonads. Further studies are ongoing to have a better understanding of the mechanisms involved in testicular and ovarian specification and how these might be regulated by Lhx2.

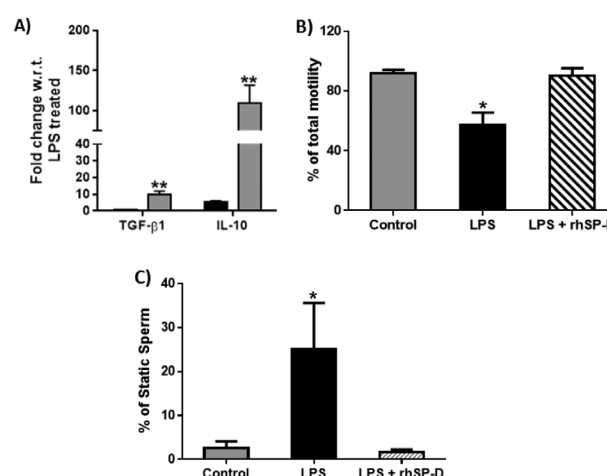
### A Study on Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene Screening and its Association with Congenital Bilateral Absence of Vas Deferens (CBAVD)

The polymorphic regions of IVS9-c.1210-12T [5] and M470V were amplified using specific primers followed by Sanger's DNA sequencing in Indian CBAVD men. A statistically significant increase in the frequency of heterozygous IVS9-c.1210-12T (39.4%) was observed in CBAVD men as compared to controls (14%). The allelic distribution of c.1210-12T, c.1210-12T and c.1210-12T in CBAVD men was 21%, 64.4% and 13 % and that in healthy controls were 7%, 73% and 20% respectively. Longest TG-repeat c.1210-34TG was found in association with c.1210-12T with an allelic frequency of 5.9% in CBAVD men. Study found a significant association of c.1210-34TG /c.1210-34TG - c.1210-12-V470 allele in CBAVD men.

Twelve female partners harboured a heterozygous c.1210-12T allele. The study emphasizes the need to screen both partners for the polymorphisms M470V, poly T, TG tract repeats in addition to population specific known *CFTR* gene mutations.

### Deciphering the Roles of Collectins (SP-A, SP-D & MBL) in Testicular Immunoregulation

The study highlighted the involvement of SP-D in maintenance of testicular immune privilege and its indirect contribution to male fertility. SP-D<sup>-/-</sup> mice sperm showed decreased vigour parameters, LPS challenged SP-D<sup>-/-</sup> mice showed a ~4 fold decrease of caudal sperm count, degree of protein tyrosine phosphorylation was significantly low in the sperm preparation from SP-D<sup>-/-</sup> mice, Exogenous supplementation with rhSP-D, salvaged the WT mice from LPS-induced pro-inflammatory immune response and impairment of sperm motility by upregulating the levels of TGF- $\beta$ 1 and IL-10. As an extension of this project, a new proposal entitled, "Deciphering the role of Surfactant Protein D in protecting male fertility during infection and inflammation" has been initiated.



**Fig. 3:** Protective effect of exogenous rhSP-D on LPS induced inflammation in testis and sperm motility. High dose LPS challenged WT mice were treated with exogenous rhSP-D for 24 h and the levels of intra-testicular cytokines were assayed by real time RT-PCR, while the sperm motility was assessed by CASA. (A) Comparing the levels of cytokines in rhSP-D treated LPS challenged mice to that of LPS alone challenged mice showed a predominance of anti-inflammatory milieu in rhSP-D treated group. (B) Significant decrease in the % of total sperm motility and (C) increased % of static sperm in LPS challenged mice compared to the saline control group.

Treatment with rhSP-D restored the total motility of sperm and reduced the percentage of static sperm that was hampered by LPS challenge to control levels.

### **In vivo Study Deciphering the Role of HDAC6 in Spermatogenesis**

It was previously documented the role of HDAC6 as a tubulin deacetylase in sperm with experimental evidences for its role in sperm motility. While exploring the specific tubulin acetyltransferase (TAT) in sperm, it was observed that there was presence of Chromodomain Y-Like (CDYL), on the principal piece of rat spermatozoa. Presence of CDYL on rat sperm flagella and its colocalization with  $\alpha$ -tubulin suggests the role of CDYL beyond spermatogenesis. Occurrence of CDYL in sperm axonemal fraction supports the hypothesis of a putative CDYL-tubulin interaction. *In silico* analysis, biophysical analysis (Microscale Thermophoresis) and biochemical analysis (*in vitro* assay) together validate the CDYL-tubulin interaction and its ability to acetylate  $\alpha$ -tubulin thus revealing its new function as a tubulin acetyltransferase in sperm. Study has overexpressed rat *hdac6*, experimentally validated the sequence and performed its structural and functional annotation bioinformatically. Study also demonstrates that the cloned *hdac6* is bioactive.

### **Studies to Elucidate the Molecular Mechanism of Estrogen Action in Spermatogenesis**

Estrogen, through its receptors (ER)  $\alpha$  and  $\beta$ , regulates different aspects of spermatogenesis thereby affecting fertility. Using selective receptor specific agonist, estrogen action through epigenetic mechanism was studied. The results demonstrated that estrogen signalling through its two receptors regulates different spermatogenic processes and epigenetic mechanisms. ER $\beta$  regulates spermatocyte survival, sperm release and DNA methylation, while ER $\alpha$  mediates histone modification and chromatin remodelling. The epigenetic defects observed in sperm, could affect the subsequent embryogenesis and contribute to the decreased male fertility after these treatments. In addition, it was demonstrated that process of sperm release was regulated by both estrogen

and androgen. Estrogen through its beta receptor, regulated genes involved in actin remodelling whereas androgen regulated genes involved in endocytosis, the two mechanisms important for sperm release.

### **RTI/ STIS/HIV/ MICROBICIDES**

#### **Deep Sequencing based evolution and diversity analysis of HIV-1 clade C**

Using a combination of next generation and sanger sequencing methods, primary (uncultured circulating and archival) sequence data from approximately 50 HIV-1C infected individuals encompassing near full length genome coverage have been generated. This data is expected to provide critically needed information including:

- Broadly Neutralizing Antibody Epitope characterization to enable design of next generation HIV-1 clade C vaccine candidates.
- Analysis of drug resistant mutations associated with current anti-retroviral therapy (ART) regimens in co-evolution and prediction of acquired resistance enabling optimal management of ART.
- Comparative analysis of selective pressures operating on pathogenic determinants of HIV-1 clade C in geographically disparate areas such as South Africa and India.

#### **Dysregulated Cellular Immunity in Immune Reconstitution and Disease Progression in HIV Infection**

Analysis of homeostatic markers (IL-2 receptor and IL-7 receptor) expressed on CD4 T cell subsets in HIV-1 clade C and HIV-2 (described for the first time) infected individuals from a tertiary hospital setting in Mumbai has resulted in:

- a. Identification of homeostatic dysfunction of IL-2 (CD25) and IL-7 (CD127) receptor expression on CD4+T cell populations including Treg, memory/naive and effector subsets in both HIV-1 and HIV-2 infected individuals.
- b. This dysregulation was found to be associated with HIV disease progression and provided an immune



signature (circulating CD4<sup>+</sup> Treg frequency) that correlated with absolute CD4<sup>+</sup> count in ART naive conditions, highlighting its utility as a prognostic marker for disease progression.

c. HIV induced immune dysfunction of CD4 subsets (Treg and Memory/Naïve populations) remained unchanged even after prolonged ART, suggesting the need for immunomodulatory therapy as a supplementary modality.

### Association of Host Immunogenetic Factors with HIV Infection

This completed study on HIV discordant couples and mother child pairs has highlighted possible association of specific HLA alleles with infection acquisition (HLA-A\*01, HLA-B\*14;\*35;\*39 and DRB1\*08) or with protection (HLA-B\*40, B\*18 and DRB1\*04 and DRB3\*02).

Cytokine gene analysis in discordant couples have shown significant association of IL1RA mspA 11100 CC, IL4 -33 TT and TNF- $\alpha$  -238 AG with HIV infection. Among the children, CC genotype at IL1R1 (rs2234650) and GG genotype at TNFA (rs1800629) acted as a protective factor.

A significant increase was observed in KIR 3DS1 and 2DS5 activating gene frequency in exposed uninfected individuals/infants. KIR 2DS1 was associated significantly with low viral load while a 22 bp deletion in the exon 5 of KIR 2DS4 gene was associated with high viral load. Among the total of 199 individuals tested, 118 KIR genotypes were identified. Thirty nine genotypes were identified for the first time in Indian population. Twenty nine genotypes were identified as unique/ novel, not reported earlier in any population of the World.

### Characterization of Antimicrobial Peptide (AMP) Isolated from the Indian mud crab, *Scylla serrata*

Autophagy is known to be involved in host defense against invading pathogens. Hence, the role of autophagy was evaluated during *in-vitro* *C. albicans* infection in vaginal epithelial cells (VECs). Study observed a significant increase in transcript levels of autophagy marker LC3 and lysosomal marker LAMP1 in VECs infected with *C. albicans*.

### Characterization of AMPs Isolated from Rabbit Vaginal Fluid and their Role in Vaginal Innate Immunity

An anti-HIV peptide, HbAHP-25 has been designed against CD4 binding domain of gp120 of HIV. The *in-vitro* toxicity studies of this peptide revealed that it did not affect various strains of vaginal lactobacilli and also did not disrupt monolayer integrity of HEC-1A cells.

Hemoglobin (Hb) is a major protein involved in transport of oxygen (O<sub>2</sub>), and normally expressed by cells of erythroid lineage. However, till recently, it was not known whether non-erythroid cells like vaginal epithelial cell (VEC) synthesize Hb and whether it has any functional significance. Study determined the molecular mechanism regulating the expression of Hb- $\alpha$  and Hb- $\beta$  in hPVECs. The results revealed the expression of Hb- $\alpha$  and Hb- $\beta$  at both mRNA and protein level in hPVECs. The expression was significantly upregulated following LPS treatment. The results of EMSA and ChIP revealed for the first time the presence of putative binding sites of NF- $\kappa$ B in the human Hb- $\alpha$  promoter.

### Studies on the Modulation of Vaginal Immunity during Host-pathogen Interactions in Response to Microbicide

MicroRNA, let-7f is a key regulator of immunity in immortalized endocervical epithelial cells (End1/E6E7). The transcription factors involved in regulation of let-7f expression are not known. The upstream sequence of let-7f was analyzed for transcription factor binding sites. C/EBP- $\beta$  was found to have six binding sites in this region. Knock down and over expression of C/EBP- $\beta$  affected the expression of let-7f. Poly (I:C) treatment increased the binding of C/EBP- $\beta$  at three of the six identified binding sites.

### Approaches for Controlling Biofilm Formation by *Gardnerella vaginalis*

Effective control of polymicrobial biofilm formation is thought to be important for curtailing the recurrence of bacterial vaginosis (BV), a

common female genital tract infection. Towards this, study aimed to develop anti-biofilm agents and identified two peptides, 1018 and 1037, which exhibit dose dependent inhibition of biofilms produced by *Gardnerella vaginalis*, the major pathogen, associated with BV. Combination studies with the commonly prescribed antibiotic, metronidazole suggest that these peptides do not display a synergistic effect on anti-biofilm activity. Further testing revealed that peptide 1037 shows a better *in vitro* safety profile compared to peptide 1018 on account of the absence of cytotoxicity against both commensal lactobacilli and human vaginal epithelial cells. Thus, peptide 1037 could be a good candidate for development as an anti-biofilm agent.

#### Studies on HIV-1 gp120 Mediated $\alpha_4\beta_7$ Integrin Dependent Signaling in T Cells and its Role in HIV-1 Pathogenesis

Integrin  $\alpha_4\beta_7$ , a receptor for HIV gp120 has been implicated in promoting mucosal transmission of HIV. Recent literature suggests that antibody mediated blocking of integrin  $\alpha_4\beta_7$  could be a potential strategy for controlling HIV infection. The main objective of the project is to attain a mechanistic understanding of the role of HIV-1 gp120 mediated integrin  $\alpha_4\beta_7$  signaling in HIV infection. Towards this, phosphoproteomics analysis of HIV gp120 treated  $\alpha_4\beta_7^hi$  CD4+ T cells was carried out. In all phosphorylation sites, mapping to 953 proteins were identified, of which 184 (19.5%) were responsive to gp120. Amongst the proteins identified, were those involved in pathways such as leukocyte transendothelial migration, regulation of actin cytoskeleton etc. Validation of these candidate proteins is expected to yield interesting targets for controlling HIV infection.

#### Development of a Multi-strain Probiotic Formulation Effective Against Reproductive Tract Infections

Lactobacilli are important component of vaginal microenvironment, preventing the outgrowth of undesirable pathogens. Hence, it is necessary to

characterize the vaginal lactobacilli from Indian women. The study was undertaken to characterise vaginal lactobacilli, and urogenital *Candida*. *Candida* was isolated in 30.73% women with 71.4% showing prevalence of Non-albicans *Candida* (NAC). Virulence was indicated by their Phospholipase activity and biofilm formation in 31.7% and 7.3% respectively. Virulent strains of *C.albicans* and *C.glabrata* were used to study the antagonistic effect of *Lactobacillus* isolates.

High throughput screening of 327 *Lactobacillus* isolates was carried out to determine their growth inhibitory effects on *C.albicans*, *G.vaginalis*, *N.gonorrhoea* and Group B *Streptococci*. Majority of the *Lactobacillus* strains exerted 100% inhibition on pathogens. Auto-aggregation and co-aggregation by lactobacilli form vaginal barrier and prevent pathogens colonization. Around 80% of isolates could aggregate *C.albicans* and most isolates showed high auto-aggregation.

Based on these properties, 38 strains were tested for their adherence to VK2/E6E7 cells *in-vitro*. Of the strains evaluated, most of them showed high level of adherence (70-95%).

#### Implementing Linked Services between HIV and Family Planning at District level in Maharashtra to Improve Dual Method Use among HIV positive people

Based on the experiences gained in linking HIV and FP services at tertiary care public hospitals in Mumbai, this NACO supported study is being implemented at two district hospitals in Pune and Parbhani districts of Maharashtra. Training providers developing IEC material, assessing unmet need for contraception, providing information on use of dual methods and facilitating linkage strategies were key interventions apart from maintaining Management Information System (MIS). All eligible HIV positive couples attending ART Clinics, were given a comprehensive counseling on use of dual methods of contraception and referred to Family Planning services, where their medical eligibility to use a specific contraceptive method was assessed and suitable method offered.

## MENOPAUSE AND OSTEOPOROSIS

Osteoporosis is a major health problem worldwide and need for simple approaches for its diagnosis and management has markedly grown. Imaging technique and biomarkers play an important role in the assessment of bone health.

### Elucidating the Differences in Monocyte Proteome from Pre and Postmenopausal Women with Varying Bone Mass

While elucidating the differences in the proteome of monocyte (MO), it has been observed that phosphorylated heat shock protein 27 (pHSP27) is upregulated in MO in low BMD conditions in pre- and postmenopausal women. In order to see the utility of pHSP27 levels in sera as marker of low bone mass, study examined the association of serum pHSP27 with BMD and compared its predictive ability with C-telopeptide fragments of collagen type I or CTX-1, a standard marker of bone resorption. The pHSP27 and CTX-1 levels were significantly elevated in low BMD groups in both groups of women (pre and post-menopausal women). Further, it was found that pHSP27 and CTX-1 exhibited significant odds ratios to predict low BMD condition. However, the odds ratio exhibited by pHSP27 was higher than CTX-1. These observations show that pHSP27 may be a potential marker for diagnosis of osteoporosis/osteopenia in women; however, its usefulness needs to be addressed through larger studies.

### A Study of Links between Osteoporosis and Cardiovascular Status in Women

Many epidemiological studies, mostly on Western populations, support a link between skeletal health and cardiovascular status as they share many common risk factors. The abnormal lipid parameters known to have adverse effects on cardiovascular system; also have adverse effects on skeletal system. Since, consumption of tobacco and alcohol is high in tribal populations of Northeast India; a study has been initiated to see association between tobacco and alcohol consumption and lipid parameters and Bone Mineral Density (BMD). Lipid parameters like Low Density Lipoprotein

(LDL), Triglycerides (TG), Total Cholesterol (TC) and High Density Lipoprotein (HDL) correlated significantly with BMD at spine and femur. In multiple regression analysis of the data, age, BMI, HDL and LDL contributed significantly, explaining 44.3 % ( $R^2=0.443$ ) the variation in BMD at spine whereas age, BMI & LDL contribute significantly, explaining 35.3% variation in BMD at femur. Prevalence of low BMD was significantly higher in alcohol and tobacco consumers.

### Identification of Estrogen-Regulated Proteins in the Proteome of Osteoblast and Osteoclast Differentiation

Estrogen ( $E_2$ ) exerts its action on osteoclast, the bone resorbing cells to maintain bone mass. Unravelling mechanism of its action may lead to identification of novel therapeutic targets for better management of bone diseases. Centre therefore, studied the changes in the proteomes of osteoclast during different stages of differentiation in response to Estradiol ( $E_2$ ) using 8plex-iTRAQ. Study observed upregulation of Lyn, a member of Src family tyrosine kinase, in response to  $E_2$  during commitment stage of osteoclast differentiation. Lyn is reported to interfere with RANK-RANKL signalling, which is indispensable for osteoclastogenesis. Further, we observed estrogen significantly decreases Lyn phosphorylation at Y507, thereby releasing Lyn from its inactive conformation. The study thus proposes a novel mechanism by which estrogen negatively regulates osteoclastogenesis by triggering overexpression and increased activation of Lyn leading to decreased osteoclastogenesis.

## MATERNAL AND CHILD HEALTH

### Review of Traditional Childbirth Practices Among Various Tribal Communities in Maharashtra

Review of childbirth practices among tribal women of Maharashtra showed low utilization of MCH services due to strong traditional beliefs. Common practice was to take less food during delivery to ensure small baby and easy delivery, which contributed to deliveries of premature and



undernourished babies. No colostrum feeding among few tribal women, may also influence the child health. Overall, strong traditional beliefs are barriers to the MCH services.

### **To Assess Magnitude and Factors Associated with Vitamin D Deficiency in Children between 1 to 5 Years**

The study aims to assess magnitude and factors responsible for Vitamin D deficiency in 1-5 year age group. The study was initiated in October 2015 in the slum strata of the community surrounding our Family Welfare Clinic at Abhyudaya Nagar, Kalachowki. Meetings were organized with Anganwadi workers, from whom the list of 1-5 year children was obtained for the study, which was followed by household visits. Healthy children in 1 to 5 years age group were included in the study and children with chronic illness, skeletal diseases, receiving anticonvulsants, steroids, anti-tubercular drugs and vitamin D supplementation were excluded from the study.

After the screening, clinical examination and blood investigations of the enrolled children were carried out at the clinic. During household visits, information on Socio Demographic profile, Physical activity profile (Sun Exposure, time spent outdoor) and Dietary Profile was collected. Total 334 children were screened and 194 children were enrolled in the study, till March 2017. Study is ongoing and standard level of care in form of treatment (as per US endocrinology society guidelines) and health education is being provided to children with deficient 25(OH)D levels.

Preliminary analysis of 101 children showed high prevalence of Vitamin D deficiency among 1-5yrs (82.4%). 92.3% deficient children were having normal growth standards and 53.5% children with vitamin D deficiency did not have any clinical signs. Relatively, higher incidence of recurrent respiratory tract infections (65.3%) was seen among children with vitamin D deficiency.

### **Improving Health and Nutritional Status of Vulnerable Segment of Population by Implementing Multi-component Health**

### **and Nutrition Education Intervention as a Sustainable Model of Intervention**

NIRRH is one of the participating centres in this ICMR task force study which is funded by DHR. This Pre and post intervention study is being conducted in two blocks, Dahanu and Palghar, in Palghar district of Maharashtra. Total number of participants included in the baseline survey: 240 each of pregnant women and adolescent girls and 480 children of age under-five. Baseline survey was completed in August 2016. Data entry and analysis is ongoing. Intervention phase is initiated in the end of September 2016 as per the intervention plan provided by ICMR. Intervention phase will be for 18 months duration, after which post intervention survey will be done.

### **GENETIC RESEARCH CENTRE**

#### **Identification of Genetic and Biochemical determinants of Autism Spectrum Disorders (Funded by Indian Council of Medical Research)**

The number of children diagnosed with Autism Spectrum Disorder (ASD) has substantially increased over the last decade. It has been estimated that 2 million such cases are prevalent in India. Therefore, this research study is planned to identify the genetic causes specially Copy Number Variations (CNVs) of ASDs using microarray technology.

One of the major pathways that are affected in Autism is the synaptic plasticity. Therefore, we will carry out the whole exome sequencing to identify any novel mutation/variants in genes responsible for synaptic plasticity. Strong indications of different causative factors are not available for Indian cases of ASDs.

Till date, we have recruited 42 study participants with primary autism. Karyotyping carried out for all the participants showed no abnormalities. Lymphoblastoid cell lines have been generated for all the participants.

Centre has carried out whole exome sequencing of 4 families (trio samples). The entire exons and exon-intron boundaries have been amplified and sequenced. A total of 1354 various single

nucleotide variants have been identified. However, pathogenicity of these mutations is yet to be determined. Online tools like Mutation taster, SHIFT, Polyphen have been used for determining pathogenicity of these mutations. Mutations identified in the genes such as NRXN, GABRB3, SHANK3 are implicated in causation of autism.

### Functional study of voltage-gated calcium channel gene mutations in Schizophrenia using induced Pluripotent Stem Cells (iPSCs): A new approach for developing a cellular model

The objective of the study is to identify mutations in voltage-gated calcium channel genes that are implicated in the etiology of schizophrenia and subsequently, developing induced pluripotent stem cell (iPSCs) cellular model to study functional effect of these mutations. This would be a first attempt from India to generate cellular model using iPSCs.

Fifteen participants, including 7 familial cases of adolescence onset Schizophrenia have been recruited. Whole exome sequencing has been carried out in 3 familial cases of Schizophrenia. A total of 980 single nucleotide variants have been identified. The 80% of the mutations are single nucleotide variants, rest 20% are of small deletion and duplication. The pathogenicity of the identified variants is being investigated using *in-silico* approach.

For derivation of iPSCs, fibroblasts have been isolated from skin biopsy of the control sample. Fibroblasts were transduced with Sendai virus containing OCT4, SOX2, KLF4 genes. The transduced cells were grown in fibroblast feeder cells for derivation of iPSCs. The iPSCs colonies were picked-up and grown in feeder free condition. Characterization of these colonies will be further characterized for expression of pluripotency markers.

## STRUCTURAL BIOLOGY AND BIOINFORMATICS

### Role of Specific Residues in the Follicle Stimulating Hormone Receptor in its Function

The interaction of follicle stimulating hormone with its cognate GPCR, the follicle stimulating

hormone receptor (FSHR), triggers events essential for folliculogenesis and spermatogenesis. Thus, delineation of the epitopes involved in FSH-FSHR interaction is important. Investigation of the naturally occurring mutations in FSHR is also essential as they are associated with pathophysiological conditions. Using site directed mutagenesis as a tool, functional characterization of two such naturally occurring mutations in FSHR was carried out to establish a genotype-phenotype correlation. The V<sup>221</sup>G mutation in the woman with primary amenorrhea was due to the diminished receptor function in terms of FSH binding and cAMP signaling. The T<sup>449</sup>N mutation identified by study team in a patient, presenting symptoms of spontaneous ovarian hyperstimulation syndrome, was due to an enhancement in the FSHR function.

### Purification and Characterization of CRISP-3, a PSP94-binding Protein, from Human Seminal Plasma

Prostate secretory protein of 94 amino acids (PSP94) is secreted from the epithelial cells of the prostate and plays a functional role in seminal plasma and sperm. To understand the biological role of PSP94, centre carried out studies to identify its putative binding proteins in semen and on sperm. During the purification, PSP94 was found to be present in some RP-HPLC fractions, in addition to the one containing PSP94. Hence, efforts were made to identify and isolate PSP94 binding protein/s from these fractions as well. Cysteine rich secretory protein-3 (CRISP-3) was identified to be one such interacting protein of PSP94. Earlier studies from centre's laboratory have demonstrated the ability of CRISP-3 from human seminal plasma to bind to PSP94. In this direction, affinity chromatography was further used to purify CRISP-3 from the RP-HPLC fraction; wherein centre obtained a yield of ~1 mg of CRISP-3 from 100 mL of human seminal plasma with 90% purity. The purity and molecular mass of this preparation was authenticated using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis. A homogenous preparation of CRISP-3, without CRISP-1/CRISP-2 (other CRISPs which are known to be present in the semen and on sperm)

contamination was obtained and the binding kinetics of affinity purified CRISP-3 with PSP94 was validated using surface plasmon resonance.

### *Callithrix jacchus* FSH: Production of Recombinant Protein and Understanding the Gene Regulation

Follicle stimulating hormone (FSH) is essential for mammalian gametogenesis. Common marmoset (*Callithrix jacchus*) is a New World primate monkey which exhibits certain FSH-related features which are different than in other primates. These features indicate that marmoset FSH is different than other primate FSH including human FSH in terms of structure-function and synthesis. The present study aimed at understanding these differences. Centre previously reported synthesis of recombinant marmoset FSH and development of anti-marmoset FSH antibody. During the reporting period, the promoter of marmoset and human FSH $\beta$  were compared using *in silico* tools and *in vitro* reporter assay. An alternate distal promoter, identified in marmoset FSH $\beta$  gene, was absent in the corresponding region of human FSH $\beta$  gene. The alternate distal promoter of marmoset FSH $\beta$  was found to be functional. This indicated a differential regulatory mechanism of marmoset FSH $\beta$  gene expression.

### Developing an Online Server for Identification of Drug Targets for Infectious Diseases

A unified, web-based application for use of concepts and databases developed for drug target identification is lacking. To address this lacuna, study has developed an online webserver called PBIT (Pipeline Builder for Identification of Targets). The proven concepts for target identification have been coded for high-throughput human drug target prediction, in a user-friendly pipeline format. Users can effortlessly integrate the modules available in PBIT to customise their protocol for target identification (Fig. 2). The tool has been validated by analysing the experimentally proven targets currently identified for *Candida albicans*. PBIT can be freely accessed online at [www.pbit.bicnirrh.res.in](http://www.pbit.bicnirrh.res.in)

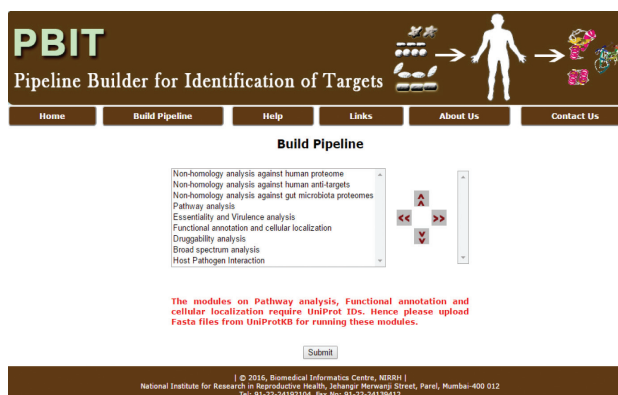


Fig.4: Snapshot of the Build Pipeline page of PBIT server.

### Developing a Gene Based Database on Infertility

A manually curated database with genes associated with reproductive conditions, leading to infertility, is being developed. The database would integrate gene-related information, literature references and links to other external databases like KEGG, Gene Ontology, OMIM databases etc. A manually curated list of 2200 unique genes associated with infertility in humans has been compiled. Database interface design and data analysis is ongoing. This repository would prove beneficial to researchers interested in understanding the genetic aetiology of the different reproductive disorders leading to infertility.

### Multiple Genes Involved in Controlled Ovarian Hyperstimulation: Applications in Predicting Prognostic Biomarkers

The management of ovarian stimulation has been difficult due to the high variability in clinical outcome among women undergoing follicle stimulating hormone (FSH) stimulation during IVF. More than 1500 genes have been reported to be involved in FSH signaling network. Series of genetic markers have been reported to be involved in controlled ovarian hyperstimulation (COH). Although, FSHR SNPs, are the most promising genetic markers available to date, several other promising genetic markers are being identified. Presently, a knowledge base of these genes, associated with FSH signaling pathways and the key genes associated with ovarian response is being developed. Data mining is in progress. The information organized in the database could be used further to improve the understanding of the ovarian response during controlled ovarian



hyperstimulation. This database will aid to select genes which need to be screened as prognostic markers for predicting ovarian response during IVF.

### STEM CELL BIOLOGY

A novel population of pluripotent stem cells, termed very small embryonic-like stem cells (VSELs), has been characterized in mouse bone marrow and reproductive tissues (ovary, testis and uterus). Study work shows that VSELs express pluripotent markers and exhibit the ability to differentiate into 3 germ layers – thereby, satisfying criteria to be classified as pluripotent stem cells in adult tissues. They also differentiate into germ cells and hematopoietic stem cells (HSCs) *in vitro*. Thus, the true regenerative potential in the bone marrow lies with VSELs, whereas HSCs are committed stem cells that can only reconstitute the bone marrow. Work done in the reporting year also shows that VSELs survive oncotherapy in the mice gonads and restored gonadal function, when healthy niche cells (Sertoli or bone marrow derived mesenchymal cells) were transplanted directly into the chemoablated gonads. This work has translational potential, as it will allow restoring gonadal function early in life of pediatric survivors and also allow normal hormonal support for proper growth and development. VSELs have also been detected in adult mouse uterine perimetrium for the first time by centre and evidence generated to show that they differentiate into myometrial cells and based on published work in humans, VSELs are also the possible tumor initiating cells leading to the development of leiomyomas. FSH receptors expression on stem/progenitors in bone marrow, testis, ovary and uterus suggests a direct stimulatory effect of FSH on wide variety of organs, rather than a restricted indirect function only on germ cells via Sertoli cells in testis and granulosa cells in the ovary.

### NATIONAL CENTRE FOR PRECLINICAL REPRODUCTIVE AND GENETIC TOXICOLOGY

#### MicroRNA Regulation in Prostate and Ovary upon Exposure to Endocrine Disruptors

The present study was undertaken to investigate the effect of exposure of BPA (25µg/kgbw) on miRNA

expression in prostate and ovary using perinatal rat model. Preliminary observation suggests that there was significant decrease in sperm count and motility in F1 male. A significant decrease in the levels of testosterone and estradiol was noted in treatment group in F1 male and female rats. A significant increase and decrease was observed in the prostate and ovarian weights, respectively. Multifocal reactive and functional hyperplasia of prostate glands with presence of fingerlike projections of hyperplastic epithelium was observed in treatment group. Differentially expressed miRNA were selected from prostate and ovary of F1 animals and further validation is in progress.

#### Deciphering the Effects and Mechanism of Action of Butyl Paraben on Fertility

With exposure to n-butyl paraben, the F1 male rats showed delayed age of balano-preputial separation, a reduced caudal sperm motile activity and reduced sperm count; suggesting an impaired spermatogenesis. F1 female rats showed delayed age of vaginal opening, a perturbed oestrus cycle with F1 reduced oestrus phase and reduced number of corpora leutea. Moreover, the adult F1 male and female rats showed a reduced fertility with an increase in pre- and post-implantation loss. These cumulative effects of n-butyl paraben exposure on reproductive functioning of F1 generation, also caused impaired steroidogenesis.

The given study highlights the effects of perinatal exposure of n-butyl paraben on sensitive window of development, which could lead to impaired endocrine homeostasis, reduced fertility with an impaired spermatogenesis in F1 male offspring and oogenesis in F1 female offspring.

#### Cellular and Molecular Effects of Cypermethrin on Reproductive Functions of Male and Female Rats

Cypermethrin (CYP) is a widely used insecticide and known endocrine disruptor due to its estrogenic and anti-androgenic activities. The study observed that perinatal exposure to 25 mg/kg bw/day cypermethrin from gestation day 6 to postnatal

day 22, has adverse effects on the fertility of F1 male rats and leads to developmental defects in F2 fetuses. The CYP treated F1 male rats were subfertile, as mating with normal cycling females resulted in significant decrease in copulation index and increase in the time taken for copulation. The increase in preimplantation (PIL%) and postimplantation (POL%) losses led to decrease in litter size.

Also, physical malformations such as under developed body with deformed limbs, and lack of tail in F2 fetuses indicated that cypermethrin does have the potential, to bring about teratogenic and embryotoxic effects, even at low levels of exposures. This necessitates further detailed teratogenic investigation in F2 and subsequent generations.

## REPRODUCTIVE CANCERS

### Evaluating the Potential of Trop2 as Immunotherapeutic Target for Ovarian Cancer

Trop2 (Trophoblast Protein 2), overexpressed in epithelial ovarian cancer cells, is emerging as an important immunotherapeutic target. Trop2 monoclonal antibody is already in trials. In order to overcome the disadvantages associated with the monoclonal antibody based approach, which include high cost as well as requirement of repeated injections, active immunotherapy is considered as a suitable and cost-effective alternative. Towards this objective, two peptides, corresponding to the extracellular domain of Trop2 were synthesized and their potential to elicit immune response against whole protein was determined. One, out of these two peptides generated antisera, capable of detecting native protein by ELISA, Western blotting as well as immunofluorescence. Ability of these anti-peptide antibodies to prevent ovarian cancer cell invasion and to induce antibody dependent cell cytotoxicity (ADCC) will be investigated.

### PSP94 as an Adjunct Marker for Serum PSA for Differentiating between Benign Prostatic Hyperplasia and Prostate Cancer

PSP94 has emerged as a promising serological marker along with PSA in differential diagnosis of

BPH and PCa. The study had developed a specific, sensitive and cost-effective in-house ELISA for measuring sPSP94 concentrations. Preliminary results suggested that sPSP94/sPSA ratio is a better diagnostic indicator to differentiate between BPH and PCa, than sPSA or sPSP94 alone. The centre's earlier exploratory study was done in biopsy confirmed PCa and BPH cases wherein it was observed that unnecessary biopsies could be avoided in BPH cases using sPSP94/sPSA ratio. The present study has been initiated to validate the in-house developed ELISA for measuring the levels of PSP94 in serum of lower urinary tract symptom (LUTS) patients and to ascertain whether serum PSP94/PSA ratio has a diagnostic potential to differentiate between BPH and PCa in patients with PSA values between 4-20 ng/ml.

### Differential Expression of Host Immunogenetic Factors in Development of Cervical Cancer in Indian Women with Human Papillomavirus (HPV) Infection

Results of this completed study, highlighted the following information on cervical cancer in Indian women. Infection with multiple types of HPV is associated with cervical cancer. High resolution analysis of HLA class I revealed association of HLA-A\*02:01, B\*35:01, B\*37:01 and B\*58:01 with increased risk of cervical cancer, while HLA-A\*11:01, -A\*24:02, -B\*07:05, -B\*35:03 and -B\*40:06 were associated with decreased risk. The positive association of HLA B\*37:01 and negative association of B\*40:06 with cervical cancer was validated by *in silico* analysis. Analysis of HLA class II alleles, further revealed presence of HLA- DRB1\*03, DQB1\*02, and haplotype HLA-DRB1\*03-DQB1\*02 in significantly ( $p$  value < 0.05) high frequency in cervical cancer cases. Among the HPV infected women with normal cervix, HLA-DRB1\*13, DQB1\*06 and haplotypes DRB1\*13-DQB1\*06 were observed, significantly, ( $p$  value < 0.05) in high frequency. Results further confirmed the assessment of DRB1\*13 as a protective marker in HPV infection outcome.

### Identification and Characterization of Membrane Bound Estrogen Binding Proteins in Prostate Cancer Cell Lines

The laboratory is engaged in elucidating the role of estrogen in prostate cancer pathogenesis. Towards this, lab has carried out detailed investigations to identify and characterize the cell surface bound proteins which mediate estrogen signaling in prostate cancer cell lines. Lab previously demonstrated the presence of such proteins on the cell surface of tumorigenic and non-tumorigenic prostate epithelial cells and their similarities to the conventional nuclear estrogen receptors. Lab also demonstrated their entry into biosynthetic pathway, as indicated by their localization in endoplasmic reticulum and Golgi complex. Investigations conducted during the reporting year, demonstrated that activation of cell surface estrogen receptor (csER) in tumorigenic prostate cancer cell lines-LNCaP and DU145 leads to modulation in the expression of epithelial to mesenchymal associated proteins such as E cadherin and Vimentin. Also, AKT protein was found to be phosphorylated in response to estrogen signaling at the cell surface of prostate cancer cell lines. Collectively, these investigations highlight the relevance of csER in prostate cancer pathogenesis.

### Workshops/ Training programmes conducted

- Sensitizing health care providers to provide HIV-FP linked services at district hospitals in the NACO funded project.
- Network meeting of WHO collaborating centres in the area of Reproductive, Maternal, Newborn, Child and Adolescent Health, Mumbai, June 7-8, 2016.
- Science Academies Lecture Workshop on Applications of Flow Cytometry in Health and Disease at National Institute for Research in Reproductive Health, July 14-15, 2016 (Course Co-ordinator).
- Organised International Immunology Day, 3rd May 2016, in collaboration with Enterovirus Research Centre (EVRC), with financial support from Indian Immunology Society. The program comprised of a lecture entitled

Immunomodulators for Immune-prophylaxis and Immunotherapy by Prof. K.B. Sainis, Ex-Director, Bio-Medical Group, BARC, Mumbai.

- Organised 9th International Annual Conference of Clinical Pharmacology in Maternal and Child Care, 28-30 April 2016 as part of the Organising committee, South Asian Chapter of American College of Clinical Pharmacology.
- CME on Mental Health in Tribal Community at Sub-District Hospital (SDH), Dahanu, Dist. Palghar, held on 18th June 2016, at MRHRU Dahanu 18.06.2016.
- Non Pneumatic Anti-shock Garment (NASG) Workshop cum Training of Master Trainers at MRHRU, Dahanu, District Palghar, on 30.01.2017.
- A Symposium on Vitamin D Paradox: Real or Virtual?, 8<sup>th</sup> March 2017.
- CME on Early detection of common cancers for Medical Officers of tribal area of Dahanu, Dist. Palghar, Dahanu, March 23, 2017.
- Workshop on “Basics of Bioinformatics” for PhD students of NIRRH and Haffkine institute and M.Sc. Clinical Pharmacology students of Maharashtra University of Health Sciences (MUHS) from January 23<sup>rd</sup> - February 2<sup>nd</sup>, 2017.
- Workshop on “Systematic Reviews and Meta-analysis” from September 19 – 21, 2016.
- Workshop on “Basics of Bioinformatics” for PhD students of NIRRH and M.Sc. Clinical Pharmacology students of Maharashtra University of Health Sciences (MUHS) from April 18<sup>th</sup> – 22<sup>nd</sup>, 2016.

### National Animal Resource Facility for Biomedical Research

#### BACKGROUND INFORMATION

Established on 1<sup>st</sup> Jan 2016, this institute deals with Animal experimentation in Bio-medical research which continues to remain crucial to find out better ways to understand, prevent, treat and cure diseases, as currently there are no existing alternatives to substitute the biological systems. World over, new drug research as well as tests meant for assuring



the quality, safety and efficacy of pharmaceutical products/vaccines/recombinant products are based on experiments involving animals. The use of animals for research is essential for the development of new and more effective methods for diagnosis and treatment of diseases that affect both humans and animals. Virtually, every breakthrough in new drug / vaccine development, has been the direct result of research in animals.

For testing of products, there is a need of state of the art facilities with GP standards and international accreditation. At present, there is no GLP accredited institution in the country to certify and authorize testing of products on behalf of the Government of India.

### VISION

To build state of the art infrastructural facility as a single stop solution and to make available all the resources for basic, applied and regulatory research to bio-tech, bio-pharma and bio-medical institutions in the country.

### MISSION

- To pursue and support basic biomedical and clinical research utilizing laboratory animals, with emphasis in the areas of developmental biology, reproductive biology, cardiovascular physiology, stem cell, molecular cell biology, neurobiology, behavioural sciences, development of animal models, immunology, virology especially AIDS-related research etc.
- To create resources of Specific Pathogen Free (SPF) quality, large and small experimental animals for research & affiliated programs, complying with all applicable regulations of ethics and rehabilitation by breeding them in barrier and natural habitat environment.
- To provide comprehensive, worldwide, bibliographic information on all laboratory animals that enable investigators to communicate information on research findings, availability of animals, tissues, blood and specimens throughout the world.
- To provide and expand access to the resources by way of collaboration with the universities and institutes on a National and International level.

- To meet the demands, especially the requirements of academic institutions, universities, research institutions, biotech, biopharma and biomedical research organizations for developing diagnostics, new therapeutics for human and animal health, with its state of the art infrastructural facilities.
- To function as testing and authorizing body of drugs, devices and products prior to human clinical trials and for further submissions.

### MAJOR RESEARCH AREAS

Use of innovative methods for testing of new molecular entities and modern medical devices, development of alternatives and validated testing approaches for treatment of human and animal diseases.

### ACTIVITIES UNDERTAKEN

During this year, based on the suggestions of the PMO, three committees viz Programme Management Committee (PMC), Programme Evolution Committee (PEC) and Core Committee (CC) have been formed, with members having expertise and specialization in the identified areas, to evaluate different facilities, specifications and the progress of establishment of NARF institute.



Fig. 5: 1<sup>st</sup> committee meeting held in Hyderabad on 3<sup>rd</sup> March 2016.

The first joint committee meeting of ICMR-NARFBR PMC, PEC and CC has been held in Hyderabad on 3<sup>rd</sup> March 2016. The meeting was organised to introduce the objectives of institute and discuss the mandate of the above committees to describe processes and steps involved in the development of the institute. The committee discussed the modalities for the appointment of agency to take up construction activity and to create the best resource facility as per international standards in the country. Secretary DHR & DG ICMR, Dr. Soumya Swaminathan and Sr. FA ICMR, Ms. Ritu Dhillon and Dr. R.S.Sharma Scientist-G, ICMR had participated in the meeting. They also had interactive meeting with Shri. BP Acharya, Principle Secretary of Planning, Government of Telangana and discussed on the strategies and modalities for developing this institute at the same venue.

Subsequently, about 35 meetings including six PEC meetings, have been held during the year as part of the development and evaluation of the progress for undertaking the construction works and for establishing the institute soon.

During the year, the institute launched a website ([www.narfbr.org](http://www.narfbr.org)) by DG ICMR, Dr. Soumya Swaminathan on 27<sup>th</sup> April 2016, during the Directors meeting in the ICMR Head quarters.



**Fig. 6:** Website launched ([www.narfbr.org](http://www.narfbr.org)) by DG ICMR, Dr. Soumya Swaminathan.

The institute had organized a program of the State Government of Telangana's "Haritha Haram Programme" and planted more than 2000 saplings in the allocated land of NARF on 23<sup>rd</sup> July 2016. More than 200 staff members of NIN & NCLAS institute along with local MLA and other political leaders had participated in this programme.



**Fig. 7:** "Haritha Haram Programme".

In addition, there were several meetings organized in the year, including a meeting with international expert Dr. Krishnamurthy, from South West Foundation for Biomedical Research, Texas, USA, to discuss about the specifications of ABSL3 facilities of NARF institute on 29<sup>th</sup> November, 2016.

The institute had organized a programme of "Unveiling of Model" during - Bio Asia Inaugural Function, organised by Federation of Asia Biotech Associations of the Telangana State on 6<sup>th</sup> February 2017. Secretary DHR & DG ICMR, Dr. Soumya Swaminathan, and the Honorable Industry Minister, Government of Telangana, Shri. K.T. Rama Rao graced the occasion along with Dr. Poul Stoffels, MD, Johnson & Johnson. They had also planted the saplings on this occasion.





**Fig.8:** Unveiling of Model, by Honb'l Industry Minister, Government of Telangana Shri. K.T. Rama Rao and Secretary DHR & DG ICMR, Dr.Soumya Swaminathan.

**Extramural Research**

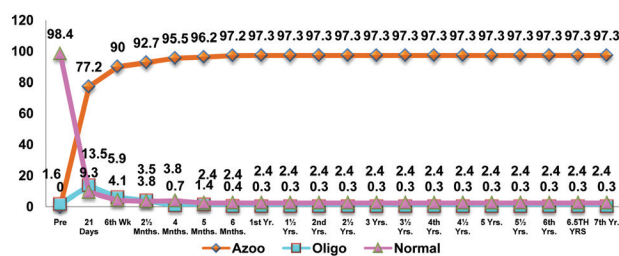
**Division of Reproductive Biology, Maternal & Child Health (RBMCH)**

**FERTILITY REGULATION**

**Phase-III Clinical Trial with an Intravasal Injectable Male Contraceptive – RISUG®**

To study the safety and efficacy of once injectable non-hormonal, intravasal male contraceptive RISUG (Reversible Inhibition of Sperm under Guidance), a Phase-III Clinical Trial is going on at five centres in the Country. Total 313 subjects have received RISUG injection and all these subjects have been followed for their efficacy and safety. The interim data analysis indicates the following: People from all major religion (i.e. Hindu 82%,

Muslim 9%, Sikh 8% and Christian 1%) have accepted the RISUG injection. People from all major caste (General 31.0%, SC 43.5%, OBC 16.1% and ST 6.8%) have accepted the RISUG injection. Majority of the RISUG subjects injected were having either primary education (43.6%) or Graduation (28.8%). No adverse side effect was reported and observed on clinical evaluation of these subjects, even up to 7 years of post RISUG injection. No adverse trend was observed in any parameter related to haemogram, liver function test (LFT), kidney function test (KFT), blood sugar, urine examination of the subjects up to 7 years, post RISUG injection. Over all data indicates that 92.7% subjects achieved azoosperma at 2½ month post injection and it reached to highest level (97.2%) at 6<sup>th</sup> month post RISUG injection. 1.2% method failure was observed. Over all failure of the drug RISUG was 1.6%. Hence, over all contraceptive efficacy of the drug RISUG was 97.2%.



**Fig.9:** Efficacy of RISUG Injection (%).

The dropout rate of the subjects observed in the trial was 20% after one year. The reasons of dropout were:- personal reasons, subjects got transferred, family problem, residence shifted away from the participating centre and subjects could not be located. The data analysis clearly indicates that RISUG is a safe and effective male contraceptive and has been accepted by people of all religion.

**Phase I-II Clinical Trial on Safety, Immunogenicity and probing efficacy of the Revived Recombinant Vaccine against Human Chorionic Gonadotropin (hCG)**

The Phase-I & II clinical trial protocol for prevention of pregnancy by Recombinant hCG β-LTB vaccine has been approved by the Drugs Controller General (India), based on the



recommendation of the RCGM of Department of Biotechnology, Government of India. The trial is going to be initiated at 2 clinical centres i.e. AIIMS, New Delhi and Sir Ganga Ram Hospital, New Delhi shortly.

### National Registry of ART Clinics and Banks in India

Around 1736 ART Clinics and Banks have been identified. Out of these, 1084 ART Clinics and 190 ART Banks have confirmed their contact details and remaining are in the process of confirmation. As the registration under the National Registry is a three step process, therefore, based on the prescribed performa, the complete information from 1084 ART Clinics has been received about the minimum infrastructure facility, trained manpower and procedures being under taken at the respective ART Clinic. Out of 1084 ART Clinics, 396 ART clinics have been enrolled under the National Registry. An enrolment number has been given to 385 ART clinics out of 396, and their contact details are available on ICMR web site.

The data analysis of the registry indicated that out of 396 approved ART clinics, only 7 ART clinics were IUI clinics whereas out of 436 under process ART clinics, 29 ART clinics were performing IUI. Only two ART clinics from Govt. sector were approved in comparison to five ART clinics in under process category. All ART clinics were allopathic in approved category whereas 13 ART clinics did not provide any information about their nature of clinic under process category. Eighty ART clinics, out of 396 approved ART clinics were registered under CE Act. All approved ART clinics were registered under PCPNDT Act, whereas out of 436 under process ART clinics, 360 ART clinics were registered under PCPNDT Act. Out of 396 approved ART clinics, 33 ART clinics were sub-clinics of main ART clinic. In approved ART clinics category, all gynaecologist were specialized in obst. & gyn. whereas in under process category, 2 ART clinics did not have the qualified gynecologist and 22 ART clinics did not provide any information about their qualification. Similarly, all approved ART clinics were having andrologist and 206 were on regular basis, whereas

in under process category, 21 ART clinics did not have any andrologist. All approved ART clinics were having clinical embryologist and 71.7% were on regular basis, whereas in under process category, 36 ART clinics did not have qualified embryologist. All the approved ART clinics were having regular counselor whereas in under process category, 14 ART clinics did not have counselor and 125 ART clinics did not have regular counselor. All the approved ART clinics were having sterile area whereas 4 ART clinics in under process category did not have sterile area. All the approved ART clinics were having semen processing lab and clean room for IUI. In under process ART clinics, 14 ART clinics did not have Operation Theatre for carrying out surgical endoscopy and vaginal ovum pickup. In approved ART clinics, 385 ART clinics were doing IVF-ET, whereas in under process ART clinics, 357 ART clinics were doing IVF-ET. In approved ART clinics, 314 ART clinics were doing surrogacy whereas in under process ART clinics, 232 ART clinics were doing surrogacy. In approved ART clinics, 203 ART clinics were not doing cryo-preservation of ovarian tissue.

A systematic review and meta-analysis was also carried out to find out the Safety and effectiveness of Letrozole compared to Clomiphene Citrate (CC) or any alternative treatment for ovulation induction in infertile women. Letrozole is more effective in inducing ovulation and pregnancy rate in infertile women as compared to CC or any other alternative treatment. There was no significant difference in miscarriage rate and congenital abnormalities in children who were born from women who were administered letrozole as compared to those given CC or other alternative treatment. The concern that letrozole use for ovulation induction could be teratogenic, is unfounded, based on the present systematic review. In Indian woman, Letrozole is more effective in all out comes, even at lower dose with no congenital anomalies.

To develop minimum standard provisions for ART Banks, in terms of trained manpower, physical infrastructure facility, procedure being undertaken and ethical, social, legal, technical & moral issues,

a draft National Guidelines for Accreditation, Supervision and Regulation of ART Banks in India has been developed.

## MATERNAL HEALTH

### Changes in human gut flora and in immune functions following probiotic administration

Probiotics are living organisms that are known to positively affect human health. The mechanism of their action remains unclear, but is believed to be mediated by a change in gut flora or through their effect on immune cells. This study was conducted to see the effect of probiotic administration on gut flora and immune responses. Twenty healthy women, who had not taken any drug that could alter immune responses or gut flora (antibiotic, probiotic, drugs that influence gut motility, immunosuppressive agents) were included in the study. Each of them received one capsule twice a day of VSL3, a probiotic preparation containing several bacterial species, for four weeks, and were followed up for further 4 weeks. Blood and stool specimens were collected at 3 time- points each, before, after 4 weeks of probiotic and 4 weeks after stopping the probiotic. Gut flora were assessed using parallel sequencing of V3 region of 16S rRNA, and severe immune parameters were assessed.

The profile of gut flora at the three time points was similar, with no change in alpha diversity or the composition. The proportion of Th1 and Th2 cells was similar at the three time points. Probiotic administration led to an increase in the proportion of Tregs as well as that of Th17 cells. The concentration of cytokines (unstimulated) in the peripheral blood did not show any change after probiotic administration. Similarly, the production of cytokines from whole blood cultures, following stimulation of T-lymphocytes, did not show much change in cytokines. However, the production of cytokines on *in vitro* monocyte stimulation was reduced. These data suggest that the effect of probiotics is unlikely to be mediated by change in gut flora but may be mediated by some changes in immune response.

### Participatory health facilitation intervention to promote maternal health, feeding attitude and adherence to recommended IYCF practices in tribal Gujarat

An interventional experiment was carried out in the tribal region of Dahod, Gujarat after need assessment of 1239 mothers, where prevalent practices was assessed. Dahod block was selected as control-arm and Jhalod block was selected as intervention-arm in consultation with Chief District Health Officer. Mothers were recruited in two cohorts, accounting for high migration in the region: (i) mothers in their third trimester; (ii) mothers having infant <4 months of age. In intervention-arm, mothers received counseling after questionnaire assessment. Age-appropriate and culturally acceptable IYCF counseling messages were developed and pre-tested in presence of FHSs and CHWs. In addition, audio-visual counseling leveraged Gujarati videos, relevant to tribal context, from YouTube channel HealthPhone (developed by Indian Academy of Pediatrics and partners; The videos were shown using 8 inch tablet devices. Additionally, group counseling and recipe demonstration was also conducted at all anganwadi centres in the intervention villages by the study team members trained. Results and Conclusion: The BCC intervention helped improve breastfeeding practices in terms of early initiation of breastfeeding and colostrum feeding. It also helped improve timely introduction of complementary feeding in 6-9 month age-group. However, the intervention did not show any significant impact in improving minimum meal frequency, minimum dietary diversity and minimum acceptable diet. The simplicity of this contextual intervention enhanced its replicability by leveraging the existing healthcare system. Given the low literacy, poverty, limited livelihood opportunities and high out-migration, complementary feeding practices, need further exploration to understand social determinants of health.

### **Health Account Scheme (<http://healthaccountsscheme.nic.in/account/login>): Empowering people for health care through inter-sectoral coordination- an operational evaluation-ICMR task force study**

Lack of community and district level data, connection between community and health care providers has been observed in monitoring reports of NRHM. Though conceiving and implementation of various health programs starts from the specific needs of the community and their unique circumstances, lack of effective communication channel and absence of feedback mechanism between healthcare provider and population may lead to suboptimal implementation of schemes and may result in unwarranted effort. Health Account Scheme was started as a pilot project with objective to gauge the lacunae and address it, by introducing a physical health diary at family level and encouraging them to write/record their health needs. This information was channeled by health workers monthly, in electronic data system. Observations on health status and solutions to bridge implementation gaps of various Health programs are becoming possible while involving community for maintaining their health problems and feedback in a Diary.

500 households at 3 sites (i.e. a rural and urban site at Uttar Pradesh, and tribal region of Arunachal Pradesh) were surveyed with a questionnaire which served as baseline data. A health diary with unique identification number was designed. Health volunteers were recruited and trained to approach each household directly and to distribute the health diary to each household. Each member of every family was encouraged to record (or taking help of health worker/volunteer to record) the health related problems in their own language. Periodic visit of health workers ensured the building of rapport with community and recording of health related data, perception, need and situation at the ground level. The required logistic were put in place to transfer these physical recordings to electronic database for analysis.

It was found that unmet needs of community exist due to lack of health related information, knowledge and education among the public, also

due to absence of reliable feed back to health planners about the pattern of health issues of the public and their redressal mechanism. Lacuna of communication channel came out as focal point, for example, active and sustained communication, feedback mechanism between the citizen and health policy maker could make rapport and communication was built during diary filling and updating the health problems.

Based on feedback in health diary, following interventions could be undertaken: at tribal site- health education imparted to the community curbed high prevalence of typhoid, 70 patients at baseline, 30 at end term. At Rural/ urban site: geriatric patients got support for transport to visit hospitals; severely malnourished children were provided solutions as per guidelines at urban slum site. A shift in addiction, non communicable disease reporting, was observed. Contraception, targeted home delivery of medicines, was explored for bridging gaps among consumers and care givers. This way available logistics could be used to meet the unaddressed needs of target population. As a consequence of monthly updating, the health problems in the diary by the community with the help of health literate worker, a rapport and communication can be built and gaps may be bridged for effective implementation of health programs at ground level.

### **Development of protein nanoparticles based microbicidal formulation in combination of curcumin and antiretroviral agent (NanoCurcARV) (An ICMR-DBT collaborative project)**

A combination of Efavirenz (EFV) and Curcumin (Cur) loaded lactoferrin Nanoparticles (ECNP), Tenofovir (TNF) and curcumin loaded lactoferrin Nanoparticles (TCNP), Dapivirine (DPV) and curcumin loaded lactoferrin Nanoparticles (DCNP) showed significant anti-HIV-1, anti-inflammatory and spermicidal activities, qualifying these formulations as topical microbicide under multi protective technologies. Nano form of individual drugs (curcumin, EFV, TNF & DPV) showed effective results such as drug bioavailability and systemic toxicity, when compared to soluble



form of respective drug. Thus, ARV nanoparticle formulation act as an effective microbicidal formulation, when compared to soluble form of drugs. Combination nanoparticles such as, ECNPs, TCNPs and DCNPs can act as an effective microbicides when compared to its soluble combination forms, respectively and to individual nanoparticles. Because of curcumin, ARV and lactoferrin nanoparticles combination, these microbicidal formulation can act as MPTs (Multiple Prevention Technologies) such as both microbicidal and spermicidal activity when compared to its soluble formulation.

Three different types of Nano Particles (single drug loaded LF NP and Cur combination ARV loaded LF NP) have been prepared using sol-oil chemistry. Size of NPs 20-26nm (lacto) and 40-80nm (ARVCur lacto) were observed by FE-SEM, AFM and TEM (Fig 10). The hydrodynamic radii and zeta potential have been measured using DLS and found to be an average of 40nm (LF) 102 to 120 nm (drug loaded) and -19 to -27mV respectively. The physical stability of drug/s loaded NPs have been estimated through FT-IR study, and found that the key functional groups related to respective drugs are intact in the nanoformulation. The encapsulation efficiency percentage (EE %) was calculated for each type of NP and found as follows, Cur from CNPs =  $59.6 \pm 1.34\%$ , EFV from ENPs =  $58.4 \pm 1.79\%$ , TFV from TNPs =  $53.5 \pm 1.3\%$ , DPV from DNPs =  $61.6 \pm 1.6\%$ , ECNPs (Cur =  $63 \pm 1.9\%$ , EFV =  $61.5\% \pm 1.6\%$ ), TCNPs (Cur =  $61.75 \pm 1.6\%$  & TFV =  $64.52 \pm 1.8\%$ ) and DCNPs (Cur =  $60.33 \pm 1.3\%$  & DPV =  $62.17 \pm 1.9\%$ ). pH dependent drug release assay of NP shows that maximum drug release (40-60%) was observed at pH-5 in pH dependent drug release. Optimum drug release showed in vaginal simulated fluid (pH 4.5) Antiviral properties of all ARV drugs and Cur have been found to be improved with significant decrease in IC<sub>50</sub> (50% inhibitory concentration) of nano form as compared to its sol counterpart. IC<sub>50</sub> of Sol versus Nano are  $5.1\mu\text{M}$  Vs  $1.75\mu\text{M}$  for curcumin,  $2.56\text{nM}$  Vs  $1.1\text{nM}$  for Efavirenz,  $4.78\mu\text{M}$  Vs  $2.8\mu\text{M}$  for Tenofovir and  $28\text{nM}$  Vs  $17\text{nM}$  for Dapivirine. The combination forms (ECNPs, TCNPs and DCNPs) are found to be more efficacious in their nano form as compared to its sol form. As the percent HIV inhibition is found

to be equal or improved even after, treatment with 50% of their sol form combination (Fig 2). Main observation is that antiviral activity was enhanced in combination nano form, this gives the advantage of using drug combination in the nano form, thereby, reducing the dosage leading to the prevention of unwanted side effects and toxicities. Due to negligible toxicity to lactobacillus, exhibited by all forms of drugs; there may not to be any threat to vaginal micro flora, considered as a safety Microbicide.

The comparative in-vivo pharmacokinetics (PK) studies were performed on Wistar female rats. Results showed a significant improvement of PK profile of drug when nanoformulation is administered topically (C<sub>max</sub>, t<sub>1/2</sub>, AUC and AUMC), suggesting higher bioavailability of drugs as compared to its sol equivalents. In time course, assay drugs were present up to 8-12 hrs in nano form treated but in case of sol treated drugs were eliminated in 4-6hrs, this proved that drug retention is high in nano treated groups when compared to sol treated (Table 1).

Bioavailability studies showed that in nano form (ECNPs, TCNPs and DCNPs) treated rats, maximum amount of drugs present in the lavage,  $10^3$  and  $10^6$  fold less concentrations of drugs were observed in the vaginal tissue and in the systemic circulation, when compared to the lavage respectively. The integrity of epithelia was found heavily damaged when the combination of sol drugs were used. In case of ECNPs, TCNPs and DCNPs, the integrity of tissue was found to be same as that of control or very lesser extent. Further IL-6 and TNF- $\alpha$  levels were estimated in vaginal tissue and in systemic level, they found 1.5 to 3 fold less in NPs treated when compared to sol form treated.

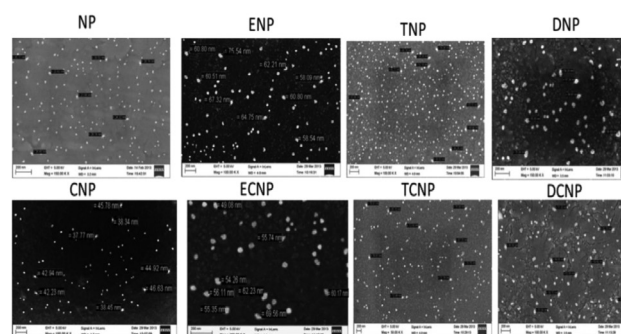
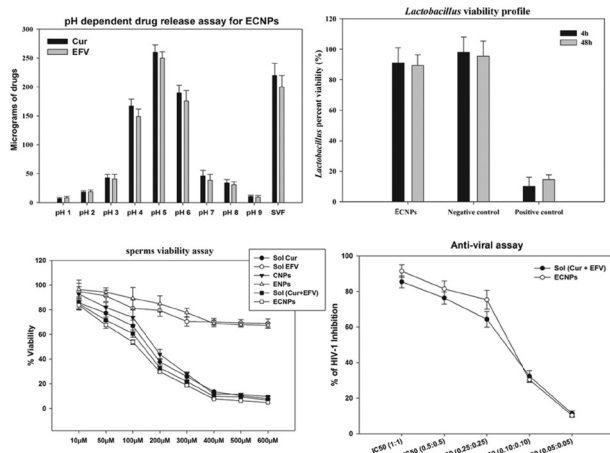
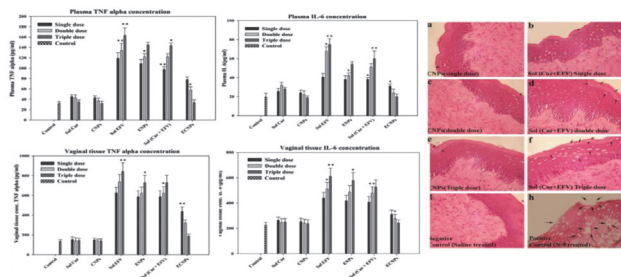


Fig.10: Nanoparticles are characterised using Fe-SEM.



**Fig.11:** Clockwise: Drug pH dependent release, wherein drug is completely released p5 and simulate vaginal fluids. ECNPs has no effect on Lactobacillus. Further, ECNPs anti-viral activity is enhanced.



**Fig 12:** Sol EFV, Sol Cur, Sol (EFV + Cur), CNPs, ENPs and ECNPs were applied topically in vagina. IL-6 and TNF alpha levels in plasma and vaginal tissue were analyzed and plotted. Value of significance \*\*\*P<0.0005, \*\*P<0.005, \*P<0.05. Tissue sections were H & E stained and observed under microscopy.

**Table 1. Pharmacokinetic profile of ARV and Curcumin in lavage when delivered in combination**

		EFV		Curcumin	
		Nano	Soluble	Nano	Soluble
AUC	(h)*(mg/ml)	20.4164	13.7581	32.4251	21.9066
AUMC	(h) <sup>2</sup> *(mg/ml)	132.482	52.448	172.922	56.496
C <sub>max</sub>	mg/ml	4.9126	4.2897	9.7794	8.9723
T <sub>max</sub>	hr	2	1	2	1
t <sub>1/2</sub>	hr	4.63222	2.14916	3.97347	1.8513

		Dapivirine		Curcumin	
		Nano	Soluble	Nano	Soluble
AUC	(h)*(mg/ml)	32.4757	15.3342	32.2141	20.1822
AUMC	(h) <sup>2</sup> *(mg/ml)	206.007	57.5105	176.238	51.5535
C <sub>max</sub>	mg/ml	6.9347	5.3746	10.1473	9.7498
T <sub>max</sub>	hr	2.5	1	2	1
t <sub>1/2</sub>	hr	4.28923	2.10652	3.64984	1.88261

		Dapivirine		Curcumin	
		Nano	Soluble	Nano	Soluble
AUC	(h)*(mg/ml)	32.4757	15.3342	32.2141	20.1822
AUMC	(h) <sup>2</sup> *(mg/ml)	206.007	57.5105	176.238	51.5535
C <sub>max</sub>	mg/ml	6.9347	5.3746	10.1473	9.7498
T <sub>max</sub>	hr	2.5	1	2	1
t <sub>1/2</sub>	hr	4.28923	2.10652	3.64984	1.88261

AUC: The integral of the concentration-time curve (after a single dose or in steady state).

AUMC: Partial area under the moment curve between t start and t end.

C<sub>max</sub>: The peak plasma concentration of a drug after oral administration.

T<sub>max</sub>: Time to reach C<sub>max</sub>.

t<sub>1/2</sub>: The time required for the concentration of the drug to reach half of its original value.

### Study of sexual dysfunction and sexual concerns among persons with disability due to myelopathy; assessment of unmet need for rehabilitation services and development of a hospital based sexual rehabilitation package.

Comprehensive neurological rehabilitation should address sexual function of affected individual to allow them highest level of function and quality of life. Sexuality of disabled people is not considered important and few studies have explored this issue in India. A cross sectional, hospital-based study was carried out among 80 men and women in the age-group 18-50 years of age, with disability due to myelopathy to determine sexual dysfunction, assess sexual concerns and unmet need for care for sexual dysfunction and develop a sexual rehabilitation package in the form of a manual (rehabilitation manual), listing the issues and possible options for addressing sexual concerns among them. The mean age was 33.73 years, three-quarters were male and majority (92.5%) were literate. 88.75 % had perceived, there is need to provide information, 50% of participants had felt the need to discuss sexual issues after injury. Only 32.5% were able to discuss about sexual issues after injury, out of which 46% were not satisfied with the responses. Majority (88.75%) patients suggested that sexual issues should be discussed within 6 months of injury. Topics suggested by the participants that should be taken into account include effect of spinal cord injury on sexual function, managing bladder and bowel function, effect of spinal cord injury on fertility, pregnancy related issues in female patients, labour care and complications and contraception for couples. Also, 55% participants opined that health care provider should initiate the discussion about sexual concerns/issues. Mainly (77.5%) participants wanted to have discussion with doctor in deliberating sexual concerns/issues. The results for involvement in sexual activity from

the present study showed that 57.5% had involved in sexual activity after injury. Those who had not involved in sexual activity after injury, medical condition was given as the main reason for non-involvement in 67.6% cases. Other reasons for not involving in sexual activity included lack of interest (2.9%), no desire (2.9%), no partner (5.8%), physical limitations (11.76%), lack of opportunity (2.9%) and fear of injury (2.9%). It was found that psychogenic erection was reduced in 53.2%, absent in 24% and normal in 14% of study subjects. Reflex erection was reduced in 56.4%, absent in 22.5% and normal in 14.5% of study subjects. Ejaculation was absent in 46.7%, reduced in 24%, and normal in 19% of study subjects. Orgasm was absent in 38.7%, reduced in 27.4% and normal in 9.6% of study subjects. In the present study, genital sensation was absent in 22.2% and present in 77.7% of subjects. Psychogenic genital arousal was reduced in 61%, absent in 5.5% and normal in 11% of study subjects. Reflex genital arousal was reduced in 44.4%, absent in 11.1% and normal in 11.1% of study subjects. Orgasm was absent in 22.2%, reduced in 16.6% and normal in 22.2% of study subjects. A rehabilitation package for Indian set-up, enlisting the above findings that would help rehabilitation clinicians to address the sexual dysfunction needs of the persons with disability due to myelopathy has been developed. Sexual rehabilitation would promote sexual health, encourage positive experiences of sex and relationships for all persons with disabilities. Knowledge and access to options for safer sexual practices would also help protection against sexual abuse or contracting sexual transmitted infections.

#### **Rapid epitope mapping for neutralizing antibodies and other entry inhibitors of HIV. (An ICMR-DBT collaborative project)**

Using a large number of OD fragment, core gp120, full-length gp120 and gp160 Env mutants in a variety of expression systems such as *E. coli*, yeast and mammalian cells, it was shown that gp120 folding can be largely made independent of glycosylation and such glycan-deficient virions retain infectivity and replication fitness. A combination of frequency-based, rational and

random mutagenesis methods were employed to achieve this goal. A large number of mutations identified were charged mutations, possibly preventing aggregation in a manner similar to that of glycosylation. The ability to engineer glycan-free protein derivatives allows for protein expression in inexpensive, prokaryotic or yeast systems, where glycosylation is absent or different from mammalian cells, but has been difficult to achieve hitherto. The methodology outlined in this work can, in principle, be used to probe the role of glycans in the stability and folding of any glycosylated protein and to construct glycan-free protein variants.

An attempt was made to probe residue burial using Asp scanning mutagenesis for the stretches 512-517 of the fusion peptide, and residues 547-568 which are disordered in most Env structures, as well as a few additional residues as controls. The experiments probed the conformation of mammalian cell surface expressed JRFL trimers using FACS. Most residues (with the exception of Q562 and Q567 and the buried, positive control residues 576 and 580) are tolerant to Asp, suggesting that the stretch from 547-568 is largely exposed and dynamically flexible in native Env with the exception of residues 562 and 567. In another series of experiments, a rapid and reliable method for mapping protein: ligand binding sites and conformational epitopes was done. The method uses a combination of Cys scanning mutagenesis, chemical labeling and yeast surface display. While Ala scanning is widely used for similar purposes, often mutation to Ala (or other amino acids) has little effect on binding, except at hot-spot residues. Many residues in physical contact with a binding partner are insensitive to substitution with Ala. In contrast, the study showed that labeling of Cys residues in a binding site consistently abrogates binding. This methodology was coupled to yeast surface display and deep sequencing and demonstrate that conformational epitopes targeted by both monoclonal antibodies and polyclonal sera as well as a protein:ligand binding site can be mapped. The method does not require purified protein, can distinguish buried and exposed residues and can be extended to other



display formats, including mammalian cells and viruses, emphasizing its wide applicability.

### Effect of maternal B12 supplementation on neurocognitive outcomes in children

Several national reports have indicated that children do not achieve optimal cognitive outcomes due to various environmental reasons such as maternal nutritional deficiency during pregnancy, current nutritional deficiency and lack of cognitive stimulation at home. Few randomized controlled interventions in pregnant mothers to improve nutritive status of the mothers and its impact on early child development, exist in literature. The study examined whether the daily oral administration of 50 µg vitamin B12 to pregnant Indian women improves infant neurodevelopmental status (as measured by the cognitive scale of the Bayley scales of infant development (BSID), 3rd edition), compared to placebo. The sample included 218 mother- child dyad that were part of an earlier study that gave B12 supplementation to pregnant women. This study also explored the possible factors that could affect cognition, which include maternal depression, maternal homocysteine (Hcy) levels during pregnancy, biochemical measures, social support and socio-demographic variables. The children were assessed on BSID and Behaviour Rating Index of Executive functions (BRIEF P) to measure cognitive functions. It was seen on Mann Whitney U test that children of mothers supplemented with B12 in pregnancy had better scores on Shift subscale of the BRIEF P. Spearman's correlation analysis and Mann Whitney U tests indicated that sociodemographic factors like maternal age and gender of the child, maternal depression, social support, birth weight, food security at home, B12 levels in the plasma and maternal homocysteine (Hcy) levels had an association with cognitive variables. In a linear regression analysis, high maternal Hcy in the third trimester had an association with expressive language and gross motor skills after adjusting for infant gender, birth weight, maternal age, household income, maternal depression, food security and intervention. It was also found that the intervention group performed better on the

expressive language subscale of BSID when adjusted for maternal Hcy levels in the second trimester along with the other variables. This study points to the importance of public health policy on supplementation during pregnancy and also its impact of neurodevelopmental outcomes in children. It will also help to examine the role of food fortification as it is the most cost effective and sustainable way to improving the nutrient status in deficient populations. Fortification of staple foods, which is a relatively low cost and along with absence of side effects, is a promising strategy in bridging the nutrient gap in resource poor settings. Future work could include following up the population to childhood and adolescence to examine the effects of supplementation at later ages. It could also be important to examine the effects of supplementation using other nutrients.

### Development of a multistrain probiotic Lactobacillus formulation, effective against reproductive tract infections

Vaginal Lactobacillus dominated vaginal microenvironment is associated with lower risk of genital infection. Numerous *Lactobacillus* species have been identified in the vagina. However, the native 24 species may vary widely according to the studied population. Indian continent has a very diverse population. There is limited information about the beneficial lactobacilli which contribute to healthy vaginal status in healthy Indian women. The present study was to investigate the predominant *Lactobacillus* strains present in the vaginal microenvironment of healthy Indian women and screen isolates for their functional properties. For the study, 199 healthy premenopausal women with no specific vaginal complaints were recruited. From 147 women with normal microflora, 110 women without colonizing *Candida* were selected for further analysis. The lactobacilli were identified using Multiplex-PCR and 16sRNA gene sequencing. Qualitative determination of H<sub>2</sub>O<sub>2</sub> and lactic acid was done on TMB-HRP MRS agar and BCP-MRS agar respectively. Inhibitory activity of Lactobacillus CFC against *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Neisseria gonorrhoea*, *Pseudomonas aeruginosa*

and *Gardnerella vaginalis* was investigated. Auto-aggregation, Co-aggregation with *C.albicans* and adherence of selected lactobacillus strains to vaginal epithelial cell line was also evaluated. Synergistic activity of Lactobacilli was evaluated by growth curve assay.

Lactobacilli were recovered from 108/110 (98.2%) women. *L. iners* 70.9 % (78), *L. crispatus* 26.4% (29), *L. reuteri* 20.9% (23), *L. gasseri* 18.2% (20) and *L. jensenii* 15.5% (17) were the most frequently occurring species in the vaginas of normal healthy premenopausal women. The vaginal microflora was dominated by either homogenous (80.9%, n=89) or a heterogeneous (19.1%, n=21) combination of two *Lactobacillus* species. Though most frequently identified, *L. iners*, coexisted only with other *Lactobacillus* species. All isolates produced acid whereas 94.2% were H<sub>2</sub>O<sub>2</sub> producers. Around 91% of *Lactobacillus* isolates inhibited the growth of pathogens causing UTIs, gonorrhoea, vulvovaginal candidiasis and bacterial vaginosis. Around 75% isolates could aggregate clinical strain of *C.albicans*. When quantitatively evaluated, 41% *Lactobacillus* isolates demonstrated greater than 90% co-aggregation with *C.albicans*. Highest co aggregating (100%) ability with *C.albicans* was observed in strains belonging to *L. crispatus* followed by *L. rhamnosus*, *L. gasseri* and *L. jensenii*. Maximal scores of auto aggregation were observed in strains of *L. crispatus* (81.9%) and *L. gasseri* (77.7%). The major effective Lactobacilli showed greater than 70-95 % adherence on VK2/E6E7 cells. The five selected effective *Lactobacillus* strains (*L.crispatus*, *L.gasseri* and *L.reuteri*, *L.rhamnosus* and *L.plantarum*) did not display any antagonistic effect against itself or against each other. Further antagonism against the pathogenic species was observed by co-culture with the combined *Lactobacillus* species. The study concludes that vaginal lactobacilli from healthy women identified in the study differ from that reported in North India. Presence of heterogeneous *Lactobacillus* population highlights the cumulative effects of different *Lactobacillus* species maintaining healthy VMB. The results indicate that the effective vaginal *Lactobacillus* strains with probiotic potential could

be explored for the control of urogenital infections in women.

## CHILD HEALTH

### Estimating the burden of Pediatric HIV in 'A' Category District in India – an ICMR task force study

The study involved implementation of three different strategies to estimate the burden of paediatric HIV. While strategy 1 was longitudinal and used as a measure of annual incidence, the other two strategies were cross-sectional. The objective of strategy 1 was to facilitate early case detection in infants and young children (0-22 months) born to a HIV positive pregnant woman registered at one of the public or private health care facilities of the district, using the age-appropriate tests at 6-10 weeks, 6-9 months and 18-22 months. Strategy 2 was to detect HIV infection among children (<15 years) of HIV-infected parent(s), identified during the study period through family screening of the index persons. The objective of Strategy 3 was to detect HIV among sick children (<15 years) presenting with suspected signs and symptoms, satisfying the modified IMNCI HIV/ special screening criteria for testing at health care facilities using age appropriate HIV tests.

A total of 971 health care facilities were mapped in the district, out of which 285 facilities with a provision of HIV testing were selected for strategy 1 & 2. For strategy 3, data was systematically collected from 10 health care facilities selected by random sampling stratified to represent the various levels of health care across four talukas in the district. Time-based sequential sampling was adopted for all three strategies. All resident HIV positive pregnant women, reported from selected high volume delivery health care facilities, between 01 Jan 2011 and May 2013, were included for strategy 1, and they were followed up until delivery. Their newly born infants were followed up until 18-22 months of age. HIV test results as per national Early Infant Diagnosis protocol were assimilated.

In strategy 2, HIV positive parents detected in the district during 01 Jan 2011 and March 2013

were traced and their children (<15 years) were HIV tested. For strategy 3, a screening tool was developed and used by trained study staff to identify a “sick” child (satisfying screening criteria) from among children reaching the identified health care facilities. Those who classified as being ‘sick’ were referred for age appropriate HIV testing and counseling.

The estimation of burden of pediatric HIV in the district, took into consideration the results from the three strategies. Cumulative incidence was derived from strategy 1, while the prevalence was estimated from the cross sectional methods adopted in strategy 2 and 3. The results from the study were extrapolated to the population characteristics within the district, including total population (adult and child), estimated overall adult HIV prevalence, estimated prevalence among pregnant women and reported coverage of HIV testing among the antenatal sub-population.

A total of 487 live births from 506 mother-child pairs were included in strategy 1. Of this, 454 (92.3%) children were tested at least once, and 39 were found to be HIV positive, at different points of time during follow up till 22 months. Thus, the net cumulative incidence rates (%) of vertical transmission of HIV per 100 pregnancies were 2.1, 5.3 and 7.8 by 10 weeks, 9 months and 22 months of age, respectively. This was considered as adding to the existing pool of HIV infection among children. Cox hazard model revealed that the risk of mother-to-child HIV transmission was found statistically significant in cases where both mother and child were not covered with ARV, and when reported breast feeding duration is longer.

769 households of strategies 1 and 2 included 1388 children <15 years (Male: 676; Female: 712). Prevalence of HIV infection among children was 10.6% (Male: 12.8%, Female: 8.4%). In strategy 3, of the total 33342 children who visited the 10 health care facilities during the period 24 Feb 2014 to 30 Jun 2014, 24342 (73%) were screened by the trained field investigators, 527 (2.2%) sick children were identified, 509 completed the testing requirements. Of these, 97 children turned out to be positive (HIV prevalence 19.1%), but 86 of them knew their HIV

positive status before falling sick. That is, the study was able to identify 11 (2.16%) new HIV infections from among the total 509 sick children. Expanding these findings to district population characteristics, the existing pool of HIV infection in the district is about 3266, among a mid-study projected child population of 1401688 children aged <15 years, thus making an overall HIV prevalence among children of 0.23%. This is 2.5 times that of the reported prevalence for Karnataka state (National Institute of Medical Statistics and National AIDS Control Organization, 2012). The proportion of children among all persons living with HIV in the category A Belgaum district in this study is 10.4% (3266/(3266+28119)) against 6.54% estimated/ reported earlier for India (National Institute of Medical Statistics and National AIDS Control Organization, 2015) and 7% (Department of AIDS Control (NACO), Annual report, 2012-13).

From strategy 3, it was also observed that three clinical criteria (unexplained fever for more than one month, being orphan child and having parents with HIV) tend to be positive predictors of HIV infection in the child.

#### **Novel in-house multiplex PCR guided management of neonatal sepsis in a tertiary care hospital based setting**

Neonatal sepsis is a significant cause of morbidity and mortality and claims an estimated 1.4 million lives each year. The clinical symptoms of neonatal sepsis are nonspecific and diagnostic tools lack specificity or sensitivity. Bacterial cultures are time-consuming and lack the ability to provide rapid results that may be useful for case management. As such, the empirical treatment with antibiotics remains the mainstay of treatment in case of suspected sepsis or in those with severe presentation. In the present study, centre aimed to employ a multiplex PCR approach to characterize bacterial agents causing neonatal sepsis. To achieve the above aims, centre employed a case-control approach and recruited neonates presenting with sepsis as cases along with age-, sex- and location-matched controls. Informed consent was obtained from the parents or care givers accompanying the child. In depth multi- system clinical evaluation



of study subjects performed at recruitment, was entered into standard clinical data abstraction forms. Upon recruitment, appropriate samples were collected as per standard protocols for management of neonatal sepsis at our institution. Additionally, study employed a novel in-house multiplex PCR approach for the detection of etiological bacterial agents. The association of clinical, laboratory markers, anthropometric, socio-demographic and economic variables with risk of severe disease was evaluated using multi-variate logistic regression analyses. The accuracy indices of this approach as compared to the current gold standard bacterial culture and clinical case definitions were examined using latent class analysis.

Uniplex PCR as well as multiplex PCR was optimized in sequential steps. Multiplex PCR primers were selected based on specificity and primers concentration for multiplex PCR was finalized based on individual priming efficiency in the multiplex PCR reaction. Blood samples spiked with different loads of bacteria was subjected for above mentioned molecular techniques. Enriched samples after RBC lysis yielded better result when compared with non-enriched samples. The sequences data of specific PCR product showed 98 to 99% homologies to their respective whole genome sequence which confirmed the sources of sample being used. When multiplex PCR technique was employed for clinical samples it was successful in detecting the bacterial species in all neonates with positive blood culture and in 18 neonates with negative blood culture. The diagnosis of neonatal sepsis by multiplex PCR when compared to blood culture revealed 100% sensitivity, 64% specificity, 73.5% positive and 100% negative predictive value. The turnaround time from DNA extraction to detection of bacterial organism by multiplex PCR was about 8 hrs, compared to cultures which became positive after 24 to 72 hrs.

**Interpretation and Conclusion:** Multiplex PCR-based approach which requires as little as 8 hours of turnaround time and blood volumes as less as 100µl, correlated well with conventional blood culture reports and is relatively cost effective for the diagnosis of neonatal sepsis.

### **Surveillance of infection in neonates: ICMR taskforce study**

Sepsis is the major cause of morbidity and mortality in neonates. Limited information is available on causative agent of sepsis in the community and their antibiogram. This project was carried out at 6 sites (Assam, Himachal Pradesh, Maharashtra, Tamil Nadu, Uttar Pradesh and West Bengal) at secondary level hospitals with the objective to identify organism causing sepsis in the neonates and their antimicrobial susceptibility pattern. All babies less than 28 days old either delivered in the district hospital or referred to district hospital, from CHCs/FRUs/ community with signs and symptoms of probable sepsis, were screened. Neonates fulfilling inclusion, exclusion criteria were enrolled. On enrolment detailed history was obtained and recorded in a predesigned structured proforma. Blood sample was collected prior to administration antimicrobials for culture/ microbiological evaluation as well as for sepsis screen. Of the total 34656 neonates screened, 88.1 percent were inborn and 11.9 percent were outborn. Of them 3179 (9.2%) were enrolled. Age at onset was <3 days in 51 percent neonates, 19.7% developed in 3-<7days and around 28% developed during 7-27 days. Fifty percent neonates had birth weight <2500 gms, 12.7% had birth weight <1500 grams. Blood culture was positive in 12.3% neonates. Klebsiella sp (29.2%) was the most common gram negative pathogen followed by E. Coli(12.9%). Among gram positive, Staph aureus was most common pathogen isolated (24.8%) followed by CONS (8.3%). Most gram negative pathogens were resistant to almost all common antibiotics. Conclusion: Klebsiella spp and Staph aureus are major pathogens causing sepsis in secondary level hospitals. Antimicrobial resistance was most frequently seen among gram negative organisms. Early onset sepsis was more common.

### **Examining Cultural Meanings, Experiences And Behaviours Associated With Child Malnutrition In Tribal Communities of Maharashtra To Contribute To Malnutrition Control Strategies**

Mokhada, situated in Palghar District of Maharashtra, India is a tribal region with one of the

highest number of undernutrition cases in under 5 years children. This study focused on assessing local perceived causes of undernutrition in the Mokhada block of Palghar amongst Thakur and Warli tribal communities. Qualitative study was undertaken to understand the local terminologies used to denote undernutrition, community's perception on undernutrition, its causes and the help seeking practices of the community on account of undernutrition in children under- 5 years of age. Data was collected in two phases through focus group discussions (n=7), key informant interviews (n=12), in-depth interviews (n=15) and EMIC semi-structured interviews (n=164) which recorded notions about healthy baby, small baby size and its association with food, proscribed and prescribed food practices during pregnancy and after delivery. The perceived causes of under nutrition, nutrition related health practices during pregnancy and lactation, services provided by Anganwadi and hygiene/sanitation related practices in the household were documented.

A major cause for under nutrition in children as perceived by community was lack of proper child care practices. Data on food provisioning revealed that while the caloric needs of the community were substantially met by consumption of cereals and pulses which were grown on their farms, minimal consumption of other vegetables could lead to micronutrient deficiency. Cereals and pulses were mainly cultivated in monsoon which declined drastically in summer. The benefits of government schemes, though targeted at malnourished children were often shared by the entire household and thus got diluted. Proscriptions of food items on account of traditional beliefs and practice of 'Sidva' during pregnancy and post-delivery both by mother and the child may be other factors contributing to under nutrition in these tribal communities. Key finding was that nutrition interventions should be designed to address the entire household and emphasis should be given to appropriate nutrition education, without which distributing food, increasing income or introducing other interventions would have minimal effect.

### **Clustering of infant and child mortality among Primitive Tribes (PVTGs) in Odisha.**

The objective of the study was to assess family level death clustering by socioeconomic risk factors among various PVTGs in Odisha, examine the community level factors which influence the infant and child mortality, relative role of the individual mothers competence/genetic frailty versus environmental/community factors influence on child death clustering and assess the comparative picture of extent of clustering among various PVTGs. Out of 13 PVTGs in Odisha, four major PVTGs was selected purposively for the study. The sample was classified into two groups; case (women who have experienced multiple child deaths) and control (women who have not experienced any child deaths). Accordingly, 100 women from case group and 50 women from control group were selected from each tribal group. The quantitative survey techniques were used to collect data from 587 respondents from seven districts. Qualitative data was also collected from the key respondents through case study method. Total 10 case study was conducted to examine socio-cultural aspects of death clustering. Findings of the study indicate that the mean child death was 2.31. The mean child death was higher among the women who belonged to Hindu religion and got married at the age of 10-15 years. The women who availed private healthcare facilities have experienced more cases of child deaths compared to the mothers who availed public healthcare facilities. The study also reveals that the husbands of the control group respondents were playing an important role in child care. Study also highlights that the women in case groups were less aware of the various health programs of the government. Study also highlights on the very fact that life style indicators can significantly affect the death clustering among the PVTGs. The PVTGs who are experiencing multiple child and infant deaths were more into higher consumption of tobacco, facing more domestic violence and also less educated.

### **Comparison of the efficacy of a 7-day versus a 14-day course of intravenous antibiotics in the treatment of uncomplicated neonatal bacterial sepsis: a randomized controlled non-inferiority trial**

The standard care for neonatal septicemia is intravenous antibiotics. Most authorities advocate 14 days of intravenous antibiotics for blood culture positive neonatal septicemia. The objectives of the project are to evaluate whether a 7-day course of intravenous antibiotics has a relapse rate that is inferior to a standard 14-day course of antibiotics, in uncomplicated blood culture proven septicemia and who are in clinical remission by the end of the fifth day of intravenous antibiotics. There are 6 participating centres- Chacha Nehru Bal Chikitsalaya N. Delhi, PGIMER Chandigarh, Lady Harding Medical College, N. Delhi, Institute of Child Health Chennai, St. Jones Medical College Bangaluru, Rohtak Medical College.

### **Etiology of childhood pneumonia in India**

Multicentre study is being initiated at five tertiary care as well as involving community site. Primary objective of the study is to estimate the relative prevalence of selected bacterial and viral pathogens in cases with clinically diagnosed community acquired severe pneumonia and in healthy controls among children, aged 29 days to 59 months of age. It is expected that the study will generate valuable data on bacterial as well as viral pathogens, serotypes, antimicrobial susceptibility that will help to formulate preventive as well as case management strategies.

### **EMF HEALTH**

#### **Effect of Non-ionizing Electro Magnetic Field (EMF) on Human Health**

This is a multidisciplinary, prospective cohort study going on at seven departments of AIIMS, New Delhi and at Jawaharlal Nehru University, New Delhi. After fulfilling the inclusion and exclusion criteria, 2817 male and female subjects have been enrolled under highly exposed (978), moderately

exposed (1221) and control group (618). Currently, the follow up of the enrolled subjects is on going, on yearly basis for clinical and laboratory examinations. Simultaneously, to complete cohort of the study, the enrolment of new subjects is also going on.

### **Health survey of the people residing near cell phone tower**

A health survey of the people residing near cell phone tower was conducted. The data of 718 subjects of 18-45 age groups including 567 males and 151 females residing near cell phone towers at various distance i.e. at 10m, 50m, 100m, 300m and more than 300m was recorded through a prescribed proforma. This indicated that the Epworth Sleepiness Scale (ESS) was noticed, highest both in male and female subjects, residing at 10m distance from the cell phone tower and this score was decreasing as the distance increases. The following symptoms were reported more in subjects residing at 10m of distance from cell phone tower and the percentage of people reporting these symptoms decreased as the distance increased with minimum observed value beyond 300 mtrs.i.e. Mental stress (50-24.6%), Headache (47.6-13.1%), Depression (23.5-3.3%), Nausea (41.2-34.3%), Vomiting (23-16%), Slurred Speech (14.8-6.7%), Fever (47-29.5%), Fatigue (35.2-21.3%), etc.

### **INSTITUTIONAL STRENGTHENING AND CAPACITY BUILDING**

#### **National Animal Resource Facility (NARF) for Biomedical Research**

The activities of the Project Management Consultant of the project NARF-BR i.e. CPWD is being monitored by the Project Executive Committee (PEC) on monthly basis and Project Management Committee chaired by Secretary, DHR, review the progress after every three months. Based on the recommendation of PEC and the consultant & architect appointed by the CPWD, all the drawings have been finalized and submitted to the State Government Authorities for their approval and clearances.



### **Multidisciplinary Research Units- MRU in North eastern states- with ICMR support**

This activity is to bridge the gap in the infrastructure which is inhibiting health research in the Medical colleges, by assisting them to establish research laboratories and animal house facility with a view to improve health research and health services. Five States Medical colleges have been getting support for five years to develop research capacity. Equipment and logistic budget infrastructure is approved to carry out basic, clinical, translational research and develop innovative methods to combat health problems in North-East Region. Equipment e.g. Auto analyser, Trinocular Microscope, Fluorescent microscope imaging system & motorised stage, Table top refrigerated centrifuge, Microfuge (refrigerated) Centrifuge, Minicold lab Refrigerator etc, 4 staff, related logistics are being provided. The brief update on activities is as follows:

MRU at Nagaland: Healthcare Laboratory & Research Centre started functioning in 2015 and have done following work. Four task force studies were done on Nutrition, breast cancer, genetic research. Scrubtyphus : Survey and awareness at 4 villages under Phek and Peren districts of Nagaland was done. Paragonimiasis: Survey and awareness of Paragonimiasis was carried out at Pfutsero and Pholami villages of Phek district.

Multi-Disciplinary Research Unit, Agartala Govt. Medical College, Agartala, Tripura: The unit has identified location, established logistics and currently is in process to recruit staff and equipment.

Hospital & Medical Education, Govt. of Mizoram, Dintar, Aizawl-Started in 2014. The unit is being used for routine investigations of hospital in the region, and planning to undertake research also.

Multidisciplinary Research Laboratory Assam Medical College & Hospital, Dibrugarh: 13 PhD thesis research and 2 ICMR ad- hoc studies were initiated during last year, apart from 20 ongoing studies, utilizing this lab facility. Apart from serving routine test laboratory to hospital services, the research findings were published.

Tomo Riba Institute of Health & Medical Sciences, Naharlagun, Arunachal Pradesh: After sorting location issues, this site is now undergoing establishment.

### **Seed Grant scheme**

In order to build capacity among faculties of medical colleges, universities and research institutes, for carrying out research in health and biomedical sciences, to address local health issues in the North East states, Seed Grant Scheme is ongoing since 2011. During the year of reporting, 13 new projects were approved and 48 projects are ongoing.

# NUTRITION

The ICMR continues to be instrumental in developing public health activities with many significant contributions in the country in the field of Nutrition. It has been made possible by undertaking the laboratory and hospital based research with community based participation. The salient features of various research activities undertaken during 2016-2017 are given below.

## *Intramural Research*

### NATIONAL INSTITUTE OF NUTRITION

#### COMMUNITY STUDIES

- District level mapping of under nutrition among under five years was carried out in the state of Madhya Pradesh. The overall prevalence of under nutrition among <5 y children - underweight, stunting and wasting had declined from 52%, 49% and 26% during 2010 to 42%, 43% and 21% respectively in the present survey.
- A study was carried out among 437 subjects to estimate the 24-hour urinary sodium excretion levels, as well as spot urine examination, in three cities viz., Hyderabad (Telangana), Vijayawada (Andhra Pradesh) and Guwahati (Assam) in order to validate the sodium consumption levels assessed by 24-hour dietary recall method during 2016. There was an acceptable agreement between the two methods employed for using sodium level estimation.
- Diet and Nutrition status of urban population and Prevalence of Obesity, Diabetes, Hypertension, Dyslipidemia among urban adults was done as

part of NNMB urban surveys, 2014-16, in 16 states of the country, following standardized methodology, equipment and procedures. The prevalence of Hypertension among men and women was high in the states of Assam (46.9%), Kerala (46.6%) and Maharashtra (43.4%) and lowest in Madhya Pradesh (28.2%). The prevalence of diabetes among men was low in Madhya Pradesh (17.6%) and high in Puducherry (41.2%), while among women, low in Orissa (13.6%) and high in Puducherry (36.6%). 40% men and 28% women were observed to have high triglyceride ( $\geq 150$ mg/dL) levels.

#### BASIC STUDIES

- Indian Food Composition Tables – 2017 covers all the key foods sampled from the entire country drawn from statistically valid sampling method. About 160 discrete food constituents including bioactive compounds were analyzed for 528 foods. The database is available in the form of a book and freely downloadable e-version and a mobile app based on IFCT-2017 is under preparation, for public use.
- Studies demonstrated amelioration of neuronal cell death in a spontaneous obese rat model by dietary restriction, through modulation of ubiquitin proteasome pathway system.
- Studies demonstrated that obesity with impaired glucose tolerance impacts retinal structure and function in a rat model of metabolic syndrome, which is mediated by elevated sorbitol, through ER stress and insulin receptor pathway.

## CLINICAL STUDIES

- After 30 days of oral probiotic or placebo supplementation, clinical evaluation and microbiological evaluation showed significant reduction in BV in both the groups (probiotic and placebo) during all the three follow-ups. However, the vaginal and fecal microbiome analysis showed very encouraging results with probiotic supplementation.

## MICRONUTRIENT RESEARCH

- Studies have shown that a minimum of 2.5% dietary fat, rich in unsaturated fat, is required for optimal carotenoid absorption. Fish oils/PPAR Alpha Agonists (fibrates) down regulate the carotenoid absorption and conversion via down regulation of lipid transporter expression.
- Sodium Hexametaphosphate (SHMP), a stabilizer that is used in Double Fortification of Salt with iron and iodine was found to be a promoter of iron absorption in the intestinal cells.
- Studies identified zinc rich rice and carotenoid rich maize as part of the ICAR-CRP on bio-fortification. The bioavailability of these nutrients studied using coupled *in vitro* digestion/Caco-2 cell model was also relatively higher compared to respective controls.
- An indigenous technology for fortification of rice using extrusion technology, was developed as part of PPP project with industry. The human studies are being initiated during the current year.

## STUDY BASED TRAINING

- A study assessed health seeking behaviour, food beliefs and practices among Chenchu tribe women during different physiological phases of life. Majority (76%) women utilize the services of the government healthcare system. Millet acceptability was high, but consumption is less frequent. About 80% of the women get married before the age of 18 years. The women usually do not take any additional food intake during pregnancy. It was found that during lactation (first six months), most of the foods are avoided except

rice and spicy chilli powder, which often leads to malnutrition of both mother and child. At the end of the project, one day dissemination workshop on “Health and Nutrition Awareness” was conducted for various stakeholders. IEC materials developed, based on information collected from Chenchu tribe such as folders, charts were distributed among the participants as a source material to educate the women.

## FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

- When Organophosphorus pesticide exposure of urban children was assessed through bio-monitoring of different age group children with different socioeconomic status, it was found that the concentrations of OP pesticides (chlorpyrifos and fenitrothion) were more in High Income Group and Middle Income Group as compared to Low Income Group subjects.
- Dietary exposure assessment of Polychlorinated Biphenyls (PCBs) has shown that the estimated daily intake of PCBs through chicken consumption has exceeded the concentration levels suggested by Environmental Protection Agency (EPA, US) (1 to 4 Pico grams per day for 1 kg weight of body mass). Therefore, continuous monitoring of PCB contaminants is needed to mitigate the impact of these contaminants on human health and the ecological environment.

## NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

- Role of calorie restriction on pathophysiological changes in tongue fat and its relation with Obstructive Sleep Apnea (OSA) in WNIN/Ob obese rats was studied. The outcome showed that calorie restriction can be used as a non-pharmacological intervention therapy for obesity associated OSA.

## MAJOR ACHIEVEMENTS OF PUBLIC HEALTH IMPORTANCE

- Indian Food Composition Tables – 2017 which covers all the key foods sampled from the entire country drawn from statistically



valid sampling method is available in the form of a book and freely downloadable e-version. This data is useful for multiple stakeholders including policy makers, industry and researchers.

- District level mapping of undernutrition among children (below five years of age) was carried out in the state of Madhya Pradesh. Results would be helpful for efficient implementation and targeting of nutrition alleviation programmes.
- A study validated the assessment of sodium consumption levels, assessed using 24-hour dietary recall method and proved that it would be as useful a tool for assessing sodium consumption as examination of 24-hour urinary sodium excretion levels.
- Diet and Nutrition status of urban population and Prevalence of Obesity, Diabetes, Hypertension, Dyslipidemia among urban adults was carried out. This data would be useful for public health programmes.
- An indigenous technology for fortification of rice using extrusion technology was developed as part of PPP project with industry.

### **Extramural Research**

#### **TASK FORCE STUDIES**

Two new studies have been initiated.

- (1) Assessment of iodine status among pregnant women in selected districts of India. The study is being carried out at total 10 locations in the country. The objective of the study at six centres (Una, Himachal Pradesh; Ghatampur, Uttar Pradesh; Kamrup, Assam; Khasi hill, Meghalaya; South Tripura, Tripura; Palghar, Maharashtra) is to undertake cross sectional study to assess the urinary iodine level among pregnant and non-pregnant women. The study being done at four centres (Jaislmer, Rajasthan; Yadadribhu Nagar, Telangana; Gejapati, Orissa; Ballabgarh, Haryana) is a longitudinal study to assess the serum/plasma micronutrient level, thyroid profile and

urinary iodine level of pregnant women during first, second and third trimester as well as at the time of delivery, at 6 months and at one year after delivery and to also assess the new borns for birth weight and growth up to one year of age at four of the total ten participating centres.

- (2) Prevalence of fluorosis in the community of selected districts of India and development of an appropriate intervention model for prevention and control of fluorosis at 7 locations in the country (covering one district each in Orissa, Madhya Pradesh, Telangana, Punjab, Bihar, Rajasthan and Assam).

A significant progress has been made in other two ongoing task force studies namely; (i) Effectiveness of diet and lifestyle intervention through IEC tools with Angan Wadi Centres as the centre of knowledge dissemination for hypertension risk reduction; (ii) Improving health and nutritional status of vulnerable segment of population by implementing multi-component health and nutrition education intervention as sustainable model of intervention. The preliminary analysis of these studies indicated the prevalence of Hypertension and Diabetes in adult population is around 25% and 10% respectively. The study also revealed that while there is a progress in exclusively breast feeding practices, the problem of supplementary feeding is also at large and need to be addressed with appropriate intervention.

#### **CENTRE OF EXCELLENCE**

A new Centre of Excellence for Fluorosis Research and Mitigation of the Diseases was initiated with the main objectives of human resource development and skill development.

#### **CENTRE FOR PROMOTION OF NUTRITION RESEARCH AND TRAINING WITH SPECIAL FOCUS ON NORTH-EAST, TRIBAL AND INACCESSIBLE POPULATION**

The centre continued to support lab work of various task force studies of ICMR and research work of Ph.D and DM scholars for analysis of blood, blood

serum and urine samples for various micronutrients, lipid profile etc.

Around 70 research studies, in the form of adhoc projects and fellowship proposals, are being supported for various investigators and young

scientists of the country. These scientific studies are on nutritional status of vulnerable population, maternal and child health, food fortification and role of various micronutrients.



**Fig.1.** Proforma filling, anthropometry measurement and dietary survey at a village of Gurgaon (Haryana) under the project “Improving health and nutritional status of vulnerable segment of population by implementing multi-component health education intervention as a sustainable model”.



# ENVIRONMENTAL & OCCUPATIONAL HEALTH

**R**esearch in priority areas of occupational and environmental health relevant to national needs for various working groups is actively undertaken by the National Institute of Occupational Health, Ahmedabad and the National Institute for Research in Environmental Health, Bhopal. Major highlights of various programmes undertaken by the ICMR in the areas of occupational and environmental health during the year 2016-2017 are given below.

## *Intramural Research*

### **NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH, AHMEDABAD**

**Study of occupational diseases and hearing impairment of Coal Mine workers of BCCL directly involved in active mining operations**



**Fig.1.** An occupational health study was conducted involving different mines of Bharat Coking Coal Limited (BCCL), Dhanbad.

An occupational health study was conducted involving different mines of Bharat Coking Coal Limited (BCCL), Dhanbad. This study was undertaken among the exposed workers, mainly from active mining activity. The study covered 351 subjects from Kustore sector and 140 subjects from Bagmara sector of BCCL Collieries. The subjects were selected from workplaces identified by BCCL, Govt. of India, thus results may be restricted to the concerned workplaces.

The mean age of the workers was  $45.9 \pm 8.36$  years. Mean job experience was  $11.72 \pm 8.49$  years. Most common symptom complained by the subjects was musculoskeletal pain (34%). Other complaints were cough, difficulty in breathing, chest pain, soreness of mouth, etc. About 20% subjects had systolic blood pressure  $>140$  as well as diastolic blood pressure  $> 90$  mm of mercury. About 3% subjects had restrictive type of abnormality (FVC/PFVC  $< 80\%$ ) and 0.6 % subjects had combined type of abnormality (FVC/PFVC  $<80\%$  and FEV1%  $<70\%$ ) on spirometric examination. Mean FVC values were significantly lower among the subjects of age 45 years or above. Chest radiographic findings showed that 93% subjects had findings within normal limits and 3% subjects showed findings suggestive of opacities in the lung field on chest X-ray. Such findings may be due to pneumoconiotic changes in the lungs. Haematological and biochemical findings of the subjects were mostly within normal limits. As far as ophthalmological findings are concerned, 5%



subjects had uncorrected vision and 9% subjects had cataract in eyes. Decline in hearing ability with increasing duration of exposure was observed more at higher frequency; however, this observation is subject to adjustment for age and other probable factors.

Prevalence of musculoskeletal pain during work in workers reflects that manual work of the processes might be causing some discomfort for the workers. Training on proper method of manual material handling may prove useful to these workers. Regular proper exercise should be promoted and periodic relevant examination (lung function test, audiometry) at regular interval is recommended. Industrial hygiene survey (periodic monitoring of dust and other environmental hazards) at regular intervals should also be undertaken including noise level monitoring in different operations. Measures like using protective appliances (e.g. PPEs), pre-placement- and periodic medical examination, for the control and prevention of relevant health hazards, are to be implemented to protect the health of the workers.

#### Study on Sero-Prevalance of Brucellosis among the Abattoir workers in Kolkata, West Bengal

An epidemiological study was conducted to find out the sero-prevalance of Brucellosis involving 15 abattoir workers of two slaughter houses of Kolkata. The study components, questionnaire



Fig.2. Study on sero-prevalance of Brucellosis among the Abattoir workers in Kolkata.

survey to collect demographic and occupational details, clinical examination, pulmonary function test, complete blood count, routine and microscopic urine analysis and serological examination were performed. Serological examination was done using *Brucella* specific IgG and IgM ELISA test, and preliminary detection test like RBPT test was also performed. Seroprevalence of *Brucella* was measured in the serum samples of the subjects by *Brucella* specific IgM and IgG ELISA kit method. All the 151 subjects' serum was tested separately for detection of IgG and IgM antibodies in the serum sample.

Mean age of the abattoir workers was  $35.6 \pm 12.7$  years. With regards to the personal habits, 86% of subjects were non-smokers, 44% were tobacco chewers and 15% were occasional alcohol consumers. Mean job experience was  $11.24 \pm 12.29$  years. Mean experience was a little higher in butcher worker group than other staff. About 15% subjects reported cough. Among the other symptoms were frequent headache (30%) followed by breathlessness and chest pain while walking uphill. Mean systolic and diastolic blood pressure was  $128.87 \pm 16.47$  and  $84.29 \pm 12.19$  mm of Mercury. About 22% subjects had systolic blood pressure  $>140$  as well as diastolic blood pressure  $> 90$  mm of Mercury. 5% workers had only higher systolic blood pressure and 14% had only higher diastolic blood pressure. As far as pulmonary functional status, about 15% subjects had restrictive type of abnormality ( $FVC/PFVC < 80\%$ ) and 2.0% subjects had combined type of abnormality ( $FVC/PFVC < 80\%$  and  $FEV_{1\%} < 70\%$ ). Ten percent subjects had obstructive findings ( $FEV_{1\%} < 70\%$ ). A good number of subjects (25%) had  $FEV_{1\%}$  values between 70% - 80%. Twenty six subjects showed abnormalities in their urine sample, either one or more than one parameter studied. The IgG and IgM *Brucella* positive cases were found in 5.96% and 13.24% respectively, irrespective of their age.

**Environmental stressors and workplace hazards in wood and iron handicraft industries and identification of personal protective equipment for artisans**

A study was conducted to identify physical and ergonomic hazards at wood and iron handicraft industrial clusters in and around Jodhpur city. The exposure assessment for wood and metal dust, welding fumes, noise and heat stress was evaluated.



**Fig.3.** Environmental stressors and workplace hazards in wood and iron handicraft industries and identification of personal protective equipment for artisans.

The study found higher concentration of wood and metal dust, gaseous pollutants and also high noise level and hot environment in different work areas. Based on the study results and observations, suitable personal protective equipment for the artisans and local exhaust ventilations and proper work practice controls to prevent fire hazard and to reduce wood and metal dust and other physical stressors - noise and heat stress, were suggested to safe guard worker’s health.

**Assessment of Occupational, Environmental Health risks and challenges of migratory brick kiln workers in Gujarat**

The study was carried out in 10 brick kiln industries situated in and around Adalaj and Uvarsad villages of Gandhinagar district, Gujarat. Migratory brick kiln workers (273) were enrolled. The average age of workers was 29.8±10.0 years and about 87.5% workers were between 18 to 45 years, with majority of them being uneducated (52.0%). Most of the brick kiln workers were migratory and belonged to

Uttar Pradesh (39.9%) and Chhattisgarh (47.6%) state.



**Fig.4.** Assessment of occupational, environmental health risks and challenges of migratory brick kiln workers in Gujarat.

The personal habit data revealed that 21.0% workers had smoking habit (bidi), 58.6% had chewing habit (tobacco & gutakha/khaini) and 34.4% reported alcohol abuse (especially local liquor). The Body Mass Index (BMI) of these workers indicated 54.6% workers with lower BMI <18.0 i.e. underweight; which clearly indicates poor nutritional status. The data on duration of work showed that majority (83.9%) of workers worked continuously for 8 to 12 hrs per day and 53.9% of workers complained about suffering with bodyache. The major clinical signs and symptoms reported by the workers were headache, fever, respiratory infections, asthma, chronic bronchitis, musculoskeletal disorders, throat infections, etc.

**Effectiveness of a cooling jacket with reference to physiological responses in iron foundry workers**

The study was conducted in hot environments of iron foundry to evaluate the efficacy of a battery operated Personal Cooling Garment (PCG). Seventy-four workers were exposed to climatic conditions of 38.5 ± 3.7°C DB temperature, 35% RH during a 90 min work with PCG and without PCG (with Habitual Clothing-HC). Mean weighted skin temperature was significantly lower by 4.2±1.3°C as compared to HC 0.4±0.9°C (p<.05). A statistically significant difference was also observed for mean sweat loss with PCG group 0.447 ± 0.27 g as compared to 0.823 ± 0.42 g in HC group (p<0.05). Heart rate, back and chest



skin temperatures were comparatively more reduced in PCG group as compared to HC group. PCG provides a practical and economical way of alleviating the physiological effects of heat stress, when environmental control is not feasible.



**Fig.5.** Effectiveness of a cooling jacket with reference to physiological responses in iron foundry workers.

#### Indoor air pollution from biomass combustion and health hazards among people residing in slum areas of Ahmedabad

The study aims to find out adverse effects of air pollutants especially Polycyclic Aromatic Hydrocarbons in 43 urban slum houses where biomass is used as the cooking fuel. The pollutants SO<sub>2</sub>, NO<sub>2</sub> and particulate matter were also quantified in the selected houses. Medical examination and Pulmonary Function Test of all the family members, along with the primary cook were performed. Urine samples were collected after cooking, from the selected women subjects, using wood as a cooking fuel. 1-HOP was measured from the samples by HPLC method using Fluorescence detector.



**Fig.6.** Indoor air pollution from biomass combustion and health hazards among people residing in slum areas of Ahmedabad.

The results of the indoor monitoring showed the presence of 12 PAHs namely Naphthalene,

Acenaphthalene, 1-Methylnaphthalene, 2-Methylnaphthalene, Fluorene, Acenaphthene, Fluoranthene, Pyrene, Benzo (a) Anthracene, Benzo (a) Pyrene, Dibenzo (a,h) and Anthracene, which are reportedly hazardous for human health. The Maximum concentration (1296.9 µg/m<sup>3</sup>) was found of Naphthalene and a minimum (45.43 µg/m<sup>3</sup>) for Anthracene. Pulmonary Function Test of subjects showed 10 restrictive, 5 obstructive and 3 combined types of impairment. Results from the urine sample analysis have shown the presence of 1-HOP, a biomarker for PAH exposure.

#### Personal and biological monitoring of workplace pollutants and their potential health effect indices assessment in fuel filling station workers

This study investigated the personal and biological monitoring of workplace pollutants and their potential health effect among petrol fuel filling station workers. A total of 92 subjects (exposed 67; control 25) with the mean age of exposed and control subjects 26.7±6.1 yrs and 24.6±13.4 yrs respectively, participated. Blood and post shift urine samples were collected from each fuel filling station workers and control subjects.

About 34 percent of workers use personal protective equipment. The average working hours of exposed male and female workers were 15.2±6.1 and 8.4±1.2 hours per day, respectively. Elevated urinary 8-OHdG and high frequency of DNA damage was observed in exposed subjects as compared to control. The data indicates that continuous such exposure can lead to deleterious effects on worker's health.



**Fig.7.** Personal and biological monitoring of workplace pollutants and their potential health effect indices assessment in fuel filling station workers.



### Environmental cum epidemiological study on Arsenic toxicity from surface and underground water in and around Kaudikasa village, Rajanandgaon, Chhattisgarh

A detailed environmental cum epidemiological study has been conducted to assess the Arsenic in surface and underground water sources of Kaudikasa village, and surrounding areas of Rajanandgaon, Chhattisgarh, and also in river water at different locations at the upper, middle and down streams of the river at 0.5 KM distance. The Epidemiological study was conducted to determine prevalence of Arsenic related skin lesions in the village population. This study covered a sample size of 305 subjects selected randomly. Questionnaire survey, medical examination, estimation of drinking water Arsenic as well as hair and urinary Arsenic level was included.



**Fig.8.** Environmental cum epidemiological study on Arsenic toxicity from surface and underground water in and around Kaudikasa village, Rajanandgaon, Chhattisgarh.

Drinking water of only 6% subjects had Arsenic (As) level more than WHO recommended level (10 µg/l). Skin lesions in the form of pigmentation (15%) and keratosis (8%) was found in some subjects. No significant relation could be established with present level of As in drinking water and skin morbidities. No relation between skin morbidities and urine As level indicates that present exposure has very little effect on presence of lesions. Water samples were examined from different water sources, which showed that ground water and stream water at some places had higher As levels. This has important implications for selecting site for As removal plant for supplying As free water to community.

### Biomarkers and gene polymorphisms to predict association of chronic environmental Organo-phosphorus pesticide exposure with neurodegenerative diseases: A case control study in rural West Bengal

A study was initiated to find out an association of chronic environmental pesticide exposure with neurodegenerative diseases among directly and indirectly exposed population of rural W. Bengal. For the pilot phase, screening of subjects was taken up among directly exposed population in a high pesticide exposure block (Galsi II) of Bardhaman district. Samples were drawn from subjects of 50 and above years of either sex, living in the area for at least last five years. A set of general questionnaire based assessment of a person's (1) memory, (2) mood and (3) motor problem, observed over last 6 months as primary screening for neurodegenerative disorders, was applied. Any participant screening "positive" for either of three diseases, fulfilled criteria for recruitment as a "case"; and a negative screener for all three diseases as a "control". Following this, the socio-demographic instrument including exposure status, i.e., "direct" or "indirect" and the KAP instrument were administered. A total of 164 subjects were screened; n=51 (31%) subjects fulfilled criteria for cognitive impairment or possible depression or both, on primary screening and were designated as "cases"; remaining n=113 (69%) screening negative participants were enrolled as "controls". Screen positive for Parkinson's disease was not detected. Among the determinants of pesticide exposure, croppers (p=0.01) and agricultural activity of more than 8 hours in a day (OR=1.70) had significant associations for detection as a "case". Other significant associations were high risk pesticide use practices, e.g., not following instructions about using pesticides (OR=2.0), spraying pesticides in windy condition (p=0.04) and not using any personal protective device (OR=1.57). This study has identified a high prevalence of cognitive impairment, possible depression or both, among rural, agricultural workers. High risk pesticide use, not following instructions about use of pesticides, spraying in windy condition and not using any

personal protective device by the workers were identified as risk factors.



**Fig. 9:** Biomarkers and gene polymorphisms to predict association of chronic environmental Organo-phosphorus pesticide exposure with neurodegenerative diseases.

### Effects of lead exposure on human kidney cells ACHN using molecular methods

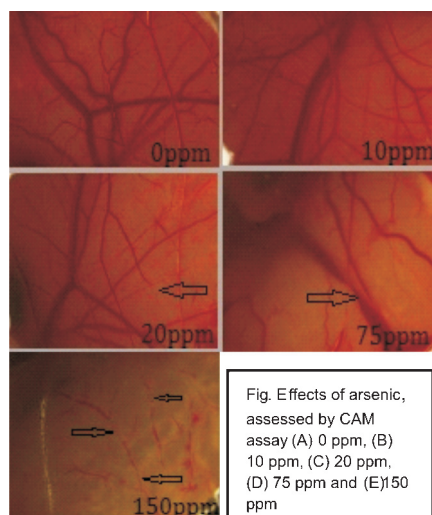
Lead is an important environmental and occupational pollutant, which can cause nephrotoxicity. Early detection of renal diseases to nephrotoxic chemicals is limited by the lack of sensitive or chemical specific tests. There are evidences that support Kidney Injury Molecule-1 (KIM-1) as a specific marker for nephrotoxicity. The effects of different doses of lead on human renal adenocarcinoma cells (ACHN) was evaluated after 24 hrs, 48 hrs and 72 hrs of treatment. Relative KIM-1 gene and protein expression, cell viability, apoptosis and necrosis were analyzed after treatment of ACHN. Dose dependent cytotoxicity of lead was observed. Down regulation of KIM-1 gene and protein expression was observed after 48 and 72-hour treatment of cells. Results also suggested dose and duration dependent apoptosis and necrosis of ACHN cells treated with lead acetate.

### Effect of Arsenic on vasculogenesis assessed by Chick Chorioallantoic Membrane (CAM) assay and determination of the molecular lesions affecting the placenta

The study was carried out to understand the effect of short and long term Arsenic exposure through drinking water on VEGFA, PIGF and HIF 1 $\alpha$  gene and protein expression in placental tissues of mice. For this purpose, female Swiss albino mice were exposed to different doses of arsenic through drinking water for 30 and 90 days. VEGFA, PIGF and HIF 1 $\alpha$  protein levels were decreased significantly in all arsenic treated groups. In placental tissues of dams on Gestational Day (GD) 12.5 treated with Arsenic for one month, expression of all four genes i.e. VEGFA, VEGFB, PIGF and HIF 1 $\alpha$  were down regulated. Expression of VEGFA, VEGFB, PIGF and HIF 1 $\alpha$  gene has down regulated in both the treatment groups, i.e. 1 and 3 months and in all doses, except 150 ppm. Similarly, protein expression is also reduced in all treatment groups as compared to control.

*In-vivo* angiogenic effects of Arsenic were also assessed by using chick embryo chorioallantoic Membrane (CAM) assay. The CAM assay revealed that the angiogenesis parameters such as number of junction, total tubule length, number of tubules and average tubule length were affected by Arsenic. The number of junction was decreased in all the Arsenic treated groups which were significantly lower in 150 ppm Arsenic exposed group. Total number of tubule significantly decreased in 75 and 150 ppm Arsenic exposed groups. The total tubule length and mean tubule length was also statistically nonsignificant in all Arsenic treated groups. The percentage vessel inhibition assessed by ImageJ software was statistically significant in 20, 75 and 150 ppm Arsenic treated groups as compared to controls. In conclusion, decreased levels of VEGFA, PIGF and HIF 1 $\alpha$  proteins, and downregulated gene expression of VEGFA, VEGF B, PIGF and HIF 1 $\alpha$  in placental tissues of As treated dams (on GD 12.5) alongwith maximum vessel inhibition observed by CAM assay at 75 and 150 ppm arsenic treated groups indicated that Arsenic caused deleterious effects on placental vasculogenesis.





**Fig.10.** Effect of Arsenic on vasculogenesis assessed by chick chorioallantoic membrane (CAM) assay and determination of the molecular lesions affecting the placenta.

**Interaction between Nrf2 and BDNF-dopaminergic circuit in brain and testis: Molecular mechanism for development of nicotine-induced drug addictive neurobehavioral disorders in rats**

The aim of the study was to explore the toxic impact of short and long-term exposure to oral nicotine and passive cigarette smoking on the adult albino Wistar rats, where doses closely mimic the human smoking scenario. The results showed dose and time-dependent loss of developing spermatogonia and spermatocytes of the seminiferous tubules, disruption of basement membrane, DNA damage, and high serum cotinine upon exposure to nicotine and cigarette smoking, resulting in low sperm count as compared to the control. Further, the results showed the up-regulation of BDNF, dopamine, tyrosine hydroxylase, and pro-oxidants, i.e., ROS and iNOS in the exposed testis; the down-regulation of antioxidants such as ascorbate and Nrf2 when compared with the controls. Thus, results highlight a potential role of the local neurotransmitter system and antioxidant depletion (Nrf2) in nicotine/cigarette smoking-induced testicular pathogenesis, which could underpin the development of therapeutic interventions targeted at oxidative stress-associated disorders and probably establish a link with the brain system contributing addiction. Further, result showed dose-dependent

association of stress/depressive behaviour, and cognitive interference upon exposure to nicotine/CS as compared to the controls.

**Workshops/ Training programmes/ Conferences /Seminars**

- National Seminar on Environmental and Occupational Health, 5-6 June, 2016 on the occasion of world Environment Day.
- Three Days Workshop on Statistical Methods in Biomedical Research, in Collaboration of National Institute of Medical Statistics, New Delhi 11-13 May, 2016.
- Three Days Workshop on Statistical Methods in Biomedical Research, in Collaboration of National Institute of Medical Statistics, New Delhi, 4-6 January 2017.
- Occupational Health & Safety Management System (OHSMS) 18001:2007/updated version and OHSAS, 9-13 January 2017 (CPCB Sponsored).
- Occupational and Environmental Health problem & solution 2017, II<sup>nd</sup> National Scientific Hindi Symposium.

**PUBLIC HEALTH PROGRAMMES**

**May Month Measurement 17: Blood Pressure awareness programme at ICMR-NIOH, Ahmedabad**

Raised BP is the biggest single contributing risk factor to global death and to the global burden of disease (*Lim et al., 2012. Lancet:380:2224*). This impact is largely mediated through increased rates of coronary artery disease, stroke and renal disease. The aetiology of raised BP is largely explicable by identified environmental factors such as overweight, excessive intake of alcohol and dietary salt, and insufficient exercise (*Poulter et al., 2015. Lancet:386:801*). Despite the availability of these antihypertensive medications, global data suggest that less than half of those classified as hypertensive, are aware of their problem (*Chow et al., 2013. JAMA: 310:959*). It is clear that a huge beneficial impact on mortality and reduction in this burden of disease can be achieved by increasing awareness through enhanced screening for raised BP.



A cross-sectional Blood Pressure (BP) survey of volunteer adults (aged  $\geq 18$  years) who had not their BPs measured for at least a year before the recruitment was done. National Institute of Occupational Health Ahmedabad had taken part in this Anti hypertension campaign awareness programme along with its regional centres (ROHC Kolkata, ROHC Bengaluru).

Total population covered under awareness programme was 7560 (male=5346, female=2214). 699 individuals were found to be hypertensive (140/90 mm Hg). Overweight (BMI>25) population was 2958/7650 (39%). Out of overweight population, 413 individuals found to be hypertensive.

539 individuals out of 699 hypertensive individuals (i.e.77%) were unaware about their hypertensive status, which is very significant value and which emphasizes the need of awareness as well as proper medication. In awareness programme, centre has given top ten tips verbally in regional language to each subject and also provided printed information in Hindi and English. This awareness programme will have definite impact on lifestyle changes among these people.

### POISON INFORMATION CENTRE

Poisoning is a major public health concern worldwide. Poison Information Centre at NIOH (PIC-NIOH) established in the year 1993, is one of the five WHO recognized poison centres in India. PIC-NIOH has been involved in public health related services like laboratory based investigations of poisoning cases, toxico-vigilance, teaching and training of health professionals related to poisoning cases. Centre also collect epidemiological data on poisoning cases in relation to occupational health and the data is provided to WHO. This includes data on human exposure to chemicals, information about the agents involved, the circumstances giving rise to exposure, and the health ill effects of exposure. The PIC-NIOH has been enrolled in the 'World Directory of Poison Centres' by WHO.

In the time frame of 1<sup>st</sup> April 2016 to 31<sup>st</sup> March 2017, a total of 583 poisoning cases were referred to PIC-NIOH. The centre investigated cholinesterase

activity in blood samples by testing the plasma as well as RBC cholinesterase activity, which is the most reliable marker for acute organophosphorus poisoning. The epidemiological data in relation to the referred poisoning cases was also collected and documented.

### Information System (ENVIS) on Environmental and Occupational Health

Environmental Information System [ENVIS] Centre, NIOH, sponsored by Min. of Environ, Forests & Climate Change, is working since 1984, with the objective to establish linkages with all information sources, & creation of data bank on selected parameters on Environment and Occupational Health and disseminate to the community. ENVIS centre promotes its theme through different outreach programmes at school, villages, colleges and at various exhibitions. Further, this centre has contributed through awareness programme at villages for flagship Programme of Government of India i.e. "Swachhh Bharat Abhiyaan" and also at Ahmedabad Railway Station with a Video presentation at the railway platforms and distribution of newsletters among the passengers.

ENVIS is publishing Newsletter in both English (Children in Hazardous occupation, Copper: Exposure & Effects, Ergonomics in Occupational Health, Vehicular pollution: A current issue and Hindi languages (निर्माण श्रमिक एवम स्वास्थ्य और मुस्कलोस्केल्टल विकार) and bibliography on selected topic, etc.

Posters on occupational and environmental health were also prepared, published and uploaded on the website. Several awareness programmes were conducted for the occupational groups - Automobile and Machinery workers. Various important days like World Environment Day, World Nature Conservation Day, World Day for Safety and Health at Work, National Safety Day, World Health Day etc. were celebrated as an awareness program among the community. ENVIS NIOH team participated in 'Science Express Climate Action Special (SECAS) held at Gandhinagar. The team members explained the visitors regarding the climate change as a strong stressor in different

occupations and also distributed stickers and posters on Climate change as an awareness tool.

## **NATIONAL INSTITUTE FOR RESEARCH IN ENVIRONMENTAL HEALTH, BHOPAL**

The National Institute for Research in Environmental Health, the 31<sup>st</sup> permanent institute of ICMR, was established on 11<sup>th</sup> October, 2010 at Bhopal. The aim of the institute is to understand the mechanisms of chemical-induced injury through basic, clinical, translational and community research and to develop diagnostic and therapeutic tools to chemical threat agents including toxic industrial, agricultural chemicals, toxins and other chemicals. Though the immediate focus of this institute is to cater to the continuing health research needs of the gas exposed population in Bhopal, the Institute is mandated to address National environmental health research issues in long term.

### **Population based long term epidemiological studies on health effects of Bhopal toxic gas exposure**

This long term epidemiological study has been continuing since 1985 (1985-1994 by ICMR under BGDRC; 1996-2010 under Centre for Rehabilitation Studies, Government of M.P; 2011 onwards under NIREH), wherein, the available persons belonging to the originally assembled cohort of toxic gas exposed and unexposed persons in 1985, are being surveyed at six monthly intervals for morbidities and mortalities following the original protocol. During 2016-2017, 52<sup>nd</sup> (January-June, 2016) and 53<sup>rd</sup> (July-December, 2016) round of surveys, were completed. In the 52<sup>nd</sup> round of survey, 23,981 individuals from exposed cohort and 6,427 individuals from unexposed (control) cohort were followed up. Any morbidity recorded was 22.4% in severely exposed, 14.6% in moderately exposed and 16.6% in mildly exposed cohort which was way higher than 9.5% morbidity recorded in the control cohort. During the 53<sup>rd</sup> round of survey, a cohort of 23,919 individuals from exposed and 6,567 individuals from unexposed cohort were followed up, which revealed 21.2% any morbidity in severely exposed, 14.4% in moderately exposed and 15.9% in mildly exposed cohort compared to 10.3% in the

control cohort. Overall mortality rates in exposed cohort were 3.8 and 3.5/1000 population in 52<sup>nd</sup> and 53<sup>rd</sup> round respectively, compared to mortality rates of 6.4 and 4.6/1000 population in the control cohort.

### **Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December, 1984**

In this completed pilot study, the cytogenetic status of gas exposed and unexposed individuals, among those screened earlier at Bhopal Centre under multi-centric genetic screening study of ICMR (1986-1990) was examined and compared with the previous genetic status to exclude the possibility of long term effects, if any, of MIC gas exposure. Among the 800 retrieved earlier screened cases (543 exposed, 257 unexposed) in Bhopal, only 174 (130 exposed, 44 unexposed) could be traced, contacted and their 3- generation pedigree prepared. Blood samples from the consenting 143 subjects (108 exposed, 35 unexposed) were collected and subjected to cytogenetic analysis. The study estimated different degrees of acquired and constitutive chromosomal abnormalities in differently exposed and unexposed populations and concluded that collectively, the chromosomal rearrangements and clonal abnormalities recorded among gas exposed survivors in this study, 30 years post disaster, can not directly be correlated to MIC exposure of 1984.

### **Cytogenetic analysis in Methyl IsoCyanate (MIC) exposed population and their progeny**

This completed study investigated the cytogenetic status of gas exposed survivors, aged >28 years, belonging to 6 sub-groups and their progeny. In this study, 1029 subjects were enrolled from the hospital registration record of 2013-2015 of Jawahar Lal Nehru Cancer Hospital and Research Centre (JNCHRC), Bhopal, following defined inclusion and exclusion criteria. Blood samples collected from 882 enrolled consenting individuals were subjected to chromosome preparations from lymphocytes and assessment of structural as well as numerical chromosomal aberrations. Both structural and numerical chromosomal aberrations such as micronucleus, chromosomal associations, minutes and deletion were found significantly

higher ( $p < 0.05$ ) among gas exposed individuals compared to non-exposed individuals. The study concluded that chromosomal instability persisted as a long term effect in the survivors of MIC gas tragedy. However, definite conclusion could not be drawn because of the multiple associated aetiologies in the present scenario.

#### **A hospital based study of congenital malformation in the neonates of gas exposed and non exposed mothers and their first generation progenies in Bhopal**

This hospital based ongoing study is comparing the prevalence of congenital malformations in 1,250 neonates borne to gas exposed /first generation progeny mothers and an equal number of neonates borne to un exposed mothers. So far, 1,250 deliveries in unexposed mothers group and 1,050 deliveries in gas exposed/first generation progeny mother group have been followed up.

#### **Effectiveness of Institutional versus domiciliary implementation of standard pulmonary rehabilitation module in Bhopal gas exposed survivors having COPD**

This newly initiated study will assess the difference in effectiveness of pulmonary rehabilitation in the management of COPD cases in two different settings *viz.* under supervision in a health facility and unsupervised at domestic level. So far, 50 COPD subjects have been enrolled under this study.

#### **Cytogenetic profiling of patients with Chronic Kidney Disease: Evaluation of genomic Instability**

Cytogenetic profiling of gas exposed and unexposed individuals with chronic kidney disease through conventional and molecular cytogenetic techniques will be prepared under this recently initiated study. So far, blood samples from 61 study subjects (36 gas exposed with CKD and 25 unexposed with CKD) have been collected.

#### **A cross-sectional study on current Health status of gas affected individuals of Bhopal : Phase I-Data triangulation to understand health status of gas exposed survivors of Bhopal**

This recently initiated pilot study is aiming at understanding the current status of morbidities

and health status of gas exposed survivors by data mining, triangulation and linking of available data bases related to the gas exposed persons in Bhopal. Mining and linking of available 3 data bases of gas survivors' morbidity from 2010 onwards is being done in order to prepare the morbidity profile of individuals who sought treatment either from BMHRC or Gas Rahat Hospitals or were surveyed under NIREH long term population based epidemiological study.

#### **Major achievements having public health importance**

NIREH is providing following community based services for the benefit of the gas exposed survivors –

- A special respiratory clinic has been functioning at NIREH in collaboration with Kamla Nehru Gas Rahat Hospital, Bhopal and Bhopal Memorial Hospital and Research Centre. Under this programme patients with chronic respiratory symptoms, identified in the field during long term epidemiological surveys, are transported to NIREH for examination and advised treatment. Till March 2017, Pulmonary Function Test of 603 respiratory patients was carried out at this clinic. In addition, a Pulmonologist of NIREH has been providing OPD services in the Kamla Nehru Gas Rahat Hospital, Bhopal twice a week since June 2016. Till March 2017, a total of 989 gas exposed patients having respiratory diseases have been examined in Kamla Nehru Hospital OPD by NIREH Pulmonologist and advised treatment.
- A total of 103 COPD patients, identified and referred by NIREH doctors from the gas-exposed community, were provided respiratory physiotherapy by a qualified physiotherapist at the Respiratory Physiotherapy Clinic run by NIREH at one of the Mini Units of BMHRC till March 2017.
- Under Community based health service programme, a total of 424 severely ill chronic patients having recurrent exacerbations of bronchial asthma and COPD, cardiac, gastrointestinal, neurological or ophthalmic morbidities who needed emergency care/



specialized care availed the benefit of referral to BMHRC for investigations and door step ambulance service till March 2017.

### *Extramural*

#### **GLOBAL CLIMATE CHANGE AND HEALTH**

- The two proposals for phase II were initiated. These were epidemiological studies of foci of visceral leishmaniasis in Himachal Pradesh.
- Vulnerability Assessment and Adaptation Measure towards Potential Impact of Climate Change on Malaria in Hot Spots of India.

Under Task Force on Respiratory Diseases, a multi centric study on, “Impact of Meteorological

Changes and Air Pollution on Respiratory Health and Morbidity”, has been formulated. The study aims to evaluate the link between climate and respiratory health and morbidity through analysis of local climate data, air quality data, and hospital statistics over a ten-year period. Based on recommendations of High Powered Committee held in January 2017; the project would be undertaken at Chennai, Kolkatta and Chandigarh centres, to begin with. Overall, disease specific, as well as temporal patterns would be explored using descriptive, graphical and time-series analysis.

# NON-COMMUNICABLE DISEASES

In the area of non-communicable diseases, the ICMR Institute of Cytology and Preventive Oncology, Noida, continues to carry out research studies for prevention and early detection of cancer. The National Centre for Disease Informatics and Research, Bengaluru, continues to focus on activities related to population based cancer registries, hospital based cancer registries, time trends in cancer incidence rates, software development, among others. Other research studies were carried out in the areas of Oncology, Diabetes, Neurological Sciences, Obesity and Metabolic syndrome, among others. Major highlights of various programmes undertaken by the ICMR in the area of non-communicable diseases during the year 2016-17 are given below.

## NATIONAL INSTITUTE OF CANCER PREVENTION AND RESEARCH (ICMR-NICPR), NOIDA

ICMR-National Institute of Cancer Prevention and Research (ICMR-NICPR) was initially established as Cytology Research Centre (CRC) by the Indian Council of Medical Research (ICMR) in 1979, and was elevated to the level of an Institute (Institute of Cytology and preventive Oncology) in 1989. It was granted national status in 2016 acknowledging its mandate and contributions towards cancer prevention.

The institute has broadened its horizon to cater to prevention of prevalent cancers in the country. The thrust areas of research include pre-cancer and cancers of the uterine cervix, breast and oral cancers. The website of the institute is <http://nicpr.res.in/>.

## PROJECTS UNDERTAKEN

ICMR-NICPR has always been expanding its research borders and learning aspects. A total of 35 research projects, both intramural and extramurally funded, are currently approved/undergoing/completed in the financial year 2016-17.

Major ones include:

- Task Force project on “Biomedical Informatics Centres of ICMR” (ICMR).
- Screening for pre-cancer and cancer of cervix, breast and oral cavity in the setting of Health Promotion Clinic in ICMR-NICPR.
- Evaluation of existing web-portal for cancer awareness for general population and level 1 care providers (ICMR).
- Implementation of cancer screening of common cancers through AYUSH doctors: A Pilot study (AYUSH).
- Policy oriented project on Smokeless Tobacco control policy (WHO FCTC).
- Exploration of NCRP data and statistical modeling (ICMR).
- Follow up of patients seen during an Indian Council of Medical Research (ICMR) study on oral pre-cancer and cancer lesions (Intramural).

## PUBLIC HEALTH IMPORTANCE

- More than 1000 patients have been screened for cervical, breast and oral cancer free of charge at the Health Promotion Clinic in the institute’s premises.



Fig.1. Health Promotion Clinic in the institute’s premises.

- ICMR-NICPR has conducted nearly 22 community outreach camps, performing cancer screenings in 1135 females and 327 males with 38 frank malignancies registered at IRCH, AIIMS.
- ICMR-NICPR has been monumental in the HPV vaccine initiative in Punjab and Delhi, targeting prevention of cervical cancer among school-going girls.
- ICMR-NICPR is also the National ECHO (Extension for Community Healthcare Outcomes) Hub for cancer prevention (<http://echo.unm.edu/>). This portal provides a platform for dissemination of training and education through an interactive online tool. Apart from routine fortnightly webinars on ECHO, a certificate course is also being provided.



Fig.2. ICMR-NICPR has conducted nearly 22 community outreach camps.

- Introduction of newer, low-cost cancer care devices such as Magnivisualizer.
- Development of Cancer Web portal (<http://cancerindia.org.in>): An interactive two-way website for providing comprehensive cancer-related information and statistics for general population.
- Bi-annual and Quarterly workshops and training programs for continual medical education and research initiatives in different disciplines.



Fig.3. National and International collaborations among Cancer centres.

- National and International collaborations among Cancer centres, leading health care organizations, Ministry of Health and other stake holders.
- ICMR-NICPR as the WHO HPV Reference Laboratory for South-east Asia for HPV Genotyping and Serology.
- Data Management Laboratory: A specialized unit developing and providing professional data management solutions/applications.

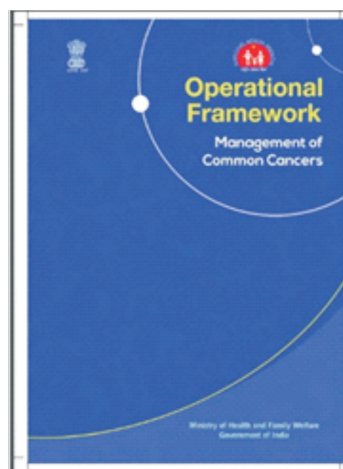


Fig.4. “Operational framework document”.



- ICMR-NICPR has been instrumental in the formulation of “Operational framework document” for cancer screening and management, recently released by Ministry of Health and Family Welfare, Govt. of India, which has been designed to carry out population based screening in 100 districts of India using cost-effective screening tools. NICPR has also been designated as the training hub for implementing the guidelines.
- ICMR-NICPR was designated as the WHO FCTC Global Knowledge Hub on Smokeless Tobacco, which is the focal centre for all information on smokeless tobacco and is responsible for providing research and evidence (<http://untobaccocontrol.org/kh/smokeless-tobacco/>).
- ICMR-NICPR hosted the “Inter-country meeting on Smokeless Tobacco Control Policy”; wherein global smokeless tobacco policy evaluation and its implementation status was discussed and recommendations were framed to be applicable for all FCTC member countries.



**Fig.5.** ICMR-NICPR was designated as the WHO FCTC Global Knowledge Hub on Smokeless Tobacco.

## NATIONAL CENTRE FOR DISEASE INFORMATICS AND RESEARCH, BENGALURU

More than thirty years journey of National Cancer Registry Programme (NCRP) has led to its enrichment and expansion into different parts of the country and also culminated in the establishment of a permanent institute of Indian Council of Medical Research (ICMR) namely National Centre for Disease Informatics and Research in 2011 in Bengaluru. NCDIR has a very high potential of leading the public health informatics as well as research in the areas of Cancer, CVD, Diabetes, Stroke and other non-communicable diseases in India.

### Population Based Cancer Registries (PBCR)

As of 2016-2017, there were 31 Population Based Cancer Registries (PBCR) in the country. These registries collect data on cancer incidence and mortality from a defined geographical region. Then they submit data to the National Cancer Registry Programme for analysis and the data represents different geographical regions in India (10% population). Three-year Report of Population Based Cancer Registries (2012-14) from 27 PBCRs was released in May 2016.

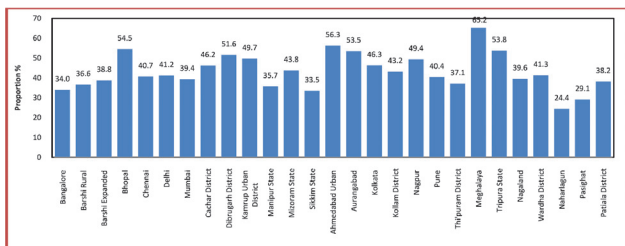
The report dealt with cancer incidence, leading sites of cancer, tobacco related cancers, childhood cancers, method of diagnosis, cancer rates for some sites of cancer and cancer mortality. The comparison of this data was also done with the data available in Cancer Incidence in Five Continents (CI 5 Vol-X) published by International Agency for Research on Cancer (IARC). The quality indices of the registries were looked into as per international norms. The trend of some sites of cancers over time was generated in addition to estimating and projecting the burden of cancer in the country till 2020. The report can be accessed from: [http://ncrpindia.org/ALL\\_NCRP\\_REPORTS/PBCR\\_REPORT\\_2012\\_2014/index.htm](http://ncrpindia.org/ALL_NCRP_REPORTS/PBCR_REPORT_2012_2014/index.htm)

### Cancers associated with the use of Tobacco

East Khasi Hills district of Meghalaya had the highest relative proportion with 69.5% and 45.0% for males and females respectively. Excluding the north east PBCRs, the highest proportion of cancers

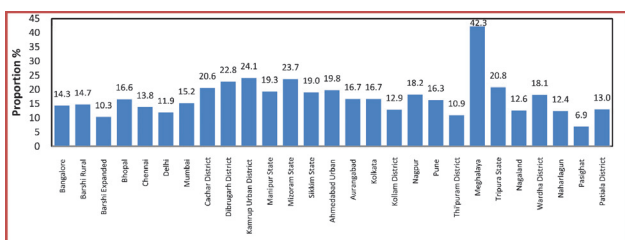
associated with use of tobacco was observed in Ahmedabad Urban PBCR for both males (56.3%) & females (19.8%).

**Males**



**Fig.6.1.** Cancers associated with the use of Tobacco (Males).

**Females**



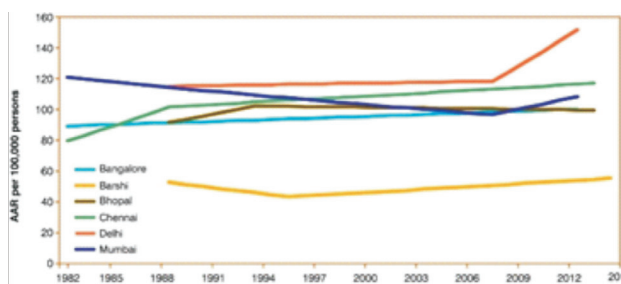
**Fig.6.2.** Cancers associated with the use of Tobacco (Females).

Childhood Cancers: Among boys, proportion of cancers in childhood relative to cancers of all ages,

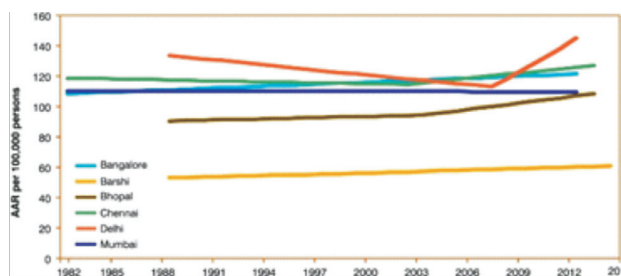
varied from 0.7% in Nagaland PBCR to 5.4% in Delhi PBCR. Among girls, it ranged from 0.5% in East Khasi Hills District of Meghalaya to 3.5% in Naharlagun excluding Papumpare district.

Trends over time – The following graph depicts the trend of all sites of cancers for 6 PBCRs.

All Sites (ICD10: C00-C97) - Males



All Sites (ICD10: C00-C97) - Females



**Fig.7.** depicts the trend of all sites of cancers for 6 PBCRs.

**Table 1:** Some of the findings from the analysis of the data based on the 2012-2014 are given below.

Leading Sites of cancer across 27 PBCRs		
Males		
Site (ICD10)	Number of PBCRs	PBCR Name
Lung (ICD10:C33-C34)	10	Bangalore, Chennai, Delhi, Mumbai, Manipur State, Aurangabad, Kollam District, Kolkata, Thiruvananthapuram District, Tripura State
Mouth (ICD10:C03-C06)	7	Barshi Rural, Barshi Expanded, Bhopal, Ahmedabad Urban, Nagpur, Pune, Wardha District
Oesophagus (ICD10:C15)	5	Cachar District, Dibrugarh District, Kamrup Urban, Meghalaya, Patiala District
Stomach (ICD10:C16)	4	Mizoram State, Sikkim State, Naharlagun, Pasighat
Nasopharynx (ICD10:C11)	1	Nagaland

**Table 2:** Some of the findings from the analysis of the data based on the 2012-2014 are given below.

Leading Sites of cancer across 27 PBCRs		
Females		
Site (ICD10)	Number of PBCRs	PBCR Name
Breast (ICD10:C50)	19	Bangalore, Bhopal, Chennai, Delhi, Mumbai, Cachar District, Dibrugarh District, Kamrup Urban, Manipur State, Sikkim State, Ahmedabad Urban, Aurangabad, Kolkata, Kollam District, Nagpur, Pune, Thiruvananthapuram District, Wardha District, Patiala District
Cervix Uteri (ICD10:C53)	6	Barshi Rural, Barshi Expanded, Mizoram State, Tripura State, Nagaland, Pasighat
Oesophagus (ICD10:C15)	1	Meghalaya
Stomach (ICD10:C16)	1	Naharlagun

**Table – 3.** Projected Incidence of Cancer Cases at India level for Selected Sites and Selected Time Periods –

Both genders							
ICD10	Site Name	2015	2020	ICD10	Site Name	2015	2020
C00-C97	All Sites	1388397	1734886	C33-34	Lung	106794	144351
C01-02	Tongue	60333	81200	C50	Breast (Females)	134214	179790
C03-06	Mouth	89645	128451	C53	Cervix	97909	104060
C12-13	Hypopharynx	19700	20948	C54	Corpus Uteri	25395	37178
C15	Oesophagus	49059	53898	C56	Ovary	45231	59276
C16	Stomach	44998	54108	C61	Prostate	43049	61222
C18	Colon	37435	51906	C67	Urinary Bladder	26886	33608
C19-20	Rectum	36290	46427	C70-72	Brain	30629	32619
C22	Liver	36255	49844	C82-85, C96	NHL etc	42273	51903
C23-24	Gall Bladder	37561	55141	C91	Lymphoid Leuk	20762	27709
C32	Larynx	33642	39636	C92-94	Myeloid Leuk	24287	27201

Projection: Cancer trend and prediction analysis serves as surveillance tools in measuring and evaluating the introduction of targeted screening or early cancer detection programmes for cancer control as well as for improving of health care services. The new number of cancer cases expected to increase from 13.8 lakhs in the year 2015 to 17.3 lakhs in the year 2020.

NCDIR encourages new PBCRS to join the NCRP and two more PBCRs have been initiated recently:

ICMR- National Institute of Cancer Prevention & Research (NICPR), Noida covering Gautam Budh Nagar (UP).

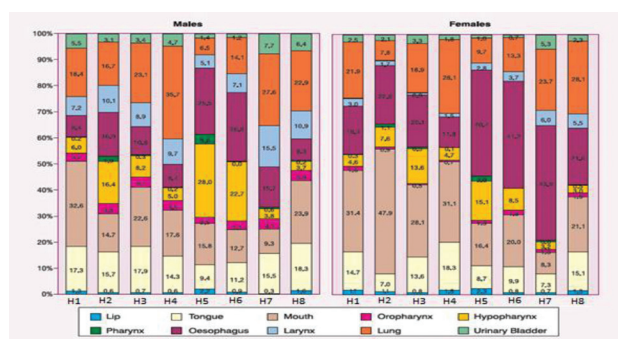
Cachar Cancer Hospital, Silchar covering districts of Karimganj, Hailakandi & Dima Hassao (Assam).

**Hospital Based Cancer Registries (HBCR)- Patterns of Care and Survival Studies**

Hospital Based Cancer Registries (HBCRs) collect information of cancer cases reported in a given hospital, regardless of where that person with cancer resides. HBCRs help in assessing cancer patient care in a given hospital and aid in hospital administration including the individual hospital’s cancer control programmes. HBCRs also collect information pertaining to clinical stage, treatment and follow-up and in planning and monitoring survival studies and clinical trials. In many instances hospital registries contribute cases to the population based cancer registry.

Due to the challenges in obtaining follow-up parameters, a separate study on Patterns of Care

and Survival (POCSS) was undertaken by the National Cancer Registry Programme in the year 2006 for three major sites of cancer namely Breast, Cervix and Head & Neck Cancers. There are 29 HBCR including the Regional Cancer Centres (funded by Ministry of Health and Family Welfare) under the network of National Cancer Registry Programme (NCRP) - NCDIR, most of which are also providing data. In addition, the following collaborating centres are also providing data on POCSS, namely; Amrita Institute of Medical Science, Kochi, Malabar Cancer Centre, Kannur, Rajiv Gandhi Cancer Institute and Research Centre, New Delhi, Medanta Cancer Institute, Gurgaon, Vydehi Institute of Medical Sciences, Bangalore and Mahavir Cancer Sansthan, Patna. The Three-year Report of Hospital Based Cancer Registries for the years 2012-14 was printed in March 2016 and released in the Workshop on Dissemination of Cancer Registry Report (2012-14) held on May 18, 2016 at ICMR headquarters under the Chairman ship of DG, ICMR.

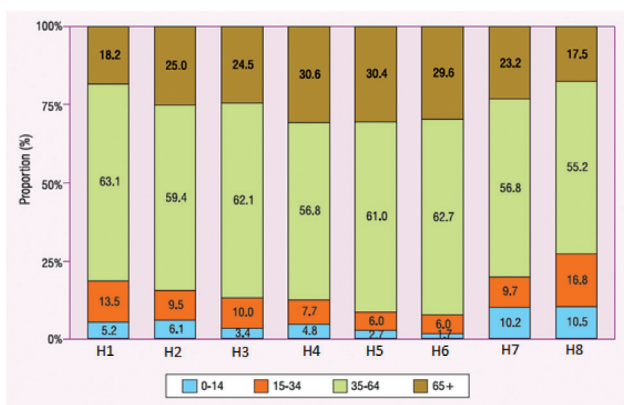


**Fig. 9:** Stack Diagram Showing Proportion of Specific Tobacco Related Sites Relative to All Tobacco Related Cancers.



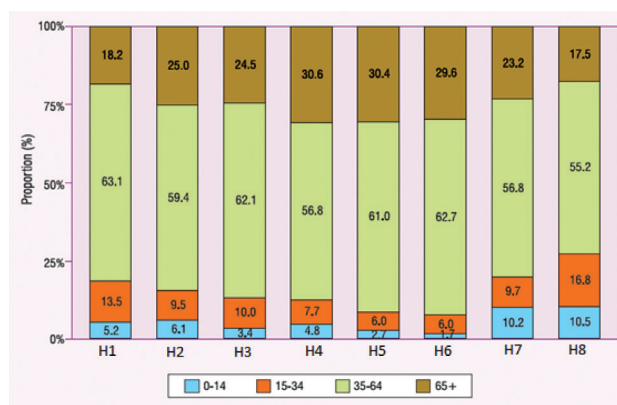
**Stack Diagram Showing Proportion of Cancer by Broad Age Groups**

**Males**



**Fig. 8.1:** Stack Diagram Showing Proportion of Cancer by Broad Age Groups (Males).

**Females**



**Fig. 8.2:** Stack Diagram Showing Proportion of Cancer by Broad Age Groups (Females).

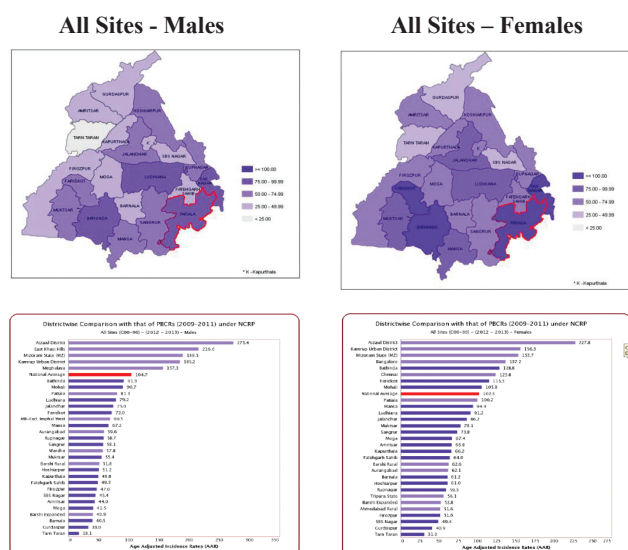
**Stack Diagram Showing Proportion of Specific Tobacco Related Sites Relative to All Tobacco Related Cancers**

The main findings were

- In locally advanced cervical cancer significant survival benefit was observed when treated with a combination of radiation with cisplatin than radiation alone. The same observation was seen in patients with locally advanced cancers of the oro and hypo-pharynx.
- The low proportion of patients undergoing breast conserving surgery in contrast to mastectomy in both Stage II and Stage III disease. Statistically significant decreased survival noted with mastectomy compared to breast conserving surgery.
- Patients who had mastectomy did better with systemic therapy (chemotherapy and/or hormone therapy) whereas patients with Breast Conservation Surgery (BCS) required just local radiation therapy for achieving best survival.

**Development of an Atlas of Cancer in Punjab State**

This project was implemented between 2011-2015 to make an assessment of patterns of cancer in parts of Punjab state under the National Cancer Registry Programme (NCRP) of ICMR. Under this project, a cost-effective design and plan using advances in electronic information technology, was used to collate and process relevant data on cancer, and wherever possible, it was also envisaged to calculate estimates of cancer incidence. All medical colleges, pathology labs, civil hospitals and individual oncologists, throughout the state were contacted for collaboration. Data was also received from Mukhya Mantri Punjab Cancer Raahat Kosh Scheme (MMPCRKS) patients. Detailed checks were done to meet international standards. The 2015 report on "Development of an Atlas of Cancer in Punjab State" covers the calendar years 2012 and 2013. In all, there were a total of 33,940 cases from 29 centres including the cancer registries under the NCRP and other functioning cancer registries. The incidence rates of multiple myeloma along with other lymphoid and haemopoietic malignancies appeared to be higher in some districts of Punjab compared to that seen in other PBCRs.



**Fig. 10:** Atlas of Cancer in Punjab State: Age Adjusted Incidence Rates for all sites of cancers.

### Atlas of Cancer in Punjab State: Age Adjusted Incidence Rates for all sites of cancers

- The incidence rates of multiple myeloma along with other lymphoid & haemopoietic malignancies appeared to be higher in some districts of Punjab compared to that seen in other PBCRs.
- Oesophageal cancer in males and females was an important leading site in many districts and was one of the five leading sites in females.
- In males, there were six districts (Bathinda, Mohali, Ludhiana, Jalandhar, Faridkot and Mansa) and in females, five districts (Bathinda, Faridkot, Mohali, Mansa and Ludhiana) that had incidence rates higher than that of Patiala PBCR under NCRP among males .

### Development of an Atlas of Cancer in Haryana State (HCA)

The rationale of such an atlas is that there are areas of population in state, particularly, rural areas that remain largely uncovered and therefore, patterns of cancer in several urban centres and rural areas remain largely unknown. The environment differs in rural and urban areas and so do dietary practices and socioeconomic status. The scientific data on cancer collected from Haryana state by the project brings out the actual picture and burden of cancer.

At present there are 127 centres registered and 96 are transmitting data. As on date, a total of 23,980 confirmed malignant cases have been transmitted with 15,384 cancer cases for the year 2016 and 7897 cases for the year 2017.

### National NCD monitoring survey

The ICMR- National Centre for Disease Informatics and Research (NCDIR) is implementing the survey at the behest of the Ministry of Health and Family Welfare, Govt of India, to monitor the progress made at the national level towards achieving the national NCD targets by 2025. It is being undertaken in partnership and collaboration of AIIMS Delhi, National Institute of Medical Statistics (NIMS) New Delhi, National Institute of Epidemiology (NIE) Chennai, AIIMS Bhopal, AIIMS Jodhpur, AIIMS Bhubaneswar, National Centre for Disease Control New Delhi, Assam Medical College Dibrugarh, BJ Government Medical College Pune, National Institute of Nutrition Hyderabad and AMCHSS Sree Chitra and Trinamool Institute of Medical Sciences, Kerala.

It is planned to generate country/national level estimates of key NCD related indicators (risk factors and health system response) identified in the national NCD monitoring framework, create a central and regional pool of resources (protocols, standard tools, training manuals etc.) and support similar surveys at state level. The survey is Cross Sectional in 12000 households, 7200 adults (18-69 years) and 4,800 adolescents (15-17 years) from 300 rural Primary sampling units (villages) and 300 urban PSUs (urban wards). The study tools after suitable revisions by experts are translated into twelve languages (English and Hindi including ten regional languages like Tamil, Odia, Assamese, Bengali, Kannada, Marathi, Malayalam, Punjabi, Gujarati and Telugu) and will be used for data collection after loading in hand held device.

Key behaviours will be assessed by face to face interview with selected sample; physical measurements (height, weight, waist and blood pressure) and biochemical (fasting blood sugar and spot urinary sodium excretion) measurement will be performed for selected sample.

### Patent/ Product filed/ Obtained

The following components have been developed for the Software which is a copyright of NCDIR.

1. Population Based Cancer Registry software application-
  - a. Annexure tabulations
    - i. Registry wise as per PBCR report (intranet)
    - ii. Religion wise (offline)
    - iii. Source of registration wise (offline)
  - b. Duplicate and matching modules (online)
2. HBCR-POCSS
  - a. Updated Data capture page
  - b. Quality check report
3. Independent Oncology Modules (Surgery, Radiotherapy, Chemotherapy)
  - a. Updated Data capture page
  - b. Output Generation for all independent modules; development is under process.
4. E Monitoring modules for data transmission for the project on Development of an Atlas of Cancer in Haryana
5. Software for TMH Mumbai Hospital data to NCRP database extractor into SQLSever, Excel, csv etc
6. Offline to online data sync software developed and deployed in JIPMER Local server

### Extramural Research

#### ONCOLOGY

Cancer Management Guidelines consensus document for management of Non Hodgkin's Lymphoma-high grade, Pediatric and Soft Tissue Lymphoma and Cervix were published during this period. A total of twelve guidelines have been printed and also available at ICMR website.

The Standard Operating Procedures (SOPs) for flow cytometer in diagnosis of haematolymphoid

malignancies are printed and also available on Council website ([www.icmr.nic.in](http://www.icmr.nic.in)).

#### Comparative Study of Genetic, Clinical and Epidemiological Factors of Breast Cancer in Rural and Urban Area of India

This study shows the role of XRCC genes, tobacco and alcohol usage and family history as risk factors. Based on leads obtained, the project has been initiated at NICPR, Noida; NIP, Delhi; RCC, Thiruvananthapuram and AIIMS, Delhi.

#### Screening and early detection of cervical, breast and oral cancer in Cachar, Assam: A pilot project

The population-based screening program has been carried out in 2 phases. The first phase of the study includes the training of the master trainers on screening and early diagnosis of common cancers by National Institute of Cancer Prevention and Research, Noida. In the second phase, the front-line workers trained by master trainers will implement the screening program in the community. The project is initiated in Cachar district of Assam state.

#### Strengthening State Non-Communicable Disease Programme for Early Detection of Breast Cancer Involving Strategic Education and Awareness among the Women: A Joint Programme of State Government and ICMR-Desert Medicine Research Centre, Jodhpur

The project is primarily aimed to strengthen state breast cancer screening programme and develop a referral system for diagnosis and treatment of suspected cases at state medical colleges/ district hospitals. The project is being undertaken in collaboration with State NCD Programme and is proposed to cover all the districts in Rajasthan.

The consolidated data of 2012-14 from various Population Based (PBCR) and Hospital Based Cancer Registries (HBCR) was released in Dissemination Workshop and Media Event held on 18<sup>th</sup> May 2016. The Director General, ICMR and Secretary, DHR in presence of SAC, Chairperson; Dr GK Rath and Dr P Gangadharan released Cancer Registry data (2012-2014).



For all sites of cancer, it was observed that there was a significant increase (Annual Percentage Change (APC)) in males in the PBCRs at Bangalore, Chennai and Delhi and in females at Bangalore, Barshi and Bhopal registries. Among males, there was a significant increase in the incidence rates of cancers of the colon, rectum and prostate in the PBCRs at Bengaluru, Chennai and Delhi. Among females, there was a significant increase in the incidence rates of cancers of the breast, uterus, ovary and lung. All the PBCRs at Bengaluru, Barshi, Bhopal, Chennai, Delhi and Mumbai showed a significant decrease in the incidence rate of cervical cancer.

### **Indian Childhood Collaborative Leukaemia (ICiCLE) Group Multicentric Study for Children with Acute Lymphoblastic Leukaemia (ALL)**

It is aimed to improve the outcome of children with ALL in India and develop a model with limited health resources. In the west, current focus is on intensification to cure those who relapse. Thus, treatment is unnecessarily toxic for those at low risk. Though the requirement for de-escalation of therapy is recognized, this is difficult to achieve when outcome approach 90%. Thus, study not only has the potential for improving outcome for children with ALL in India, but could also pave the way for less intensive curative strategies for patients worldwide.

### **CARDIOVASCULAR DISEASES**

#### **A Web Based National Network of Management for Acute Coronary Events (MACE) Registry**

The study has been up scaled to 35 centres including centres with Cath lab facility and those without cath lab facility. Preliminary analysis suggests that there is delay of 300 minutes in symptom onset to visit to hospital with cath lab facilities. Hypertension emerged out to be the most common risk factor in ACS patients in this study.

A study on “Assessment for accreditation and comparative analysis of health profile in selected accredited schools of Chandigarh” has been initiated from 1<sup>st</sup> Sep 2016.

### **STEMI TAMIL NADU PROJECT**

A novel ST elevated Myocardial Infarction (STEMI) care pathway has been developed in this project in a district in Tamil Nadu. This approach essentially used hub and spoke model. Provision of 108 ambulance facility and insurance scheme were mandatory. The hub hospitals have 24x7 (PCI) facility interventions and are connected through an indigenous built technology with the spoke hospitals. The ECG is transmitted to hub hospitals and a thrombolytic therapy is initiated at CHC level under the supervision of a cardiologist at the hub hospital. This increases time window for carrying out PCI from 90 minutes to 24 hours. The STEMI patient is then shifted to nearest hub hospital for PCI using a GPS facility. A workshop to disseminate the findings of this project was held in Jan 2017. States with 108 ambulance facility were invited for taking up the project. The major recommendation of the workshop was that the STEMI India model may be adopted as the National STEMI Programme. The states should prepare Project Implementation Plan under NHM along with Costing of the project for implementation in their respective states. The NCD cell at MOH&FW and ICMR will provide support in this activity.

### **NEUROLOGY**

#### **Centre for Advanced Research for Innovation in Mental Health and Neuroscience: Manpower Development and Translation Research at NIMHANS, Bangalore, Karnataka**

A ‘Histological Atlas of the Common Infection of CNS’, along with set of histological slides depicting the pathological features and CD containing the text & photographs in the Atlas has been prepared. Slides and CDs are provided to medical college students. The Brain Bank has made 41 posters as a public awareness initiative and these are being regularly used in local exhibitions by schools/colleges and Indian Epilepsy Association.

Under the study, “Translation research in ALS- Development of biomarkers for diagnosis, monitoring disease progression and evaluation of toxicity”, CHIT-1 has been identified. This

molecule appears to have the potential of developing a biomarker for ALS diagnosis and monitoring disease progression.

An ELISA sensitive test has been developed for quantization of 14-3-3 protein in CSF. This test can be used as a supportive biomarker to the clinical, imaging and EEG findings in suspected cases of CJD. The test has been introduced for diagnostic services at NIMHANS in Oct 2015. NIMHANS has been asked to setup an ICMR –NIMHANS National Level Facility for CJD diagnosis

### Population Based Rural Stroke Registry at Ludhiana

It covers 70 villages of Pakhowal and all 94 villages of Sidhwan Bet blocks and utilizes ASHA workers. The extrapolated incidence of stroke among males is 151 per lakh and 175.3 per lakh in females. Among the first ever stroke study in rural population, imaging by CT/MRI was done in 32-45% of cases only. Deaths due to stroke were 40.7% of total cases.

An ICMR comprehensive neuropsychological test battery has been developed to be used in Indian patients with MCI and dementia in 5 languages in both literates and illiterates. A validation study has been undertaken in 1200 subjects to test the above neuropsychological battery. Cognitive tests were validated for dementia and stroke.

## DIABETES

### ICMR Advanced Centre for Genomics in Type 2 Diabetes Mellitus

To up scale the findings obtained, a new project on “Changing treatment profile in Monogenic forms of Diabetes such as Neonatal Diabetes & Maturity-Onset Diabetes of the Young (MODY) by Translational Genomics Research” has been initiated.

The Registry of People with Diabetes in India with Young Age at Onset has shown that in all the diabetes categories, retinopathy is the most common diabetes complication (3.6% in T1DM and 10.4 % in T2 DM). This was followed by nephropathy in T1DM and neuropathy in T2DM.

The prevalence of complications increased with duration of disease. Retinopathy and neuropathy appeared to manifest within a short duration since diagnosis, among T2DM patients compared to T1DM. Among those who have lived with diabetes for more than 20 years, the prevalence of coronary disease was more among T2DM patients (9.8%) compared to those with T1DM (4.8%). 14.1 % of the YDR patients had at least one co-morbid condition such as Hypothyroidism, Dyslipidemia, Hypertension, Tuberculosis, Sepsis etc., with the most common co-morbidity being hypothyroidism (3.1%). 20.8 % of the early onset T2DM patients were reported to have co-morbidities such as Dyslipidemia (11.3%), Hypertension (7.4%) and Hypothyroidism (2.1%).

There is a substantial prevalence of Type 2 DM in young patients from North East India as reported by “Registry of Youth Onset Diabetes in India (YDR)”. The NE people are short and thin and the life expectancy of Assam is 58.9 years. So, NE is an ideal place to study the DOHaD (Developmental Origins of Health and Disease) theory which suggests that intergenerational and early life deprivation, combined with rapid socio-economic development, predisposes to a range of NCDs including Diabetes, Hypertension and Coronary Artery Disease. Hence a study on Phenotyping North East Indian Young Type 2 Diabetes (PHENOINDY-2) has been initiated.

### ICMR-India National Diabetes Study (ICMR-INDIAB)-Rest of India

Following study undertaken in 4 states during phase I; the five states [Andhra Pradesh, Bihar, Gujarat, Karnataka and Punjab] of India undertook the survey during phase II. During year under report; four states viz: Delhi, Madhya Pradesh, Rajasthan and UP initiated the field survey.

Impact of Yoga on Chronic Care: 3 projects have been initiated to study the effect.

Yoga Day Celebration: 3rd International Yoga Day was celebrated by Indian Council of Medical Research Headquarters, New Delhi. Dr Soumya Swaminathan, Secretary Department of Health

Research and Director General ICMR, welcomed the participants and highlighted importance of yoga in daily life, especially in prevention of life style diseases. The importance of scientific evidence was mentioned. The brief of the studies initiated by ICMR on studying the impact of yoga on pre-diabetes; biological markers; cognitive functions and adolescent health was presented. Dr RM Acharya, Centre for Integrative Medicine, AIIMS and visiting faculty S-VYASA University, Bangalore gave a public lecture on health effects of yoga. The audience was apprised of different yoga modules to be practiced in day to day life and cited examples how simply following yoga, one can feel healthy, both physically and mentally. The yoga practice sessions for the staff were conducted by Dr Sriloy and Mr Niranjan.



Fig. 11: Yoga Day Celebration.

### Guidelines for Management of Type 1 Diabetes

The ICMR in collaboration with AIIMS and IIPH is in process of formulating guidelines for management of type 1 diabetes and also using leads obtained from ICMR’s Young Diabetes Registry.

### Indo US Joint Statement on Collaboration in Diabetes Research

On 12th June, 2012, India’s Health and Family Welfare Minister Hon’ble Shri Ghulam Nabi Azad and U.S. Health and Human Services Secretary Kathleen Sebelius signed a joint statement to begin a formal research relationship between the National Institutes of Health and the Indian Council of Medical Research. The 5<sup>th</sup> Meeting of JSC was held in May 2016 to charter future road map for collaboration. Diabetes and Tuberculosis”, “Thin Fat Phenotype” and “Gestational Diabetes

Mellitus” were suggested areas.



Fig.12. Indo US Joint Statement on Collaboration in Diabetes Research.

## MENTAL HEALTH

### Epidemiology of Substance Use and Dependence in the State of Punjab

Household survey in Punjab was conducted in total of 44 sites (22 cities and 22 villages) in all the 22 districts of Punjab, total number of respondents being 13,295. Current use and current dependence reported highest in District Mansa (39.13% each). The current use (8.17%) and current dependence (7.95%) was reported to be minimum in District Ferozpur.

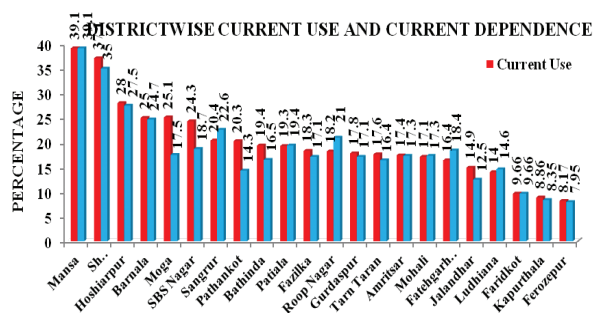


Fig. 13: Ongoing Schemes and Targets Achieved.

The Weighted Prevalence rates of lifetime use, annual use, current use, lifetime dependence and annual dependence of any substance was 20.48%, 18.01%, 15.35%, 15.75% and 14.70% respectively.

LIFETIME DEPENDENCE AND CURRENT DEPENDENCE (ANY SUBSTANCE)

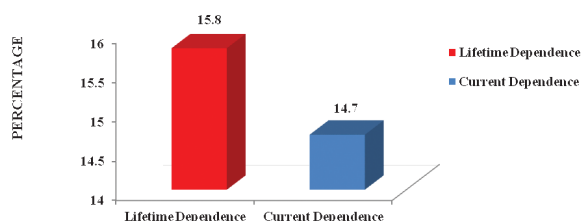


Fig.14.1: Lifetime dependence and current dependence (any substance).



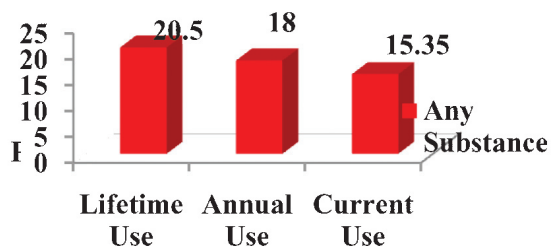


Fig.14.2: Lifetime use, Annual use.

The estimated number of lifetime users, annual users, current users, lifetime dependence and current dependence of any substance were 41.39 lac, 36.40 lac, 31.01 lac, 31.83 lac and 29.71 lac, respectively in the state of Punjab. Respondent driven sampling was used and 6600 respondents were enumerated for RAS. Lifetime rates of use of any opioids was 91.5% followed by Tobacco 70.5%, alcohol 59.5%, cannabinoids 32.2%, sedative/hypnotics 12.9%, stimulants 2.6% and inhalants & solvents 1.3%. Current rate of Dependence on any opioids was 87.9%, followed by Tobacco 69.4%, alcohol 51.2%, cannabinoids 29.5%, sedative/hypnotics 12%, stimulants 2.2% and inhalants & solvents 0.9%.

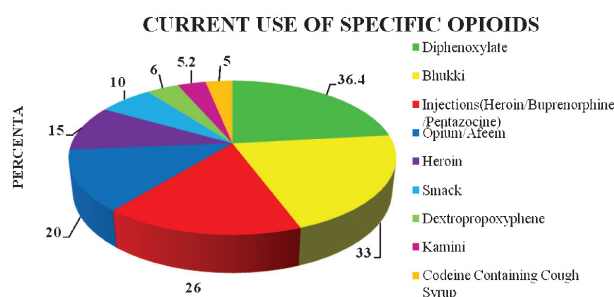


Fig. 15: Current use of Specific Opioids.

## DISABILITY & REHABILITATION

### National Disability & Rehabilitation Research Centre (NDRRC)

ICMR has in-principle agreed to establish “National Disability & Rehabilitation Research Centre (NDRRC)” under ICMR, consisting of National Disability & Rehabilitation Research System (NDRRS) and National Disability & Rehabilitation Research Network (NDRRN).

## PUBLIC HEALTH IMPORTANCE

The project entitled “Effectiveness of socio-culturally sensitive endogenously developed module administered by parents as compared to professionals for Indian children with autism spectrum disorder aged 18 months to 6 years” found out that collaborative action between parents and professionals is crucial step towards effective intervention. Parents can train their children independently after undergoing brief training under RCI approved clinical psychologist. Active professional help is as good as passive professional help for imparting intervention to children with Autism spectrum disorder. Parent-education and home based interventions are more likely to be effective, if part of a multidisciplinary intervention program.

## GERIATRICS

The Division under ICMR-FORTE collaboration in the area of Geriatrics floated a call for Research Project. Under this scheme 16 projects were received, of which, 5 were jointly agreed for support.

## NATIONAL BURDEN OF NON COMMUNICABLE DISEASES

Under the aegis of the Ministry of Health and Family Welfare, Government of India, the India State-level Disease Burden Initiative is producing robust state-level estimates of disease burden and risk factors in India. This initiative that was launched in October 2015, is a collaboration between the Indian Council of Medical Research (ICMR), Public Health Foundation of India (PHFI), Institute for Health Metrics and Evaluation (IHME), University of Washington. The work spanning over the past two years has involved about two hundred senior academics and decision makers from over 100 institutions across India. This work benefitted from the close engagement and contribution of fourteen expert groups formed as part of this Initiative. Data from all available sources over the past 25 years have been compiled and the methods of the Global Burden of Disease study are being used to analyze this data. This will lead to the production

of estimates for all diseases leading to health loss in each state of India from 1990 to 2016, along with estimates of risk factors driving this burden. In order to present the key trends of the state-level disease burden findings and risk factors over the past 25 years, and to engage central and state-level policy makers in India on the implications of the variations in these trends across the states on

how health systems should develop, the findings will be disseminated in November 2017. The dissemination will include a comprehensive report, an open access interactive visualization tool that will present the disease burden trends over 25 years across the Indian states and a detailed scientific paper describing the science behind these findings.

# BASIC MEDICAL SCIENCES

**D**uring the year, the intramural research in the field of basic medical sciences was carried out by the National Institute of Pathology (NIOP), New Delhi, National Institute of Immunohaematology (NIIH), Mumbai and also at several other centres. The extramural research was undertaken in several areas viz. haematology, biochemistry, pharmacology, human genetics, nano medicine, stem cell research, biomedical ethics.

## NATIONAL INSTITUTE OF PATHOLOGY, NEW DELHI

At National Institute of Pathology, the thrust areas of research are tumor biology, infectious diseases including Leishmaniasis, Tuberculosis and Chlamydia, stem cell biology and environmental toxicology. The scientists have been conducting both basic as well as translational research towards the development of Biomarkers for screening, diagnosis, prognosis and prediction of drug resistance for various diseases and vaccine for prevention of kala azar.

### TUMOUR BIOLOGY

Studies were conducted in breast, bladder and prostate cancer and acute lymphoblastic leukemia. Whole exome sequencing of 34 breast tumors and 8 controls showed 52,454 variants associated with early and late onset breast cancer. Among coding gene exons, 40,895 variants were found, rest 11,559 variants were present in other non-coding regions. Further, a total of 29,968 variants were found in early onset breast tumors. Among them were 22,868 variants in coding gene exons, while 7,100

variants were found in other noncoding regions and 8632 were novel variants. The expression of the long non-coding RNA, MEG3 was found down regulated in breast cancer cell lines MCF7, T47D, BT-20 and MDA-MB-231 compared to control, suggesting the loss to play a role in breast carcinogenesis.

Proteome profile by iTRAQ-LC-MS/MS in urothelial bladder cancer identified urinary PRDX1 and PRDX2 as markers of recurrence of urothelial bladder cancer. Whole transcriptome sequencing (RNA-seq) of two groups of prostate cancer patients showed 862 up regulated and 521 down regulated genes between the two groups. Pathway enrichment analysis of differentially expressed genes showed PI3K-Akt signaling pathway (32 genes), Ras signaling pathway (24 genes), Pathways in cancer (34 genes), Prostate cancer pathway (11), Estrogen signaling pathway (10 genes), Transcriptional misregulation in cancer (14) are up regulated while genes from Mismatch repair (4), cell cycle regulation are observed to be down regulated. Studies in ALL patients showed that high mTOR expression was associated with poor prognosis in Acute Lymphoblastic Leukemia (ALL) patients.

### LEISHMANIASIS

#### Mechanism of resistance towards paromomycin in *Leishmania donovani*

Microarray analysis revealed 129 modulated genes in paromomycin resistant parasite indicating probable adaptations in drug resistant mutants which



included a) reduced oxidative phosphorylation b) increased glycosomal succinate fermentation and substrate level phosphorylation c) reduced DNA synthesis and increased DNA damage repair d) decreased protein synthesis and degradation e) dependency on lipids and amino acids for energy generation f) increased ABC transporters mediated drug efflux. Based on results obtained, it was proposed that the mechanism of PMM resistance is multifactorial, involving various processes. The study provides the comprehensive insight into alteration of parasite behaviour associated with PMM tolerance in *L. donovani* that will help to design strategies to increase lifespan of this important antileishmanial drug.

#### Studies on mechanism of resistance towards artemisinin in *Leishmania donovani*

Inherent *in vitro* sensitivity of different field isolates of *L. donovani* towards artesunate was investigated and an experimental artesunate tolerant isolate was generated. A wide range of variation ranging from  $8.82 \pm 0.85 \mu\text{M}$  to  $140.33 \pm 11.43$  in  $\text{IC}_{50}$  values was observed. Experimental artesunate resistant parasite was >9 fold tolerant to NO stress, compared with the wild type parasite. Both resistant and wild type parasites were similarly susceptible towards  $\text{H}_2\text{O}_2$  (mimicking oxidative stress) with mean  $\text{IC}_{50}$  values of  $167.39 \pm 0.76$  and  $178.16 \pm 2.3$  respectively. Resistant parasite significantly ( $p < 0.0001$ ) modulated NO production upon infection to mice macrophages and induced reduced NO compared to wild type parasites. In presence of artemisinin, macrophages infected by resistant parasite produced significantly less ( $p < 0.0001$ ) ROS compared to K133 WT infected macrophages.

#### Characterization of amastigote specific gene A1 by gene knock-out and over-expression

It was observed that there was up-regulated expression of A1 gene (LdA1) at amastigote stage at both RNA and protein levels. Sequence homology search revealed that LdA1 is unique to *Leishmania* genus. Sequence and structure level functional annotations predicted the involvement of LdA1 in the biological processes critical for the

survival of parasites. Over-expression of LdA1 in *Leishmania* parasite did not affect the parasites growth, phenotype or infectivity. Mutant parasites with deleted single allele of LdA1 (LdA1+/-) showed reduced motility, size and growth rate at both the life stages, while they remained infective to macrophages. However, the capacity of LdA1+/- to survive inside the macrophages was reduced significantly ( $P < 0.01$ ) beyond 72 h of infection. Attempts to generate null mutants of LdA1 by homologous recombination were not successful indicating that the gene may have an essential role in *Leishmania* life cycle.

#### Application of LAMP for rapid and sensitive detection of *Leishmania* using direct lysis of clinical samples.

Previously, it was reported about SYBR Green I based LAMP assay using 6 primers targeting kDNA region of *Leishmania* as a diagnostic tool for VL and PKDL. In an attempt to enhance the potential of LAMP assay for field applicability, modified Direct Blood Lysis (DBL)-LAMP approach was attempted on VL and PKDL samples. The DBL-LAMP approach eliminates the step of DNA isolation which reduces both cost and turnaround time. Sensitivity and specificity of LAMP assay was evaluated using both column extracted DNA and using direct clinical specimen. In VL cases, LAMP assay with column extracted blood DNA and DBL gave sensitivity of 96.9% and 92.42%, respectively. In PKDL cases slit DNA and blood DNA gave sensitivity of 83.78% and 81.08% respectively whereas direct blood lysis gave sensitivity of 78%. The assay was negative in all healthy and other disease control samples giving it a specificity of 100%.

#### TUBERCULOSIS

It was demonstrated that PE32/PPE65 leads to production of anti-inflammatory cytokines upon interaction with macrophages. Similar results were observed with other co-operonic pair PE35/PPE68. Thus, these PE/PPE operons play distinct and complementary roles in infection process, acting in concert to facilitate adaptation to the hostile host environment. The team carried out an

in-silico analysis to look for unstructured proteins and unstructured regions with their possible relevance in members of important protein families of *M.tuberculosis* i.e. PE-PPE, Mce, MmPL along with secretome and compared it with rest of the proteome as a control. It was found that PE\_PGERS subfamily as most unstructured and enriched with ANCHOR binding sites and eukaryotic like motifs in unstructured regions. The team analysed PE32-PPE65 and PE35-PPE68 pairs as a model to study their interaction and secondary structure through Fourier transform infrared spectroscopy and the structural changes these proteins undergo after interaction. It was found that in all 4 proteins, there is significant content of unstructured regions and after interaction there is structural shift from disorder-to-order in both PE32-PPE65 and PE35-PPE68 complexes. The complex of these protein pairs attained structural stability and enhanced the responses manifold. This depicts a novel way employed by *M. tuberculosis* to survive the hostile environment of host by attaining structural order.

The observation of disorder in mycobacterial proteins led to another protein of interest i.e. PPE37. This intrinsically disordered protein (IDP) was shown to be expressed as a function of low iron stress and was cleaved by *M.tuberculosis* specific protease into N- and C-terminal segments.

### CHLAMYDIASIS

In undifferentiated spondyloarthropathy (uSpA) patients, *C. trachomatis* plasmid was detected in urine by PCR (17.3%) while chlamydial antigen was localized in urine cells (22.8%) of patients by fluorescence assay. Anti-*C.trachomatis* IgM/ IgG/ IgA antibodies were detected in serum of 11.4%, 22.8% and 25.7% patients, respectively. Urine analysis showed the same pattern as the SF analysis in ReA/ uSpA patients but with a lower incidence of *C. trachomatis* DNA PCR results. Detection of *C. trachomatis* DNA in the SF and in the corresponding urine samples showed moderate agreement between the two types of samples. Further comparison of PCR results with the serologic findings showed moderate agreement between PCR and anti-*C. trachomatis* IgA findings in uSpA patients who were otherwise asymptomatic

for genitourinary infection and lacked effusions. It was concluded that chlamydial infection, often occult, is etiologic for many patients with uSpA and non-invasive clinical samples such as serum/urine can be used for diagnosis of chlamydial IgA antibodies and DNA, respectively in uSpA patients, in whom full blown symptoms are unapparent.

In another study undertaken to elucidate the expression of matrix metalloproteinases (MMPs) in recurrent spontaneous aborters (RSA) found to be *C. trachomatis* positive (n = 21), significant up regulation of MMP-2/ MMP-9 and decreased TIMP-1/ TIMP-3 were found in the endometrial curettage tissue of infected aborters in comparison to control women. MMP-2 was found positively correlated with TNF- $\alpha$ , TGF- $\beta$ 1, TGF- $\beta$ 2 and Cox-2. Also, significant positive correlation was observed between MMP-2/ MMP-9 and *C. trachomatis* copy load. Overall data suggested that dysregulated MMPs/ TIMPs promoted excessive endometrial matrix degradation and were involved in sequelae of events leading to abortion in infected women.

### ADULT STEM CELL BIOLOGY

#### Identification of growth stimulating proteins expressed by feeder cells following a novel growth arrest protocol

In earlier study, it was found that a unique processing of Swiss 3T3 cells optimally stimulated the epidermal keratinocyte cells *in vitro* resulting in faster cultured epithelial production with potential in resurfacing the burn wounds. It was hypothesized that exclusive expression of specific proteins might be involved. A strategy was therefore, proposed to initially test whole feeder cell protein extracts on keratinocyte proliferation and to subsequently identify the potential proteins through sub-cellular fractionation and proteomic approach that could be used as a substitute for feeder-dependent stem cell culture systems. The specific sub-set of 3T3 cells termed as the 3K3D (Chugh et al 2015) was expanded.

The patent protected technology of growing cultured epithelial autograft and the subsequently accomplished characterization and analysis of

Quality Control and Quality assurance tests was proposed for undertaking issues involving culture of human epidermal keratinocytes in the presence of a specific sub-set of SWISS 3T3 feeders cells (Chugh et al 2015) which were growth arrested with low concentration of Mitomycin C using an innovative dose derivation (Chugh et al 2016). A Prototype has been prepared (Yerneni and Chug 2014). As part of the Quality Control and Quality assurance issues, earlier the team found no detectable residues of mitomycin C in the final product. The team has now completed the pre-clinical testing of the cultured keratinocytes for tumorigenesis in nude mice and Karyotyping by G-Banding in cultures established from human skin Biopsy.

### BIO INFORMATICS CENTRE

Major research focus of Biomedical informatics centre is “to implement/develop biomedical informatics techniques for assisting disease diagnosis and therapies at the point of patient care.”

- **Database of genes associated with Psoriasis (dbGAPs)** – The database includes 202 genes with genetic markers (SNPs, INDELS, CNVs etc.) associated with psoriasis (<http://bmicnip.in/dbgaps/>) collated from 344 articles. An interactive search engine is attached to the database for easy retrieval of information based on general queries such as genetic markers in exon, intron, UTR segment of a gene etc. A comparative genomic tool to assist biomedical scientist in detecting known psoriasis SNPs in an individuals’ genome/gene sequence. It also listed analytical reports on gene ontology terms and pathways controlled by the 202 genes.
- **Oesophageal Cancer Database** - The database reports genetic variations as well as dysregulated transcriptome in Oesophageal Cancer (Genetic variations in 161 genes have been compiled; Expressions of 278 genes are identified to be dysregulated in disease condition).
- **TiD: Target identification**– An automated tool for drug target mining from whole proteome of bacteria within ~ 2hrs.
- **Psoriasis patient registry** – Six years retrospective data on psoriasis patients compiled from National Institute of Pathology histopathology records (2010-2015). *Framework is developed to implement the patient registry in Safdarjang hospital in prospective mode.*
- **T-cell driven peptides as subunit vaccine candidates against Zika virus** – Twenty one subunit vaccine candidates have been identified through immunoinformatics approach.
- **Design of peptide inhibitors against IL17: *In silico*** mutagenesis, Protein-protein docking, molecular dynamics simulations.
- **Lead antibody design:** Lead antibodies are designed for IL6 and IL23
- **Workshops/training:** Three workshops/training programs organized.

### PUBLIC HEALTH

The “cultured epidermis” produced by an in-house novel processing technology has been subjected to pre-clinical safety evaluation and is qualitatively comparable to “Epicel” of Vericell Corporation, USA (<http://vcel.com>) and is now ready for clinical trial-commercialization for burns.

LAMP assay established for field application for diagnosis of KA and PKDL is ready for commercialization.

### AWARDS

- Dr. Poonam Salotra was awarded J.C. Bose National Fellowship- 2017.
- Dr. Poonam Salotra was awarded Drs. Kunti & Om Prakash Oration Awarded by ICMR.
- Dr Nasreen Ehtesham was awarded Kshanika Oration award by ICMR.
- Dr. Poonam Salotra was appointed Member of RTAG (Regional Technical Advisory Group), SEARO, WHO, 2017-2019.
- Dr. Poonam Salotra was appointed member of the WHO Advisory Panel on Parasitic Diseases, 2017 to 2018.



## NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY, MUMBAI

NIIH has projects under tribal research, intramural projects and extramural projects. Apart from this, institute also provides diagnostic services for variety of hematological and immunological disorders. Providing prenatal diagnostic services many severe hematological and immunological disorders is also a very important activity of our institute.

### PROJECTS UNDER TRIBAL HEALTH RESEARCH FORUM

- Newborn Screening (NBS) for Sickle Cell Disease and providing comprehensive care to understand the natural history of Sickle Cell Disease in Tribal Populations in Madhya Pradesh and Gujarat.
- Micro mapping of G6PD deficiency among the tribals of India and its importance for antimalarial therapy.
- Establishment of Prenatal Diagnosis of  $\beta$  Thalassemia Syndromes and Sickle Cell Disorders in Madhya Pradesh and Assam.

### Extramural projects

- Establishment of satellite centre for hemoglobinopathies at Chandrapur.
- Quality Assurance Programme for Molecular and Prenatal Diagnosis of Hemoglobinopathies.
- Molecular characterization of undiagnosed cases of congenital hemolytic anemia using a multigene next generation sequencing panel.
- Centre of excellence for Research, Diagnosis and Management of Primary Immunodeficiency disorders (PID).
- Study the role of T cell Receptor Excision Circle (TREC) assay in diagnosis of various combined immunodeficiency disorders.
- Understanding role of NK cells in immunopathogenesis of Dengue virus infection.

- Understanding the role of Neutrophil Extracellular Trap (NET) in pathophysiology of CGD patients.
- Harnessing Induced pluripotent stem cells (iPSCs) technology for understanding the pathophysiology and management of human Primary Immunodeficiency Disorders.
- Assessing the role of cell-derived microparticles in dengue virus infection.
- Tissue factor and endothelial cell microparticles as prognostic markers for monitoring bypassing agent therapy in haemophilia patients with inhibitors.
- Comparative Study of red cell antigen profile among malaria infected patients and normal population from Assam.
- Systemic Lupus Erythematosus (SLE) – An Investigation into diagnostics and disease pathogenesis.
- Cytogenetic and molecular study of Myelodysplastic syndrome.
- Study of Single Nucleotide Polymorphisms (SNPs) of Multiple Candidate Genes (ABCB1, ABCG2, CYP3A4, CYP3A5, SLCO1B3, AGP1, SLC22A1) in Imatinib resistant Chronic myeloid leukemia (CML).
- Molecular analysis of telomerase RNA component (TERC) gene and telomerase reverse transcriptase (TERT) gene and DKC1 gene in aplastic anemia.
- To determine the prevalence of serological and molecular markers of Hepatitis B virus in voluntary blood donors from Maharashtra region.

### International Projects

- Molecular study of RH gene variants in Indians **Indo-French collaboration under CEFIPRA**
- Collaborative effort to study pathophysiology and molecular characterization of congenital anemia in India **Indo-Japan collaboration JSPS-DST.**

- Collaborative effort to understanding and characterization of Novel Molecular Changes in Fanconi Anemia (FA) **Indo-Japan collaboration JSPS-DST.**
- India-Japan collaborative research aspiring for prevention and diagnosis of erythrocyte associated diseases **Indo-Japan collaboration JSPS-DST.**

### Intramural projects

- Innate immune receptors gene polymorphisms and their role as genetic determinants of infection in neonatal sepsis.
- Implication of the Presence of Globin Gene Modifiers on Fetal Diagnosis of  $\beta$ -Thalassaemia and Sickle cell disorders.
- Role of Erythroid Kruppel-like Factor (EKLF or KLF1) in Haemoglobinopathies.
- Prevalence and molecular characterization of NADH-Cytochrome b5 reductase deficiency in India.
- Oxidative stress in hereditary chronic hemolytic anemia and the protective effect of natural antioxidants- fermented papaya preparation, strawberry extract and potato peel: An in-vitro study.
- Understanding of molecular pathology of Chronic Granulomatous disease (CGD).
- Clinical, molecular and immunological characterization of common T-B+ and T-B- Severe Combined immunodeficiency (SCID) in Indian Population.
- Clinical, Immunological and Molecular Characterization of an Autoimmune Lymphoproliferative Syndrome (ALPS) and B-cell expansion with NF $\kappa$ B and T-cell anergy (BENTA) in Indian Population.
- Defining immunological markers for response to immunosuppressive therapy in patients with Aplastic Anemia (AA).
- An RNAi therapeutic targeting protein C to promote hemostasis in hemophilia.
- Importance of genetic screening for antithrombin (AT) deficiency in patients with thrombosis.
- Clinical spectrum and molecular basis of thrombotic microangiopathies.
- A prediction model of preeclampsia using a combination of biomarkers.
- Microparticles versus thrombin generation assays as predictive tests for dosage adjustment of bypassing agents in hemophilia patients with inhibitors.
- Impact of co-inheritance of interacting Human Leukocyte Antigen (HLA) with killer cell immunoglobulin-like receptor (KIR) on clinical presentation and complication of Systemic Lupus Erythematosus (SLE).
- Immune Profiling in newly diagnosed Scleroderma patients from India.
- Adipocyte-associated cytokine network in glomerular disease.
- DNA analysis of blood donors for minor blood group antigens Study on Human Neutrophil Antigens (HNAs) and associated antibodies.
- Study of Fucosyltransferases (FUT1, FUT2 and FUT3) haplotypes and their association with some autoimmune disorders.
- Genetic Determinants for Expression of Duffy Antigens on Red Blood Cells and their Interaction with *Plasmodium vivax*.
- Study on Human Neutrophil Antigens (HNAs) and associated Antibodies Non invasive prenatal RhD typing.
- Molecular genotyping of Kell, Duffy and Kidd blood group systems.
- Development of a novel and simple nano particle based red cell antigen detection system for screening rare blood donors.
- Understanding the molecular mechanism of oxidative stress and mitochondrial function in impaired Fanconi Anemia pathway.
- Molecular study of JAK2, MPL and CALR genes in BCR-ABL negative myeloproliferative neoplasm patients.
- Genetic and Epigenetic study of Myelodysplastic syndromes.
- Molecular study of SRC kinase family in drug resistance chronic myeloid leukemia.

- Glycosylation of the serum haptoglobin-beta – A potential biomarker for hepatocellular carcinoma due to Hepatitis B virus.
- Differential HCV expression in variable liver pathological conditions.
- Pharmacogenomics of hematological toxicity of Zidovudine in HIV patients.

### Public Health

- The Institute continues to offer prenatal diagnosis for various hematological disorders to couples referred from different parts of the country. Prenatal diagnosis was offered to 249 with hemoglobinopathies, 68 families with inherited bleeding disorders including hemophilia A & B, vWD and others rare bleeding disorders, 6 families with primary immunodeficiency disorders including Leukocyte Adhesion Deficiency (LAD-I), Chronic Granulomatous Disease (CGD), Severe Combined Immunodeficiency (SCID) and Familial Hemophagocytic Lymphohistiocytosis (FHL) and 1 family with GPI deficiency.
- Specialized diagnostic services are provided by different departments including transfusion medicine, hematogenetics, hemostasis and thrombosis, paediatric immunology and leukocyte biology, cytogenetics and clinical and experimental immunology. More than 12,000 patients from India and abroad have availed these diagnostic facilities.
- Large number of cases of these inherited and acquired disorders are given specific diagnosis and counseling at our institute. Hemoglobinopathies 654 cases of inherited bleeding disorders were diagnosed this year including 149 patients with hemophilia A, 36 with hemophilia B, 29 with Von Willebrand disease 32 with rare factor deficiencies, Glanzmanns thrombasthenia 19; Bernard Soulier syndrome 5; Unclassified platelet function defect 4.
- 115 cases of primary immunodeficiency disorders were diagnosed including 49 cases with phagocytic defects, 35 with antibody deficiency, 11 with severe combined

immunodeficiency and 23 with diseases of immune dysregulation.

- 554 cases of genetic defects and 738 cases of various hematological malignancies availed the kayotyping services by NIIH.
- From large number of patients referred for hemolytic anemia workup 23 cases were diagnosed as Red cell membrane defect , 4 cases of pyruvate kinase deficiency, 7 cases of hypermethemoglobinemia, 28 cases of G6PD deficiency were diagnosed.
- Under the Satellite centre for haemoglobinopathies at Chandrapur, basic facilities for diagnosis of haemoglobinopathies have been established and already 400 sickle cell patients have been enrolled which will be followed up regularly. Antenatal and newborn screening has also been initiated. During antenatal screening, high risk couples were identified and offered prenatal diagnosis.
- A total of 3477 newborns were screened from Gujarat and Madhya Pradesh. In Gujarat 649 (22.0%) sickle cell trait and 76 (2.5%) SCA/HbS -b-thalassemia babies were identified whereas in Madhya Pradesh 42 (7.9%) sickle cell trait and 9 (1.7%) SCA/HbS -b-thalassemia babies were detected. The newborn screening programme for sickle cell anemia has been very successful in providing early diagnosis and comprehensive care to infants with sickle cell anemia. Follow up of these babies suggests that the sickle related complications increase with age and they may not necessarily remain mild.
- A total of 8047 individual blood samples from 30 tribal groups were studied under the project entitled 'Micro mapping of G6PD deficiency among the tribals of India'. Prevalence of G6PD deficiency in some of the tribal groups in different states was found to be 1.9% to 18.1% with a highest frequency among the Siddhis from Karnataka.
- Laboratory for Prenatal Diagnosis (PND) of  $\beta$ -Thalassemia and Sickle cell anaemia has been established at NIRTH, Jabalpur and RMRC, Dibrugarh. In NIRTH, Jabalpur, a total of 3671 pregnant women were screened for hemoglobinopathies and 30 high risk



- couples were identified of which 4 had agreed for prenatal diagnosis.
- Centre of excellence for Research, Diagnosis and Management of Primary Immunodeficiency disorders (PID) has been established at NIIH. Under this project advanced diagnostic facilities for diagnosis of PIDs have been established. Facilities for molecular confirmation for many of these severe PIDs have also been initiated. 1386 patients from different part of the country have availed these facilities. Prenatal diagnosis is also provided for some of these severe disorders.
  - Three scientists from the institute received training in the area of next generation sequencing under Indo-Japan collaborative which can be eventually established at NIIH and then applied to variety of inherited hematological and immunological disorders referred to NIIH for molecular diagnosis.
  - The use of circulating Microparticles (cMPs) and other serum based biomarkers were tested for early prediction of Preeclampsia (PE). At 17-23 weeks of gestation, combination of Copeptin, PIGF and CD142 gave AUC of 0.979 with 94.9% sensitivity and 90.4% specificity for PE prediction. It was demonstrated that the combination of serum markers and plasma microparticles can be used for prediction and also for discrimination of PE from other pregnancy complications such as IUGR.
  - Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP) are two rare, yet life threatening Thrombotic Microangiopathies (TMA) characterized by endothelial cell injury, intravascular platelet-fibrin thrombi, and vascular damage. A project which aims to develop a national level facility for comprehensive evaluation of TTP-HUS and to characterize and classify them has been initiated at NIIH.
  - A project on evaluating microparticles as promising biomarkers which may help predict the risk of developing severe dengue virus infection has been initiated at NIIH.
  - DBT funded task force project, a basic autoimmune diagnostic laboratory was established at Assam Medical College, Dibrugah and an advanced laboratory was also set up at Tezpur University, Assam. The data from Western region (NIIH) and Eastern region (Tezpur University) is now compared for SLE clinical presentation, disease severity and immunological parameters to look for the genotypic and phenotypic diversity between two regions.
  - Molecular study of *FANCA*, *FANCC*, *FANCG*, *FANCE* and *FANCL* genes in 21 confirmed patients revealed 12 patients with *FANCA* mutations, one with *FANCC* and remaining one with *FANCL* mutation. This is the first large series of patients with FA which has given important information on the underlying molecular mechanisms in these patients. It has also helped in genetic counseling and prenatal diagnosis of these patients. The study has also shown significant oxidative stress and mitochondrial dysfunction in these patients suggesting its role in pathogenesis of these disorders.
  - Studies on spliceosomal mutations in a large series of patients with MDS will be useful in understanding the pathways involved in pathogenesis of the clinically heterogeneous disease of MDS.
  - Study on the multiple Candidate Genes Imatinib resistance chronic myeloid leukemia results revealed that the *SLCO1B3* 334TT genotype is associated with a failure of obtaining a complete cytogenetic response, thereby suggesting its potential role as a predictive biomarker in IM therapy in CML patients.
  - The work done under CEFIPRA funded project on molecular genotyping of RhD variants (identified serologically) in the Indian population has led to a discovery of novel molecular mechanism viz. exon 3 duplication predominantly responsible for weak D variant in Indian population. An Indian-specific genotyping assay has been designed and a Patent application has been filed in Europe on March 8, 2017 under number 17 305246.5.

- Study on Telomerase RNA Component (TERC) gene, Telomerase Reverse Transcriptase (TERT) and Dyskeratosis Congenita (DKC1) gene in idiopathic aplastic anemia showed telomere shortening observed in the study can be considered as biomarker for the aplastic anemia as after ATG treatment the telomere length was restored. Telomere complex gene mutations were not detected in the study, however the polymorphisms of TERT gene may have role in aplastic anemia.
- Work on DNA analysis of blood donors for minor blood group antigens has helped to know the frequency of Dombork and Diego antigens for the first time in Indian population.
- Study on Human Neutrophil Antigens (HNAs) and associated Antibodies will help us to understand the prevalence of various HNAs in Indian population and will be extremely useful for establishing diagnostic facilities for patients with suspected autoimmune neutropenia.
- Occult Hepatitis has been characterized by the absence of HBsAg in the blood. On the basis of markers tested for Hepatitis B virus, the percentage of samples negative for HBsAg marker, but positive for Anti-HBc was found to be ranging from 6% -46.7% among the various districts of Maharashtra.

### NEW CORE FACILITIES AT NIIH

#### Next generation sequencing and bioinformatics facilities at NIIH

NIIH has been working on variety of inherited hematological and immunological disorders. One of the important aspects of evaluation of these disorders is identifying the underlying genetic mutations. Due to large genetic heterogeneity seen in these disorders, genetic diagnosis by conventional Sanger sequencing becomes extremely time consuming and expensive. To establish facilities for Next Generation sequencing and Microarray systems at the institute for understanding molecular pathology of various hematological and immunological disorders and identify novel

genetic defects, three scientists and three students/ technicians were trained at USA, UK and Japan under different collaborative schemes/ fellowship programmes.

#### Patents

Patent filed in Europe on March 8, 2017 under number 17 305246.5 “RHD gene allele associated with a weak D phenotype and its uses”

### CONFERENCES / WORKSHOPS CONDUCTED

#### Diamond Jubilee celebrations of NIIH: International Conference on “Revolution of Laboratory Medicine in Modern Biology” held from 15th-17th February, 2017.

The International Conference on “Revolution of Laboratory Medicine in Modern Biology” was held at Nehru Centre on the occasion of Diamond Jubilee of the Institute from 15<sup>th</sup> to 17<sup>th</sup> of February 2017. A total of 15 International and 78 National faculty members participated and delivered lectures/chaired various sessions/participated in panel discussions. One hundred and seventy four delegates from various parts of the country attended during these three days of the Conference. The Maharashtra Medical Council has awarded 4 credit points.



Fig. 1: Diamond Jubilee celebrations of NIIH.



## WORKSHOPS CONDUCTED

- A training workshop was conducted for 15 Medical Officers, Laboratory technicians and District coordinators from Ahmednagar and Nagpur for Hemophilia, Thalassemia and Sickle cell disease from 20<sup>th</sup> to 24<sup>th</sup> March 2017. This was sponsored by Directorate of Health Services, Maharashtra State.



**Fig. 2:** workshop conducted at Ahmednagar and Nagpur for Hemophilia.

- NE India Hemophilia CME cum Coagulation Wet Workshop was conducted by NIIH team on 21<sup>th</sup>-22<sup>th</sup> Sep 2016 at Regional Institute of Medical Sciences, Imphal in which hands on training in various coagulation tests was offered to 40 Medical Officers and Laboratory technicians who attended from different parts of NE India.



**Fig. 3:** NE India Hemophilia CME cum Coagulation Wet Workshop.

- Annual training programme for Medical Officers and Laboratory Technicians from 10<sup>th</sup> April to 9<sup>th</sup> May, 2017 was conducted. Seventeen Medical officers and two technicians attended.



**Fig. 4:** Annual training programme.



- Four days training program in basic techniques in blood banking and Immunohaematology was conducted at Andaman & Nicobar Islands, at G.B Pant Hospital, Port Blair, from 7<sup>th</sup>-10<sup>th</sup> March, 2017 where 23 medical officers and technicians from different blood banks were trained.



**Fig. 5:** Four days training program in basic techniques in blood banking and Immunohaematology.

## NATIONAL INSTITUTE OF TRADITIONAL MEDICINE, BELAGAVI

### Evaluation of anti-inflammatory and analgesic activities of *Plumbago zeylanica* in osteoarthritis of knee

A number of anti-arthritis agents are available for the treatment of arthritic disorders such as Adalimumab, Infliximab, Abatacept, Rituximab, and aurothiomalate and these are neither completely safe, nor affordable for common/poor people. Recently, there is surge in the identification and validation of natural product based therapies, especially for chronic conditions including OA and RA. *Plumbago zeylanica* L. root paste (Chitrakmoola lepa) is used by local health practitioners in the treatment of joint pains in general. Hence, a study is being undertaken which aims to evaluate the safety and efficacy of this preparation against OA. The study is open label, single centric, three groups with pre-test and post-test design, and with a sample size of 120 patients. So far, 22 patients with osteoarthritis, meeting all inclusion/exclusion criteria have been included in the study. Patients

with Osteoarthritis are allotted in mild, moderate and Non-responsive to NSAIDs groups and are given test preparation. The preliminary results of the trial are encouraging.

### Evaluation of Anti-HSV activity of non-nucleoside inhibitor to prevent drug resistant HSV mutants

The centre has synthesized a series of  $\alpha$ -pyrone from natural product structure, of which  $\alpha$ -pyrone-3-carboxylic acid analogue (3d) showed significant antiviral activity against isolates of HSV-1 and HSV-2 at 17-25 $\mu$ g/ml at 2-6 h post-infection, by interfering the HSV-DNA polymerase. The chemical study using thioether Zn (II) fluorescent sensor revealed its use in DFT computation and live cell imaging; while Pyridylthioether-hydroxycoumarin Schiff base was found to be useful as selective Zn<sub>2+</sub> fluorescence sensor in life cell imaging and as secondary probe for ATP sensing (Publication No: 4,8,9). The safety and *in vivo* efficacy in mice is yet to start.

### Evaluation of anti-HSV activity of an alkaloid, its characterization, synthesis and mechanism of action

An indole alkaloid from a folk medicinal plant showed potent antiviral activity against HSV-1 and HSV-2 (Wild type, clinical isolates, and Acyclovir resistant strain) at EC<sub>50</sub> of 1.1-1.5  $\mu$ g/ml and EC<sub>99</sub> of 5.0  $\mu$ g/ml with CC<sub>50</sub> of 30 $\mu$ g/ml. The compound binds with the lysine-specific demethylase-1 (LSD-1) to block the immediate early (IE) transactional events of HSV. Animal study revealed its efficacy in cutaneous and vaginally infected mice at 0.25-0.5 mg/kg dose without any toxic manifestations. To reduce its cytotoxic dose, centre had synthesized several analogues, out of which AK-IV-14-25-T showed similar anti-HSV activity at 30.5 $\mu$ g/ml, and CC<sub>50</sub> at 300 $\mu$ g/ml with complete inhibition of plaque formation at 35-40 $\mu$ g/ml at 2-4h post-infection. This study demonstrated that AK-IV-14-25T block IE transcription by inhibiting the recruitment of LSD-1 or its association with HCF1. The safety study in Balb C mice revealed that the compound is safe up to 1200  $\mu$ g/ml.

### Evaluation of in-vivo anti-HSV activity of HM-7, to develop an antiviral formulation (Translational)

The centre has already tested the toxicity and efficacy in Swiss Albino mice model of HSV-2 and HSV-1. Vaseline based formulation has also been successfully tested. The infection model in Balb/C is in progress; while the topical formulation development is initiated in August 2017 with the help of M/S Parker Robinson Private Ltd, the Company who have applied to ICMR for its clinical trial and further development.

### Preclinical evaluation of safety and efficacy of the decoction of the plant RMRC-BM IP\_156 for anti-diabetic activity and characterization of active compound(s)

Traditional system of medicine has been practiced since historical times and traces its roots to ancient civilizations. More than 80% of the population, especially the rural population are still dependent on traditional herbal remedies for their primary healthcare. Herbal based Traditional Medicine has been practiced in several regions of India. This Institute has identified a practice for the management of diabetic mellitus, from a place nearby Dharwad, Karnataka. The traditional practitioner was treating the diabetic patients with the decoction of a plant coded as RMRC-BM IP\_156. Therefore, a study is undertaken to evaluate the safety and efficacy of the plant initially. The effect of decoction of the leaves of the plant RMRC-BM IP 156 on blood glucose level was carried out in normal rats using various doses (X/2, X, 2X and 4X). The decoction did not decrease the fasting glucose level of the normoglycemic rats after 2 hours which implies that the decoction is safe and does not cause hypoglycemia in normal rats. In Oral Glucose Tolerance Test (OGTT), the decoction at 2X and 4X doses was found effective in reducing blood glucose level by 2 hours after treatment as compared to the normal (no treatment) group and similar to the standard drug metformin, while glibenclamide showed significant reduction in the glucose level, thus causing hypoglycemia.

### Evaluation of antiviral activity of a bioflavonoid against Herpes Simplex Virus infection and its modus operandi

The centre has isolated a known flavonoid luteolin, from the flavonoid-rich extract of a traditional medicine, with significant activity against wild-type and clinical isolates of HSV-1 and HSV-2 (EC<sub>99</sub>, 81-86.5 and 37-40.2 µg/ml), and the effect was significant (p<0.001) when added at 1-4 h post-infection. The mechanistic study demonstrated that luteolin significantly inhibit HSV at 2h prior to, during and 4 h post-infection by suppressing NFκB activation and down-regulate the expression of TNF-α, IL-1β, IL-6, iNOS and COX-2 with the production of IFN-γ, essential to inhibit viral infection, in clinical isolates. Further, animal study in Balb/C mice revealed that the compound has good safety profile and animal efficacy study is yet to be initiated.

### Pharmacological evaluation of formulation containing *Emblia officinalis*, *Curcuma longa* and *Trigonellafoenum-graecum* for anti-diabetic effect in experimentally induced diabetes in rats

The prevalence of type 2 diabetes is rising globally. Due to limitations of modern medicines for the management of diabetes, there is a global demand for better herbal/traditional formulations. One such formulation containing *Emblia Officinalis*, *Curcuma Longa* and *Trigonella foenum-graecum* for management of diabetes is in practice in this region. The *in-silico* network pharmacology studies conducted by centre earlier revealed complex interaction of multiple bio-actives interacting with multiple targets involved in regulation of glucose metabolism. Hence, it was aimed to carryout *in-vivo* preliminary pharmacological evaluation of the formulation containing *Emblia Officinalis*, *Curcuma Longa* and *Trigonellafoenum-graecum* for anti-diabetic effect in rats. The results of the preliminary preclinical *in vivo* study revealed that the formulation favorably modulated the biochemical parameters such as plasma glucose and triglycerides, indicating potential anti-diabetic efficacy. Further detailed study consisting graded doses is under progress.

### Formulation of thermo-reversible gel of cranberry juice concentrate: Evaluation, biocompatibility studies and its antimicrobial activity against periodontal pathogens

A thermo-reversible gel of cranberry juice concentrate was formulated which was found to be comparable in efficacy to the widely used standard chlorhexidine gluconate gel (0.2%) against periodontal pathogens through a combination of a number of *in vitro* studies.

This could serve as a novel herbal alternative to currently available periodontal treatment modalities without the adverse effects of chlorohexidine.

### Resolving identification issues of *Saraca asoca* from its adulterant and commercial samples using phytochemical markers

*Saraca asoca* (Roxb.) De Wilde (Ashoka) is a highly valued endangered medicinal tree species from Western Ghats of India. A phytochemical fingerprinting method using HPLC of phenolic compounds (gallic acid, catechin, and epicatechin) coupled to multivariate analysis was developed to detect adulteration with *Polyalthia longifolia* (Sonnerat) Thwaites (False Ashoka) which is rampant in the crude drug market and will aid the herbal drug industry in addressing quality issue with this traditional medicine.

### Combination of DNA isolation and RP-HPLC analysis method for bark samples of *Saraca asoca* and its adulterant

A method for simultaneous DNA isolation and phytochemical extraction from dried bark powder of *Saraca asoca* was developed.

The method is simple, reliable and it requires small amount of sample with an option of integrating both phytochemical and DNA-based profiling, from the same starting material and therefore, could be useful for applications such as quality control assessment of *S. asoca* products.

### Evaluating the effect of selected *Vrikshayurvedic* preparation on yield and quality of biomedically important secondary metabolites in medicinal plants

The present work is an effort to evaluate the efficacy of *Kunapa jala* and *Panchagavya*, in two of the medicinal plants, namely *Ashwagandha* (*Withania somnifera* Dunal) and *Kalamegh* (*Andrographis paniculata* Nees.). Both the preparations have been standardized based on their pH, EC and analysis of nutrient contents. RP-UFLC method developed for simultaneous detection and quantification of Withaferin A, Withanolide A and Withanolide B in *W. somnifera*, is a first report of such protocol. The results indicated maximum yield of WFA, WIA in *Panchagavya* group and WIB in *Kunapa jala* group. The study proved the efficacy of the *Vrikshayurvedic* preparations by recording highest yield of biomass in both the plants in *Kunapa jala* group.

### Micropropagation of *Curcuma pseudomontana* and *Iphigenia stellata*, Endangered Medicinal Plants from Western Ghats

The study is aiming at developing micropropagation protocol for two endemic, threatened medicinal plants, viz. *Curcuma pseudomontana* Graham and *Iphigenia stellata* Blatter and to compare the quantity of bioactive compounds in *in vitro* grown plants with that of plants *in vivo*. So far, the protocol for micro-propagation of *C. pseudomontana* is established. The curcumin content in *in vivo* populations of *C. pseudomontana* was analysed and is being compared with that in *in vitro* grown plant.

### Plant extracts having insecticidal effect to *Phlebotomus argentipes* Annandale and *Brunetti* (Diptera: Psychodidae), and mosquitoes

Plant-based insecticides have been used in many parts of the world against different vectors, as they are environment friendly and easily biodegradable. A study in collaboration with RMRI, Patna is being undertaken on plant selected on the basis of lead findings of preliminary studies conducted



so far. The leaves of plant PK-48 have been found effective. Further, toxicity evaluation of Methanol extract of PK48 Leaves by % Haemolytic activity showed the extract to be Non-toxic (at 500 mg/L & 125 mg/l.) to the human being. The lyophilized extract of methanol leaf extract of the plant *pk-48* was subjected to the analysis of phytochemical profile and its fractions were prepared and the efficacy of the lyophilized preparations was tested at RMRIMS-Patna. A patent application has been submitted to IPR Unit, ICMR New Delhi for filing. An extract of a plant coded as *IC\_Goa* has found to have good potential in the control potential against malaria, filariasis and arboviral vectors, by the National Institute of Malaria Research, Field Unit, Goa. The extract incites growth preventing activity by way of not allowing the pupal stages of mosquito to emerge as adult flies, at a very low dose. The extract was also lethal to larval stages as well. In view of its potential in controlling mosquitoes, a study has been undertaken to evaluate the potential of this plant as a source of mosquito control tool, in collaboration with NIMR FU, Goa.

#### Identification of potential herbs against *Shigella flexneri 2a* targets by Herbal Informatics approach.

ICMR-NITM has combined *in silico* computational studies with modern big data biology with the age old knowledge of Indian traditional medicines (Herbal Informatics) to help validate traditional herbal medicines against various diseases as a precursor to wetlab and clinical studies. In one such study, *Shigella flexneri 2a*, the commonest cause of childhood shigellosis in developing countries including India was chosen as the target and plants that are traditionally used in treatment of diarrhoea were evaluated using 3D structure prediction and binding pocket analysis, annotation of SNPs from Whole Genome (WGS) data, identification of lead compound from plant sources, and subtractive genomics. Top 3 compounds present in 8 traditional medicinal plants have been identified against 5 drug targets in *S.flexneri 2a* for subsequent evaluation through wet lab and clinical studies. Use of Herbal Informatics as an initial screening tool helps in reducing cost and effort in validation of traditional medicine and also indicates possible mechanism of action.

#### Immunoepidemiology of lymphatic filariasis in endemic districts of Karnataka

Currently the 'Global programme for Elimination of Lymphatic Filariasis (GPELF)' tools for detecting exposure to filarial infection. Hence, a study on immunoepidemiology of LF is being carried out in collaboration with NVBDCP and other ICMR centres for identifying biomarkers. Two genes of *Wuchereria bancrofti*, RMRC-BLG1 and G2, and a peptide (RMRC-BLP1), were identified as biomarkers of exposure. RMRC-BLG1 and G2 genes were cloned, expressed and the recombinant proteins purified. The RMRC-BLG1 rec.protein and RMRC-BLP1 peptides were investigated for sero-reactivity and both assays were able to differentiate the filarial infected individuals from uninfected ones. A lateral-flow Point-of-Care (P-o-C) diagnostic for detecting antifilarial antibodies was developed and evaluated for its efficacy in detecting the infection in mf+ve individuals.

#### Immunomodulation by parasitic Macrophage Migration Inhibitory Factor (MIF) in Type2 Diabetes Mellitus

Parasite Macrophage Migration Inhibitory Factor (MIF) homologues are reported to play a role in immunomodulation of inflammatory diseases. This study was undertaken to investigate immunomodulatory (pro- and anti-inflammatory) effects of MIF of *Wuchereria bancrofti* (Wb-MIF) in diabetic rats. The WbMIF was cloned in the expression Vector pRSET B and transformed into salt inducible *E. coli* strain GJ1158 and checked for expression. Enzymatic activity of the purified recombinant protein was checked for tautomerase assay and found to have good activity. The rec.MIF protein is being tested for immunomodulation in diabetic rats.

#### ProSnip: Development of a GUI based tool for the protein annotation of SNPs from prokaryotic Whole genome NGS data

A tool called ProSnip was developed by ICMR-NITM that automates the workflow of SNP annotation from the Whole genome NGS short read data from resequencing projects. The tool will help users with further ease of analysis and automation.

### First detection of human parechovirus infection with diarrhoea

Clinical cases of HPeV have been detected for the first time in children with diarrhoea in India by studies carried out by ICMR-NITM in Karnataka and Maharashtra. Circulation of at least four genotypes has been documented, of which genotype 1 was found to be more common. The study recommends consideration of HPeV as one of the emerging aetiological agent of acute diarrhoea in children.

### Resurgence of Diphtheria in Rural Areas of North Karnataka

An ICMR-NITM study during 2012-2015 identified an upsurge in cases of diphtheria in North Karnataka, particularly in Vijayapura District and reported the emergence of penicillin resistance for the first time in India. The study called for enhanced surveillance for the disease and making antidiphtheritic serum available in key hospitals in the region.

### Tribal Health Research Unit

In the present year, the THRU undertook two major activities: (i) Assessment of Health Status of Kathodi-Katakari Tribe (ii) Micro mapping of G6PD deficiency among the tribals of India and its importance for anti-malarial therapy and initiated one more on Assessment of Nutritional Status and Health Seeking Behavior among Siddi Tribe in Karnataka, funded by ICMR. Assessment of present health status of Kathodi-Katakari tribe indicated the deficiency of Vitamin-A and Microcytic hypochromic anaemia with Eosinophilia in majority of the population. The detailed report has been submitted to State Government recommending appropriate interventions. In addition, documentation of 46 species of ethnomedicinal plants from tribes for various health conditions has also been made, which will be considered for their scientific evaluation.

### Micromapping of G6PD among tribals in Karnataka with reference to malaria

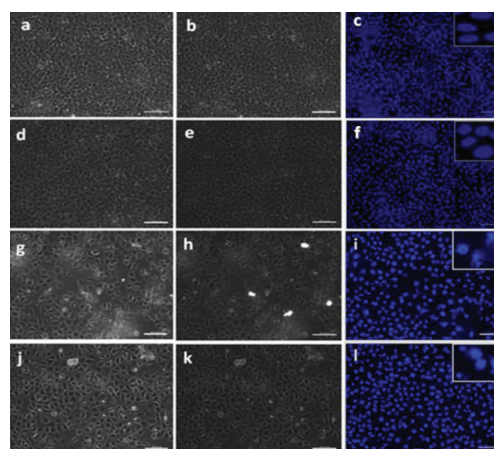
Glucose-6-phosphate-dehydrogenase (G6PD) deficiency has direct bearing on the treatment of malaria and most of the tribal populations live in malaria endemic areas. Inappropriate treatment of antimalarial drugs may result in drug induced

haemolysis. The present study was undertaken to micro-map the deficiency among tribes of Karnataka state as there is no information available so far. About 3320 blood samples have been collected from the Bedar, Siddi, Gouli, Valmiki, Bhovi, Kathodi-Katkari and Kunabi tribes residing in different parts of Karnataka. The samples were screened for G6PD deficiency and samples of Siddi community, a tribe that migrated from Africa, showed high prevalence (8.35%) of deficiency. This is higher than national average (around 7.0%). The mutation in the deficient enzyme was similar to that found in African population. The G6PD deficiency has also been detected in Soliga tribes (6.5%).

### Extramural

### BIOCHEMISTRY

A study on mass spectrometric identification of matrix protein(s) from calcium oxalate stones in nephrolithiasis and assessment of their activity on calcium oxalate crystal-mediated stress in renal epithelial cells, conducted at Amity University, Noida. Flow cytometric analysis and fluorescence imaging ascertained that matrix proteins decreased the extent of apoptotic injury caused by COM crystals on MDCK cells. Moreover, the electron microscopic studies of MDCK cells revealed that matrix proteins caused significant dissolution of COM crystals, indicating cytoprotection against the impact of calcium oxalate injury.



**Fig. 6:** Effect of kidney stone matrix protein extract on calcium oxalate monohydrate induced renal injury in MDCK cells, visualized under phase contrast (Lane 1), polarizing (Lane 2) and fluorescence microscope (Lane 3).

## CLINICAL PHARMACOLOGY

**Regulatory science, capacity building for subject experts:** Applications for approval of clinical trials and for marketing permission, which are made by the industry and the regulatory review, done by subject experts, has recently been criticized for its quality and delays. Handbook for applicants and reviewers of application for clinical trials and marketing permission has been prepared in collaboration with CDSCO and USFDA. It was released in 23<sup>rd</sup> Jan 2017 during the workshops for subject experts who were held to apprise them of the handbook and the evaluation process. Second such workshop was held on 28<sup>th</sup> April 2017 at the SAC ACCP conference in Mumbai which was also focused on regulations and review of fixed dose combinations.

**Regulatory changes and Court decisions:** Impact of regulations on clinical research and practice of medicine is being evaluated in collaboration with Queen Mary University, UK, and Vidhi Centre, Delhi. The work is in progress.

**Package insert for FDC:** An analysis of package inserts of 5 commonly used Fixed Dose Combinations showed that the package inserts do not have any FDC specific information. The Drugs and Cosmetics Act does not provide any FDC specific guidance for package insert. This issue was mentioned by ICMR National Chair at the Drug Technical Advisory Board (DTAB) meeting held in June, 2017 for necessary action.

**Clinical trials centres Network:** There is need to develop Clinical trial sites with good infra-structure, trained staff and SOPs at Govt. institutions to fulfill the demand of trials for new drugs, repurposed drugs, vaccines, implementation studies. A guidance document outlining requirement for clinical trial sites has been prepared. A call for proposals for clinical trials for tuberculosis was sent out. Applications received have been reviewed by committee chaired by ICMR National Chair.

**Rational use of drugs:** Appropriate selection and prescribing of medicines is a basic requirement for rational practice of medicine. Assessment is

considered as best motivator. Assessment conveys what is valued most. Hence, in collaboration with BPS, workshop on prescribing skills assessment was held in Mumbai in April 2017. It was attended by 89 participants, teachers and senior professors, from all over the country. In collaboration with IPS and GSMC KEM hospital a road map for adopting/ adapting it in the teaching and assessment program in India has been prepared for implementation.

**Human Resource development:** In April 2017, the 10<sup>th</sup> annual conference of South Asian Collage, an Affiliate of American College of Clinical Pharmacology was organized in Mumbai, with theme “Clinical Pharmacology for Healthy Ageing”. An IUPHAR-SAC ACCP workshop on “Managing drug induced adverse events in the elderly: can we do better?” and panel discussion on “Are we doing enough for the elderly regulatory challenges and solution” were held on 30<sup>th</sup> April 2017. Recommendation from the panel discussion have been accepted for publication by Lancet Global Health as Blog. The conference was attended by 194 delegates.

In collaboration with British Pharmacology society, workshop on prescribing skills training and assessment was held. It was attended by 89 participants teachers and senior professors from all over India.

In collaboration with CDSCO and USFDA, workshop on regulatory review of application for clinical trials and marketing approval was held on 29<sup>th</sup> April, 2017 in Mumbai, with focused on Regulatory and clinical requirements for fixed dose combination. It was attended by 90 participants from Industry, academia and regulatory agency.

ICMR National Chair delivered 13 invited lectures, chaired sessions, coordinated panel discussions at National and International meetings, workshops, conferences.

## HEMATOLOGY

A study was carried out to investigate the association of KIR gene polymorphisms with childhood leukemia at PGIMER, Chandigarh. 100



children with acute lymphocytic leukemia and an equal number of healthy controls were recruited. Of the 19 KIR genes evaluated, 18 were not protective or susceptible for childhood acute lymphoblastic leukemia. 2DS4\* DEL KIR gene was identified as a protective KIR gene, particularly for B-lineage ALL and for male patients. The frequency of 2DS4\*DEL was significantly greater in patients with a Day 14 M1 marrow as compared to patients with a M2 or M3 marrow. Bx-ID6 was identified as protective genotype for childhood ALL.

### HUMAN GENETICS

A study on molecular (DNA) analysis of Epidermolysis Bullosa Simplex and Dystrophica was carried out at Department of Dermatology, AIIMS, New Delhi. The study showed the clinical application of a recent sequencing technique [Whole Exome Sequencing (WES)] in the diagnostics of rare genetic skin diseases. The manually curated mutational database is an invaluable contribution for understanding future phenotype-genotype correlations in this disease. Another exceptional and an original contribution is the development of a novel clinical diagnostic tool to accurately classify the disease. A new technique of Immunohistochemistry (IHC) using frozen skin tissues, highlighting a novel application of a pre-existing technique, was developed as an alternative to the widely used costly Immunofluorescence (IFM) technique.

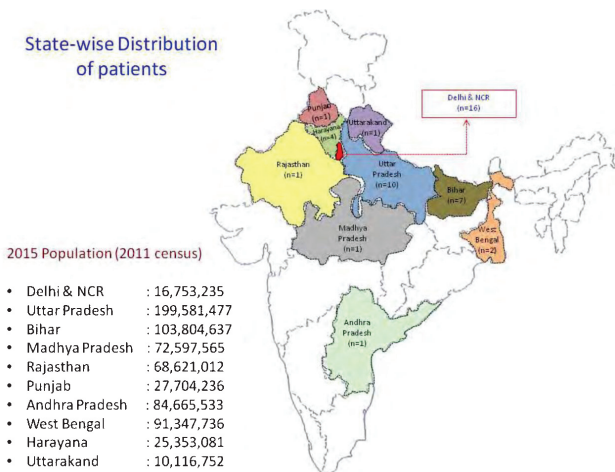


Fig.7 A: Statewise distribution of EB patients.

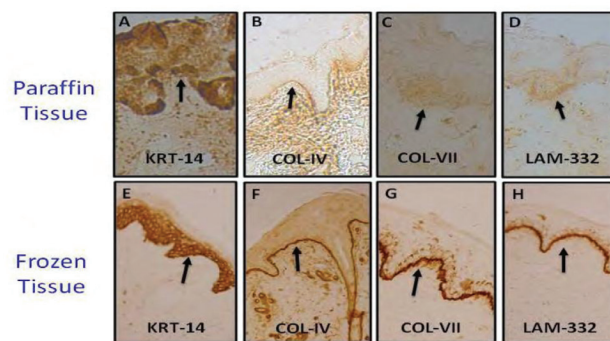
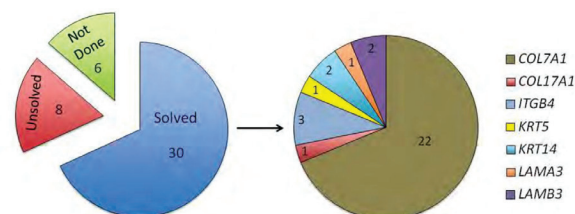


Fig. 7B: Immunohistochemistry-Paraffin embedded tissues vs Frozen tissues.



Diagnosis (Major Type)	Phenotype (Sub Type)	Gene involved (no. of patients)
EB Simplex	EB Simplex_Localised	KRT5 (1)
	EB Simplex_Autosomal Recessive	KRT14 (3)
Junctional EB	Junctional_EB_Generalised Severe	LAMB3 (1)
	Junctional EB_Generalised Intermediate	LAMB3 (3), ITGB4 (1), COL17A1(1)
	Junctional EB with Pyloric Atresia	ITGB4 (1)
	Laryngo-onycho-cutaneous syndrome	LAMA3 (1)
Dystrophic EB	Dominant Dystrophic EB	COL7A1 (7)
	Recessive Dystrophic EB_Generalised Severe	COL7A1 (5)
	Recessive Dystrophic EB_Generalised Intermediate	COL7A1 (6)

Fig. 8: Summary of mutational spectrum in EB patients.

Another study on detection and follow up of germline BRCA 1 & 2 gene mutation in breast cancer families in west Bengal was undertaken at Netaji Subhash Chandra Bose Cancer Research Institute, Kolkata. BRCA1 mutation was detected by AS-PCR and direct sequencing. Functional effects of mutations were confirmed by in-silico analysis and m-RNA folding. Mutations were identified in 7.35% (n=17) patients. The data confirms a noticeable contribution of BRCA1 mutation in BC development and establishes a correlation between the BRCA1 mutation status with clinical characteristics.

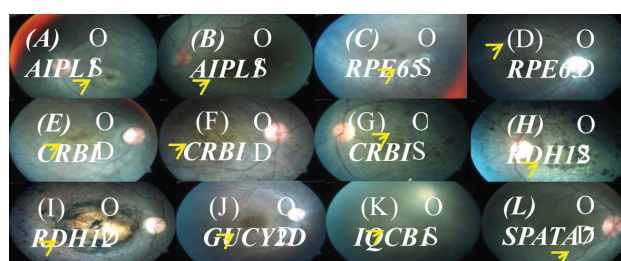
Another study was done aiming at establishing and characterizing experimental models (*in vivo* and *in vitro*) of muscle damage and degeneration and testing of therapeutic drugs in the same, with implications for human muscle pathologies

at NIMHANS, Bangalore. Mass spectrometric analysis of the mitochondrial proteome indicated widespread alteration in the expression of mitochondrial proteins, which mainly included subunits of the respiratory complexes. Curcumin treatment in CTX mouse model significantly prevented muscle damage, improved muscle strength, hastened muscle regeneration, reduced infiltration of inflammatory cells, hastened restoration of the ultrastructural changes and mitochondrial function and prevented oxidative stress in CTX-injected muscle.

A molecular genetic study in Indian patients with tuberous sclerosis was undertaken at Division of Genetics, Department of Pediatrics, AIIMS, New Delhi. MLPA for TSC2 gene showed 30% sporadic cases having either deletions or duplications or having both deletions and duplications. Sequencing of 42 exons of TSC2 gene showed variants in 87% sporadic cases in which MLPA testing for TSC2 gene was normal. Of the 23 familial cases, 23 exons of TSC1 gene were sequenced and variants were found in 17% cases and 1 case had multiple deletions in TSC1 gene in MLPA testing. For the remaining 18 TSC1 mutation negative cases, sequencing of TSC2 gene was done and variants were found in 94% familial cases. In 12% of the definite TS cases, no mutation was identified in both the genes..

Another study was conducted to perform homozygosity mapping in inherited retinal degenerative cases like LCA, arRP and CRD in order to identify the causative mutations in known gene or novel locus at Vision Research Foundation, Sankara Nethralaya, Chennai. Screening the candidate gene(s) by Sanger sequencing, identifying the causative gene(s) and/or mutations, performing the segregation analysis in the families studied and confirming the pathogenicity of the mutation by doing control screening and in-silico prediction in possible cases and to correlate the observed phenotype with the genotype determined in the study. Twelve LCA families, two arRP families and one CRD family were included in the study. For the

eleven LCA families homozygosity mapping was followed by Sanger sequencing of the candidate gene and identified the causative mutation in ten families (90%). In the arRP family, a novel nonsense mutation in MERTK was identified. In one LCA family no causative mutation in known LCA candidate gene(s) screened were identified. The phenotype recordings were correlated with the genotype determined from the study, certain classical phenotype correlation such as para-arteriolar preservation of the retinal pigment epithelium (PPRPE) in CRB1, macular atrophy with bony spicules in AIPL1, bony spicules with salt and pepper fundus for RPE65, pronounced maculopathy and bony spicules for RDH12, macular atrophy with bony spicule pigmentation in ABCA4 positive patients were observed.



**Fig. 9:** Fundus photographs of proband from LCA and arRP families.

A study on Indian patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD) was carried out at Centre of Medical Genetics, Sir Ganga Ram Hospital, New Delhi. Potentially pathogenic mutations were identified in 76 families (60.3%) and at least one unique mutation was present in each family, which included 39 frameshift insertions/deletions (27 novel), 18 nonsense mutations (9 novel), 11 missense (5 novel) and 6 novel splice site variations. In addition, 40 non-pathogenic missense variations and 50 synonymous variations were observed. Most of the mutations that are deleterious to protein function were located on exon 12.

## IMMUNOLOGY

A study on determination of Plasma and Urinary levels of catecholamines in patients with vitiligo and their correlation with disease stability and outcome

of melanocyte transplantation was carried out at AIIMS, New Delhi. Responders showed significant decline in plasma and urinary E and DA values pre- and post-intervention. Persistent low levels of  $\alpha$ -MSH are probably interfering with repigmentation treatment. The recalcitrant low levels of alpha MSH, not improving with intervention, were associated with poor repigmentation as compared to those whose alpha MSH levels increased with intervention, transplantation and phototherapy for 6 months.

Another study to check the association of polymorphism related to cytokines and cytokine genes (TNF-  $\alpha$ , IFN- $\gamma$ , IL-17, IL-20 and IL-22) with psoriasis and analysis of cardiovascular stress in psoriatic patients was undertaken at Department of Human Biology, Punjabi University, Patiala. A positive correlation between PASI and serum level of these cytokines were observed. Decreased frequency of IL-17,4. (rs10484879) G allele and IL-17F (rs763780) C allele in psoriatic patients was observed as compared to healthy controls. A significant association between IFN-1 and IL-22 and IL-20(1053), 1380, 3978 and TNF-c-238 and 308 respectively, with psoriasis was observed.

Another study on possible role of glycosylated & glycoxidated histones in SLE induction and progression was conducted at Aligarh Muslim University, Aligarh. The physicochemical alterations in glycoxidated-histone HI were monitored by UV, fluorescence, CD and FT-IR spectroscopy. Amadori adducts formed during early glycation were detected using nitroblue tetrazolium colorimetric method. Glycoxidated-histone HI exhibited hyperchromicity at 276 nm, decrease of tyrosine fluorescence accompanied by increase in pentosidine, AGE-specific and ANS fluorescence, shift in amide bands I and II, increase in melting temperature, compared to native HI. Autoantibodies in SLE sera showed strong binding with glycoxidated-histone HI compared to native or glycosylated HI.

## INDO-FOREIGN

A study was undertaken to evaluate the role of Early Growth Response-1 (Egr-1) in impaired arteriogenesis in the Peripheral Circulation during Diabetes Mellitus (DM) at SASTRA University, Thanjavur. The present study showed decreased expression of Egr-1 at the transcript and protein level and downstream targets of Egr-1 namely ICAM-1, uPA in a mice model (streptozotocin) which correlated with decreased collateral vessel diameter. Treatment of the diabetic mice with insulin improved the expression of Egr-1, increased the collateral vessel diameter and normalized the expression of downstream targets of Egr-1.

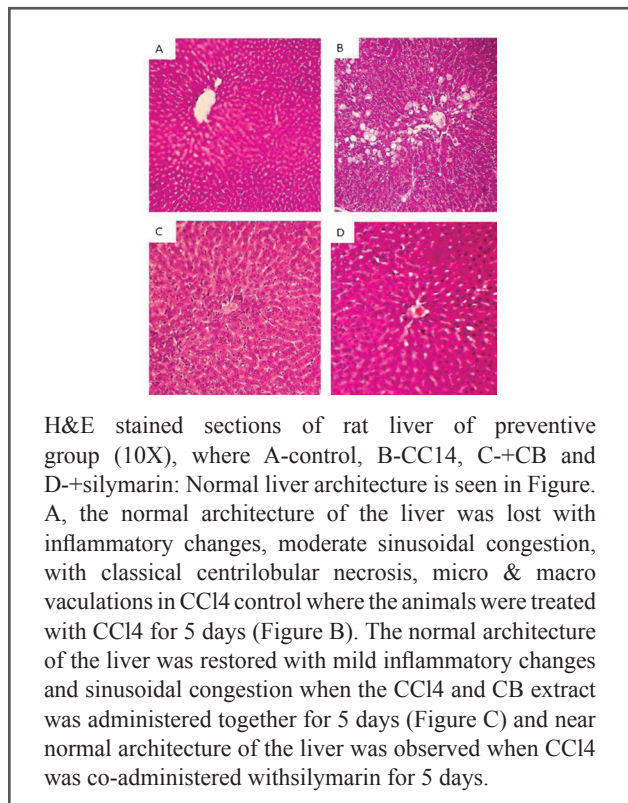
## MEDICINAL PLANTS

**Liver disorder** is a prime issue in the health care sector worldwide, which involves array of liver pathologies like- fatty liver, hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. The availability and efficiency of treatment modalities for liver diseases in modern system of medicine are questionable. The treatment largely relies on herbal therapy for regeneration of hepatocytes. *Caesalpinia bonduca* (CB) is a prickly climber seen in the tropics and subtropics. Its different parts are extensively used in folk medicine to treat various disorders including liver ailments. However, there is a deficiency of scientific evidences in the available literature that would support the same. Thus, a study on functional, cellular and molecular evaluation of hepatoprotective property of *Caesalpinia bonduca* (Kantakikaranjah) was conducted at Department of Anatomy, Kasturba Medical College, Manipal. In the present study, the hepatoprotective efficacy and efficiency of the aqueous, alcoholic and petroleum ether extracts of leaves of CB were scientifically evaluated in *in-vitro* and *in vivo* hepatotoxicity models. In both *in vitro* and *in vivo* hepatotoxicity models, the crude aqueous extract of CB expressed better hepatoprotection amongst the three tested extracts. Interestingly, the crude aqueous extract when subjected to fractionation, the efficacy of

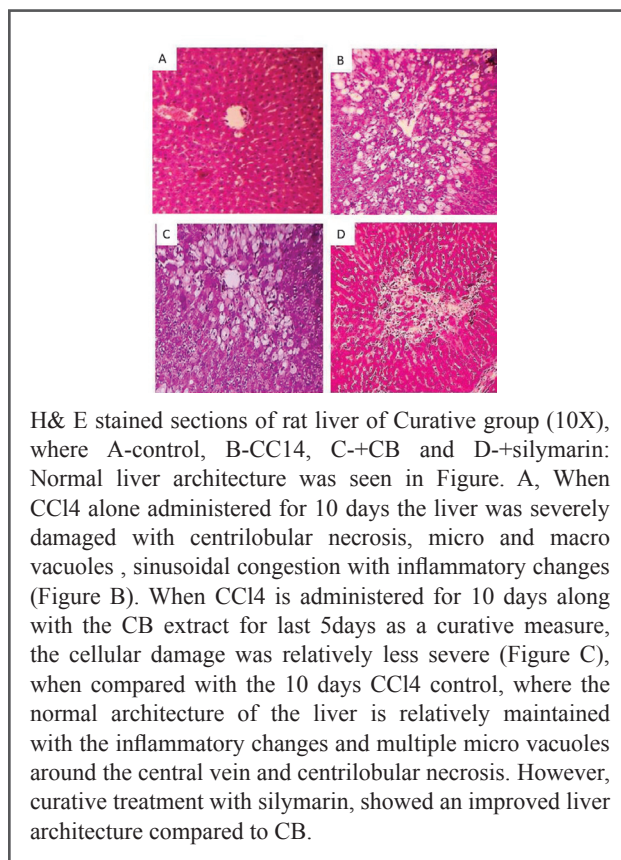


the extract was lost in *in-vitro* model and it offered minimal hepatoprotection. The aqueous leaf extracts of CB showed maximum cytoprotection in *in vitro* study against PCM induced toxicity on HepG2 cell lines in preventive model.

The cytoprotection offered was almost similar to the cytoprotection offered by standard drug – silymarin. In *in vivo* study against CCl<sub>4</sub>-induced acute and chronic hepatotoxic models, the aqueous extract of CB showed most prominent hepatoprotection against to CCl<sub>4</sub> toxicity in preventive model. The hepatoprotection offered in the aforesaid model was almost similar to the protection offered by the silymarin. Among the nine fractions of aqueous extract of CB, the aqueous extract was found to be more active. The bioactivities (hepatoprotective effect) of the fractionized samples were less effective than the crude aqueous extract of CB indicating synergistic effect constituents in aqueous extract of CB. The aqueous CB extract increased cellular levels of GSH and reduced the level of MDA.



**Fig.10.1:** H&E stained sections of rat liver.



**Fig. 10.2:** H&E stained sections of rat liver.

This suggests that the hepatoprotection was due to the synergistic effect/ activity of the individual components of CB extract. Further, cellular and molecular evaluation of the crude extract of the CB is also suggestive of its hepatoprotective activity. Thus, the aqueous extract of CB may act as a novel /promising hepatoprotective agent with further scientific exploration.

**Shikimic acid (SA)** is the primary precursor for the biosynthesis of drug against H5N1 virus (especially Tamiflu). Until now, SA was commercially extracted from fruits of Chinese star anise (*Illiciumverum*). However, with increased demand for SA, it is necessary to search other alternative to enhance the productivity. In view of this, a study on production of Shikimic acid from *in vitro* cultures of selected medicinal plants from Western Ghats was completed at RMRC, Belagavi. The research work was proposed to develop viable alternative sources and to increase the yield of SA through plant biotechnology approach. During the study, highest content of shikimic acid was reported in *Mammeasuriga* (family- Clusiaceae)

followed by *Calophyllum* (18.20 mg/g) and *Garcinia morella* (8.52 mg/g) of the same family. It forms the first report of Shikimic acid contents in these plants. This provides the lead to consider Clusiaceae as the potential family for further evaluations. It is also noted that, in all these cases, the part of potential yield was leaves, which is viable for scaling up studies for being non-destructive collection and source of large quantity of biomass.



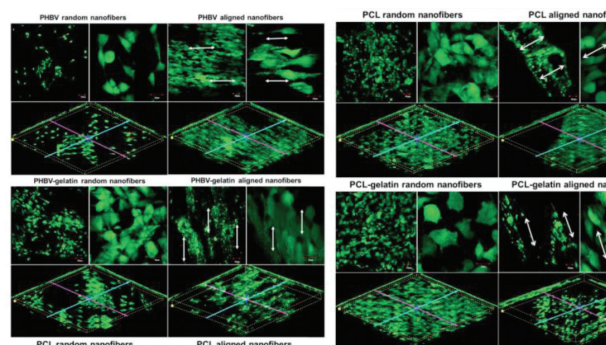
**Fig.11:** *Clematis gauriana*, a) Growing in green house; b) initiation of callus on leaf explant; c) formation of callus; d) 3 months old callus.

The protocol for callus production was developed for *Clematis gauriana* (Ranunculaceae), which found to have highest content of Shikimic Acid (3.171 mg/g) among herbaceous species. The further studies by using elicitors will help to standardize the *in vitro* protocol for viable and enhanced production of shikimic acid.

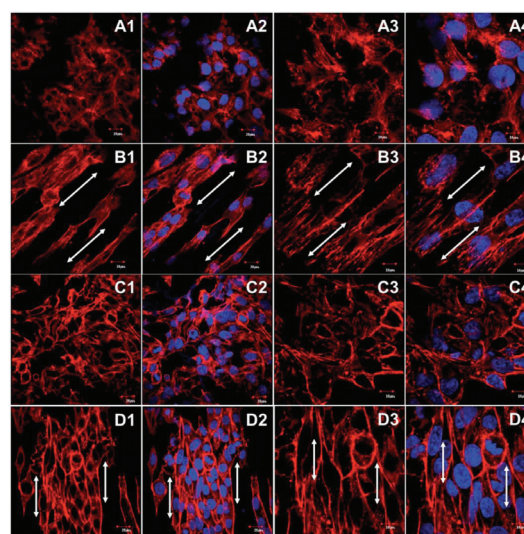
### NANO-MEDICINE

SASTRA University, Thanjavur is working towards development and characterization of novel scaffolds for the regeneration of liver and esophagus. 3-D porous galactose containing PVA/Gelatin (P/G) hydrogel scaffold were developed, which showed better HepG2 adhesion, spheroidal morphology and significantly higher ( $p < 0.05$ ) viability, proliferation and albumin secretion. The presence of mitogen (HGF), asialoglycoprotein recognition motif (galactose) and integrin recognition motif (gelatin) in 3D porous hydrogel had positive influence in hepatocellular specific functions, essential for liver tissue regeneration. Random and aligned

electrospun nanofibres of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), PHBV-gelatin, poly( $\epsilon$ -caprolactone) (PCL) and PCL-gelatin were developed and characterized for esophageal tissue regeneration. *In vitro* co-culture of epithelial and smooth muscle cells on aligned nanofibres was successfully achieved with two cell types segregating and forming distinct layers. The functional phenotype retention of both cells in co-culture was confirmed by cell-specific genes expression.



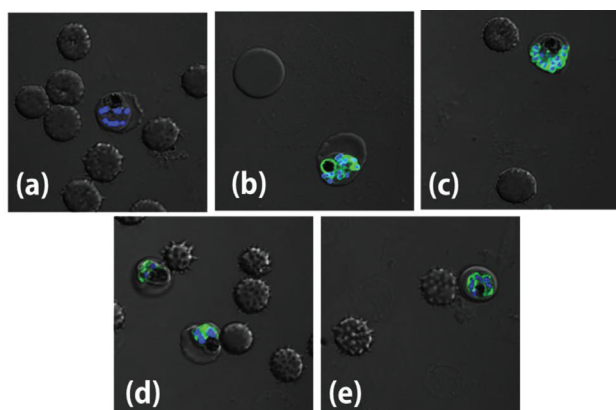
**Fig.12:** CLSM images depict the viability of human non-keratinized, stratified, squamous epithelial cells cultured on PHBV, PHBV-gelatin, PCL and PCL-gelatin random and aligned nanofibrous scaffolds after 3 days respectively.



**Fig.13:** CLSM images of human non-keratinized, stratified, squamous epithelial cells cultured on random and aligned nanofibrous scaffolds of PHBV, and PHBV-gelatin after 3 days of culture. Cell nuclei (blue) and actin filaments (red) were stained by Hoechst and rhodamine-phalloidin respectively on [A1 & A2] random PHBV nanofibres (low magnification); [A3 & A4] random PHBV nanofibres (high magnification); [B1 & B2] aligned PHBV nanofibres (low magnification); [B3 & B4] aligned PHBV nanofibres (high magnification); [C1 & C2] random PHBV-gelatin nanofibres (low magnification); [C3 & C4] random PHBV-gelatin nanofibres (high magnification); [D1 & D2] aligned PHBV-gelatin nanofibres (low magnification); [D3 & D4] aligned PHBV-gelatin nanofibres (high magnification).



In this study, investigators have developed stearylamine (SA) based cationic liposomes for delivery of potent antimalarial drugs and studied its efficacy in *Plasmodium falciparum* in culture and murine malaria model. Stearylamine in liposome alone exhibits potent antiplasmodial activity. Delivery of monensin, a carboxylic ionophore through stearylamine liposome exhibits enhanced killing of the parasites under *in vitro* and *in vivo* condition. The SA liposome formulations were found to be more effective than a comparable dose of other liposomal formulations (without SA) and free monensin. The efficacy of monensin in SA-liposomes was significantly enhanced in combination with various potent antimalarial drugs like chloroquine, FR900098, or piperazine, in free form, in culture and murine malaria. Other cationic liposomal formulations (DIDAB, DTAB, DDAB) without drug, showed growth inhibition of cultured parasites. In addition, curcumin delivered through SA-liposome substantially reduced the parasite growth as compared to free form. Also, delivery of monensin or curcumin through PLGA nanoparticles exhibited marked reduction in parasite load relative to free drug. The prepared nanoformulations either liposomes or PLGA nanoparticles showed no hemolysis of erythrocytes.

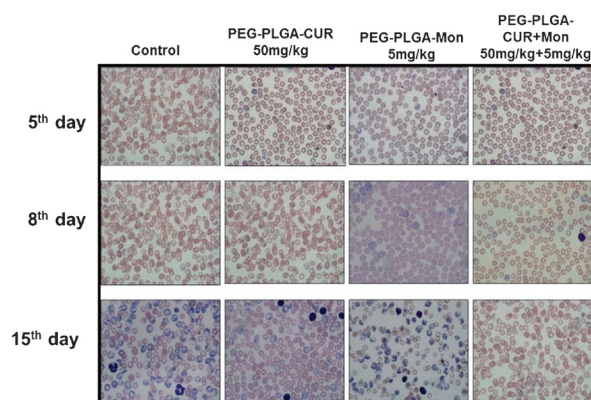


**Fig.14:** Uptake studies of SA-PLGA nanoparticles in erythrocytes (a) PLGA nanoparticles (b) Coumarine-6 loaded PLGA nanoparticles (c) Coumarine-6 loaded PEGylated PLGA nanoparticles (d) Coumarine-6 loaded Stearylamine-PLGA nanoparticles (e) Coumarine-6 loaded PEGylated-Stearylamine-PLGA nanoparticles. DAPI (Blue in color) was used to stain the nucleus of malarial parasite. Coumarine-6 (Green in color) was used to stain the PLGA nanoparticles.

Nanocarriers exhibited sizes ranging from 90 to 120 nm, with spherical morphology as measured

by dynamic light scattering and high resolution electron microscopy.

However, the enhancement of antimalarial activity was dependent on preferential uptake of nanosized vehicles into infected red blood cells as compared to uninfected ones. Monensin/Curcumin in long-circulating liposomes of stearylamine with 5mol % DSPE-m PEG 2000 in combination with free artemisinin resulted in enhanced killing of parasites, prevented parasite recrudescence, and improved survival.



**Fig.15:** Determination of parasitemia from the blood smear of treated versus untreated group stained with Giemsa on day 5, 8 and 15. The treated group mice were administered with Curcumin or Monensin loaded in PEG-PLGA nanoparticles alone and in combination of both at different dosages.

Overall, PEGylated stearylamine-liposomal monensin having 5 mol % DSPE-mPEG-2000 may act as a potent chemotherapeutic agent against human malarial infection. Three potent antimalarial compounds having IC<sub>50</sub> values in nanogram range against *P. falciparum* were identified from 127 potential inhibitor, which were identified through virtual screening of NCI compound libraries against *P. falciparum* PF<sub>1</sub>DHFR.

## PHARMACOLOGY

**Adverse drug reactions [ADRs]** are important to monitor, when medications are administered for chronic illnesses such as epilepsy/migraine particularly with recently approved widening indications for Antiepileptic Drugs [AEDs]. Therefore, it is essential to recognize potential of these AEDs to unduce bone loss, as an adverse effect. A comparative evaluation of effects of



newer antiepileptic drugs [Levetiracetam/Topiramate] on bone health using biological markers was completed at Sri Rajarajeshwari Medical College and Hospital, Bangalore. Since osteoporosis is one of the well-known complications following administration of older generation AEDs, the aim of the study was to quantify and compare the effects of newer AEDs - levetiracetam [LEV] and topiramate [TPM] monotherapy, on bone health using bone biomarkers viz. - serum cross laps, osteocalcin, vit. D and parathormone [PTH]. 76 patients with epilepsy/migraine between 18-45 years of age, receiving LEV or TPM monotherapy for < 1 month with no co-morbid conditions were included. Serum samples were collected at baseline, 3rd and 6th month. Samples were analyzed for serum cross laps, osteocalcin, Vit D and PTH using electrochemiluminescence. The levels of bone biomarkers were compared across time. The mean age of patients was  $28.60 \pm 6.60$  [Mean  $\pm$  SD] years with 60.78% females. Patients who received TPM complained of bone related side effects which was significantly greater compared to LEV. There was no significant difference in levels of bone biomarkers over a period of six months and no difference between LEV and TPM.

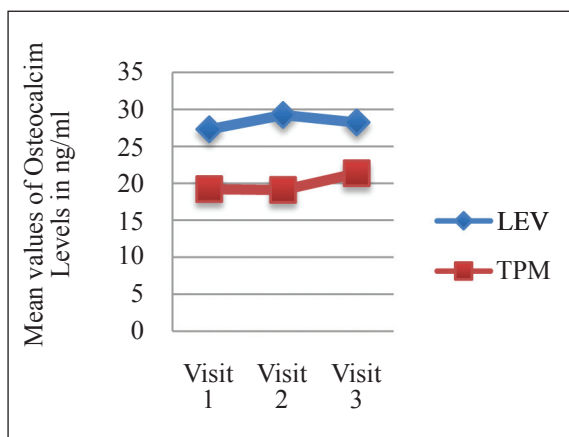


Fig 16: Effect of LEV and TPM on Osteocalcin levels.

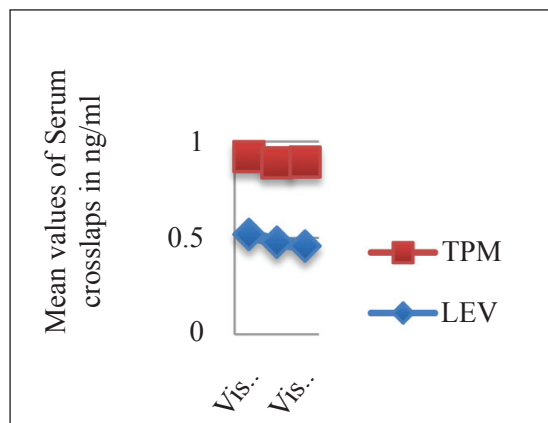


Fig 17: Effect of LEV and TPM on Serum crosslaps levels.

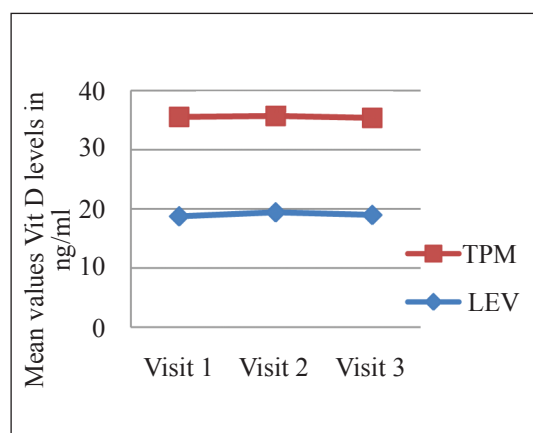


Fig 18: Effect of LEV and TPM on Vit D levels.

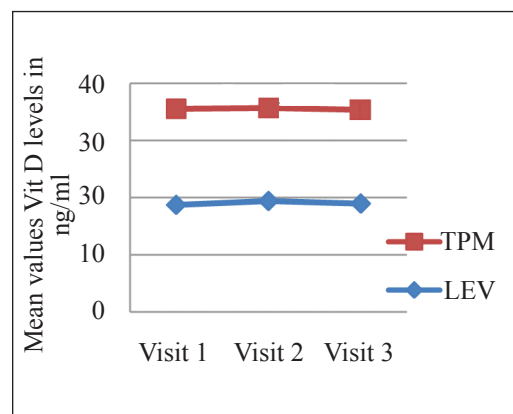


Fig 19: Effect of LEV and TPM on PTH levels.

It is concluded that the use of LEV and TPM as monotherapy in treatment of epilepsy/ migraine over a period of six months was not associated with adverse influence on bone health.

Increasing drug resistance in malarial parasites at an alarming rate is impeding the therapeutic efficacy of existing antimalarial drugs. To circumvent drug resistance, there is an urgent need to develop new antimalarial agents and an efficient drug delivery system. A study on evaluation of Soya phosphatidylcholine-stearylamine liposome as antimalarial agent, was completed at University of Delhi, South Campus, New Delhi, with the objectives to study the mechanism of internalization of Soya phosphatidylcholine-stearylamine liposomes into the *Plasmodium falciparum* infected erythrocytes followed by into the parasites vacuoles; Study the synergistic action of Soya phosphatidylcholine-stearylamine liposomes along with chloroquine and artemisinin in free and liposomal form in killing *Plasmodium falciparum* in culture; Develop murine model of malaria by injection *Plasmodium berghei* into Swiss Albino mice; Study the therapeutic efficacy of Soya phosphatidylcholine-stearylamine liposomes in the treatment of malaria in animal model; Study the synergistic action of Soya phosphatidylcholine-stearylamine liposomes along with chloroquine and artemisinin in free and liposomal form in the treatment of murine model of malaria and study pharmacokinetics and biodistribution of Soya Phosphatidylcholine-stearylamine liposomes in normal and malaria infected mice.

## PHYSIOLOGY

**Preoptic Area (POA)** is one of the most important neural sites involved in body temperature regulation. TRPV channel is no-selective cation channel and its role in thermosensation has been observed in *in vitro* study but the role of TRPV (1-4) channel in thermoregulation has not been observed. In view of this, a study on role of preoptic area thermo Transient Receptor Potential Vanilloid (TRPV) channels in thermoregulation in rats was completed at Department of Physiology, AIIMS, Delhi with the objective to study the effect of microinjection

of TRPV(1-4) channels, agonist and antagonist in the reoptic area on brain and body temperature to localize TRPV(1-4) channels in the preoptic area using immunohistochemistry.

The study was conducted in 54 male Wistar rats. Under thiopentone sodium anesthesia (40 mg/kg BW), a bilateral guide cannula (24G) was implanted at two mm above the preoptic area as per De Groot's atlas. A radio transmitter TA10TAF-40 (Data Science international, USA) for the telemetric recording of body temperature was implanted in the abdomen. A K - type thermocouple wire was inserted near the hypothalamus to measure the brain temperature. TRPV1-4 agonist and antagonist (Sigma-Aldrich) (0.4µg/0.2µl) injections were given bilaterally at the POA at a rate of 0.1µl/minute using an injector cannula at 12 h in all groups except immunohistochemistry group. The site of injection was confirmed histologically. Immunohistochemistry was performed for the localization of TRPV1-4. The statistical comparison was made between pre and post injection record using repeated measure ANOVA. The injection of TRPV1 and TRPV4 agonist (0.4µg/0.2µl) into preoptic area, produced significant decrease in body and brain temperature while the injection of TRPV2 agonist (0.4µg/0.2µl) into preoptic area produced significant increase in body and brain temperature. The injection of TRPV3 agonist and antagonist (0.4µg/0.2µl) into preoptic area produced no effect on body and brain temperature. It can be concluded that TRPV1, TRPV2 TRPV4 channels in the preoptic area play an important role in thermoregulation.

## STEM CELL RESEARCH

A Study to Investigate the Mitochondrial Network Dynamics during Differentiation of Human Mesenchymal Stem Cells to Cardiomyocytic and Adipocytic Lineage *in vitro* was done at PGIMER, Chandigarh. The study showed that mitochondrial biogenesis was essential for both adipogenic and cardiomyocyte differentiation

A Study was conducted on Neurogenic potential of naïve and astrocyte-like differentiated cells from human mesenchymal stem cells in an *in vitro*

Parkinson's disease model at Manipal Institute of Regenerative Medicine, Bangalore. The study showed that DPSCs exposed to glial commitment cues show a substantial differentiation towards astrocyte-like cells and these cells attain functional maturity with respect to their interaction with dopaminergic neuronal cells to bring about neuroprotection. The data indicates that astrocyte differentiated DPSCs may be appropriate candidates for further evaluation as stem cell therapy-based treatments using animal models representative of neurological diseases and injury involving modification of midbrain microenvironment. *In vitro* PD model is successful in establishing this fact.

## OTHER ACTIVITIES

### Coordinating Cell Biology Based Therapeutic Drug Evaluation Committee (CBBTDEC) Meetings

CBBTDEC was constituted on 1st September, 2010 to advise DCG(I) in matters pertaining to regulatory pathways leading to the approval of clinical trials and Market Authorization for the "Therapeutic products derived from Stem Cell, Human Gene manipulations and Xenotransplant technology" and chaired by DG, ICMR. CBBTDEC meetings are held at ICMR and the organization of these meets is co-ordinated by ICMR. So far, 10 meetings have been conducted in which 25 clinical trials have been approved. The committee has either approved/ conditionally approved marketing of cell based therapeutic products including Stempeucel, Ossoron and Chondron.

### Strategy to curb the rampant practices of banking and use of stem cell for therapeutic purposes: Inter-Ministerial/Inter-Agency Dialogue

There is premature use of stem cell therapies without obtaining adequate data on their safety and efficacy and also a trend for banking of different tissues as potential source of stem cells. In order to address the issue, ICMR took an initiative to organize "Interministerial / Interagency meeting to discuss the strategy to curb the rampant practices of banking and use of stem cell for therapeutic

purposes". Several issues were discussed including prevention of abuse of stem cells and promotion of ethical clinical research, path and process to review retrospective clinical data, regulations for stem cell banking and harmonization of existing guidelines and regulatory requirements.

### National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT)

NAC-SCRT has been regularly deliberating on the issues and developments in the field and accordingly brings necessary amendments/revision of the NGSCR. The guidelines are finalized and will be released soon. Formats for IC-SCR registration and annual reports were further modified as per the revised guidelines. During 2016-17, a total of 33 new applications were received and 8 IC-SCRs were registered. At present 28 IC-SCRs are registered with NAC-SCRT and necessary information is awaited for the remaining for further consideration. Their annual reports are being evaluated by the committee members. Representations from various stakeholders / government agencies were addressed as per the existing policies.

### Guidance Document for Regulatory Requirement for Banking of Cord Blood/ Other Tissues as a Source of Stem Cells

Two meetings were conducted to draft Guidance Document for Regulatory Requirement for Banking of Cord Blood/ Other Tissues as a Source of Stem Cells which is being adapted from Net-Cord FACTS guidelines.

### Public Health Significance

## CLINICAL PHARMACOLOGY

### Pharmacovigilance

Considering the impact of adverse drug reactions on patients' compliance and outcome, patient guide for anti TB drugs was prepared. The patient guide has been field tested in patients with drug resistant TB to evaluate its effect on knowledge, attitude and action taken by the patients. In 162 patients, a questionnaire was administered before and after giving them the patient guide. Patients were



initially unaware of the adverse drug reactions and preventability and their knowledge improved by 80% after reading the patient guide. However, as per the patient feedback, the patient guide needs to be shorter.

The ready reckoner for health workers and doctors for ADR prevention and monitoring is being widely used by the RNTCP as well as private practitioners.

### **Bedaquiline**

Bedaquiline, a new anti TB drug has been made available through conditional access in the RNTCP program. ICMR Chair as Chairperson of the bedaquiline DSMC evaluated its safety and effectiveness using various indicators. Update on bedaquiline was presented to the WHO ACSOMP committee in Geneva. QTC prolongation is a known ADR to Bedaquiline. Evaluation of genetic polymorphism of the ion channel genes is likely risk factor is under progress..

### **ADRs in elderly:**

Demographic transition has resulted in an increase in elderly population. There is limited data on elderly tuberculosis patients and their treatment outcomes in India. Retrospective analysis of data from patients admitted during 2016 showed that elderly (> 60 years) suffered from more comorbid conditions and ADRs particularly CNS and otovestibular, compared to younger patients.

### **ICMR PvPI collaborating centres :**

ICMR-PvPI collaborating centres at NIRRH, Mumbai NIRT, Chennai, NARI, Pune NICED, Kokata NIMS, Delhi NIE, Chennai and NIN, Hyderabad have been approved with focus on reproduction, tuberculosis, HIV/AIDS, hepatitis, malaria epidemiology and herbal drugs respectively.

DMPA has been recently introduced in the National Family Planning Program. Unmet need for spacing pregnancies has been estimated to be 5-10% as per NFHS 2015-2016, which can be met by DMPA. However, it has disadvantages such as risk of sexually transmitted illness and increased bone loss. The data on need, efficacy, effectiveness and

safety of Depot Medroxyprogesterone acetate is being analyzed.

### **Tropical Diseases:**

*Visceral Leishmaniasis* is planned for elimination by 2017. However, PKDL which contributes to continued transmission is of concern. Liposomal Amphotericin developed indigenously by Delhi University and GSMC KEM Hospital, Mumbai is being evaluated for PKDL for efficacy, safety, pharmacokinetics and dose searching and the study is in progress.

### **MALARIA**

Despite many efforts malaria continues to remain a public health problem. Under the Foundation for disease elimination a demonstration project has been started at Mandla.

**Pharmacoepidemiology:** National Institute of epidemiology was identified as nodal centre for pharmacoepidemiology as part of the PvPI-ICMR collaboration.

### **Drug utilization research:**

National chair was invited to participate in WHO workshop on drug utilization research and delivered 3 lectures on basic principles of DUR, indicators and DUR in developing countries. In response to call for proposal and as per recommendations of expert group subcommittees, collaborating centres have been formed for studies in elderly, diabetes, childhood diarrheas, antiinfectives, tribals and childhood tuberculosis. The drug utilization research to improve case management of childhood diarrhea is in progress.

### **TRADITIONAL MEDICINE**

Director General, ICMR (Indian Council of Medical Research) and Executive Vice Chairperson, National Innovation Foundation (NIF, a DST Institute), has a collaborative programme on validation of innovative claims of herbal healer. This collaboration will strengthen the synergy achieved between informal, uncodified and non-classical health related people's knowledge and the apex institution for Medical Research in the

country. The collaboration focuses on grassroot traditional knowledge based practices that incorporate either use of new medicinal plants not reported in any Indian codified literature or new use of already mentioned medicinal plants or multi-herb formulations for drug development. This cooperation will also serve to recognize, respect and reward indigenous people which may have made innovations in traditional knowledge. Under this program, the following protocols were finalized for validation of the innovative claims of herbal healers

- 1) Pain inflammation and arthritis: carrageenan induced rat paw; edema model, wistar rat groups = 5 each group (n=8); Collagen induced arthritis DBA/1 mice and Groups= 6 (n=8) tail flick method
- 2) Anti-diabetic activity: Db/db (Type II Diabetic) Mice, Db/db mice Groups = 6 (n=8)
- 3) STZ induced (Type I) diabetic rats and spraguedawley Groups=6 (n=8)
- 4) Liver disorders & Jaundice: Carbon

tetrachloride (CCL4) induced hepatotoxicity wistar rat, Groups= 6 (n=8); Paracetamol induced hepatotoxicity, Sprague dawley group 6 (n=8) and antifibrotic effect (HSC-T6) rat liver stellate cell line human, (LX-2) hepatic stellate cell line;

- 5) Obesity: diet induced obesity C57BL/6 group 6 (n=8).
- 6) Osteoporosis (bone fracture): human osteoblast MG-63 (Human bone osteosarcoma cells) and female ovariectomised rat model SD Rats,
- 7) Diarrhoea: mouse model swiss albino mice group 6 (n=8).
- 8) Dysentery: mouse model BALB/c mice group 6 (n=8).

An invention entitled “5-[4-(2-biphenyl-4-yl-2-oxo-ethoxy)-benzylidene]-thiazolidine-2,4-dione derivatives, Synthesis and uses thereof” with patent application No vide 201721025305 for Guru Ghasidas Vishwavidyalaya, Bilaspur and ICMR, Delhi are co-applicants.

# REGIONAL MEDICAL RESEARCH CENTRES

To deal with health problems of the regional and marginalised population, ICMR has established a total of 4 Regional Medical Research Centres at Port Blair (Andaman Nicobar), Bhubaneswar (Odisha), Jodhpur (Rajasthan), and Dibrugarh (Assam). The effort of these institutes is to focus on the regional health problems and find suitable solutions with the help from the respective state governments. The significant outcome of the research activities carried out by these centres during 2016-17 is mentioned below.

## REGIONAL MEDICAL RESEARCH CENTRE, BHUBANESWAR

During the year 2016-17, the centre had undertaken several outcome oriented activities to address various research issues on vector borne diseases (Malaria, Dengue, Chickungunea, Lymphatic Filariasis), Tuberculosis, viral infections, nutrition, diarrhoeal disorder, non-communicable diseases (hypertension and diabetes) zoonotic diseases and strengthened health research capacity in the region, in accordance to the prescriptions of National Health Policy 2017.

### Strengthening of Health Research Capacity

To develop and sustain research capacity of this region, the centre has been undertaking academic as well as training programs. In this period, the centre had conducted Pre-PhD course (6 month duration, 8 students), provided supervision and lab facility to 1 Post Doc fellow, 9 PhD scholars (ICMR, CSIR, Lady TATA), M. D/ MPT (4 Students) and 16 MSc dissertation students in different fields of medicine,

life sciences and biotechnology. A dedicated unit had been constituted, which conducted a series of workshops/training for students/young scientists/research scholars/faculty from all over the country. Accordingly, the centre had also conducted workshops on Good Clinical Practices (GCP) and Bio-ethics, Systematic review and Meta-analysis, Clinical trials, Research protocol development for Multidisciplinary Research Units, Mixed method research, Developing and operating clinical data registry wherein professionals and research scientists from different institutions and research centres have been trained. Further, the centre conducted two Research Methodology workshop at MKCG Medical College & Acharya Harihar Regional Cancer Centre for faculty. A series of training on Research Methodology lectures on weekly basis was initiated for inhouse scholars of RMRC, Bhubaneswar. The IEC of the centre received DCGI approval this year. The construction of Model Rural Health Research Unit building at Tigriria has been completed.

### Organise data systems and platforms for research

Under intramural grant, an electronic dynamic health research inventory for the region was being set up. The centre's library had been up graded/digitalised with establishment of e-library and consortia. The biomedical-informatics cell has been assigned to develop clinical data registry in collaboration with medical colleges and research institutes of this region on diseases of National interests including rare diseases.



## ENABLE EVIDENCE TO POLICY TRANSFORMATION

### Lymphatic Filariasis

A longitudinal study conducted in high LF endemic area reveals that supervised MDA therapy can be introduced at the child bearing age of the women to accelerate the elimination programme.

### Malaria

A study undertaken in Kalahandi district has shown that *Culiseta tritaeniorhynchos* is the major vector throughout the year, followed by *Anopheles fluviatilis* and both the vectors are susceptible to DDT and Cyfluthrin.

### Diarrhoeal Disorder including Cholera

Two epidemic investigations on diarrheal disorder carried out by the centre (Balasore: coastal district, Raygada: tribal district) during this period, has checked the epidemic. Another hospital study on diarrheal disorders has generated evidence for national rotavirus vaccination in the country to include Odisha among the first phase stage (April'2016). The quadruplex PCR developed by the centre to detect *V. cholera* O1, validated by third party, can be used as rapid diagnostic tool compared to conventional method in DHQ hospital set up. During 2017, a large outbreak of cholera was reported in the Anandapur block of Keonjhar district and *V. cholerae* O139 was detected, which reappeared after 11 years of quiescence in Odisha.

### JE & other vector borne diseases

A year round investigation by the centre has shown that indigenous JE transmission is going on in the state and identified the reservoirs. This has been communicated to the state health authority for necessary intervention. A significantly large outbreak of Acute Encephalitis causing around 100 deaths in tribal population of Malkangiri district was proven to be largely due to JE. Based on the evidences, JEV vaccination was introduced by NVBDCP in four districts of the state in December 2016. To strengthen laboratory surveillance, technicians were trained from 12 districts on sero diagnosis & vector control measures recommended to the state.

Dengue infection was the other public health challenge for the region. Endemic hospital based surveillance and outbreak investigation revealed dengue in around 20 or 30 districts of the state. This included two major outbreaks from industrial (Jagatpur, Cuttack and Mining, Keonjhar district) areas. 40-50% of the patients investigated, had shown evidence of dengue infection (NS1 antigen or IgM). The centre also undertook sero-typing of dengue virus infection as the apex referral lab of the state, and received samples from different district labs of the state. Serotypes I, II and III were found to be major causes of dengue infection in the state.

### Virology Network Laboratory (Grade I)

The centre is rendering diagnostic services to the referral cases from medical colleges, tertiary hospitals and IDSP in addition to outbreak investigations. HPV infection and its genotypes associated with carcinoma cervix were recorded in context to the HPV vaccine strength. The centre has also established laboratory surveillance for the newly emerging Zika virus infection. So far, training has been imparted to 51 laboratory technicians from 12 district headquarter hospitals and NVBDCP on Dengue, Chikungunya and JE virus diagnosis, which created independent facilities at the respective districts. Laboratory surveillance network services for H1N1 infection could diagnose at the earliest, preventing mortality and spread. The public health recommendation, after identification of the environmental risk factors for the investigated outbreak, prompted public health action.

During this period, total 5456 patients were provided diagnosis for around 50 viruses of public health importance. Among the enteric virus, rotavirus was found in 40% of cases, hepatitis-A in 28% cases, hepatitis- E in 40 % cases; among the respiratory viruses, Flu A in 5.5% cases, H1N1 in 2.0%, para influenza in 22.6% and HMPV in 3.2%; among other air borne viral diseases, measles in 58.8%, varicella in 32% and rubella in 54.4% of cases and among encephalitis, herpes simplex in 8.8% and JE in 14% of cases. HPV infection and its genotypes associated with carcinoma cervix were recorded in context to the HPV vaccine strength. Possibility

of a superior rota vaccine is also explored, using animal experimentation.

### Outbreak Investigations of viral diseases

During this period, around 150 outbreaks (Measles, Hepatitis A & E, Dengue, JE, Varicella, etc) were investigated which covered 30 districts.

During this period, around 1000 suspected cases of pandemic H1N1 2009, from almost all districts of the state were investigated and in around 50% cases, the virus was detected among hospitalized patients.

Measles outbreak was investigated in a tribal dominated area (Nagada) of Jajpur district, where, in 92% of affected children, Measles IgM antibody was detected.

### Tuberculosis

As a NRL for TB, this centre has been providing training and quality control measures and technical support to Odisha, and 10 adjoining states of North Eastern region. Installation of CB NAAT in NRL, RMRC, Bhubaneswar has enhanced the involvement of private sector in TB detection. The centre has demonstrated that transportation of sputum samples by public bus services from peripheral laboratories helps in filling the gap of providing early diagnosis services to the patients staying in remote inaccessible areas. Centre is part of ICMR Tribal Task force (multicentric) to estimate the burden of TB among the tribal population and develop an innovative health system model to strengthen TB control in the tribal areas. During 2016-17, the centre surveyed 1454 households having 5194 individuals in the 6 tribal villages of 4 districts (Balangir, Dhenkanal, Kandhmal and Mayurbhanj). Out of these, 126 were found with symptoms related to chest and 27 were found sputum positive for TB, out of which 13 were culture positive. Appropriate treatment to all the MTB patients was initiated through DOT centres, after referral. Project entitled "Targeted intervention to expand and strengthen TB control in Tribal populations under the revised National Tuberculosis Control Programme, INDIA (TIE – TB Project)" has been initiated during 2016-17, to improve the Standard of Care through intervention as the main objective, funded by "Global Fund".

During reporting period, deployment of 7 mobile vans equipped with Digital X-ray and sputum microscopy services (Mobile TB Diagnostic Van, MTDV) were put into service in three districts of Jharkhand. The centre screened 668 presumptive cases, out of which 26 were found sputum positive and 65 patients were X-ray positive. Further, attempts are made to involve the community to bridge the gap between the health systems and the community in providing RNTCP services as well as bringing and involving traditional healers into the RNTCP ambit. A Prospective study to determine the comorbidity of Tuberculosis among patients with Type 2 Diabetes Mellitus was initiated and 1200 patients with Type-2 Diabetes were screened and 13 were found having active TB.

### Nutrition

The concurrent monitoring and impact evaluation of mid-day meal (MDM), status of iodine among pregnant women, assessment of fluorosis at district level in Odisha is being undertaken to guide the policy. A community based intervention is under evaluation to prevent hypertension and CVD risk reduction in tribal clusters of Kalahandi district through diet and life style modification. This has potential for incorporation in NCD programme. A project to improve the health status of under 5 children was initiated in Raygada district of Odisha as per the MOA between Govt. of Odisha and ICMR. Baseline study revealed that undernutrition is as high as 40% out of which, 10.4% are severely underweight. Major cause of morbidity was found to be respiratory infection (36.3%) followed by Malaria (22.3%), diarrhoea (8.8%), skin infections (2.5%) and Measles (1.8%). The immunisation coverage is 60-70 % & Vit. A supplementation is < 60 %.

### Zoonotic Diseases

The centre has initiated work to prepare the road map for control of anthrax in the state, prevalent particularly in some tribal pockets. A project entitled "Anthrax in Odisha: Road map for prevention" was done with the objective to assess the magnitude, identify the correlates, explore both supply and domain barrier and to reduce the morbidity due to Anthrax by implementing novel control measure in high risk areas of Odisha. Initial study reveals poor

knowledge about carcass handling and disposal, treatment of patients and absence of IEC material are the main impediments in control of Anthrax in these districts coupled with extreme poverty and inaccessibility, further compound the problem. Preparation of SOPs for all levels of care givers and training of grass root level staff are initiated.

Further, an epidemiological study examining the risk factors of Scrub Typhus has been initiated by the centre in collaboration with the state IDSP and two private health care facilities.

### Leveraging Traditional Medicine

A pilot study was carried out to screen 54 phytochemical compounds from *Carica papaya* to identify potent phytochemical compounds against potential dengue drug targets and identify a multi-epitope vaccine candidate, targeting the important non-structural protein (NS1). A Project is being undertaken to study the structure and role of different RND efflux pumps of the gram negative bacilli, isolated from major hospitals of Assam and to find suitable herbal inhibitors.

### Strengthening of programme implementation

The Centre has been assigned by the Department of School and Mass Education, Govt. of Odisha to evaluate the Midday Meal Program of the state. The centre is the designated apex laboratory for JE diagnosis by the state and undertaking Laboratory surveillance for Antimicrobial resistance and antimicrobial stewardship. The project activities have been aligned with different components of the state NVBDCP and have been supporting the implementation of RNTCP.



**Fig. 1:** Community Participation on the occasion of World TB Day 2017, RMRC Bhubaneswar.

## RMRC, DIBRUGARH

### CANCERS

#### Study of risk factors of Hepatocellular Carcinoma (HCC) in Sikkim and Arunachal Pradesh

It was observed that “HCC, NOS (C22.0)” is the most common type of carcinoma in both the states. Heterozygous variant of CYP1A1 (OR=3.25, 95% CI=1.06-9.97) and GSTM1\_Null (OR=2.60, 95%CI= 0.99-6.79) and rare homozygous variant of CYP2E1 (OR=5.25, 95%CI=0.85-32.43) and GSTT1\_non-Null (OR=2.60, 95%CI= 0.99-6.79) were associated with increased risk of HCC. Consumption of alcohol (adjusted OR= 5.01, 95% CI= 1.92-13.13) smoked meat (crude OR= 5.54, 95%CI= 2.23-13.76), dried meat (crude OR= 5.88, 95%CI= 2.31-14.96), and non-fermented bamboo shoot (crude OR= 6.25, 95%CI= 1.33-29.37) were found to be significantly associated with high risk of HCC.

#### Study of the pattern of survival and quality of life of the oesophageal & stomach cancer patients in North Eastern Region of India

This study revealed that the overall median survival time of the oesophagus and stomach cancer patients was 17.9 months and 14.57 months respectively. In both the cancers, patients under symptomatic or palliative care experience a lower survival time as compared to those undergoing chemotherapy, radiotherapy, surgery, chemo-radiation or chemo-surgery.

### New studies initiated

- (i) Germline mutation spectra of BRCA1 and BRCA2 genes in multi-ethnic breast cancer patients from N.E. region based on direct sequencing.
- (ii) Molecular epidemiology and risk factors of gastric cancers in Tripura and Nagaland.

### DIABETES

The NE component of ICMR INDIAB study was carried out in Nagaland and Sikkim during the year.



Data was generated on the prevalence of Diabetes, Prediabetes, Hypertension and Dyslipidemia in the community

### CARDIOVASCULAR DISEASES

**Effectiveness of diet and lifestyle intervention through IEC tools with Angan Wadi Centres as the centre of knowledge dissemination for hypertension (including hypercholesterolemia and diabetes) risk reduction - a cluster randomized controlled trial**

In this study, so far, lifestyle intervention in 6 intervention clusters were completed. Counseling of the community as well as IEC meeting for different groups were organized at these clusters.

**Health system preparedness for interventions for Diabetes, Hypertension, chronic respiratory diseases and cardiovascular disease and deaths due to non-communicable diseases (NCD) among the tribal population in India**

This is an ongoing project, and the study was completed in four north eastern states viz. Sikkim, Tripura, Meghalaya and Mizoram. It was observed that NCDs accounted for half of the overall deaths (52.8%) followed by communicable diseases (24.6%), other causes (19.4%) and maternal death (3.1%).

### MALARIA

**Molecular epidemiology study in India and Qatar with an emphasis on parasite diversity, drug resistance and immune response**

*Plasmodium falciparum* Pfs25 genes were successfully sequenced and analysis revealed the existence of 8 haplotypes with reference to the Th2R and Th3R regions.

**Malaria Evolution in South Asia (South Asia-ICEMR):**

This is a dynamic cohort study being carried out in collaboration with University of Washington, USA. During the active surveys, malaria prevalence was observed to be declining from 6.5% SPR in 1<sup>st</sup> survey to 0.3% SPR in 4<sup>th</sup> survey. It was observed

that males, student as an occupation, persons with recent malaria infection and bed net non-users were found to be significantly associated with malaria.

### ACUTE ENCEPHALITIS SYNDROME (AES)

**Surveillance of Chikungunya virus activity in Assam and Meghalaya**

During the study period, out of the 799 samples screened for Chikungunya IgM antibody, 114 of them (14.27%) were positive. The 16-30 years age group was mostly affected and fever was the most common symptom (100%) followed by headache (72.2%). Vector incrimination study revealed that *Aedes aegypti* (91.1%) was the predominant species in the study sites and maximum container index value was for tyre and cement water tank (100%).

**Effectiveness of single dose of live attenuated SA 14-14-2 vaccine against Japanese encephalitis (JE) in adults over a period of three years in two districts of Assam, India**

It was observed that a single dose of the vaccine elicited 90% effectiveness for one year in adults. Midterm analysis shows the vaccine coverage to be only 33% (65/198) in Dibrugarh and 40% (163/404) in Sivsagar district.

A study on the bionomics of the potential vector species of JE virus transmission in Assam revealed *Culex vishnui* as the most predominant species (32.13%). Of the 902 pools screened for vector incrimination, 8 pools were found to be positive for Japanese encephalitis virus viz. one pool of *An. hyrcanus* group, 3 pools of *Cx. tritaeniorhynchus*, 2 pools of *Cx. vishnui* and the other of *Cx. pseudovishnui*.

**Adenoviral Vector based diagnostics for Japanese encephalitis virus, (JEV)**

This study was carried out was continued to carry out cloning of surface glycoprotein and non-structural protein genes of JE virus in adenoviral vector, expression analysis of JEV proteins in different mammalian cell lines and optimization of ELISA developed using the viral neutralizing glycoproteins with different clinical serum samples.

### **A systematic study of Acute Encephalitis Syndrome (AES) in North-eastern states of India for clinical, etiological and epidemiological aspects**

Of the tested sera, 24.7% (46/186) were found to be JE ELISA positive, of which 12.5% (3/24) were found to be WN positive. Another 17.2% (32/186) and 31% (31/100) demonstrated antibodies against Scrub typhus and Spotted Fever group Rickettsia respectively.

### **LYMPHATIC FILARIASIS**

#### **Studies on Lymphatic Filariasis: A new focus of lymphatic Filariasis in a non endemic district (Tinsukia) Assam**

About 4.32 % (57/1320) individuals from tea garden worker population were found to be microfilaraemic. The mf density ranged from 1- 80 mf showing the low density of the parasite in the population. Indoor resting mosquito *Cx. quinquefasciatus* showed presence of larval stages of the parasite. The infection and infectivity rates recorded were 5.88 and 1.96 respectively.

### **VISCERAL LEISHMANIASIS**

#### **An exploratory study of Visceral Leishmaniasis in endemic areas of Assam**

*Leishmania donovani* infection are being maintained and demonstrated in animal models. The key innate and adaptive immune molecular signaling markers were studied in infected BALB/C mice and control mice at different time period post infection. The mean level of NOS evaluated by using flow cytometry was found to be down regulated in the infected group of animals while it was higher in the control. However, the TLR2 levels in the infected group were increased almost up to two fold levels.

### **TAENIASIS**

#### **Molecular typing of *Taenia solium*/cysticerci and comparative analysis of human and animal isolates from North and North East India**

The study confirmed the existence of *Taenia solium* (metacestodes and proglottids) in the region, based

on ITS2 sequencing. Seven genetic variations in *COX1* gene of *T.solium* were observed among the isolates. Based on the phylogenetic analysis of *COX1* gene sequences, 2 different clusters are evident. Four polymorphisms in ND4 gene was observed among which polymorphism at nucleotide position 161 (G->A) was most frequent.

### **BACTERIAL DISEASES**

#### **Study of Genetic diversity and drug resistance pattern of *Mycobacterium tuberculosis* in the tribal state of Sikkim**

MDR-TB was detected in 23.2% (n=99) and 25.8% (n=93) of the new and previously treated TB cases respectively. Spoligotyping revealed the existence of isolates belonging to Beijing family.

#### **Epidemiology, disease burden and vectors of Rickettsial diseases in the states of Nagaland, Meghalaya and Mizoram in Northeast India**

The first phase of surveillance carried out in two states of North East region viz., Assam and Arunachal Pradesh revealed that the major rickettsial diseases viz., Scrub Typhus, Spotted Fever Group and Typhus Group Rickettsiae are widely prevalent in these states.

#### **Prevalence of bacterial pathogens colonizing the genital tract of pregnant women and comparison with those causing early onset neonatal sepsis**

The colonization rate of Group B *Streptococci* (GBS), *Escherichia coli* and *Enterococcus* at 35-37 weeks was found to be 15.1%, 7.8% and 33% respectively The bacterial isolates obtained from neonates with sepsis were GBS (n=4), *E. faecalis* (n=5), *E. coli* (n=2), *Streptococcus* spp. (n=1), and *S. epidermis* (n=1), Both maternal vaginal isolates and neonatal blood GBS isolates had shown absolute sensitivity to penicillin, ampicillin, vancomycin and linezolid.

#### **Study of interepidemic survival of *Vibrio cholerae* in outbreak prone areas of Assam**

*V. cholerae* was isolated from human cases collected from Lakhimpur and Sibsagar districts during outbreaks. The *ompW* gene specific for *V. cholerae*

was successfully amplified by PCR technique in 30 environmental samples but were non-culturable.

#### **Epidemiological typing of *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* isolated from septicaemic patients in North East India**

Ten isolates from clinical samples were confirmed as *S. maltophilia* and MLST was performed for 4 of them.

#### **Breast milk microbiome and neonate's gut-flora enrichment: The possible predictors for survival of pre-term-birth associated Low Birth Weight (LBW) Infant**

So far, the study has demonstrated that the breast milk microbial signature is dynamic and gets reduced with time. Dominancy of Proteobacteria in breast milk reduced with time while the dominancy of Firmicutes elevated with time.

### **VIRAL DISEASES**

#### **Enhancing Biorisk mitigation awareness in public health community and creating laboratory networks for enhanced diagnostic capabilities to deal with surveillance and outbreaks of high-risk group viral pathogens causing viral hemorrhagic fevers and respiratory infections**

The overall virus positivity was detected in about 52% of the collected specimens. The highest virus positivity was observed with rhino virus (88/284) followed by respiratory syncytial virus (66/284).

#### **A multi-site epidemiological and virological survey of Nipah virus with special emphasis on North East region of India**

The overall Nipah virus IgM antibody was detected in 6.95% (24/345) cases; of which, in [Assam:8.36% ; Arunachal Pradesh :1.42%. Most of the Nipah IgM positive cases 62.5% (15/24) were from Dhubri district (near Bangladesh border) of Assam. Nipah virus IgG antibodies were detected in 3 fruit bats (*Pteropus giganteus*) samples from Dhubri district and one sample from Cooch Behar.

#### **Immune exhaustion in chronic hepatitis B and malaria: focus on $\gamma\delta$ T cells**

It was observed that a significant proportion of  $\gamma\delta$  T cells from chronic hepatitis B patients express a higher level of early activation marker-CD69 than healthy controls. The expression of CD69 and Tim3 on  $\gamma\delta$  T cells was found to be positively correlated. The  $\gamma\delta$  T cells were significantly increased in *P. vivax* positive patients as well as in asymptomatic people from malaria endemic regions compared to healthy controls and people from malaria non-endemic regions with a past history of malaria during the last one year period.. The expression of central memory ( $T_{CM}$ :CD27<sup>+</sup>CD45RA<sup>-</sup>)  $\gamma\delta$  T cells was significantly decreased in these individuals compared to healthy controls.

#### **National hospital based rotavirus (RV) surveillance network**

The overall prevalence of RV was 37.8% (490/1295) with a slightly higher prevalence among males (n=315, 39.3 %). Genotyping revealed that G1P [8] was the predominant (66.75%) rotavirus genotype observed in the year 2013 and 2014, and G9P [4] in 2015 (46.3%) whereas in 2016, both G1P [8] and G9P [4] was prevalent (35.4% and 32.3% respectively).

Establishment of a network of laboratories for managing epidemics and natural calamities: A total of 3102 samples (including the outbreaks) collected from different parts of Assam and other states of North-east India were subjected to 11,554 number of tests for different viruses.

#### **Upgradation of the virology division to nodular molecular Virology Lab dedicated to focus on HIV, Hepatitis and Influenza viruses in Northeast India**

During the period, 75 patients were provided HBV DNA viral load free of cost. Vitamin D receptor polymorphism –Bsm1 and TNFa 308 polymorphism studies were carried out. Preliminary study reveals that genetic polymorphisms at TNFa 308 and Vitamin D receptor play important role in genetic susceptibility to hepatitis B infection.



### Biomedical Informatics Centre

The centre was engaged in different research activities including identification of natural lead molecules of *Centellaasiatica* and *Azadirachtaindica* targeting cholera toxin through structure based drug designing, *in silico* identification of natural lead molecules from *Phyllanthus* against Hepatitis B Virus reverse transcriptase, immunoinformatics screening of prospective MHC class I restricted cytotoxic T-cell based epitopes in Zika virus.

### DBT-ICMR animal house facility for Biotechnology Research in North-eastern Region

The process for construction of an advance animal house facility is under progress.

### Outbreak Investigations

The centre investigated outbreak of diarrhoeal disease in Manjushree TE, Sibsagar in June 2016. *Vibrio cholerae O1 El Tor*, Serotype *Ogawa* were isolated and were found sensitive to ciprofloxacin, norfloxacin, gatifloxacin, amikacin gentamicin, doxycycline, tetracycline and chloramphenicol and resistant to ampicillin and cotrimoxazole.



Fig. 2: Collection of *Taenia* Metacestodes from Infected Pig.



Fig. 3: Mosquito collection during dusk hours & using dropnet to study the bionomics of the potential vector species of Japanese encephalitis (JE) virus transmission in Assam.

### RMRC, PORT BLAIR

### ELIMINATION/RISK REDUCTION OF INFECTIOUS DISEASES

#### Effectiveness and operational feasibility of mass DEC fortified salt as a supplementary intervention to mass drug administration towards elimination of the lone foci of diurnally sub-periodic *Wuchereriabancrofti* in Andaman & Nicobar islands.

A study to demonstrate the efficacy of mass delivery of DEC fortified salt as a supplementary measure to the on-going annual rounds of MDA (DEC+albendazole) towards elimination of the lone foci of diurnally sub-periodic *Wuchereria bancrofti* has been completed. The study was conducted jointly by the centre, Vector Control Research Centre, NVBDCP and Andaman and Nicobar Administration. Impact assessment undertaken post one year of salt distribution; indicate that in the intervention arm, out of 12 villages, 9 villages had cleared microfilaraemia while in three villages, the *mf* rate was < 1% (0.3%-0.5%). Whereas in the MDA arm, out of the 14 villages, 4 villages had cleared microfilaraemia, in the remaining villages the *mf* rate ranged between 0.1%-7.89%. The antigenemia prevalence in 6-7 years age class, in the MDA arm(3.6%, 95% CI: 0.8,10.2) was found to be three times more compared to the fortified salt combination arm (1.2%: 95% CI: 0.0,6.4). The study shows that DEC fortified salt is operationally feasible and effective in accelerating the liquidation of parasite load below the threshold level.

#### Operational feasibility of elimination of malaria in Car Nicobar Island

The declining trend of malaria at Car Nicobar Island was investigated by entomological study as well as by tracing the reported malaria cases. Four villages were longitudinally surveyed for Anopheline breeding. The role of salinity concentration in the water habitats for the breeding of Anopheline was analysed. All the four villages, where entomological studies were conducted, showed evidence of Anopheline breeding and the breeding was observed to be maximal during May-July months. 100%

survival of III instar larvae up to 0.5% salinity, post 3-days of exposure was observed. Preliminary investigation indicate that anopheline larvae were abundant in habitats with salinity <200 ppm. This indicates salinity plays a role in water bodies like creeks and lagoons. For strengthening case detection, the traditional healers in three villages were trained to maintain register of fever cases being reported to them for treatment. The API in 2016 was 0.05 and only one case was reported.

### **Estimate the burden of TB among the tribal population and develop an innovative health system model to strengthen TB control.**

To estimate the prevalence of TB among the Nicobarese of Car Nicobar, to find out the health seeking behaviour of symptomatics and to develop feasible interventions to improve case finding and compliance for TB treatment through a community based approach was undertaken in four phases. During the period under report, Phase III was completed in three village clusters, by enquiring for chest symptomatic through door to door visit. A total of 82 chest symptomatics were identified for sputum collected, of which 4 were positive in both smear and culture while 9 were positive only in culture. The estimated prevalence of sputum positive pulmonary tuberculosis was 394/100,000. Six workshop/awareness programmes were organized in the three clusters. These included a workshop on the burden of pulmonary tuberculosis, for the medical personnel and paramedical staff of the hospitals and subcentres.



**Fig. 4:** Workshop at District Hospital in which Secretary Tribal Council participated.



**Fig. 5:** Awareness programme including painting competition in different villages in progress.

### **SURVEILLANCE OF DISEASES-HOSPITAL/ COMMUNITY BASED STUDIES.**

#### **Enhancing Biorisk mitigation awareness in public health community and creating laboratory networks for enhanced diagnostic capabilities to deal with surveillance and outbreaks of high-risk group viral pathogens causing viral haemorrhagic fevers and respiratory tract infections.**

The study targeted different viral aetiologies responsible for VHF (dengue, chikungunya and ZIKA) and respiratory tract infections. A total of 108 patients were enrolled. Dengue was confirmed in 30 cases (27.7%), Chikungunya infection was confirmed in 2 cases (1.9%). None of the VHF suspected cases were positive for Zika Virus. Infection rate was more in the 18 years and above age group than the other age groups. Viral pathogens were detected in respiratory specimens of 15 of 35 (42.9%) patients. The main viral causes of lower ARI identified were PIV-3 in 17.1% (6/35), followed by RSV-A in 11.4% (4/35), H1N1pdm09 in 8.5% (3/35), Inf B and Rhinovirus each one (2.9%, 1/35). The number of cases were more in the <2years age group (42.8%), followed by 2-13 years group (37.1%), 14-17 years (17.1%) and 18 & above age group (2.8%) respectively. The present study is an indication that viral haemorrhagic fevers are emerging as an important public health problem in these islands and warrants extreme vigil from



public health perspective for its effective control. However, no threat due to ZIKA virus has been reported.

### **HEALTH AND NUTRITION OF MARGINALIZED COMMUNITIES**

#### **Phytochemical investigation and anti-malarial activity evaluation of medicinal plants used by indigenous tribes of Andaman and Nicobar Islands**

The centre continued the studies on traditional medicine and health care practices among the indigenous tribal communities. As a part of this, crude extracts from 15 medicinal plants were tested for malarial activity, of which 8 indicated anti-malarial potential. The extracts from these eight plants were purified and characterized. Seven major fractions (F1, F2, F3, F4, F5, F6 and F7) were obtained from the crude chloroform extract. Further subjection of F3 and F4 yielded sub fractions B1 and B2 respectively. B1 and B2 sub fraction yielded compound 1 and 2. Similarly, repeated fractionation of F4, yielded compound 3 and 4. Structure of these purified compounds has been elucidated using NMR spectroscopy. Alkaloids were found to be major components in plant AN-K-511.

#### **Disease Surveillance and Health profiles of Particularly Vulnerable Tribal Groups (PVTGs) of the Andaman & Nicobar Islands**

The Andaman & Nicobar Islands are home to six indigenous tribal communities viz. Nicobarese, Onges, Great Andamanese, Shompens and Sentinelese. Although the disease and health status of Nicobarese is known, no systematic studies on health profile for Onges, Jarawas and Shompens is available. A collaborative project between DHS, AAJVS, Dept. of Tribal Welfare ANTRI and RMRC was initiated for this. It was proposed to have immunological follow up of the vaccinated children, considering that the Jarawa population had no prior exposure to vaccines. Health profiles of the Onges have been completed. Vaccination for Jarawa

is in place. A total of 92 Jarawa children have been vaccinated. The vaccines covered are BCG, OPV-1, OPV-booster, DPT-Booster, pentavalent and measles. In addition, eight pregnant women were given tetanus toxoid. It was recommended to take up pro-active steps for undertaking immunological follow up of the Jarawas.

### **ECOLOGY AND ENVIRONMENTAL MICROBIOLOGY**

#### **Leptospiral biofilms and its role in transmission dynamics and pathogenesis**

The centre's previous studies on leptospiral biofilms showed that theme is leptospiral biofilms abundance in the paddy field and walls of the sewage canals and it plays an important role in the transmission dynamics of leptospirosis. The information generated is novel. An attempt was made to understand development of newer strategies such as DNA antibody as a therapeutic vaccine to prevent severe complications of leptospirosis.

#### **Leptospira biofilm and its role in biofilm formation and integrity.**

The study showed the presence of eDNA in the extracellular matrix. The eDNA was bound to the other components of the matrix, which is essential for the integrity of the biofilm. The association of eDNA with the matrix was dependent on matrix proteins. Both proteinase and DNase treatment severely reduced the clumping of re-suspended biofilms, highlighting the importance of both proteins and eDNA in connecting cells together. By adding EPS material and an excess of exogenous DNA to DNase-treated biofilm, clumping was restored not only to parent leptospiral strain, but also to other non-biofilm forming strains, thus confirming the crucial role of eDNA in the interconnection of cells across species/strains. On the basis of the results, it is evident that eDNA acts as an electrostatic net, interconnecting cells surrounded by positively charged matrix proteins of *Leptospira*.



## BASIC AND APPLIED RESEARCH

### Study of genomic diversity and expression of genes among endemic leptospiral strains and isolates recovered from patients with different clinical syndrome

A total of 24 strains recovered from various clinical syndromes were studied to understand the geographic genomics and syndrome based pathogenesis. The whole genome analysis of seven strains revealed that there is an acquisition of genes in the range of 548 – 670 and a loss in the range of 383 – 424 genes among the endemic leptospiral strains. This observation enabled us to understand the adaptation of causative agent against host defence mechanisms and causing multi-organ injury (Lung, kidney, liver and gastrointestinal injuries). Results of the expression study showed the up-regulation and down-regulation of various genes among the strains recovered from various clinical syndromes. The results also indicate that the expression of strain specific genes or sharing genes could have played an important role in the pathogenesis and leads to multi-organ injury. This information is novel and would help to understand the syndrome based pathogenesis and designing tools for the clinical management of leptospirosis. These differentially expressed genes would provide the fundamental basis for hypothesis-driven research to determine virulence factors and candidate genes for future vaccine development, both therapeutic and curative.

### SUPPORT TO NATIONAL HEALTH PROGRAMMES AND WHO

The centre is extending support and works in collaboration with various National Programmes viz. A) NVBDCP - for elimination of LF and malaria and risk reduction of dengue/chikungunya. B) RNTCP- to eliminate TB at Car Nicobar and as well as to support the RNTCP programme for follow up of the non-responders to the treatment and drug susceptibility testing.

### DMRC, JODHPUR

DMRC has been carrying out studies on Sickle Cell Anaemia, early detection of Breast Cancer,

Hypertension, assessment of nutritional status of vulnerable population including elderly.

The screening of tribal students residing in hostels and Maa-Baadi centres has been carried out in five districts of tribal sub-plan area of Rajasthan viz Sirohi, Udaipur, Banswara, Pratapgarh and Dungarpur. The study has jointly been completed with State Health Department. The training was imparted for screening test to 78 medical officers and 245 para medical staff of study districts before initiation of the study. The scheduled tribe residing in the areas are mainly Garasiya, Meena, Patel and Gujjar. A total of 37299 students were screened, out of which 2139 (5.73%) were found positive. The study to assess effective modality for making self breast examination as an acceptable practice among women, resulted in a development of mega project on self breast examination for early detection of cancer and development of a referral system. The study is to carry out in existing health system with the goal to impart training to medical and paramedicals, who in tran, trained community for self breast examination. The various projects are being carried out to assess nutritional status of vulnerable population including elderly and to identify bottlenecks in implementing existing government programmes. These studies are being carried out in Jodhpur, Jaipur, Chittorgarh, Churu and Kota districts of Rajasthan. The study reveals high prevalence of low birth weight and poor complimentary feeding. The study on elderly also demonstrated micronutrient deficiency among them. An appropriate intervention is being carried out covering over a lakh population. Impact of the intervention will be evaluated. A study was carried out in Nagaur district of Rajasthan aiming to develop IEC modality for promotion of three local pearl millet preparations viz Rab1 (Pearl millet Grains), Kadhi and Sogra.

A study has been carried out with a aim to record the impact of irrigation change on the ecological conditions with respect to vector prevalence and malaria incidences. Findings revealed that the canal irrigated areas of both the districts i.e. Banswara and Jaisalmer, exhibited higher mosquito densities during pre-monsoon season, which is the time for the transmission process of *Plasmodium vivax*.

In Banswara, *An. annularis* also recorded its presence during this season, which is also a potential vector of malaria and should be given attention while planning vector control activities in the region. In Jaisalmer *An. stephensi*, which is an important vector of arid areas, was also recorded. The data on temperature range variation indicated that the variation in both intra- as well as extra-domiciliary temperatures were more suitable for the survival of the malaria vectors, in canal irrigated and non-canal irrigated areas of Banswara district than Jaisalmer district.

Determination of the current status of insecticide resistance against the compounds being used under national control programme for adults and larval forms in the state was studied. The studies have been carried-out in five districts viz., Baran, Bharatpur, Pratapgarh, Rajsamand and Udaipur.

In Baran district, *An. culicifacies* against Etofenprox and *An. stephensi* against Alpha-cypermethrin exhibited intermediate resistance, which need further verification and is a matter of concern. All the three species exhibit resistance or intermediate resistance against DDT, except *An. annularis*, which was found susceptible to DDT in Pratapgarh district. The mortality of all the tested vector species against DDT was recorded more than 50.0 percent, except two occasions - first in case of *An. culicifacies* and second in case of *An. annularis*, where the mortality was less than 50.0 percent, hence for the reduction of vector population, DDT can be still used in these areas. Synthetic pyrethroids viz., Alpha-cypermethrin, Lambda-cyhalothrin and Etofenprox, as a substitute of DDT, can be judiciously used for the control of vector species in the study areas.

A study was conducted to identify suitable interventions to bring individuals in Jodhpur district of Rajasthan with knee pain to a lower level of discomfort. Under the study, 502 individuals were traced from five villages namely Kaparda, Binawas, Ramasani, Rawar and Holpur in Bilara tehsil of Jodhpur district which were included in an earlier study by ICMR. 502 individuals were interviewed to note for their present condition of knee pain associated with risk factor of smoking, alcohol, opium consumers & non-consumers,

history of knee injury, wearing, non-wearing of heels, vegetable intake per week, ghee intake per day, liquid intake/day, consumption of dal per week and type of grain consumed. This was measured using WOMAC Index (Modified CRD Pune Version). Results show that maximum shift from 'Normal' to 'Moderate' level of discomfort occurred in younger age group of 35 -55 and females in this age group being the most affected. Thus the highest percentage of 'No Pain' condition corresponds to higher intake of liquid/water. Infact, intake of more than 2 litre of liquid/water provides 70% chances of being in 'No Pain' condition. This is suggestive of higher intake of liquid to avoid painful condition and with increased chances to be under 'No Pain' condition. The distribution of severity of knee pain and Dal consumption per week was studied. It is noted that 239 individuals (68.3%) who were the sufferers, consumed Dal for less than 4 days a week as compared to only 111 number of sufferers (31.7%) who consumed Dal for more than 4 days a week i.e. the number of sufferer with less frequent consumption of Dal is twice the number of sufferers with frequent consumption of Dal. The study suggests an affordable and sustainable dietary modification for the poor rural people in a desert area (study subjects), which may help them in sustaining 'No Pain' and addressing 'Painful Condition' of knee joint. These modifications include, (1) Restriction of intake of Ghee to less than 10 gm/day (2) Enhanced liquid Intake for more than 2 lt/day (3) Increased frequency of consumption of Dal/week with use of Urad Dal (4) Additional use of Bajra along with wheat and (5) Increased frequency of consumption per week of vegetables, used by the people in routine. An intervention study based on the findings of the above study may be planned to help the people of the area with joint knee pain.

The centre is also having Biomedical Informatics and tribal research centre. The objective of bioinformatics centre is to develop the capacity building of medical professionals of various colleges of Rajasthan and scientists of different universities. The tribal unit has been working in only primitive tribes of Rajasthan i.e. Saharia, for assessing their nutritional status, for developing appropriate intervention module.





**Fig. 6:** Screening of Sickle Cell samples at Abu Road.



**Fig. 7:** Observing result of solubility test by CMHO, Pratapgarh.



**Fig. 8:** Training Programme on sickle cell disorder for Medical & para medical staff of Dungarpur & para medical staff of Udaipur.



**Fig. 9:** Training Programme on sickle cell disorder for Medical & para medical staff of Pratapgarh.



**Fig. 10.** Training Programme on sickle cell disorder for Medical & para medical staff of Banswara.



**Fig. 11:** Preliminary survey carried out at Udaipur district by team of Desert Medicine Research Centre, Jodhpur by DMRC, Jodhpur, TADD, Udaipur and State health Department officials regarding initiation of work of screening of sickle cell disorder.



**Fig. 12.** Mid Term Evaluation meeting on sickle cell screening work at TADD, Udaipur.



# SUPPORTING FACILITIES

During the reported period, statistical assistance was provided to all ICMR institutes by the National Institute of Epidemiology (NIE), Chennai, and the National Institute of Medical Statistics (NIMS), New Delhi. Several new projects have been undertaken. A new division Informatics, systems and research Management (ISRM) came into existence with a mandate to support informatics in medical research and to develop research management on International pattern.

## *Intramural Research*

### NATIONAL INSTITUTE OF EPIDEMIOLOGY, CHENNAI

#### ICMR School of Public Health

The ICMR School of Public Health at the National Institute of Epidemiology has been conducting two-year Master of Public Health (Epidemiology and Health Systems), two-year M.Sc. (Biostatistics) and 8-week online course for the conduct of human bio-medical research.

#### Master of Public Health (Epidemiology and Health Systems)

- During 2016, 17 trainees graduated, making the total number of graduates to 229.
- 15 abstracts of 11 MPH trainees were selected for presentation at the 8th TEPHINET Bi-regional Scientific Conference held in Siem Reap, Cambodia, 2016.
- 4 research articles published.

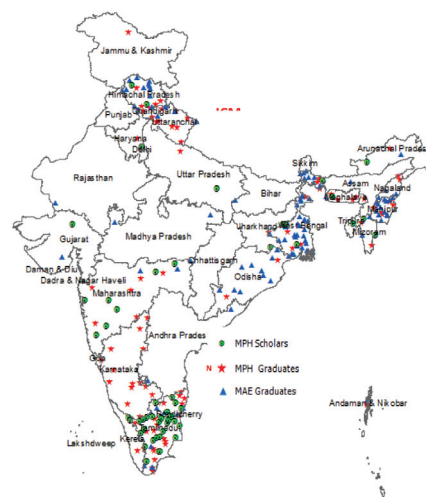


Fig. 1: ICMR SPH graduates and scholars, India, 2017.

#### MSc (Biostatistics)

- NIE has commenced Master program in Biostatistics in collaboration with Periyar University, Salem, in August 2016.

#### ICMR-NIE’s Massive Open Online Courses (MOOCs)

- NIE has started its first MOOC on Health Research Fundamentals to strengthen bio-medical research capacity in the country. A total of 12,000 health and allied professionals across all parts of the country were enrolled in four rounds of the course.

#### Operational research on implementing public health surveillance for mass gatherings in notified festivals in Tamil Nadu

ICMR-NIE coordinated with Tamil Nadu Directorate of Public Health and Preventive Medicine to (1)

describe the public health preparedness for mass gathering (2) establish real time surveillance for selected syndromes and/or disease conditions (3) document the effectiveness of real time surveillance system during the following notified festivals under the Public Health Act of Tamil Nadu.

**International open trial of uniform multidrug therapy regimen for leprosy patients**

- Efficacy of six-month multidrug therapy (MDT), currently recommended for multi-bacillary (MB) patients, as uniform MDT (U-MDT) was evaluated in a single-arm open trial under program conditions. Primary objective was to determine efficacy to prevent five-year cumulative five per cent relapse. Secondary objectives were to assess acceptability, safety and compliance.
- A total of 2091 pauci-bacillary (PB) and 1298 multi Bacillary (MB) leprosy patients were recruited. Event rates per 100 person years as well as five-year cumulative risk of relapse, were calculated.
- The relapse rate among PB and MB patients were 0.023 per 100 per person years and 0.07 person years respectively. The five-year cumulative risk of relapse among PB and MB cases was 0.11% and 0.37% respectively. The compliance to U-MDT was 99 per cent.
- The findings of this trial supports introduction of U-MDT in national leprosy program.

**Demographic and Health Surveillance -Ayapakkam Cohort Study**

- Demographic and health surveillance has been initiated in Ayapakkam, a peri-urban area in Chennai.
- GPS mapping (Fig-2.) and listing of 12,537 households has been completed. Socio-demographic profile of 10,389 households and self-reported morbidity and healthcare seeking behavior of 36,350 individuals has been collected so far.

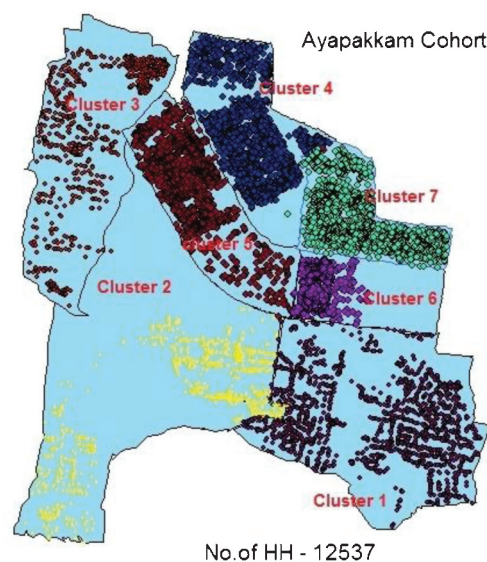


Fig. 2: Map of Ayapakkam Cohort, Chennai.

**Health systems preparedness for interventions for Diabetes, Hypertension, Chronic Respiratory Diseases, Cardiovascular disease and cancers and deaths due to non-communicable diseases among the tribal population in India**

- Study findings covering >2000 deaths investigated from 7 districts [Sikkim; Koraput (Odisha), Nicobar (A & N Islands) Kinnaur (Himachal Pradesh), Dhalai (Tripura), Lunglei (Mizoram) and East Garo Hills (Meghalaya)] indicate that non-communicable diseases are the leading cause of death, accounting for nearly three fourth of the deaths (Fig-3).
- Data collection is ongoing in Dhimaji (Assam), Mokokchung (Nagaland), Senapati (Manipur), East Kemang (Arunachal Pradesh) and Mandla (Madhya Pradesh).

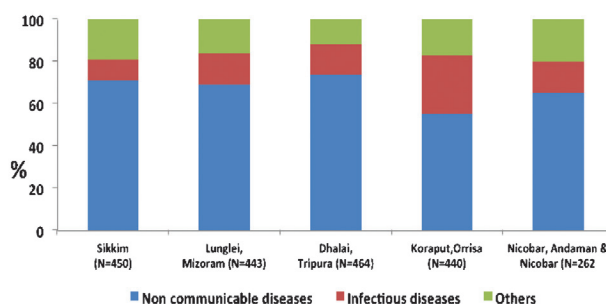


Fig 3. Proportion of deaths due to NCD, infectious diseases and other causes at five study sites, India.

### National Rotavirus Surveillance Network (2012-2016)

- Surveillance for rotavirus among children aged < 5 years has been ongoing in 28 hospital sites.
- Rotavirus was detected in 36.3% (7783/21421) of children with acute gastroenteritis enrolled in surveillance during 2012-16.
- Rotavirus infections usually occurred more commonly during the cooler months of December - February, followed by September – November.
- The highest positivity (43.1%) was observed among children between 12 and 23 months of age (Fig-4).
- G1P [8] and G9P [4] were the commonest genotypes, accounting for 53% and 9% of all strains respectively.
- Rotavirus disease burden data from NRSN contributed for the decision by policy makers to introduce rotavirus vaccine in the UIP in 2016 (Fig-5).

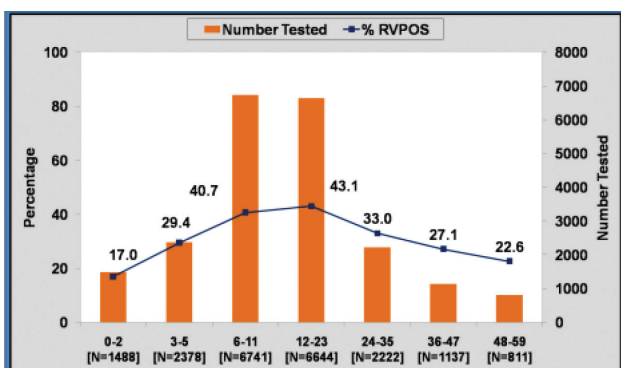


Fig 4: Age-group wise distribution of Rotavirus Positivity (%).

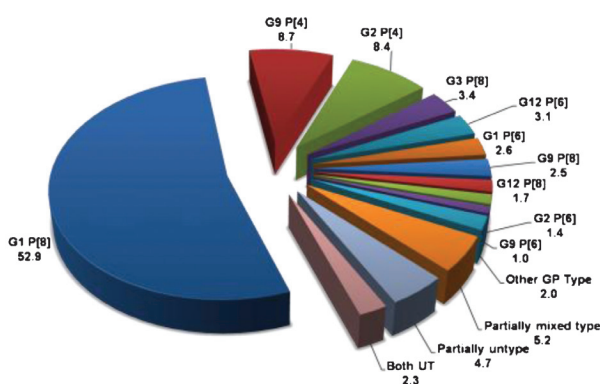


Fig. 5: Rotavirus genotypes.

### Program implementation review following Rotavirus vaccine introduction in India – A Rapid Assessment.

- Prior to expanding the rotavirus vaccine to five new states, NIE conducted a rapid program implementation review following Rotavirus vaccine introduction in four states viz. Himachal Pradesh, Haryana, Andhra Pradesh and Odisha.

### Hospital based surveillance for bacterial meningitis

- As per the surveillance data from 11 sentinel hospitals from 6 states, during 2012 – 2016, a total of 312779 cases were admitted to inpatient wards of 11 sentinel sites. Among them, 95418 patients were enrolled for fever, with 13566 cases suspected for bacterial meningitis and 12959 (13.6%) cases were found to be eligible for enrollment.
- Lumbar puncture was done in 11643 cases, of whom 1287 (11%) cases were categorized as probable meningitis cases and 587 (4.5%) were confirmed as bacterial meningitis cases either by latex agglutination test or CSF culture or blood culture or PCR.
- *S. pneumoniae* is the most frequent (74%) causative organism, followed by *H. influenzae* type *b* (22%) and *N. meningitidis* (4%).

### Hospital based sentinel surveillance of S.pneumoniae and other IBD – Pneumonia surveillance

- Hospital based sentinel surveillance for *S. pneumoniae* and other Invasive Bacterial diseases was initiated in six sentinel sites.
- During December 2016 - March 2017, 5229, patients were hospitalized; of these 2389 patients were enrolled for fever. A total of 1389 were suspected for pneumonia, 425 cases were suspected for bacterial meningitis and 382 cases were suspected for sepsis.
- From 2389 enrolled patients, 701 blood samples, 383 CSF samples and 49 other specimens (Throat swab, Sputum, BAL, Pleural fluid etc.) were collected and tested.



- Among the 701 tested blood samples, 8 were positive for *S. pneumoniae* isolate. Of the 383 CSF samples, 3 samples were positive for *S. pneumoniae* isolate and by latex agglutination test 3 samples positive for *S. pneumoniae* and one sample positive for *H. influenzae* type B. 49 other specimens were collected from enrolled patients, among them 4 (3 pleural fluid and 1 sputum) were positive for *S. pneumoniae*.

**Prevalence of leptospiral infection among fever-case-patients seeking referral public health facilities in the peri-urban areas of Chennai and distribution of leptospiral genotypes and serovars**

- During the year-round surveillance for acute febrile illness in 2 sub-district hospitals in Poonamalle Health Unit district (Avadi Taluk Hospital and Poonamalle Taluk Hospital), 924 patients were enrolled.
- Overall sero-prevalence of recent infection was 9.2% (95% CI and 1% precision index).
- Leptospire were isolated from 60 patients, being culture maintained and molecular characterization is under process.

**Virus Research and Diagnostic Laboratory Network**

- DHR/ICMR has established a network of 46 virology laboratories for providing timely diagnosis of disease outbreaks.
- During 2016-17, VDLs provided diagnosis to 329 disease clusters/outbreaks. Measles (42%; n=138), dengue (21%; n=69) and Varicella Zoster virus (12%; n=39) were the commonest etiologies.

**Health-needs assessment of selected hill tribes (Palliyar and Muthuvan) in Western Ghats of Tamil Nadu - Tribal Health Research Unit**

- A survey to assess the health status of Palliyar tribes was conducted in 17 villages in Dindigul district.
- Prevalence of Hypertension in adults, above 18 years of age, was around 25% and 11% were in pre-diabetic stage.

- Nearly 31% of the under five children had severe protein energy malnutrition and another 11% were under weight.
- Focus Group Discussions and in-depth interviews revealed that domiciliary delivery was most preferred because of the opportunity cost involved in institutional deliveries. Also, lack of ration cards, aadhaar cards and bank accounts were some of the reasons given so, could not be benefited from the cash transfers under the scheme meant for pregnant mothers. Immunization for both maternal and child was low because they had to trek long distances in forest to reach sub-centres for immunization and this involved loss of income as well as also the belief that children will fall sick following immunization.

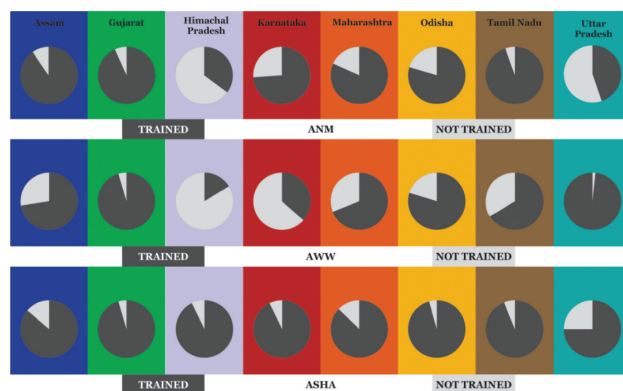


Fig. 6: Training status of frontline health workers.

**Process evaluation of Integrated Management of Neonatal and Childhood Illnesses (IMNCI) in India. Funded by Department of Health Research, Government of India**

- NIE conducted process evaluation of the IMNCI in 8 states. Information about (a) training status of Facility based service providers and frontline health workers (b) availability of essential drugs at the facilities (c) adequate placement of staff at the health centres (d) supervision mechanisms (e) presence of a swift and efficient referral system and (f) knowledge and awareness levels of both service providers as well as beneficiaries, was collected from different stakeholders including program managers (n=34), facility based service providers (n=990), community-

based service providers (n=3,031), and beneficiaries (n=4,858) (Fig-6).

Key findings of the evaluation include:

1. IMNCI Training coverage of frontline workers (ANM, AWW, and ASHAs) was higher when compared to F-IMNCI training coverage of Facility-based service providers (Medical officers and Staff nurses).
2. Frontline workers' IMNCI kit was poorly equipped to render services at the community whereas facilities were equipped in terms of adequate equipment and drugs with minimal lacunae.
3. Level of awareness of danger signs/illness recognition related to under five health was low among mothers of under five children whereas awareness of exclusive breast feeding and immunization was high.
4. Collaboration and coordination activities with ICDS, local agencies, PRIs, were weak.

#### **National NCD Survey for Monitoring the National NCD Targets-201: Principal Investigator for Mobile application and data management**

- mHealth initiative for field based research: Under the project National NCD Survey for monitoring the national NCD targets, NIE developed the android mobile application using an open source data package utilizing the existing institutional resources. Thus android mobile data acquisition application was developed for a nationwide survey at Zero cost with complex algorithms including capture of geo-tags during data collection.

#### **Model District for Public Health Preparedness, Surveillance and Response: Multi-Strategic Integrated Approach in Tiruvallur District, Tamil Nadu, India**

- Designed, developed and demonstrated electronic daily fever surveillance system (eDFSS) in Tiruvallur district of Tamil Nadu. eDFSS is a hospital based surveillance where

information about febrile patients hospitalized in health facilities are collected, analyzed, reported using improved electronic data management system. Directorate of Public Health, Govt of Tamil Nadu and NIE plans to scale up this system to corporations of Tamil Nadu under Tamil Nadu Innovative initiative project.

- Establish demographic and health surveillance in rural area under Model Rural Health Research Unit, Tirunelveli, Tamil Nadu.
- Establish surveillance networks to generate data for decision making.
- Plans to expand surveillance for pneumonia, congenital rubella syndrome and influenza.
- Disease burden estimation studies for diseases such as dengue and chikungunya.

#### **NATIONAL INSTITUTE OF MEDICAL STATISTICS, NEW DELHI**

National Institute of Medical Statistics (NIMS), situated at ICMR, New Delhi, aims to promote and undertake research in statistical techniques and methodology in the field of health, exercise surveillance to ensure the statistical adequacy and validity in various programmes of the ICMR and Government of India. During the year of report, the Institute carried out the activities relating to health research capacity building, research projects and research publications in journals of repute.

#### **Strengthen Health Research Capacity**

The Institute conducted following training/workshops/brain storming sessions and Orator Lectures.

1. Summer Training Workshop for PG Students of Banaras Hindu University, Varanasi and Kurukshetra University, Kurukshetra, (20 students).
2. Winter Training Workshop for PG Students of Kurukshetra University, Kurukshetra, January, 2016 (20 students).

3. Internship to PG Students of AMITY University NOIDA, January-March.
  4. A two-day workshop on Economic Evaluation: Introduction, Concepts and Applications on March 3-4, 2017 at NIMS, New Delhi. The objectives of the workshop was to bring together a combination of interested scientists and researchers of ICMR who are trained in epidemiology, statistics and economics to understand the basics of economic evaluation of health interventions and health care services and stimulate interest among the researchers to undertake simple cost effective studies in India.
  5. Prof. P.P. Talwar Oration Lecture on “Value of Measurements in Health: Impact on Forecasting and Evaluation” was organized at ICMR on 1<sup>st</sup> January 2017. The lecture was delivered by Prof. N.K.Ganguli, former DG, ICMR and distinguished Biotechnology Fellow & Advisor, THSTI.
- Disease Burden due to Malaria.
  - Burden of Disease due to Cancer in India.
  - Clinical Trial Registry - India (CTRI)
  - Survey for Monitoring the National Non-Communicable Diseases Targets, 2014-15.
  - Improving the Maternal and Child Health through Male Participation among Saharia Tribe in Gwalior District, Madhya Pradesh.
  - A Study on Gender Inequity in Health Seeking Behavior among Santhal Tribes of Jharkhand.
  - NACO’s HIV Sentinel Surveillance.
  - Development of Data Hub of Health Surveys and Use of Information for modeling and projection of various diseases.
  - National Burden of Non-Communicable Diseases and associated Risk Factors (NCD-BOD).
  - Comparing Methods for Assigning Causes of Death.
  - Decomposing Socioeconomic Inequality in Health among Older Population in India.
  - Exploratory Geo-spatial analysis to study the Utilization of RCH Services in North-East States.

Apart from the above capacity building programmes, the institute has been recognized for the Ph.D. programme in Medical Statistics of GGS Indraprastha University. Two students are registered and working for their Ph.D.

Also, the institute provided statistical consultancy to faculty and PG students of different Medical Colleges in Delhi including Lady Harding Medical College & Smt. Sucheta Kripalani Hospital, Kalawati Saran Children Hospital, MAMC, RML Hospital, Safdarjung Hospital, B.L.Kapoor Hospital, etc.

### Projects Undertaken

- Baseline & End line Household Malaria Survey in World bank Districts.
- Factors Associated with Under-Five Mortality among Scheduled Tribes and Other Social groups in India.
- Identification and prediction of fall among elderly.
- Application of Bongaarts model to study the proximate determinants of human reproduction process in India.

### *Extramural Research*

#### **SOCIO-BEHAVIOURAL AND HEALTH SYSTEMS RESEARCH**

Eight projects and one fellowship were completed, nine projects and four fellowships (RA= 2, SRF=2) continued during given time period. Six ICMR ICSSR joint proposals commenced during 2016-17.

#### **Unwanted sexual experiences and victimization among children (online and offline): An observational study.**

An observational, questionnaire based study was carried out to examine the rates of online and offline unwanted sexual experiences (USE) among adolescents. The study was carried out in both government and private schools. Results have shown alarming status. 60% of the adolescents have reported having faced either online or offline unwanted sexual experiences or both. 36%



adolescents reported having faced online unwanted sexual experiences while 52% reported having offline unwanted sexual experience. One-fifth of the adolescents who have reported to have faced online or offline USE are still having such ongoing experiences.

The study concluded that there is urgent need of parental supervision of internet access among adolescents. Parents need to keep a check on their child's activities on social networking and discuss, if any, problem being faced by their wards.

#### **A Cross sectional study evaluating the socio-demographic and psychosocial profile and motivations for use for individuals with prescription opioid abuse**

The project was aimed at exploring the socio-demographic as well psychosocial profile of individuals with prescription opioid dependence and their motivation to use. To compare the finding of the project, individuals with heroin dependence were taken. The motivation for first use of oral and injectable opioids was comparable between the two groups. The prevalence of co-morbid psychiatric disorders was comparable except for dysthymia which was significantly more common among individuals with heroin dependence. The individuals with prescription opioid dependence were more likely to report, never shared the injecting equipments with a sexual partner, which was seen in individuals with heroine dependence. The individual with prescription opioid dependence were using the condoms less frequently.

The study concluded that awareness about sexual relations, safety behavior and harm reduction behavior/activity has links with opioid abuse.

#### **A study of knowledge attitude and perception of young Indian men towards females and about gender discrepancy at work**

The subjects showed positive attitude towards women with total attitude score of  $64.4 \pm 0.4$  and  $65.4 \pm 0.4$  for workers and students respectively. On gender stereotyping, workers responded for gender equality in occupation compared to students.

Regarding 'crime perception', the students seemed a little more concerned towards crime against women as compared to workers. However, workers were lenient for domestic and psychological crimes against women.

The current study highlighted the scope of improvement of the overall attitude and perception of the young population regarding gender equality. There is a need to make a program on various stakeholders who can participate for a healthy and holistic environment for women.

#### **Make hand wash a healthy habit: A community Intervention trial in a rural area of Varanasi District.**

A community based intervention study was carried out to assess the knowledge, attitude and practices regarding hand washing among women having under five children and to measure the effects of behavior change communication related to standard hand washing practices.

In the intervention, rural mothers were selected who would be trained as per the standard protocol of hand washing developed by WHO, World Bank & UNICEF. Many (71.8%) mothers had excellent knowledge regarding good practices of hand washing during household activities while majority of respondents i.e. 87% had either incomplete or wrong knowledge regarding diseases occurring due to improper hand washing. A very few number of respondents had knowledge about proper steps of hand washing and mostly had learnt these proper steps from their school going children as well as other family members.

Diarrhoea is leading cause of child death and hence improvement in hand washing with soap in the household, can reduce the incidence of the diarrhoea. An intense community based intervention program was suggested in the current scenario to control future epidemic condition.

During the year, the Health Systems Research (HSR) Division has funded few ad-hoc projects on understanding and strengthening of health systems in the country. Some of these projects have

been concluded during this year. In addition, two national task force projects on road traffic injuries have been initiated in 10 cities.

### **Study on assessment of approaches and factors in increasing attraction and improving retention of physicians in rural and remote areas of North India**

This study adopted the mixed-method approach covering samples of general physicians, specialists, final year MBBS students, interns and health care administrators. It analyzed the movements of general medical officers and specialists from urban to rural area & visa-versa; exploring various push and pull factors for retention of physicians and analyzing the perceived attraction factors and deterrents of final year MBBS students and interns regarding rural area posting. The three factors such as education, regulation, financial incentives and personal & professional support cut across the traditional dichotomy between intrinsic and extrinsic motivation and uncover a novel three faceted motivation construct based on scientific factors, societal expectations and humanitarian needs. The motivation to choose medical career was rated higher in the study. Three main factors of motivation emerged were to serve the poor and needy, desire for respectable living and job security. The MDs posted in urban areas are less willing to shift in rural areas as compared to MDs who are posted in rural areas. Top barriers acting behind the unwillingness of medical students to work in rural areas are lack of proper education (64%), more political interference (59.6%), limited access to updates in medical field (55.8%), interference of traditional helpers (54.7%) and limited access to medicine (53.3%). Seven broad types of interventions, personal and professional, financial, enhanced scope of practice, improved supervision and management, compulsory rural service, continuous professional development and rural focused education in curricula, emerged out in present analysis. Study concluded that by providing only one type of intervention could not motivate health workers but the policies considering a mixture of intervention must be implemented in order to tackle the problem.

### **Study on geo-spatial analysis of accessibility of health care services by migrants in the city of Mumbai, based on Geographic Information System (GIS)**

This model focused largely on the location aspect of health care facilities and its awareness to the migrants. In certain cases, the health care facilities are not adequate to cater the booming population and also the technologies used are not advanced. Thus, it requires expansion and up gradation of the services. Access to primary health care is recognized as an important facilitator of overall population health, particularly for migrants in Mumbai. However, little about other barriers to healthcare affecting utilization rates and population health, is known. Among these less understood barriers are geographic availability and accessibility of primary care providers. This knowledge deficit can now be more aggressively addressed due to recent advances in the field of geospatial analysis coupled with the decreasing cost and improving usability of GIS. This study warranted to assess the situation and incorporate measures to ensure a balance between risks and benefits through the following steps: (i) inclusive planning of health service delivery in urban areas to ensure that the care of migrant workers is given special emphasis; (ii) the allocation of a dedicated budget for the welfare of migrant labourers; (iii) inclusion of an occupational health unit at the primary care level to cater to the needs of the migrant workforce; and (iv) dedicated outreach clinical services for migrant labourers at their worksites.

It is observed that in certain wards, where growth rate is high, healthcare accessibility is not available. The access to various services is limited to migrants. Demand is more but supply is less. In some wards, private healthcare facility is available but not affordable to the migrants. Migrants can select the service according to their type of demand and affordability. Usually, they are engaged in jobs which are low paid and also taking a day off means they lose their wage, which they do not want to do in most cases. This exercise through GIS projects, indicates for decision making for location of healthcare services at an optimal distance with

respect to time and cost. Although, disparities in health care access are a key factor underlying disparities in health outcomes, naturally other factors also play a role in outcomes. The study limited the focus of the framework presented here to health care access and how public health programs can influence it. However, the role of public health in reducing health disparities goes beyond improving health care access. Migrant labourers are at a significant disadvantage in the community into which they have migrated. They are in unfamiliar territory amidst strangers. They are also not familiar with the language and culture of the new place. In addition, they are discriminated against by the host society, who feel that they “belong to another culture”. As a result of these factors, migrant labourers may be deprived of access to healthcare.

#### **A model initiative for vital registration and communication by the use of mobile application among health care service providers was taken up in Uttrakhand**

The objective was to validate the innovative mobile based MIS application for timely and complete registration of vital statistics (i.e. birth and death). Mobile based application was operational in whole block with the help of 195 ASHA workers, 9 village development officers and one block development officer. Data was collected as per government approved birth and death certificate formats. All the required data for the certificates was collected. ASHA workers were involved in collection of birth and death related data in their registers and this was reported in monthly meeting at their respective PHCs. All ASHA workers were trained for sending birth and death related information via SMS in definite pre-decided format. Each ASHA worker sends SMS, based on defined data recording pattern to the server and data is digitalized and can be retrieved in Excel format for analysis or report can be generated at CHC/PHC/SC level or by census office through dedicated website ([www.crvs.in](http://www.crvs.in)). Beneficiaries can collect birth/death certificate from respective village development officer. Timely registration of data by ASHA avoids delay. The

diagrams illustrate the capturing of information (Fig. 7 & 8).

It can be concluded that data collected through ASHA workers is to be complemented with public and private hospital based birth and deaths as well as police station data (for deaths) for completeness with web based application (website). Data collected through either by existing system of civil registration or by ASHA workers only, is not complete and so all hospitals of the area in addition to health workers (ASHA/ANM) where birth and death takes place should also be included to necessitate complete recording of data. As per above proposed model, extension of current work is advised, for tracking of births up to 5 years for anthropometric indices to rectify avoidable deaths, immunization coverage and births can be tracked health centre wise. It can gather epidemiological data regarding individual child for morbidity pattern, anthropometric indices (weight, height, MUAC, BMI, growth charts), cause of death or still birth and simultaneous preventable/rectifiable measure can be taken. It is recommended for ICDS for decreasing morbidity, mortality and will help in child growth and development. These models have been illustrated in Fig. 9.

#### **Study on health systems responsiveness during mass gathering is going on in Kerala's Sabarimala temple**

The study undertook various epidemiological, environmental and socio-cultural issues into consideration. The study warranted that an effective mass gathering and safe pilgrimage (MGSP) policy requires an evidence base from a multidimensional platform, a committed and flexible monitoring mechanism, and a body that is ready to take suggestions. In many cases, the “we know it all” syndrome precludes constructive and critical examination of the faults and shortcomings that are pointed out. There is also a general apathy among the authorities towards creation of scientific evidence and an empirical base, which are required for the safe and satisfactory conduct of pilgrimage. Any efforts towards creation of such evidence are faced with immense road blocks.



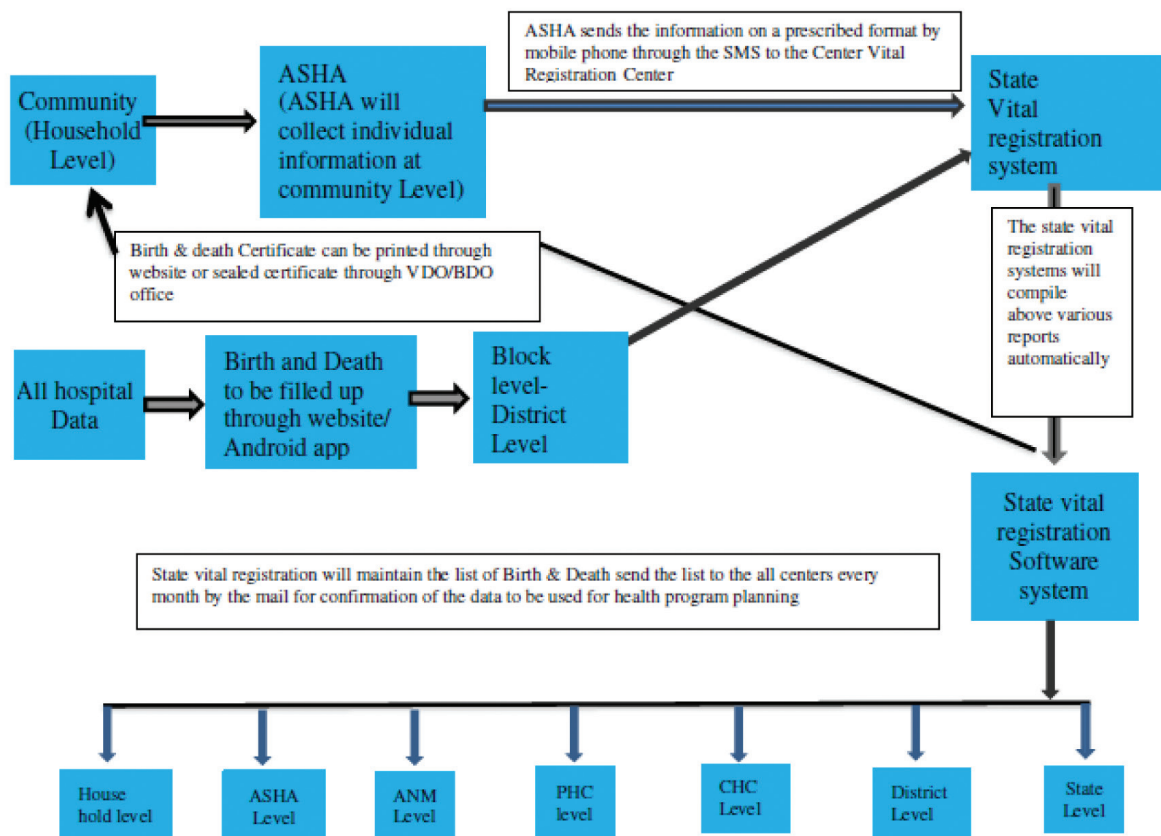


Fig. 7: Information Mechanism for Birth & Death Registration: Proposed at State

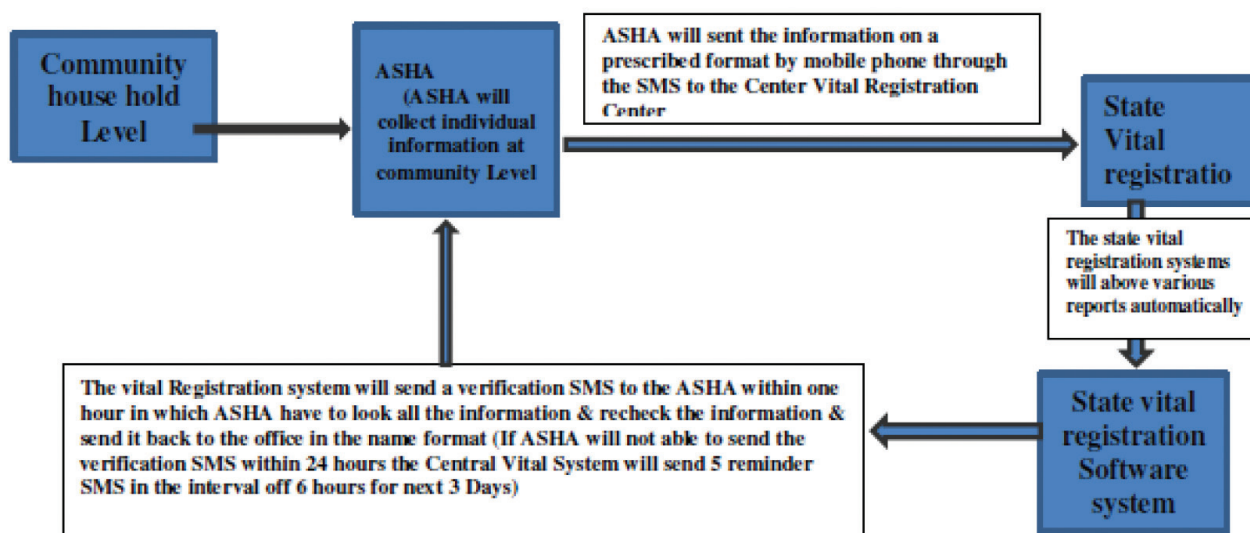


Fig. 8: Feedback Mechanisms of Birth & Death Registration.

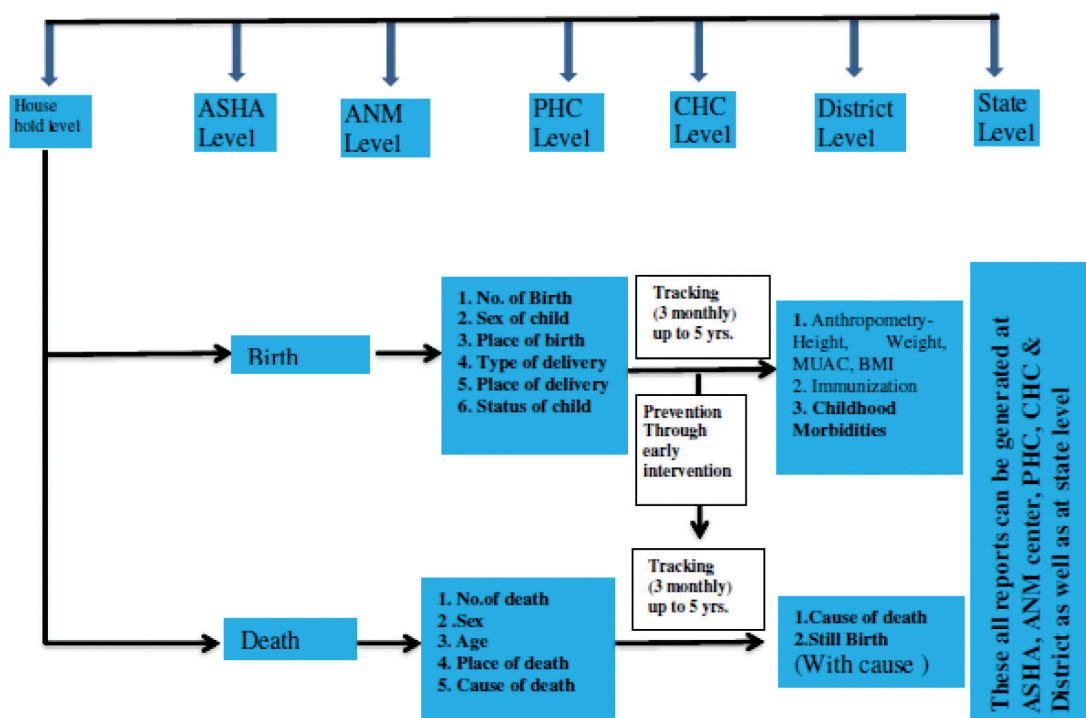


Fig. 9: Proposed model for tracking of child up to 5 years.

Pilgrim’s responses form an essential starting point for developing a MGSP policy. One issue with respect to Sabarimala pilgrimage is the multiple entry points which make it difficult to monitor and control pilgrim’s behaviour and practices while making it difficult to manage crowd entry to the main hill. The group pattern typical of Sabarimala pilgrimage creates its own problems such as their camping style, use of spaces especially for toilets and food consumption. Such groups, mostly from Tamil Nadu and Andhra regions, prefer to cook their own food. This also generates considerable waste and harms the ecosystem apart from threat of forest fires. With respect to Sabarimala, it is not possible to address infrastructural limitations as any further constructions could be deleterious to the fragile ecosystem. The environmental, public health and psychosocial factors could be handled by continuously monitoring and analyzing data on different parameters. The health profile of the pilgrims reported in this study from interviews as well as health institution data shows that cardiac care is the most important service required during the pilgrimage. Evidently, the most important issue is developing a simple cardiac kit which

could be handled by Para-professionals. There are one or two cardio-thoracic units along the trail of the pilgrimage which are not properly managed. Cardiac care needs to be improved at the converging point of the main queue before they ascend the 18 steps to the shrine. Paramedical help to be deployed here to handle breathlessness, and heart related problems. Apart from cardiac care, food hygiene needs to be strictly enforced to prevent major outbreaks of diarrhoeal diseases. The Pemba river system gets unusually polluted due to wastes generated by the eateries, shops, pilgrims’ rituals etc. The whole trail is polluted due to urine and faecal matter. Two documents spell out the Standard Operating Procedures in mass gatherings and these largely focus on crowd management and safety issues to be followed by the stakeholders (Government of India, 2014; Government of Kerala, 2015). Crowd management is certainly an issue at such gatherings but in case of Sabarimala and even other mass gatherings, a multi-sectoral approach is required. Data with respect to safety, health and environment need to be continuously assessed to understand changes and trends. Safety and security are certainly important issues which

were the focus of earlier reports. However, health, hygiene, ecology and environment, transport, pilgrim's behaviour patterns etc. are also important domains.

A national status for the pilgrim centre, especially for Sabarimala given the size and extent of the pilgrimage, can help in making the pilgrimage safe and healthy, not just from the pilgrim's point of view but equally from the point of view of ecology and sustainable development. If that happens, the state will be able to develop a sound mass gathering and safe pilgrimage policy, and this can serve as a model for other pilgrimages in India as well.

**A study has been initiated in Karnataka to assess the effectiveness of Panchayat Raj Institutions (PRIs) in managing the rural healthcare system, with special reference to the implementation of the National Health Mission (NHM) programmes in the selected districts of Karnataka and to find out effectiveness of administration of institutional quality health care delivery system.**

Based on the initial findings, the study concluded that health decentralization brings government closer to people, thereby allowing them to respond more effectively to the local health needs and preferences. In the health sector, decentralization has been explicitly conferred a crucial role in the chain of service delivery under the NHM. However, the triangular study on the position of decentralized governance in diarrhoeal the role of the grassroots health system is based on the opinion and insight of the health providers, Panchayats and the beneficiaries under NHM programmes. There are varying views on the central issue of commenting on the impact of decentralized governance on the variety of health issues; there is a definite acceptance of the change by all the three stakeholders. However, there are contrasts and disagreement in the views expressed by the independent stakeholders on the issue along with certain causal bearing with their individual attributes and behaviour. An analysis of information obtained from respondents reveals that stakeholders' perception regarding PRIs' role

in betterment health care provision is reasonably strong and there is a greater awareness regarding the change which has taken place in decentralized governance in relation to control and jurisdiction of health providers at the local level. There seems to be a greater degree of information regarding the restrictions of functioning of Panchayat, its autonomy, including roles and responsibilities and satisfaction in its service delivery.

**From Gujarat, a study to understand the perceptions of physicians, pharmacists and patients about prescription of generic drugs has been initiated**

It further evaluates the practice of prescription of generic drugs and identify the factors that prevents the prescription of generic drugs.

**A study to validate yoga programme for caregivers of schizophrenia is going on**

Support to develop self-help yoga manual with a CD was given to National Institute of Mental Health and Neurosciences (NIMHANS), Bengaluru. This study further tests the efficacy of this manual on burden, stress, quality of life and anxiety-depression of caregivers of persons with schizophrenia. Validation of the manual and pilot testing are completed during this year.

**A study from Rajasthan for assessing and managing mental health problems through frontline health workers has been initiated on pilot basis**

Improving the capacity of health workers with regard to knowledge and skills in assessing mental health is part of this study. An enabling environment for health seeking for mental health issues among rural set up will be created in this pilot project.

**A community based intervention for community engagement in control of some Vector Borne Diseases (VBDs) is going on in Alappuzha urban area, Kerala**

Formative research has been done, first phase, with the major objectives of understanding the



perception of the community and the ways by which the community engagement is done and examining the factors influencing the engagement with the social environment to prevent vector borne diseases. Along with, ecological, entomological, human socio-behavioural factors and the health system's mechanisms as well the responsiveness towards community engagement associated with prevention of vector borne diseases have been considered in study objectives.

After observing and discussing the issues with the community members and key informants, the study revealed the need of community based intervention programme by connecting the community resources. The perception of community members to engage in community based programmes are high in percentage which make the sense that it is a social need too. The first phase of the study (formative research) paved solid evidence for the need of the second phase (community based intervention). The formative research has exclusively covered public health aspect of the selected wards in connection to the VBDs. The formative research could bring out the status of existing health system under Alappuzha Municipality, its responsiveness to control VBD, characteristics of the community, perception of the community regarding the health system, community engagement etc. and the willingness of the community to involve in a community intervention programmes to tackle VBDs. On the basis of formative research, an intervention program is designed with a participatory approach by community mobilization. The basic concept of the intervention is to empower the community through an engagement programme and to make them aware of their strengths and weaknesses to tackle a public health issue like VBD. The plan is to divide the wards into clusters and to make 2-3 formal groups (community health team) of intervention participants in each cluster. Each group will be vested with the responsibility to carry out different programmes related to vector control. The support facilities will be provided and NIV research team will facilitate, monitor and evaluate their activities.

### **A study has been initiated in Jammu to monitor hand hygiene compliance of health-care workers working in government medical college hospital before, during and after the implementation of the multimodal hand hygiene improvement strategy**

It also evaluates the health-care worker's knowledge and perception of health care-associated infections and to perform gap-analysis. Actions were taken to ensure availability of alcohol-based hand rub at the point of care. Checklists and questionnaires were designed, tested and validated. Roster for direct observation was prepared after taking into consideration the working shifts of health-care workers on duty. The observers received one month training based on WHO Guide for the Implementation of the WHO Multimodal Hand Hygiene Improvement Strategy. Baseline assessment comprised of health-care workers' perception survey, a hand hygiene knowledge questionnaire and the observation of health-care workers' hand hygiene compliance. In addition, the impact of hand hygiene compliance on the infection rate in the ICU was assessed using surveillance cultures. The intervention, a combined multimodal hand hygiene improvement strategy was carried out, on the basis of the WHO guidelines for hand hygiene in healthcare, which includes training of healthcare workers by teaching sessions, implementation of preparations, and installation of reminder posters. Seven days workshop was conducted with lectures on scientific evidence of hand hygiene. Practical issues of the implementation of the multimodal hand hygiene improvement strategy were explained. The workshop addressed all the study participants. Four further half-day trainings were conducted to specifically address health-care workers who could not attend the workshop. Health-care workers who could not attend any training session, were handed a pocket bottle with alcohol-based hand rub after a short explanation of the concept "My five moments in hand hygiene". Posters emphasizing the importance of hand hygiene were placed at strategic sites within the ICU. Alcohol based solutions were placed prior to the workshop at the point of care.

**A study from Karnataka is to assess the impact of awareness programme on symptom management among advanced cancer patients and their caregivers**

This study is hospital based and patients diagnosed as having breast or head or neck cancer (stage 3 or 4th) who are 30 years of age & above and have undergone radiotherapy or chemotherapy or surgery or combination of them are first population of the study. The second population of the study is family caregivers, who are closely related to cancer patients (spouse, parents, children or siblings) aged above 20yrs and mostly involved in patient care at least 2-3 hours per day). The study is ongoing and intervention is to implemented.

**Another study from Karnataka is to help nursing students become aware of their perceptions on Reproductive and Sexual Health (RSH) issues and build the skills and abilities of adolescent or young adult nursing students to introspect and address personal and clientele RSH issues effectively**

It is anticipated that students will develop a neutral attitude and gain appropriate skills to deliver key and tailor-made information to their clientele on these issues. This study has been initiated recently and educational sessions are going on.

**In Uttrakhand, a study has been initiated to study the causes that lead to work related stress among the police officers and its impact on their health**

This study adopted mixed-methods approach and collects data related to stress, mental health and organizational support. The finding will have implications on strategies to reduce the stress and improve the health and well-being of the police officials.

Road Traffic Injuries (RTI) are the sixth leading cause of deaths in India and about 400 deaths take place every day due to road traffic accidents. Health Systems Research Division has initiated two Multi-Centric National Task Force Projects on RTIs. First study is to establish an electronic-based comprehensive and integrated RTI surveillance system. It will establish both passive and active surveillances comprehensively, to capture all RTI

and related deaths in a particular geographical area. This study is being implemented in five places in India, viz., Chennai, Chittoor, Dehradun, Delhi and Jaipur. The second issue is timely and quality care of RTI patients, as many deaths occur either at the scene or en route to the hospital. There is a clear survival and functional benefit for critically injured patients to receive appropriate care within the first 60 minutes of injury ('golden hour'). Hence, this multi-centric study has been initiated to standardize structured evidence based intervention for safety, efficacy and quality of post-crash pre-hospital and in-hospital trauma care services to improve the outcome in RTI victims. This study is being implemented in five cities, viz., Bengaluru, Delhi, Karamsad, Lucknow and Thrissur. An android-based trauma registry is being built and will be used to assess the impact of interventions. These studies will provide first comprehensive estimates on various epidemiological issues related to RTI. Also, an evidence of improvement through quality post-crash pre-hospital and in-hospital trauma care services will emerge.

## **INNOVATION AND TRANSLATIONAL RESEARCH**

### **Technologies transferred to Industry**

- Development of IgG assay for detection of anti CCHFV antibodies in Sheep and Goat – Technology transferred to M/s Cadila healthcare pvt. Ltd, Ahmedabad.
- Development of IgM assay for detection of anti KFDV antibodies - Technology transferred to M/s Cadila healthcare pvt. Ltd, Ahmedabad.
- Development of IgG assay for detection of anti CCHFV antibodies in Bovine - Technology transferred to M/s Cadila healthcare pvt. Ltd, Ahmedabad.
- Development of IgM assay for detection of anti CHPV antibodies - Technology transferred to M/s Cadila healthcare pvt. Ltd, Ahmedabad.
- Detection of Japanese encephalitis virus from mosquito using MAb based antigen capture ELISA. - Technology transferred to M/s Cadila healthcare pvt. Ltd, Ahmedabad.

- Recombinant protein based assay for diagnosis of Hepatitis E - Technology transferred to M/s Cadila healthcare pvt. Ltd, Ahmedabad.
- Development of Multiserotype OMV vaccine against Shigella - NICED signed an agreement with MSD Welcome Trust Hilleman Laboratories on 29<sup>th</sup> March 2017 to develop and commercialize a vaccine against Shigella.
- Nylon knitted seamless gloves for tobacco Harvesters - Bureau of Standard (BIS), New Delhi has finalized the 'National Standard' for these gloves and allotted an IS No. 16390:2015 and its Specifications have been gazetted for public use w.e.f 6<sup>th</sup> November, 2015. The technology was released on 29<sup>th</sup> January, 2016 by Shri Santosh Kumar Gangwar, Hon'ble Minister of State for Textiles (Independent Charge), Govt. of India in an event on 'Curtain Raiser of Technotex 2016' organized by the Ministry of Textiles, Govt. of India and FICCI.

**Patents filed during April, 2016- March, 2017**

Table 1: Filing of Indian patent application

S.no	Title of invention	Application no.	Filing Date
1.	A device for detecting cancerous lesions of uterine cervix.	201611014610	27.04.2016
2.	Nano-engineered biodegradable polymer-composite for bone-soft tissue fixation application.	201611012973	13.04.2016
3.	Alginate Chitosan nano formulations of Omp A – a Shigella subunit vaccine	201611015219	02.05.2016
4.	A novel fumigation methodology for antibacterial action	201611016409	11.05.2016
5.	Efficacy of 2-ethyl hexyl trichloacetate isolated from Acacia Arabica against multi drug resistant bacterial pathogens	201611016878	14.05.2016
6.	Formulation of Gluten free and Casein free cookies	201611020407	15.06.2016
7.	Rapid detection of drug resistant <i>Plasmodium falciparum</i> through a novel approach using loop mediated isothermal amplification	201711009381	17.03.2017
8.	A Bivalent outer membrane vesicles (BOMVs) based vaccine against typhoidal salmonellae	201711011707	31.03.2017

Table 2: Filing of PCT as national phase applications.

S. N.	TITLE	APPLICATION NO. & COUNTRY	Date of filing
1.	A test strip for holding reagents to determine blood glucose level.	US 15/318,772 UK 1619388.0 EP15759551.3	14.12.2016 16.11.2016
2.	Sensitization of thermo luminescent dosimeter CaSo4:Dy by co-doping with Mn in particular proportion for measurement of low radiation doses and the method of preparation of CaSo4:dy, Mn	US 15/115,430 EP 15711887.8 CN 201580009712.2	29.07.2016 ----- 22.08.2016

Table 3: Filing of Non PCT applications abroad

S.no	Invention	Patent Application no.	Date of filing
1.	A novel molecular diagnostic technique for detecting the different species of Plasmodium.	Myanmar 4/8587/2016	08.07.2016
2.	A novel molecular diagnostic technique for detecting the different species of Plasmodium.	Democratic Republic of Congo 373/2016	16.04.2016

Table 4: Patents granted during April, 2016- March, 2017.

S.no	Invention	Patent no.	Date of Grant
1.	A culture system for the growth of stem cells.	274456	26.07.2016
2.	Rare earth activated phosphor compounds and the process of preparation of phosphors thereof.	277495	23.11.2016
3.	A process for preparation of holey/lacey films.	279193	13.01.2017
4.	A pharmaceutical composition and a process for preparing the same thereof.	280638	23.02.2017
5.	Probes and primers for identification of mycobacterial protein useful as potential drug targets	280840	28.02.2017
<b>Foreign Patent</b>			
1.	A compound, transimycin, effective against bacterial and viral pathogens.	South Africa SA 2015/06655	09.01.2017
2.	RNAi agent for inhibition of chikungunya virus"	USA US 9574195	21.02.2017



### ICMR- IIT Kharagpur MedTech Internship Award

The ICMR - IIT Kharagpur MedTech Internship Award provides support for a supervised internship experience to individuals undertaking MBBS, clinical postgraduate degree courses (e.g., M.D., DNB), Ph.D. in clinical/life sciences subjects or working as Scientist B from ICMR institutes for formulation of innovative designs/prototypes to resolve a healthcare problem at IIT Kharagpur. Vice a versa, individuals undertaking BE/ BTech, engineering postgraduate degree courses (MTech), PhD (engineering) from IIT Kharagpur are provided support for a supervised internships at ICMR institutes.

The proposed internship intends to provide opportunities for creative expressions and foster collaborations from an early stage. This may pave way towards entrepreneurship in medical technology sector so as to solve the inherent problems of the health sector. In 2017 summer internship, 13 students were supported. Four students are being provided further support for developing prototypes over a duration of 9 months.

### IMPacting Research Innovation and Technology (IMPRINT)

This is a flagship national initiative of the Government, launched by the President, Prime Minister and Human Resource Minister on November 5, 2015. The program aims at addressing and providing solutions to the most relevant engineering challenges faced by the nation by translating knowledge into viable technology. Healthcare is one of the ten technology domains identified under IM PRINT. Twenty five projects with a budget of Rs 46 Crores have been sanctioned.

#### A platform for exhibiting medical technologies

This platform is for technologies developed by different government agencies (ICMR, DBT, DST, DRDO, AEC, Ministry of textiles), Institutes (Medical colleges/institutes, IITs, IISC, Universities) and Industry (Start Up Companies)

- i. At Rashtrapati Bhavan (March 2017) in collaboration with National Innovation Foundation and BIRC during 'Festival of Innovations': Round table meetings at Rashtrapati Bhavan for promoting innovations in medical research were also carried out.
- ii. Indo African Health Science Summit (Sep 2016): African countries showed an interest in displayed technologies. Two Laboratories in a briefcase/motorcycle given to African countries. Business talks initiated between few innovators.

### RESEARCH METHODOLOGY CELL (RMC)

Research Methodology Cell (RMC) has been established in ICMR to propagate the research at peripheral level in Govt. Medical Colleges. The objective of the Research Methodology Cell in the ICMR Headquarters is to train the young faculty, professionals and students of medical science, especially those belonging to state medical colleges and other academic institutions located in the periphery of different states through research methodology workshops for writing a good research proposal, which includes formulating valid hypothesis, drafting appropriate study design, collecting, documenting and analyzing data as well as communicating the research findings in a scientific journal.

#### Capacity Building workshops

Two Research Methodology Workshops were done in year 2016 at Indira Gandhi Government Medical College, Shimla, Himachal Pradesh and ICMR-NIN workshop for Telengana state under MERIT Scheme. In the IGMC workshop, approx. 35 faculty members from various disciplines had participated. The ICMR-NIN Workshop on Research Methodology for the faculty of the Medical Colleges of Telangana state under the MERIT scheme was conducted by the National Institute of Nutrition in association with the Directorate of Medical Education, Government

of Telangana State during 21 to 23" March, 2017. There were 52 participants, belonging to different medical institutions under Directorate of Medical Education, Government of Telangana and Research scholars from NIN.

The Workshop program is specifically designed for medical researchers in Telangana state to carry out their health research in India. This Workshop provides young researchers, a holistic view of the research methodology in the medical field. The major content of the workshop includes: Necessity of medical and health research in India; Research process and principles of epidemiology; Research Study designs and methods of Collection of research data; Sampling methods and sample size determination; Data analysis, statistical testing of hypothesis and use of statistical software in data analysis

### PROJECTS UNDERTAKEN

#### **A study of correlation between Clinical assessment and MRI findings in Temporomandibular disorders (TMDs) at B.J. Govt. Medical College & Sassoon General Hospital, Pune.**

The study aimed to correlate clinical findings of pain and sounds with MRI findings of changes in mandible structure in TMJ disorders. Among 20 subjects (10 male and 10 female), it was observed that more incidence of right ear pain was among 31-40 year age group. Other than this, right facial pain, right opening and closing, right joint sound were also studied and were predominant among females, homemaker as well as professional occupation group and in younger age group.

#### **Molecular Profiling Studies on CNS (Brain and Spinal) Tumors at Pt. B.D. Sharma PGIMER, Rohtak.**

Among 100 patients, 35 were having glial tumors of different grades. The glial tumor included astrocytoma, oligodendroglioma, ependymoma. In the non glial tumors category, meningioma cases were higher with 37 patients out of 100 showing clinical features. The 66% patients were having astrocytoma (Gr I & II), 23% glioblastoma (Gr IV). The percentage of other glial tumors e.g.

oligodendroglioma and ependydoma were 8 and 3 % respectively. Expression of several genes in different types of brain tumor samples through PCR and Microarray was undertaken. Gene polymorphism was studied using certain primers with the idea that polymorphic state of certain genes could serve as diagnostic and prognostic markers for certain tumor types. Preliminary microarray results show a substantial up regulation and down regulation amongst large number of genes regulating various cellular and molecular pathways.

#### **Project entitled "Epidermal Growth Factor Receptor (EGFR) Mutation Status in Non-small Cell Lung Cancer (NSCLC) patients in Jaipur", at SMS Medical College, Jaipur**

A total of 332 blocks were serially examined by naked eyes having unequivocal cytological diagnosis of NSCLC and complete patient history including age, sex, smoking habit, etc. To study the correlation of EGFR mutations with epidemiology, mutations were highest in age group 40-70 yrs (43/47; 91.49%) followed by age group >70 yrs (3/47; 6.38%) and least in age group < 40 yrs (1/47; 2.12%); higher in smokers (29/47; 61.70 %) in comparison to non-smokers (18/47; 38.29%); in adenocarcinoma patients (23/47; 14.74%) followed by squamous patients (13/47; 14.28%) and then (2/47; 28.57%).

#### **Acute Febrile Illness in Kerala - A Multicentre Study, at Govt. Medical College, Thrissur, Kerala**

During the study period, the commonly identified causes of acute febrile illness were Dengue and Leptospirosis. In 29.91% of patients, exact etiology couldn't be identified. Most common cause of mortality in AFI was Leptospirosis. Acute Kidney Injury, Hepatic dysfunction(OR 11.03;p 0.007) and ARDS were associated with mortality.

#### **Clinical, Biochemical and Cellular Correlates of Transcriptome of Adipose Tissue among Type-2 Diabetics, at SMS Medical College, Jaipur.**

Genome - wide gene expression profiling adipose tissue biopsies obtained from thigh (n= 15 male

and female diabetics and controls respectively, total 60) and abdomen (both subcutaneous and visceral adipose tissue (15 diabetic and control subjects, respectively, total 60 biopsies) was done using Affymatrix Primeview 16 arrays. The other parameters studied were: (1) Clinical: BMI, W: H ratio, (2) Biochemical: Glucose, lipid profile, HOMA-R, HOMA- $\beta$ , FFA, Leptin, adiponectin, TNF- $\alpha$  and IL-1, (3) cellular: adipocyte size, infiltrating macrophage. It was found that despite high insulin resistance, hyperglycemia and dyslipidemia, both male and female diabetics had comparable body weight than non-diabetics of both the genders respectively. However, diabetics in both the genders had higher BMI and waist circumference. Female diabetics had higher total body and regional body fat content, leptin level and adipocyte size than controls. Male on the other hand didn't have excess fat, instead had higher lean mass and almost double liver fat content. A large number of genes and metabolic pathways showed differential expression in thigh fat of both the genders. However, among diabetic and non-diabetic males on comparison of transcription profiles of subcutaneous vs. visceral fat, a large number of genes showed differential expression. It was interesting to note that on comparison of transcription profiles of diabetics vs. non-diabetics, the differentially expressed genes were similar for the subcutaneous and visceral adipose tissue.

#### **A multi-centric surveillance for prevalence of Carbapenemase producing gram negative bacteria with special reference to NDM1 in Maharashtra B J Govt Medical College, NCCS Pune.**

64.85% of carbapenem resistant gram negative bacteria were members of the family Enterobacteriaceae. Escherichia coli and klebsiella pneumonia were the major carbapenem resistant pathogens among Enterobacteriaceae isolates. 53.96% of Carbapenem resistant isolates showed presence of Metallo Beta lactamases. 17/202 (8.4%) isolates showed presence of NDM1 gene, 52% (13/25) of carbapenemase producers have NDM1 gene, 10/17 (58.8%) NDM1 positive isolates were from urban area while 7/17 I e (41%) were

from Miraj, a semi urban area and none from the rural centre Dhule. Highest NDM1 positivity was observed in Miraj (14%) followed by Pune (10.9%), and then in Mumbai (i.e.8%). Data published from Pune centre in the year 2010-11 showed that non-fermenters were Predominant NDM1 positive isolates while in the present study 50% of NDM1 positive isolates were found to be member of the family Enterobacteriaceae. This change in predominant pathogen over a period four indicates that there is need of continuous surveillance of (2010-2014) carbapenem resistant pathogen. In the present study, only presence of NDM1 gene was tested and not its variants like NDM2,3,..20. Hence to rule out possibility of presence of NDM1 variants, further study is required.

#### **DIVISION OF HUMAN RESOURCE PLANNING & DEVELOPMENT**

##### **Fellowships**

**JRF (Junior Research Fellowship)** - ICMR JRF Examination is the first step in the process of admission to the Ph.D./Research Programme through Council's support. The written examination for ICMR-JRF fellowship is conducted in collaboration with Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh at the 12 Centres viz. Bengaluru, Bhopal, Bhubaneswar, Chandigarh, Chennai, Delhi, Guwahati, Hyderabad, Kolkata, Mumbai, Srinagar (J&K) and Varanasi, once a year in 1<sup>st</sup> or 2<sup>nd</sup> week of July [usually Sunday]. Frequently Asked Questions (FAQs) for JRF fellowship were also added this year on the ICMR website. In 2016, a total of 6806 JRF applications were received and presently, 70 out of 150 JRFs who qualified the 2016 exams, have joined in different institutes. A total of 650 JRFs are on-going fellowship (2012-2016) at various national level institutions.

**ICMR Centenary PDF (Post-Doctoral Fellowship)**- ICMR Centenary PDF Scheme is being instituted to foster high quality research opportunities to promising fresh PhD/ MD/MS holders in the cutting edge areas of basic science, communicable and non communicable diseases, and reproductive health including nutrition at



ICMR Institutes/Centres. ICMR offers 50 such fellowships every year for working in 32 ICMR Institutes/Centres with state-of-art R&D facilities.

So far, 368 (2010- March 2017) applications were received by the ICMR, 339 short listed candidates were called for personal discussion and 147 were selected. Out of 147 PDFs, 106 have joined different ICMR institutes, 81 PDFs completed their studies and 25 PDFs are still undergoing their study for the year 2016-17. However, a total of 89 PDFs proposals were received by the Division during 2016-17. Of the 89 proposals, 85 were considered for personal discussion and 30 were approved for funding (joining awaited form 11 new approved PDFs) and 19 PDFs are ongoing (Figs. 10.1 & 10.2).

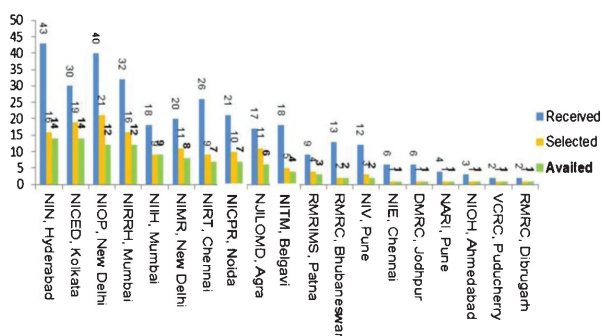


Fig. 10.1. Status of PDFs. Status of PDFs at ICMR's participating institutes 2010-2017 (n=339).

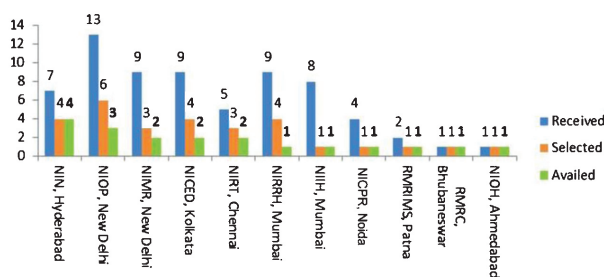


Fig. 10.2. Status of PDFs. Status of PDFs at ICMR's participating Institutes 2016-2017 (n=85).

**MD-MS/Ph.D. Fellowship-** The programme is designed to identify young medical graduates with brilliant academic records for pursuing postgraduate qualifications & to motivate them to opt for career in its research cadre. The selection of the candidates is made on the basis of a competitive

National level examination consisting of a written test conducted at 3 or 4 centres in the country. The candidate passing all MBBS examination in the first attempt with 60% or more aggregate marks will be eligible for the examination.

Under this programme, the selected medical graduates would be provided financial assistance for 4-5 years and a total of 25 fellowships are available per year. Programme is being carried out at three Centres viz; King George's Medical University (KGMU), Lucknow, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru and Sri Ramachandra University (SRMU), Chennai. During 2016-17, out of 15 allotted slots 12 were selected. So far, 105 candidates have joined the MD/PhD programme. Sixty four candidates have completed their studies in various areas.

**ICMR Visiting Fellow Scheme-** It is being instituted to foster high quality research opportunities to promising PhD/ MD/MS holders in the cutting edge areas of basic science, communicable and non communicable diseases, and reproductive health including nutrition at ICMR Institutes/Centres. Special focus is on fundamental research in areas of science and other priority areas identified by ICMR from time to time. ICMR intends to offer five (5) such fellowships. Selection of the ICMR – Visiting Fellows' is made through interview of short listed candidates. Two Fellows joined during reporting period.

**ICMR Chairs for Sr. retired medical/biomedical teachers/scientists**

i. **Dr. C.G. Pandit National Chairs-** These prestigious Chairs of ICMR have a provision of remuneration of Rs 1.00 lakh per month & contingency grant of Rs.7.50 lakh/year per Chair. The duration of Dr. C.G. Pandit National Chair is for five years (three years extendable by another two years after assessment of the progress and plans). Retired medically qualified persons are eligible for one Chair and retired non-medical/ bio-medical/ professors/ bio-medical teachers are eligible for the other Chair. The persons should preferably be the

Fellows of all the National Science Academies. At a given point of time, only two such Chairs will exist. At present Clinical Chair is vacant.

- ii. **Distinguished Scientist Chairs of ICMR-** Distinguished Scientist Chairs are open to retired scientists/ medical teachers who may belong to Medical/Bio-medical/Life Sciences with the excellent track record in the field of medical application. The duration of Chair is for five years (three years, extendable by another two years after assessment of the progress and plans). Usually two Chairs are established at one point of time.

**Adjunct Professors/Scientists of Universities/Colleges/Institutes-** This is a new Scheme of HRD, launched in 2016, which aims to enhance, strengthen and improve the quality of teaching, training and research in the ICMR network of institutes by utilizing the services of superannuated academics, reputed scientists including skilled professionals, both serving and retired. Ideally, they should be medical professionals, scientists, doctors and researchers in service or retired from national and international agencies / universities/ institutes.

**STS (Short Term Studentship)-** The ICMR initiated the Short Term Studentship Program in 1979 in order to promote interest and aptitude for research among medical undergraduates. This program is only for undergraduate MBBS students.

A total of 919 research reports were approved and awarded for the year 2016-17 (Fig. 11).

For STS-2016, a total number of 7425 undergraduate medical students got registered from all over the country. In addition, the student and the guide are now required to sign an Undertaking that there is no plagiarism in the proposal or the report submitted to ICMR. To increase human resource in medical research and inculcate research accumen in medical students, this year ICMR has selected applications from each participating States/UTs. The applications were received from 28 States/UTs, among which the top ten States are Karnataka followed by Maharashtra, Tamil Nadu, Puducherry, Andhra Pradesh, Telangana, Delhi, Gujarat, West Bengal and Haryana. There were 36 different subject categories in which top 15 subject areas were: Microbiology/Immunology, Physiology, Social & Preventive medicine, Biochemistry, Pharmacology, Oncology, Cytology & Pathology, Public Health & Epidemiology, Gynaecology & Obstetrics, Psychology, Mental Health, Pediatrics, Anatomy, Hematology, Surgery, Ophthalmology and Endocrinology. There is continuous increase in the number of STS applications from medical colleges. This included 341 medical colleges. On successful completion of the projects, a large number of students are also able to publish their papers in National/ Internal indexed journals (Fig. 12).

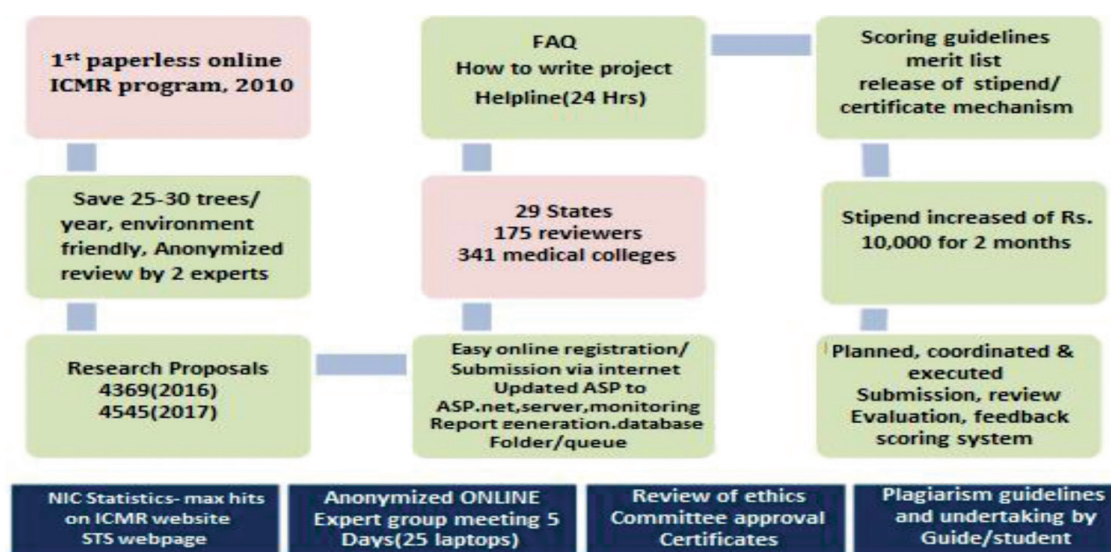


Fig. 11: Flow chart of details of STS activities pursued.

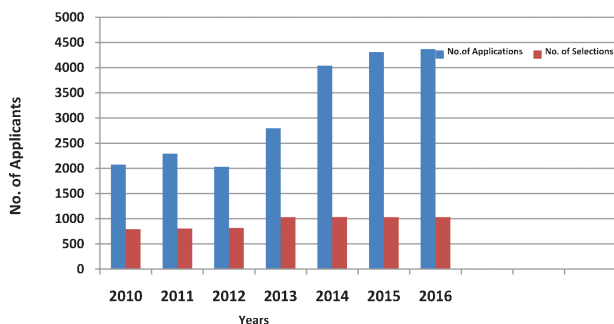


Fig. 12: STS Applications and selection data from 2010-2016.

**EMS (Emeritus Medical Scientists)-** Scientists, who have retired or are about to retire and who hold/have held before their retirement, the posts of the status of a Professor/Associate Professor in a medical college or of Director/Deputy Director, in a Institute of an all India character or scientists with comparable scientific experience and achievements in an organization of the Council and have been actively engaged in biomedical research of a high standard are eligible for appointment as Emeritus Medical Scientists. In the last five years (2012-2016), twenty EMS are working for ICMR in different areas of biomedical research.

**FINANCIAL SUPPORT**

**MD/MS/DM/MCh/MDS thesis support-** This scheme is primarily aimed at promoting good quality research in medical colleges through students pursuing post graduation courses as well as to improve visibility and accessibility of their research work to larger research audience. The Selection Committee recommended financial assistance to a total of 668 MD/MS/DM/MCh/MDS thesis out of 2944 proposals received so far. Out of 615-thesis protocols, 54 protocols/candidates were awarded financial assistance during the reporting period. Out of 54 awarded thesis protocols, 40 were medical disciplines and rest were dental sciences (Figs. 13.1 & 13.2).

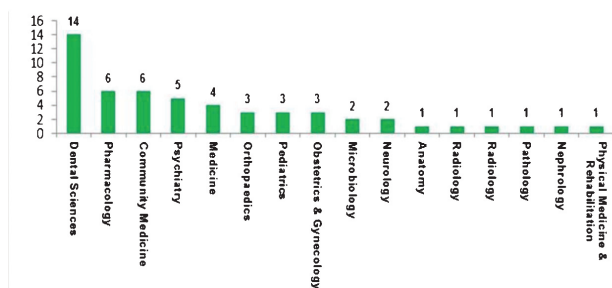


Fig. 13.1. Preferred Medical Research Areas (n=54) awarded MD/MS thesis 2016-17.

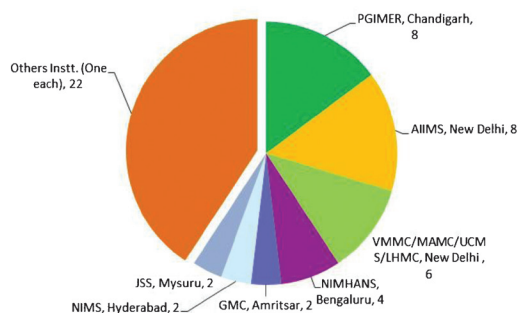


Fig. 13.2: Participating Institutes - 29 (n=54).

**International Travel Grant to non-ICMR scientists-**

The data collected from 2009-2016 was studied to identify the distribution of applications by country/state, area of biomedical science, designation; institution etc and inferences have been drawn from the study. In terms of the number of applications submitted, New Delhi led all other States with a maximum number of 2293. Uttar Pradesh ranked second with the 815 applications submitted followed by Karnataka (785), Maharashtra (711), Union Territory of Chandigarh (606), Tamil Nadu (480), Punjab (337), West Bengal (331), Gujarat (266), Andhra Pradesh (234), Kerala (139) & Rajasthan (117).

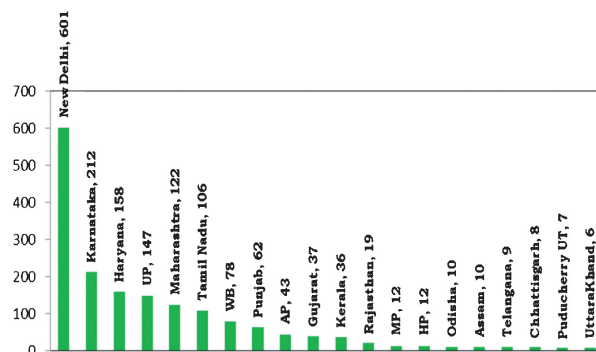


Fig. 14: Top 20 States data of scheme grants awarded. Top 20 States of scheme grants awarded.

In terms of the bio-medical science discipline, it was noted that the applications were received in wide range of areas such as Pharmaceutical Sciences-87, Cancer/Radiation-76, Neurosciences-68, Vision and Ophthalmology-51, Nuclear Medicine-36, Drug Discovery & Therapy-55, Paediatrics-37, Infectious & Emerging Infectious Diseases-30, Human Genetics-24, Psychiatry-28, Respiratory Diseases-26, Immunology-23, Environmental



Sciences and Engineering—16 and Others-1183. There were 300 disciplines/research areas under which scientists had submitted applications. Out of these, the top 24 research areas where highest number of applications were availed have been shown in Fig. 15.

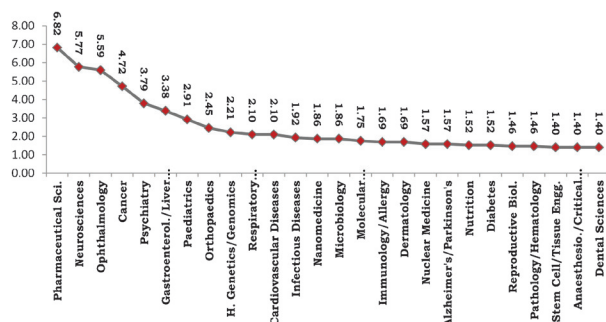


Fig. 15. Top 20 Research areas of scheme grants availed (%).

### Support for Seminars/Conferences/Workshops-

The sanction of grants by ICMR depends on the importance of the topic /subject of the Seminar/Symposium and its relevance to ICMR. Out of total 987 applications, 470 were approved.

### MISCELLANEOUS PROGRAMS

**ICMR Awards & Prizes-** ICMR offers an array of awards in biomedical sciences. In addition to the awards given for the meritorious work done by the scientists in a particular field of science, there are number of awards also given to recognize and adorn the scientific talent of the young scientists.

**Memorandum of Association between ICMR & Symbiosis International University (SIU), Pune-** MoU was signed between ICMR and SIU on 14<sup>th</sup> January 2017, (for five years *w.e.f.* January 23, 2017 to January 22, 2022) for working together to promote biomedical science using facilities, expertise, knowledge and resources at both the places by enrolling PhDs at 32 institutes of ICMR as well as Technical Divisions of ICMR Hqrs, as also to work jointly on common research grounds at both national and international level.

**ICMR Oration-** In its endeavor to promote excellence in health research, ICMR has instituted an annual oration. It acknowledges distinguished

and eminent scientists who have done exemplary work and contributed significantly to the understanding of disease prevention and treatment. For the Year 2017, this Oration was given by **Dr. Glenda Gray**, President of the South African Medical Research Council (SAMRC); she has expertise in mother to child transmission of HIV, HIV vaccines and Microbicide and **Dr. Salim Abdool Karim**, Director, Centre for the AIDS Program of Research in South Africa (CAPRISA), his major contributions to Microbicide for HIV prevention spans two decades and culminated in the CAPRISA 004 tenofovir gel trial which provided proof-of-concept that antiretroviral drugs can prevent sexually transmitted HIV infection and herpes simplex virus type 2 in women. The oration was held on March 27<sup>th</sup>, 2017 at the Jawaharlal Nehru Auditorium at AIIMS, New Delhi.

### INTERNATIONAL HEALTH DIVISION

The International Health Division (IHD) in ICMR co-ordinates international collaboration in biomedical research between India and other countries as well as with national & international agencies such as Ministry of Science & Technology, Indian and foreign missions and WHO etc. There are few specific agreements signed by the Ministry of Health and Family Welfare with other countries and rest are those signed directly by ICMR/DHR with international organizations/ institutions such as INSERM in France, German Federal Ministry of Education and Research (BMBF) and Helmholtz Association (HGF) in Germany; National Institutes of Health (NIH) in USA; University of Minnesota (UoM), USA; International AIDS Vaccine Initiative (IAVI), USA; Swedish Research Council for Health Working Life and Welfare (FORTE) in Sweden; Canadian Institutes of Health Research (CIHR) and International Consortium on Anti-Virals (ICAV) in Canada; National Health and Medical Research Council (NHMRC) and University of Sydney in Australia; London School of Hygiene and Tropical Medicine (LSHTM) and Medical Research Council (MRC) in UK; Foundation for Innovative New Diagnostics (FIND) and Drugs for Neglected Diseases Initiative (DNDi) in Switzerland;

Academy of Finland (AF) in Finland; Global Alliance for Chronic Diseases (GACD); Research Council of Norway (RCN) in Norway; Russian Foundation for Basic Research (RFBR) in Russia; National Institute of Infectious Diseases (NIID) in Japan; The Department of Health Research (DHR) has signed a Memorandum of Understanding with National Institute of Health & Care Excellence (NICE), UK.

### **PURPOSE OF INTERNATIONAL COOPERATION**

The purpose of these Memoranda of Understanding (MoU) and Joint Statements has been for exchange of scientific information; exchange of scientists/technicians; joint execution of scientific projects and organization of joint scientific meetings, seminars, workshops and symposia in identified areas of cooperation.

### **The following MoUs have been signed during this period**

1. A Letter of Intent (LoI) between ICMR and National Institute of Infectious Diseases (NIID) of Japan was signed on 16<sup>th</sup> April 2016 at Japan.
2. A Memorandum of Understanding (MoU) between Department of Health Research and The International Consortium on Anti-Virals (ICAV) of Canada was signed on 10<sup>th</sup> June, 2016 at New Delhi.

### **Exchange Visits**

The IHD supports and coordinates the international travel of Indian scientists engaged in approved bilateral collaborative research projects under various MoUs and Joint Statements with other countries. A total of 42 exchange visits of scientists / officials to and from India were arranged under various international collaborative programmes / projects.

### **Health Ministry's Screening Committee (HMSC)**

The research projects involving foreign assistance and/or collaboration in biomedical/health research

are submitted by the Indian investigators to ICMR for approval of Govt. of India through Health Ministry's Screening Committee (HMSC). The International Health Division of ICMR acts as the Secretariat for HMSC. The projects are peer reviewed by the concerned Technical Divisions at ICMR and then placed before the HMSC for consideration and decision. During the year 2016-17, 4 meetings of Health Ministry's Screening Committee were organized, wherein 149 projects were considered, out of which 128 projects were approved for international collaboration / assistance with agencies from USA, Germany, France, Canada, Australia, UK, WHO, European Union and several other foundations and foreign universities. Out of these, seven projects are co-funded by ICMR under ICMR-BMBF (Germany) and ICMR- RFBR (Russia) collaboration.

### **Online submission of International Collaborative projects at ICMR**

For facilitating online submission of International Collaborative projects for consideration of Health Ministry's Screening Committee (HMSC), a prototype for online submission of HMSC projects was prepared and the online submission of projects was opened with the support of ISRM division online team in March, 2017.

### **International Visitors / Dignitaries**

The Division also organized visits by various visitors to ICMR from foreign countries / agencies such as British High Commission, Australian High Commission, UK, USA, RCUK, HGF-Germany, Tokyo, Japan, French Embassy in India, BMBF-Germany, South Korea, Australia, Canada, France and Switzerland.

### **International Fellowship Programme**

The ICMR International Fellowship Programme for Indian biomedical scientists aims to augment capacity strengthening of institutions involved in basic, applied, epidemiological and clinical sciences through exposure of Indian researchers to the latest international advancements in knowledge, to understand the disease and find strategies for

their prevention and cure. The ICMR International Fellowships were awarded to six Senior and twelve Young Indian scientists during the year 2016-17.

### **Directorate General of Foreign Trade (DGFT) notification related to transfer of human biological material for commercial purposes**

ICMR formulated a 'draft notification' in consultation with the DGFT and DCGI to facilitate import/export of human biological samples for commercial purposes by the Indian Clinical Research Organizations (CRO'S)/Diagnostic Laboratories. After due approvals of the Hon'ble Minister of Health and Family Welfare and Hon'ble CIM, DGFT issued a notification dated 4<sup>th</sup> August, 2016 laying down Policy condition for Import/Export of human biological samples for commercial purposes: Amendment Schedule – 1 (Import Policy) and Schedule – 2 (Export Policy) of ITC (HS), 2012.

DGFT notification is available on ICMR and DGFT websites for reference of applicants.

### **India Africa Health Sciences Platform**

The landmark India Africa Forum Summit (IAFS) III in October 2015 under the aegis of the Hon'ble Prime Minister of India set the tone for reinvigorated and forward-looking partnerships between the two regions. Health was among the key areas identified for furthering collaboration, with India committing significant resources towards enhancement of health capacities in Africa through the proposed Africa Health Fund. To carry forward this vision of IAFS III in the area of public health, the Indian Council of Medical Research partnered with five Indian ministries and several African regional scientific and research agencies to organize the first India Africa Health Sciences Meet (IAHSM) in September 2016. The Meet saw participation from 11 cabinet ministers and 400 delegates including senior government representatives, technocrats, industry leaders and scientists. The deliberations highlighted the need for India and Africa to conduct

joint biomedical and health research to address diseases of common concern through indigenous development of affordable drugs, diagnostics and vaccines, and also enabling knowledge sharing and capacity strengthening. Discussions also focused on boosting pharmaceutical trade and manufacturing cooperation to foster affordability of essential drugs and harmonization of regulatory & intellectual property policies.

To formalize this partnership as the India Africa Health Sciences Collaboration, a Memorandum of Understanding (MoU) has been developed by ICMR (through the Department of Health Research) and the African Union (AU).



**Fig.16:** Letter of Intent between ICMR and National Institute of Infectious Diseases (NIID), Japan, signed by the Ambassador of India to Japan and DG, NIID on 16<sup>th</sup> April 2016 at Tokyo during the visit of Hon'ble HFM to Japan.



**Fig.17:** MoU between Department of Health Research, Ministry of Health & Family Welfare, Govt. of India and The International Consortium on Anti-Virals (ICAV), Canada signed at Canada House, New Delhi on 10<sup>th</sup> June, 2016.





**Fig.18:** Some glimpses of the Indo Africa Health Sciences Meet organized during 1\_3 September, 2016 at Vigyan Bhawan, New Delhi.

### INFORMATICS, SYSTEMS AND RESEARCH MANAGEMENT (ISRM) DIVISION

Established in 2017, ISRM is the youngest Division of ICMR. The division has a mandate to nucleate and support informatics in medical research through focused programs and services and to develop research management on International pattern. During 2016-17, the Division has provided wide

range of services to the scientific fraternity as well to the administration and finance. The activities of the ISRM Division during 2016-17 can be grouped into three categories.

### RESEARCH MANAGEMENT

#### ICMR electronic Project Proposal Management System (e-PPMS)

ICMR has shifted from paper based receipt and processing of extramural adhoc projects, to interactive web-based system since January, 2012. The system encompasses the complete life-cycle of a proposal funding including online submission, evaluation, processing and closing. During the year, the division introduced modules for complete paper-less review of submitted concept notes. The system processed 708 concept proposals. These proposals were completely reviewed online by 752 registered experts through the system and of these, 177 concept proposals were shortlisted for submission of detailed proposals.

To create awareness and enhance in-house acceptance of the developed system, the division organized a 6 days training session / orientation program cum hands on workshop from 1st – 5th and 8th Aug. 2016. A total of 140 participants attended the 6 days training sessions, comprising of 12 sessions, including all Division Heads, Program Officers of all Divisions, AOs / SOs / Dealing assistants of all Divisions and all Finance officials with Sr. FA.



**Fig.19.** Training session of online research management system.

In addition to adhoc projects during current year, the Division developed modules for online submission of proposals against specific 'Call for proposals' programs. The module has been used for a). Call for Proposals for Participation in the Health Systems Research on Road Traffic Injuries(91 proposals), b). Call for proposals in the field of Ageing and Health (58 proposals), c). Call for Proposals on Indo-Norwegian Co-operation on Antimicrobial resistance (21 proposals).



**Fig. 20:** Home page of ePPMS proposal submission portal.

The division also initiated development of module for online submission and processing of Senior Research Fellowships/Research Associate ship. The Division also facilitated development and launching of module for online submission and processing of proposals for Health Ministry's Screening Committee (HMSC).

### Task-Force on Biomedical Informatics

The Division coordinated second Phase of Task-Force on Biomedical Informatics. The task-force has a mandate to support informatics tools and techniques in medical research. Under the task-force, the ICMR established 20 Biomedical Informatics Centres at premier medical research institutes across India including RIMS, Ranchi; Pt. JLNMC, Raipur; SKIMS, Srinagar; RMRC, Port Blair. The Centres are helping medical researchers to capture biomedical data, assisting medical researchers in developing proposals involving modern technologies (Genomics, Proteomics,

etc.), providing data analysis services to medical researchers and initiating collaborative projects. The focus of these Centres is to initiate Association Studies, development of solutions for controlling infectious diseases through latest techniques such as RNA-seq, NGS etc.

During 2016-17, the Centres worked on 32 collaborative projects on various communicable and non-communicable diseases including Cancer, Tuberculosis, HIV, Sickle Cell Anemia, Malaria, Typhoid, Diabetes and rare diseases such as Progressive Familial Interhepatic Cholestasis, Corneal Dystrophy, Hypertrophic Cardiomyopathy. Approximately 17 projects have potential for translation research (Drug Development and Diagnostic/Prognostic Biomarkers). The Centres have published 21 research papers and conducted 7 training programs for the benefit of medical professionals.

### Extramural Research projects and fellowships in the area of Bioinformatics and Medical Informatics

During 2016-17, ISRM processed 21 adhoc projects and 41 SRF and RA fellowships. Majority of the projects are in the area of structural biology, however, recently the Division has taken steps to strengthen medical informatics and other areas of Bioinformatics.

### SERVICES TO ICMR

#### ICMR website and Social Media

The division is maintaining the ICMR website at <http://icmr.nic.in>. The site hosts information about various programs/activities, notifications and information different stakeholders. This year number of visitors per day to the site has increased from approx. 31K to 38K. The division has initiated a project for revamping the website with latest technology to enhance user-friendliness, enrich content dissemination, mobile application and security. The existing website was reorganized and several new features were added to it like audio-video section, publication section, ICMR News



clipping section, expedition in day to day event-reporting from HQ & other ICMR institutes, new guidelines' tabs, etc.

- The Division continued providing the Internet, intranet and videoconferencing facilities to ICMR Headquarters and institutes. During the year, the Division facilitated signing of MoU with the project ECHO wherein ICMR has been provided with software based Video Conferencing Licenses by ZOOM. The facility has been used extensively by ICMR for dissemination of educational and scientific session to wider-audience.
- The Division worked as a Nodal Point for all ICMR institutes and the Headquarters for e-Publishing of tenders on Central Public Procurement Portal of Government of India.
- The Division continues to provide programming services to different Technical Divisions. The division has developed an e-Recruitment portal for online submission and processing of applications for various posts/vacancies at ICMR. The portal has been used for online processing of 25 scientific and technical posts.
- The Division is managing the social networking interface of ICMR. The Division worked tirelessly to increase the virality and outreach of ICMR social media through Facebook & Twitter and successfully achieved it. Also, division initiated the process of ICMR Website revamping, Mobile Application development and Social Media management including Youtube/Instagram/Linkedin.
- ISRM division maintains the DBT portal of GOI for which all transaction data from other divisions and Account section is collected and then entered on DBT portal in first week of every month. Direct Benefit Transfer (DBT) is a scheme of Govt. of India to ensure that benefits go directly to individuals' bank accounts electronically, reducing delay in payment, ensuring accurate targeting of

the beneficiary and curbing pilferage and duplication. There is a total of 6 Schemes of ICMR registered under this mission.

- ISRM division is involved in co-ordination, implementation, internal committee meetings and training sessions of PFMS scheme. Public Financial Management System (PFMS) is a scheme of Ministry of Finance, Govt. of India for releasing the grants through the PFMS software and making it mandatory to record all Transfers, Advances and Expenditures in PFMS software.
- ISRM Division is a part of Communication cell of ICMR that has been mandated to disseminate the goals, guidelines, events and research achievements of ICMR.
- ICMR English bulletin e version development was initiated and quarterly issues for the year 2017 were planned.
- Annual report compilation, editing, publishing, timely laying in parliament and distribution was successfully achieved by the Division.

## RESEARCH ACTIVITIES

### ICMR Computational Genomics Centre

The Division got approval for establishing ICMR Computational Genomics Centre, which will be a centralized facility, intended to provide infrastructure and expertise in using Genomics tools and techniques for medical research. Having a centralized infrastructure, has advantages to ICMR, as it will reduce redundancy of hardware, software and the expertise and allow ICMR to develop skills and expertise to an extent that is difficult to achieve in individual laboratories/institutes.

The intended users of ICGC will be research groups from ICMR laboratories. The Centre will also provide support and services to medical researchers from Universities, Hospitals and Medical Colleges where infrastructure, expertise or awareness restricts the application of high-throughput Genomics in medical research for high impact publications and translation into products.



### Collaborative Projects

The Division has core strength in Bioinformatics and Data Management. The Division is assisting other divisions of ICMR in developing web-enabled data collection, management and analysis portals. The Division has successfully developed and launched portal for collection, management and analysis of data on antimicrobial resistance. The data is being submitted by four tertiary care hospitals across India. Presently, the system has data for nearly 40,000 clinical samples with an increase rate of approx. 3000 – 4000 samples every month. The Division has also developed

websites for Indian TB Research Consortium (ITRC), India Battles Cancer – providing India-centric patient centric information and contributed significantly to ‘FCTC Global Knowledge Hub on Smokeless tobacco.’ The Division established a ‘Data Management Laboratory’ at NICPR, Noida. The mandate of lab is to bring standardization and uniformity in developing web-enabled data collection, management and analysis portals for various ICMR programs. The Division is also contributing scientifically to the Task-Force projects initiated by other Divisions of ICMR. Prominent projects include ‘Prevalence and Etiology of Hearing Impairment’ and ‘e-Partograph – a tool for labor-pain decision support’.

# PUBLICATION AND INFORMATION

During the year under report, the Division of Publication & Information continued its routine activities to disseminate the information among not only medical scientists and students but common man also. The Division continued to bring out its periodicals like Indian Journal of Medical Research, *ICMR Patrika*, Annual Report of ICMR *Varshik Prativedan* in Hindi. Dissemination activities were also continued by organizing a number of exhibitions at various places in the country displaying the activities and achievements of ICMR.

## PERIODICAL PUBLICATIONS

### Indian Journal of Medical Research

The Indian Journal of Medical Research (IJMR) had maintained its regular publication of high quality Original research articles, Narrative review articles, Systematic reviews/Meta analysis, Commentaries, Editorials, Research correspondence, Perspective/View-points, Student IJMR, Clinical images, etc. Special reports, Status reports and Policy guidelines were also published occasionally. Special issues and Supplements were also published in addition to regular issues. The IJMR is available full text free on the website [www.ijmr.org.in](http://www.ijmr.org.in) and IJMR Archive is available on <http://ijmr.in>. The impact factor of IJMR had increased to 1.532 in 2016 from 1.446 in 2015.

IJMR is brought out in two volumes and 12 issues each year and continues to be covered by all major abstracting and indexing services available world-wide. Since January 2017, IJMR has also

been made available live on IJMR App both on Android and iPad devices. This user-friendly app has made IJMR readily available, thus helping in dissemination of information. Production, printing and subscription of IJMR have been outsourced to MedKnow/Wolters Kluwer India Pvt Ltd. from August, 2016.

During the year 2016-2017, a total of 280 articles were published under various categories/sections; 53.6 percent were original research articles, followed by 11 percent review articles. Correspondences accounted for 9.6 percent and Clinical Images 7.9 percent (Fig.1). Eighteen percent articles were contributed by authors from countries other than India (Fig.2). Countries such as France, UK, South Korea, Spain, Afghanistan, Pakistan, PR China, Iran, Turkey, USA, Poland, Brazil were major contributors (Figs.3,4). The IJMR has stringent policies for checking plagiarism, conflicts of interest and ethical issues related to biomedical publication.

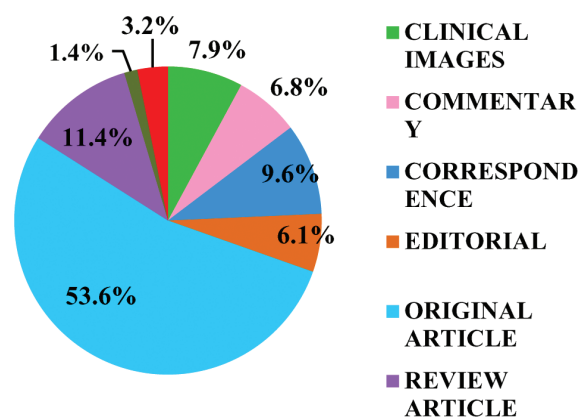


Fig. 1. Articles published in IJMR under different categories (2016-2017).

The process of peer review, as always, had been rigorous and double blind and the quality being maintained by involving reviewers from both India and abroad. Overall, during the period under report, a total of 909 reviewers had peer reviewed for the IJMR and of these, 18 percent were from countries other than India (USA, UK, Brazil, Turkey, Germany, Iran, Bangladesh, France, South Africa, etc.) (Fig.2).

During 2016-2017, a total of 2108 articles were submitted to the IJMR for consideration for publication. Of these, 42% were contributed from countries other than India such as Turkey, Iran, Iraq, PR China, Taiwan, Poland, Pakistan, Brazil, UK, Germany, Mexico, Croatia, etc. (Fig 2).

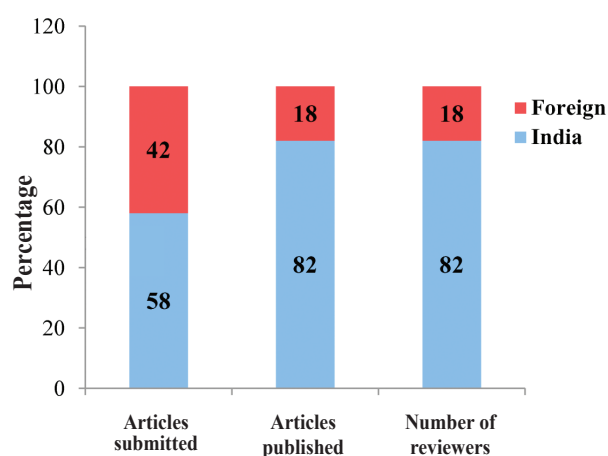


Fig. 2. Indian and Foreign contribution in terms of number of articles submitted, published and number of reviewers participated in peer review process during 2016-2017.

A new cover page of IJMR was launched in July 2016 (Fig 3). A supplementary issue on “Biomedical Research: Oncology” was brought out in May, 2016 (Fig. 3) that included 17 original articles and one systematic review article.



Fig. 3. IJMR – regular and supplementary issues.

## HINDI UNIT

The work done by the Hindi Unit of the Publication and Information Division during 2016-17 is as follows:

### ICMR PATRIKA

During the period, the publication of *ICMR Patrika* continued to be brought out. The articles on Tuberculosis, Thalassemia, Japanese Encephalitis, Diabetes, Sickle Cell Disease, Viral Hepatitis, Vehicular Pollution, Malnutrition, etc. were published. These included: *Thalassemia : Ek Anuvanshik Rog*, (April, 2016); *Japanese Encephalitis : Ek Gambhir Swasthy Samasya* (May, 2016); *Vatavarniya Pradushan evam Prajanan Swasthya* (June, 2016); *Madhumeh – Ek Mahamari aur Arthik Bhar* (July 2016); *Khatarnak Vyavsay aur Bal Mazdoor* (Aug., 2016); *Bharat mein Shigellarugna ki Sthiti* (Sept., 2016); *Bharat mein Janjatiya Abadiyon mein Sickle Cell Rog* (Oct., 2016); *Bharat mein Vishanuj Yakritshoth ka Samna* (Nov., 2016); *Bharat mein Alp Poshan aur Kshayarog ki Sthiti* (Dec., 2016); *Vahnon se Utpanna Pradushan : Ek Jwalant Swasthya Samasya* (Jan. & Feb, 2017); *Ab Samay hai Kshayarog Samapt Karne Ka* ( March, 2017).

### Varshik Pratedan 2015-16

The Hindi version of ICMR Annual Report 2015-2016 as *Varshik Pratedan 2015-16* was brought out.

### NIN Book in Hindi : *Bhartiya Khadyon ke Poshak Maan*

The Hindi Unit brought out the Hindi version of NIN book ‘Nutritive Value of Indian Foods’ as *Bhartiya Khadyon ke Poshak Maan*. (Jan, 2017).

### Awards for Popular Medical Books in Hindi for the Biennium 2014-15

A total of 9 books were received. During the screening Committee meeting held on 6<sup>th</sup> March, 2017, the following popular books were selected for the awards:



- *Genome evam Genomiki* by Dr Dinesh Mani : First Prize ( Rs 100000); and
- *Pradushan Janit Rog* by Dr Sunanda Das : Second Prize (Rs 50,000)

## **LIBRARY & INFORMATION SERVICES**

The Division continued to provide the library services to ICMR Hqrs and its institutes. The subscription to core bio medical e-journals like Lancet, Science, Nature, and NEJM for all ICMR Library & Information Centres has been continued under ICMR e consortia. Subscription for full text electronic data base, ProQuest Health & Medical Complete (which covers more than 2,107 full text and archive volumes for most of the titles are available from 1998 onwards) has been renewed for one more year for six ICMR institutes including ICMR Hqrs. ICMR has joined as a member of ERMED Consortia, an initiative taken by DGHS and MOHFW to develop nationwide electronic information resources in the field of medicine for delivering effective health care. The consortium will be coordinated through it's headquarter set up at the NML. It provides 244+ medical online e-journals access from five leading Publishers.

The subscription to J-Gate Plus has also been renewed for one year. Subscription to J-Gateplus is continued on the basis of satisfactory usage report. J-Gate provides access to millions of journal articles available online offered by 12,288 Publishers. It presently has a massive database of journal literature, indexed from 45,047 e-journals with links to full text at publisher sites. The J-Gate @ ICMR is a customized solution for the ICMR Consortium to enable 30 members across India, to access consortia subscribed journals, individual library subscribed journals and full text journals available at J-Gate, through a single search discovery platform and a DDR (Document Delivery Request) functionality, for resource sharing among the consortia members. This service is being provided by Informatics India agency. It can be accessed by all ICMR libraries & Information Centres.

## **Training Programmes**

1. Two days Training workshop for Heads of ICMR Libraries and senior library professionals was organized at NIV, Pune during 11<sup>th</sup> and 12<sup>th</sup> August, 2016.
2. Two days Advanced Training workshop for Heads of ICMR libraries and senior library professionals was organized at NIV Pune during 24<sup>th</sup> and 25<sup>th</sup> November, 2016.

## **Dissemination of Biomedical Information**

ICMR carried out large number of education and extension activities during the year in different parts of India, to disseminate the activities and achievements of ICMR at various platforms as well as to enhance the outreach of ICMR. A brief description on the same is as follows

### **ICMR Participation in Science Expo during Destination Uttarakhand Mega Fair, 24-26th Oct. 2016 at Dehradun**

ICMR participated at Science Expo in Destination Uttarakhand, Mega Fair, 24-26<sup>th</sup> October, 2016 at Dehradun. Spot nutrition quiz was conducted and prizes were given away to boys and girls.

### **Pride of India Mega Science Expo during 104th session of the Indian Science Congress held at SV University, Tirupati from 3-7th January, 2017**

ICMR participated in Pride of India Mega Science Expo during 104<sup>th</sup> session of the Indian Science Congress held at SV University, Tirupati, from 3-7<sup>th</sup> January, 2017 and showcased its activities and achievements through 135 attractive and vibrant posters on the major research activities and achievements of public health importance, which included the research highlights of various ICMR Institutes and Head quarters. These posters include the posters on Nutrition, Environmental and Occupational health, malaria, leprosy, tuberculosis, cancer, cholera and enteric diseases, HIV/AIDS and regional health problems. New posters on outbreaks/ epidemics, ICMR Research output, Antimicrobial Resistance, Rotavirus network, Schemes of DHR,

Vision 2030 etc were also prepared and displayed. There were also posters on different indigenous and affordable technologies developed by the ICMR Institutes, ICMR Institutional network, policies and programmes, guidelines/bills, human resource development, Infrastructure Development as well as posters on maternal and child health etc. Prototypes of few of the technologies developed by ICMR that include the JE Vaccine, Magnivisualizer, kit for beta thalassemia, non-invasive procedures for kalazar detection, ferritin estimation, sperm detection kit *etc* were also displayed. Video films on the activities and achievements of the ICMR and its institutes were also shown throughout the period in Hindi as well as in English.



**Fig. 4:** Pride of India Mega Science Expo during 104<sup>th</sup> session of the Indian Science Congress held at SV University, Tirupati, from 3-7<sup>th</sup> January, 2017.

### Participation in World Book Fair

ICMR participated in World Book Fair held in New Delhi during 7-15<sup>th</sup> January, 2017 and put up the display and sale of ICMR publications.

### ICMR Participation in Festival of Innovations

Indian Council of Medical Research (ICMR) organized a one day Exhibition during Festival of Innovations on the lawns of Rashtrapati Bhavan, New Delhi, during 4-10<sup>th</sup> March 2017. The theme of the exhibition was “Innovations in Medical Science and Biotechnology”. ICMR also organized a ‘Round Table Discussion on Technologies’ Chaired by DG, ICMR. A booklet on “The Innovative Medical Technologies, 2017” was also brought out.



**Fig. 5:** ICMR Participation in Festival of Innovations Rashtrapati Bhavan, New Delhi, during 4-10<sup>th</sup> March 2017.

### MEDICINAL PLANTS DIVISION

**During the year, volume 16 (with botanical names Mi-My), covering monographs on about 175 medicinal plants species carrying multidisciplinary**

Information with 1900 citations were published. This volume includes regional names of the medicinal plants, their sanskrit synonyms as well as the Ayurvedic description (wherever available), ethnobotanical studies, the habitat and the parts used, properties and uses on one hand, and the details of botanical, pharmacognostical, chemical, pharmacological and clinical data on the other, backed by complete references and bibliography on each aspect of the information cited, besides the colour photographs of important medicinal plants.



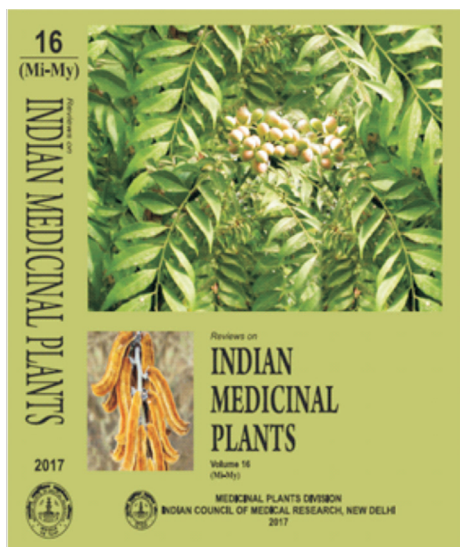


Fig. 6: Indian Medicinal Plant, Volume 16.

### QUALITY STANDARDS OF INDIAN MEDICINAL PLANTS

During the year, the Quality standards on 35 medicinal plants were developed, monographs prepared, finalized, technically reviewed and published as Vol. 14, as part of series on “Quality Standards on Indian Medicinal Plants”.

The monographs are on the pattern of WHO guidelines and focus on the diagnostic features and phytochemical studies, including markers, besides having information on Pharmacological, clinical, toxicological aspects along with dosage, adulterants, substitutes etc.

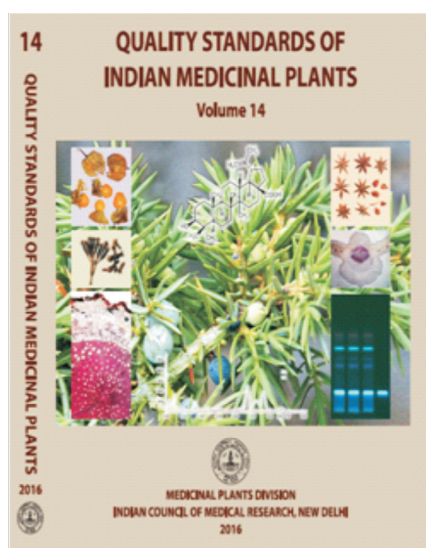


Fig. 7: Quality Standards of Indian Medicinal Plants, Volume 14.

### Generation of Phytochemical reference standards and Development of Repository of Reference Phytoconstituents of Important Indian Medicinal Plants

Phytochemical reference standards (PRS) are ideally those compounds in the plant which are therapeutically active. However, in many cases the therapeutic activity of the plant is attributed to a number of phytoconstituents present in the plants. Under such circumstances, any compound that is unique to the plant or the major compound or the major chemical constituent can be regarded as the PRS. The fourth volume of Phytochemical Reference Standards of Selected Indian Medicinal Plants containing monographs of 30 PRS was published.

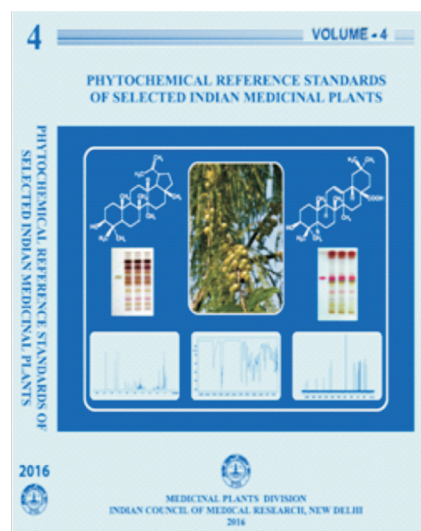


Fig. 8: Phytochemical Reference Standards of Selected Indian Medicinal Plants containing monographs of 30 PRS was published, 4<sup>th</sup> volume.

### Development of a website exclusively dealing with Medicinal Plants

A website has been developed, exclusively showcasing unit’s activities and has been hyperlinked with the ICMR main website.

### TASK FORCE PROJECTS

1. Development of a standardized formulation for preventing or delaying the development of Type-2 Diabetes in subjects with Pre-Diabetes.



2. Development of a formulation for treatment of sleep disorders.

Two important MoUs between ICMR and healthcare division of Emami Limited, Kolkata, were signed for the development of phytopharmaceutical product for the prevention and delay of Type-2 Diabetes in subjects with Pre-Diabetes and another aimed to develop herbal product for the treatment of sleep disorder. The ICMR will mainly support and manage the pre-clinical, toxicological and clinical development phase of the product development programs; whereas Industry will look after and undertake the Chemistry, Manufacturing and Control (CMC) part of the product development phase. The ownership of developed products will be solely of ICMR; whereas the Industry will have the first right of refusal on these developed products. The Secretary DHR and DG ICMR, Dr Soumya Swaminathan and Dr C.K. Katiyar, CEO Emami, signed these MoUs.



**Fig. 9:** Important MoUs between ICMR and healthcare division of Emami Limited, Kolkata, were signed for the development of phytopharmaceutical product for the prevention and delay of Type-2 Diabetes.

# ICMR INSTITUTES/CENTRES

1. National JALMA Institute for Leprosy and Other Mycobacterial Diseases  
P.O. Box No.101, Dr. M. Miyazaki Marg,  
Tajganj  
Agra-282001
2. National Institute of Occupational Health  
Meghani Nagar, Near Raksha Shakti University,  
Ahmedabad-380016
3. National Institute of Epidemiology  
R-127, 3rd Avenue  
Tamil Nadu Housing Board  
Ayapakkam, Near Ambattur  
Chennai-600077
4. National Institute for Research in Tuberculosis  
No. 1, Mayor Sathiyamoorthy Road,  
Chetput  
Chennai-600031
5. National Institute of Malaria Research  
Sector 8, Dwarka  
New Delhi-110077
6. National Institute of Nutrition  
Jamai Osmania, Tarnaka  
Hyderabad-500007
7. Food and Drug Toxicology Research Centre  
National Institute of Nutrition  
Jamai-Osmania  
Hyderabad-500007
8. National Centre for Laboratory Animal Science  
National Institute of Nutrition  
Jamai Osmania  
Hyderabad-500007

9. National Institute of Cholera and Enteric Diseases  
P-33, CIT Road, Scheme XM  
Beleghata  
Kolkata-700010
10. Centre for Research in Medical Entomology  
4, Sarojini Street  
Chinna Chokkikulam  
Madurai-625002
11. Enterovirus Research Centre  
Haffkine Institute Campus  
Acharya Donde Marg  
Parel  
Mumbai-400012
12. Genetic Research Centre  
National Institute for  
Research in Reproductive Health  
Jehangir Merwanji Street  
Parel  
Mumbai-400012
13. National Institute for Research in  
Reproductive Health  
Jehangir Merwanji Street  
Parel  
Mumbai-400012
14. National Institute of Immunohaematology  
13th Floor, New Multistoreyed Building  
K.E.M. Hospital Campus  
Parel  
Mumbai-400012
15. National Institute of Medical Statistics  
ICMR Head Quarters Campus  
Ansari Nagar  
New Delhi-110029
16. National Institute of Cancer Prevention and Research  
I-7, Sector-39, P.O.Box.No.544  
Near Government Degree College  
Opposite City Centre  
NOIDA-201301



17. National Institute of Pathology  
Safdarjang Hospital Campus  
P.O. Box No. 4909  
New Delhi-110029
18. Rajendra Memorial Research  
Institute of Medical Sciences  
Agam kuan  
Patna-800007
19. Vector Control Research Centre  
Medical Complex  
Indira Nagar  
Puducherry-605006
20. Microbial Containment Complex  
MCC 130/1, Sus Road  
Pashan  
Pune-411021
21. National AIDS Research Institute  
73, 'G'-Block  
MICD Complex, Bhosari  
Pune-411026
22. National Institute of Virology  
20-A, Dr.Ambedkar Road  
P.O. Box No.11  
Pune-411001
23. ICMR Virus Unit (Regional Infectious Disease Laboratory)  
GB4, Ist Floor , ID & BG Hospital Campus  
57, Dr. S.C. Banerjee Road, Beliaghata  
Kolkata-700010
24. National Institute for Research in Environmental Health  
Kamla Nehru Hospital Building  
Gandhi Medical College Campus  
Bhopal-462001
25. National Centre for Disease Informatics and Research  
Nirmal Bhawan-ICMR Complex (II Floor)  
Poojanhalli Road, Off NH-7  
Adjacent to Trumpet Flyover of BIAL  
Kannamangala Post  
Bengaluru-562110

26. Regional Medical Research Centre  
Nehru Nagar  
National Highway No. 4  
Belagavi-590010
27. Regional Medical Research Centre  
Nandankanan Road  
Chandrasekharapur  
Bhubaneswar-751023
28. Regional Medical Research Centre  
N.E. Region, East-Chowkidinghee  
P.O. Box No. 105  
Dibrugarh-786001
29. National Institute for Research in Tribal Health  
Medical College Campus  
Nagpur Road  
P.O. Box No. Garha  
Jabalpur-482003
30. Desert Medicine Research Centre  
P.O. Box No. 122  
New Pali Road  
Jodhpur-342005
31. Regional Medical Research Centre  
P.O. Box No.13  
Dollygunj  
Port Blair-744101
32. National Animal Resource Facility for Biomedical Research  
Biotech Park, Genome Valley  
Turkapalli  
Hyderabad

# ICMR CENTRES FOR ADVANCED RESEARCH

1. Advanced Centre For Newborn Health Research,  
All India Institute of Medical Sciences,  
New Delhi.
2. Centre for Evidence Based Child Health Advance Pediatric Centre,  
Postgraduate Institute of Medical Education and Research,  
Chandigarh.
3. Centre for Advanced Research on Environmental Health: Air Pollution,  
Sri Ramachandra University,  
Chennai.
4. Emerging Areas In Molecular Medicine,  
Jawaharlal Nehru University,  
New Delhi.
5. Centre for Molecular Medicine, Sanjay Gandhi Postgraduate  
Institute of Medical Sciences,  
Lucknow.
6. Centre of Excellence In Molecular Medicine,  
All India Institute of Medical Sciences,  
New Delhi.
7. Centre for Advanced Research for Innovations in Mental Health and Neurosciences:  
Manpower Development and Translational Research Phase-A,  
National Institute of Mental Health and Neurosciences.  
Bengaluru.





## **INDIAN COUNCIL OF MEDICAL RESEARCH**

### **Department of Health Research**

(Ministry of Health & Family Welfare)

V. Ramalingaswami Bhawan, Post Box 4911,  
Ansari Nagar, New Delhi-110029

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Website: <http://www.icmr.nic.in>