

Annual Report

2015-16

2015-2016

Indian Council of Medical Research



Indian Council of Medical Research
New Delhi

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New Delhi

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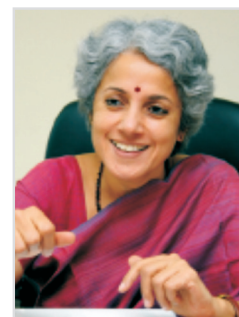
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DIRECTOR-GENERAL'S MESSAGE

It gives me immense pleasure to present the Annual Report of ICMR for the Year 2015-16. The year has been remarkable, with the world shifting from the Millennium Development Goals (MDGs) to the Sustainable Development Goals (SDGs) with 17 ambitious goals and 169 targets. Within the broader scope of a global developmental agenda, Goal 3 states, '*Ensure healthy lives and promote well-being for all at all ages*'. The Draft National Health Policy of the government highlights Universal health coverage. ICMR's research efforts have been aligned to national health priorities and aim at achieving overall well being of the Indian population.



Major Initiatives

During the year, ICMR has taken an initiative to create an India TB Research and Development Consortium, a mechanism to address TB in Mission Mode. It aims to address overarching scientific questions. In addition to the various science ministries, the consortium has a number of national & international organizations as partners. The consortium aims to deliver new, cost effective, diagnostic kit(s) for TB and MDRTB, test new TB vaccines, develop more effective, safer and shorter treatment of MDRTB, and develop models incorporating most effective interventions into RNTCP for reducing TB related mortality & catastrophic health expenditures.

To help achieve the goal of lymphatic filariasis elimination in India, ICMR initiated a new triple mass drug administration demonstration project with NVBDCP in Yadgir district of Karnataka. If successful, this would be scaled up in all highly endemic districts of the country. Similarly, a district in Bihar was identified to demonstrate kala azar elimination by implementing multiple interventions including case detection and treatment and sandfly vector control. This demonstration project proved that it is possible to reduce incidence and eventually eliminate kala azar in India.

The ICMR-INDIAB study is a landmark study as this 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 was completed in 14 states and one UT and the data obtained has been shared with the State health departments. Dissemination Workshop on ICMR-India DIABetes (ICMR-INDIAB)" was held on 15th March 2016.

In the area of infrastructure development, the Cabinet approved Establishment of National Animal Resource Facility for Biomedical Research (NARFBR) at Hyderabad. The Institute will be the first of its kind for quality laboratory animals for basic and applied biomedical research in the Country.

ICMR in partnership with Public Health Foundation of India (PHFI), & the Institute for Health Metrics and Evaluation (IHME) at the University of Washington, USA in collaboration with the Min of Health and Family Welfare, Govt of India launched State- Level Disease Burden Estimation study in India to

generate State-Level Disease Burden and Risk Factors estimates to improve health programmes and planning.

India launched National Framework for Malaria Elimination (NFME) 2016-2030, which outlines India's strategy for elimination of the disease by 2030. National Institute for Research in Tribal Health (NIRTH), Jabalpur was involved in a Brain storming meet on 21st December, 2015 for finalizing the 'Strategies for Malaria Prevention and Control' and 'Control of Malaria in special groups and situations, including cross border and regional coordination'.

ICMR's National Institute of Nutrition, Hyderabad organized the International Food Data Conference at Hyderabad from 3rd to 5th Nov., 2015. More than 350 delegates from 54 countries including India participated. The process was started for disseminating information about the upcoming new Indian Food Composition Database. In view of the importance of food Biodiversity, Indigenous food festival was also organized wherein 16 indigenous groups from different parts of the country which showcased especially the less familiar foods. A mobile App on dietary guidelines for Indians and important nutrition books in braille for visually challenged was also launched.

ICMR's-National Institute of Malaria Research (NIMR), New Delhi was certified as WHO's recognized malaria RDT lot testing laboratory for quality assurance and inaugurated by Dr Poonam Khetrpal Singh, Regional Director of WHO South-East Asia Region, on 7th March, 2016.

ICMR Flagship Programs *viz.*, Tribal Health Research Forum and Vector Borne Disease Science Forum continued to focus on the health problems of the rural communities and projects in the areas of malaria, filariasis, JE/AES, nutrition, hypertension and TB, were initiated. Indigenous production of insecticide impregnated papers for insecticide resistance was developed by VCRC, Puducherry.

Satellite Centre of NIIH at Chandrapur Maharashtra has started working on screening and management of sickle cell diseases. ICMR also set up a NIRTH Field Unit at Regional Hospital, Keylong in tribal dominated remote and inaccessible Lahaul & Spiti areas of Himachal Pradesh. Chief Minister of HP visited and chaired the stakeholder consultation on 19th June, 2015.

During the year, global pandemics like Ebola and Zika raised serious health concern. Being apex laboratory NIV Pune provided Ebola diagnostic services to the country during the Ebola threat. ICMR also demonstrated preparedness to handle Zika virus outbreak. NIV, Pune has capacity to test the samples received during the acute phase of the disease by RT-PCR. Training facilities of DHR/ICMR VRDLs and concerned ICMR Institutes for Zika virus testing have been strengthened.

National Anti Microbial Resistance Surveillance Network (AMRSN) continued to enable compilation of National Data of AMR at different levels of Health Care. Under National Hospital Based Rotavirus Surveillance Network, the study is being carried out at 4 Major referral labs, 7 ICMR's Regional labs and 23 hospital sites to see the trend in burden of rotavirus diarrhoea as well as impact of Rotavirus vaccine under Universal Immunization Program (UIP). A Research cum Intervention project on AES/ JE was strengthened.

Under Management of Acute Coronary Event Registries, structured data capture of acute coronary syndromes is the first step in improvement of quality of treatment. The feasibility study was undertaken in 13 public and private hospitals across India which recruited 1515 confirmed ACS patients \geq 18 years.

A Centre for Advanced Research for Innovations in Mental Health and Neurosciences was initiated with focus on Manpower Development and Translational Research at NIMHANS, Bangalore. A stroke

registry was set up in rural and urban population of Bangalore and Ludhiana. DHR/ICMR guidelines for diagnosis and treatment of rickettsial infections in the country were finalized and a TF study was launched towards establishment of National Institute of Zoonosis at Nagpur. ICMR's National Institute of Cancer Prevention and Research (NICPR), Noida was Designated by WHO as Global Knowledge Hub on Smokeless Tobacco (SLT). The NICPR also developed a Cancer Web portal (India Battles Cancer) for creating awareness.

As per the directives of PMO and Department of Space, ICMR created a working group on space technology and initiatives were made to promote use of space technology tools like Remote Sensing and GIS for disease mapping and developing early warning systems particularly for vector borne diseases. ICMR also established an Evidenced to Policy Unit at Hqrs for Research Synthesis and Policy briefs for implementation.

During the year, ICMR participated in various education, extension and outreach activities to disseminate activities and achievements. ICMR Participated in Festival of Innovations at Rashtrapati Bhawan, 12th-18th March, 2016 jointly organized by National Innovation Foundation and also took part in the Round Table discussions on innovations and bio-technology. Around 50 technologies targeting mother and child care, tuberculosis, disability, safe drinking water, mosquito destroyer *etc* were displayed in the Exhibition.

ICMR also participated in Mega Science Technology & Industry Expo 2015 as part of India International Science Festival from 4th to 8th Dec, 2015 at IIT New Delhi, and in the 'Pride of India' Mega Science Expo as part of the 103rd Indian Science Congress held at Mysore during 3-7th January, 2016 and won the 'Most Informative Pavilion Award'.

ICMR continued to support various capacity building schemes like JRF, SRF, STS *etc* and various national and international fellowship programs. During the year, ICMR supported 1030 Short Term Studentships projects of undergraduate medical and dental students from 29 States and 7 union UT of India. National Institute of Epidemiology (NIE), Chennai launched an 'NIE-ICMR e-Certificate' course in collaboration with National Programme on Technology Enhanced Learning (NPTEL). This course will explain the fundamental concepts in epidemiology and bio-statistics related to human bio-medical research.

Under international collaboration various Ongoing partnerships in Health Research (under 8 MOUs) with various international organizations/agencies continued. Total 11 exchange visits of Scientists were arranged for various international collaborative programmes/projects. For International Research Co-operation – 102 projects were approved by Health Ministry's Screening Committee. 12 Junior Scientist and 6 Senior Scientists were selected for ICMR International Fellowship during 2015-16.

ICMR Scientists published total 965 papers during 2015. In all, 33 patents were filed and 2 patents were granted. Efforts were also made for transfer of technologies developed by ICMR to Industry for commercialization. ICMR funded a total of 1745 research projects including fellowships during the year, while 292 new research projects including fellowships were approved.

To award excellence in biomedical research, ICMR distributed awards and prizes to 43 scientists in a function presided over by Hon'ble Union Minister of Health and Family Welfare Shri J P Nadda on 19th January, 2016.

Many of the ICMR Institutes celebrated Swachh Bharat Abhiyan, Institutes Foundation day/annual day, Vigilance Day, International Yoga Day, *etc* by organizing lectures, workshops and other activities.

ICMR has made steady progress in various fields of biomedical and health research. Scientists of ICMR have been successful in developing indigenous and cost effective technologies for public use and tackling outbreaks/epidemics. I congratulate the ICMR Scientists for their hard work, dedication and commitment. I am confident that ICMR will move forward with new vision and re-energized vigour to face new challenges to solve the health problems of India.



Dr Soumya Swaminathan
Secretary, Department of Health Research
& Director General, ICMR

OVERVIEW

It is a matter of great pride and pleasure to present the highlights of achievements and activities of Indian Council of Medical Research during the year 2015-16. It is an opportunity to glance through the achievements/contributions of ICMR during the glorious past year and plan strategies to dedicate ourselves to alleviate the suffering of Indian public at large through research. The Council has been working for opening new possibilities and opportunities to deal with persistent and over emerging medical and health problems of our countrymen. A vibrant network of 32 permanent institutions and approximately 100 field stations/units of the Council are engaged in pursuing the national health objectives and channelize its research endeavors towards control and management of communicable and non-communicable diseases, health of mothers, infants and children, control of nutritional and metabolic disorders/diseases, mental health as well as drug research.

ICMR initiated programmes for elimination of three major public health problems among the Nicobarese tribe. The project on elimination of diurnally sub-periodic filariasis from Nancowry is mid-way through its implementation of double fortified salt (DEC+iodine) as a supplementary measure to the ongoing MDA programme. Generated information on the current prevalence of TB among the Nicobarese of Car Nicobar developed a project to achieve TB control through 4 ways: 1. Active case detection of latent TB 2. Develop strategies for treatment of such cases. 3. Impart training to TKPs, as a small proportion of the patients approach them 4. To screen and follow up Hepatitis B carriers among the TB patients and implement appropriate treatment strategies among TB patients with drug induced hepato-toxicity. The other one being the malaria elimination in Car Nicobar are in their preliminary stages.

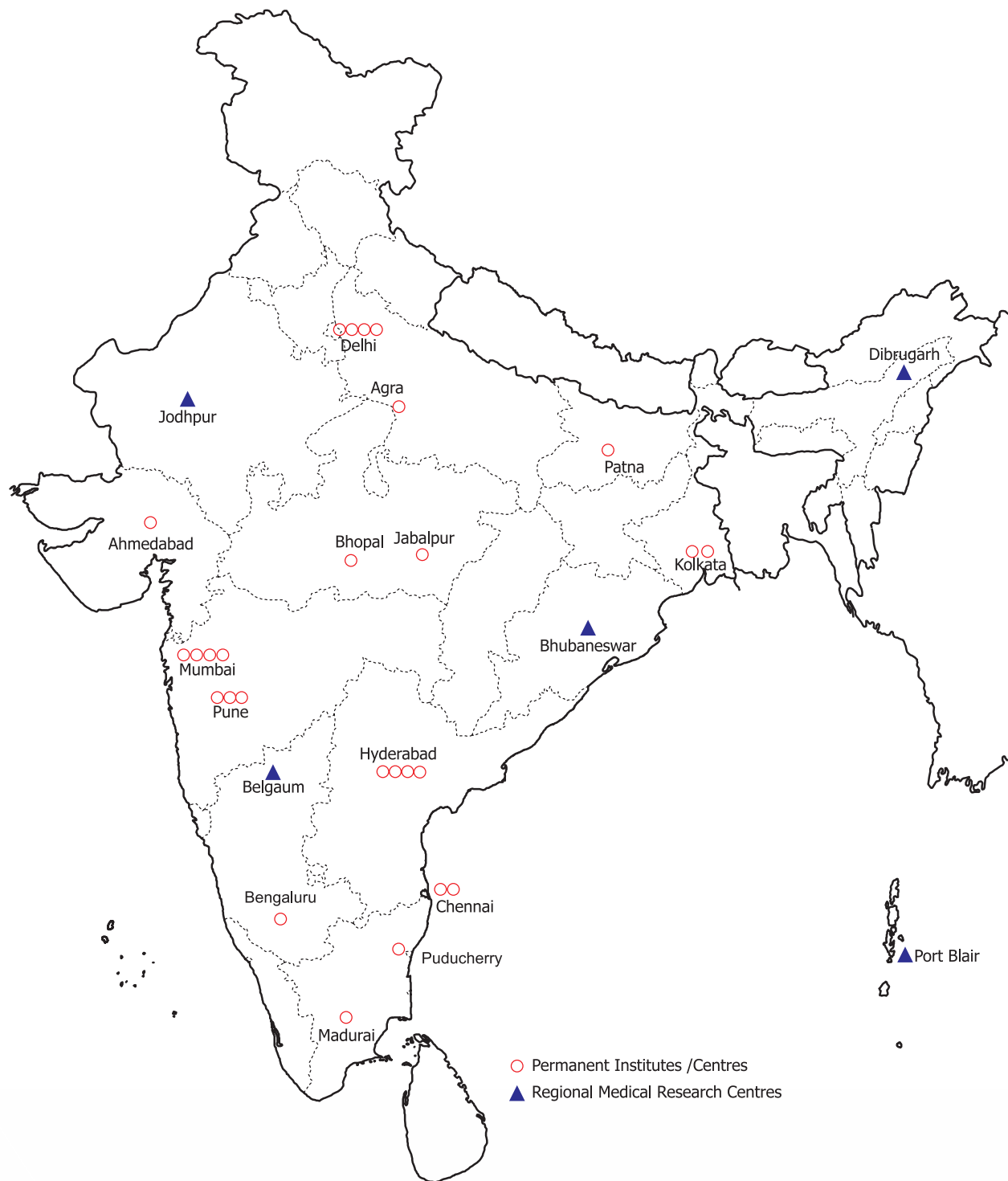
A significant progress has been made in understanding the role of biofilms in the transmission and pathogenesis of leptospirosis. A few proteins that may be involved in biofilm formation process have been identified. Biofilms' role in prolonging the survival of *Leptospira* in the environment by making them resistant to stress has been established through laboratory based studies.

Child Health services were initiated at Family welfare clinic located at Abhyudaya Nagar in the year 2015. Parents were advised mainly on Newborn care and breastfeeding, growth and development of children, nutrition, issues related to menarche and puberty.

Specialized Multidisciplinary clinic for Polycystic Ovary Syndrome (PCOS) was inaugurated at NIRRH, with a team of IVF specialist, endocrinologist, dermatologist, dietician and yoga expert.

Containment of wild poliovirus 2 has been achieved successfully. No wild poliovirus was isolated from Acute Flaccid Paralysis (AFP) cases from AFP surveillance in India. No wild poliovirus was isolated from sewage samples collected in Mumbai, Delhi, Kolkata, Patna, Punjab and Ahmedabad from Environmental surveillance.

Long term non-progressors: viz Three 'Tat' regions (The amino acid stretches ; a.a. 29-43, a.a.45-63 and a.a.77-101) were frequently recognized by Indian LTNPs. This is first report from India. These findings would be useful for HIV vaccines & novel prevention interventions.



ICMR INSTITUTIONAL NETWORK

HPTN 052: At the end of 10 years of follow up, it is found that antiretroviral therapy reduces the risk of transmission of HIV in HIV discordant couple setting by 93% and maintains the protection.

World Health Organization Framework Convention on Tobacco Control (WHO-FCTC) has designated the Institute of Cytology and Preventive Oncology (Now NICPR) as Global Knowledge Hub on Smokeless Tobacco.

The characterized “cultured epidermis” produced by ICMR novel processing technology was found qualitatively comparable to the international product “Epicel” of Vericell Corporation, USA (<http://vcel.com>) and is now ready for clinical trial-commercialization for burns.

Three courses of Paromomycin and Miltefosine combination for treatment of PKDL showed complete disappearance of nodular and papulo-nodular lesions, though few macular lesions remain.

Evaluation of the GIS, geo-statistics and remote sensing based software model in predicting sandflygenic hotspots confirms its prediction accuracy. A significant linear relationship was observed between man-hour-density and predicted density of *P. argentipes* ($R^2 = 0.72$, $P < 0.001$).

Dengue virus was propagated in cell line and further RNA extraction and RT-PCR were conducted. The amplified PCR product was exploited in the cloning of dengue virus NS1 gene for the expression of virus protein for the development of indigenous diagnostic kit. The recombinant protein was used for the development of monoclonal antibodies. The clones were successfully developed by fusion of recombinant viral protein immunised mouse spleen with mouse myeloma cells.

The hospital-based surveillance on bacterial meningitis preliminary data indicated that *S. pneumoniae* contributes to a 72% of the confirmed meningitis cases in children below 6 years of age in comparison with *H. influenza* type B and *N. meningitides* put together (28%). This data would be useful to assess the impact of Pentavalent vaccine with its phase rolled out through the Universal Immunization Programme in India. It has provided the required platform to set up pneumonia surveillance network across India with a view to generate data and information to assess the impact of initiation of pneumococcal vaccine in India.

NIE launched ICMR’s first online course – Health Research Fundamentals –3000+ registrations across India.

Establishment of School of Traditional Medicine for undertaking research and validation of traditional medicines through integrative approach was successfully demonstrated by RMRC, Belagavi.

Advanced diagnostic facilities have been established for diagnosis, genetic counseling and management of Primary Immunodeficiency Disorders.

ICMR study on molecular characterization and mitochondrial DNA variations in Fanconi anemia patients has given important information on the underlying molecular mechanisms in these patients and has helped in genetic counseling and prenatal diagnosis of these patients.

A rapid and cost-effective xenomonitoring protocol developed (gravid trap for vector collection, PCR assay for detecting vector infection, and sampling strategies) at VCRC could identify the low level of infection following eight annual rounds of MDA in PHC level and validated as a surveillance tool for assessing post-MDA situation of lymphatic filariasis elimination programme at district level.

Mosquito faunistic survey carried out in Jammu & Kashmir indicated first time record of species of mosquitoes, *Coquillettidia perturbans*, which is significant as it is a new country record for India.

The secondary metabolite of an indigenous isolate of *Bacillus amyloliquefaciens* was found to have antibacterial, mosquito larvicidal and pupicidal activity.

During the year 2015-16, ICMR received 1392 pre-proposals using Web-Based Extramural Project Management System. Many Call for proposals programmes were also launched during the year which resulted in receipt of several proposals. These included a). Call for proposal on Social determinants and management of Children in difficult circumstances (39 proposals), b). Call for proposal under North East Seed Grant Scheme (164 proposals), c). Call for Proposals on Interventions for Prevention of Prematurity (12 proposals), d). Call for proposals on Quality of care in Pregnancy and Childbirth (86 proposals), e). Call for proposals for Focused Research on Management of Arsenic Related Diseases (73 proposals), f). Call for Proposals on Drug Utilization Research (51 proposals), g). Call for Proposals on Stillbirth (52 proposals).

During the period under Report, a total of 282 articles were published in IJMR. The original articles contributed maximum to 48.6%, followed by correspondence (16%) and review articles (9.9%). Clinical Images accounted for 8.5%. A special issue on Tribal health was brought out in May 2015. Eighteen original articles and six review articles contributed by various experts were published in this special issue. A supplementary issue on Vector borne diseases was brought out in December 2015 with 15 original articles and two correspondences.

During this year, a total of 33 patent applications were filed, two in India, one each from intramural and extramural institute. Two patents granted during this period belonged to NIRT, Chennai and ERC, Mumbai.

Every year 150 JRFs (i.e. 120 for Life sciences and 30 for Social sciences including biostatistics) are selected for doing Ph.D. in Biomedical Sciences in different institutions. Number of candidates appeared in the year 2015-16 was approx. 8,000. A total of 720 JRFs is on-going (2011-2015) at various national level institutions.

Financial assistance of Rs.25, 000/- is provided to MD/MS/DM/MCh students who are in the 2nd year of MD/MS course. The Selection Committee recommended financial assistance to a total of 614 MD/MS/DM/MCh thesis, out of 2329 proposals received so far. Out of 432 -thesis protocols, 82 protocols/candidates were awarded financial assistance during the reporting period.

ICMR carried out large number of education and extension activities during the period 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 : I). Indian Science Congress Pride of India: Frontier Science & Technologies Mega Science Expo organized during 3-7th January, 2016 held at Mysore. ICMR Pavilion was given the Best Informative Pavilion Award. II). 2nd Vision Jammu & Kashmir 2016 – a mega Science Exhibition : held at Jammu from 22-24th January, 2016. III). Science, Technology & Industry Expo 2015: during 4-8 Dec., 2015 at IIT New Delhi. Dr Harsh Vardhan, Hon'ble Union Minister of Science & Technology and Earth Sciences visited the ICMR Pavilion on 4th Dec., 2015. IV). Indo-Africa Forum Summit, held in New Delhi from 27-29th October, 2015. V). 13th Infra Educa 2015 : Educational Fair, at Solan, Himachal Pradesh: from 26-29th June, 2015. V). *Festival of Innovations at Rashtrapati Bhawan, New Delhi* during March 12-18, 2016. VI). Mega science expo organized at Daman in 2015 for creating awareness among the rural population as well to disseminate useful information to students. VII). World Book Fair: held during 9-17 January, 2016 in coordination with the National Book Trust (NBT), New Delhi.

The following MoUs have been signed during this period :

- A Memorandum of Intention (MoI) between ICMR and Swedish Research Council for Health Working Life and Welfare (FORTE), Sweden was signed on 2nd June, 2015 at Stockholm.
- MoU was signed on 25th June, 2015 at New Delhi to promote and develop cooperation in the field of Environmental and Occupational Health, injury prevention and control research, education and training, infrastructure development and capacity building.
- 3. The Indo-US MoU among the NCI (AIIMS), MOH&FW, ICMR (DHR), DBT (M of S&T) and NCI (NIH), DHHS, Govt. of USA for Cooperation on Cancer Research Prevention Control and Management was signed on 25th June, 2015 at New Delhi for establishment of the general framework of collaboration for promoting and conducting high quality research to strengthen evidence base necessary for cancer prevention, treatment and management.
- Letter of Intent (LoI) between ICMR, New Delhi and Department of Biotechnology, Ministry of Science and Technology of the Republic of India and the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, DHHS, USA on Anti- Microbial Resistance (AMR) research was signed on 25th June, 2015.
- MoU between ICMR and Drugs for Neglected Diseases Initiative (DNDi), Switzerland was signed on 15th October, 2015 by the Secretary, DHR, MOH&FW, GoI & DG, ICMR and the Executive Director, DNDi at New Delhi for cooperation in neglected diseases.
- MoU between ICMR –Medical Research Council, UK was signed by DG, ICMR on 10th Nov. 2015 at ICMR Hqrs. New Delhi.
- MoU between ICMR and The National Health and Medical Research Council (NHMRC), Australia was signed on 18th Feb, 2016.
- ICMR-London School of Hygiene and Tropical Medicine (LSHTM), UK was signed on 8th Dec. 2015 at London.

ICMR scientists published a total of 965 research papers in various National & International Journals.

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COMMUNICABLE DISEASES

Communicable and infectious diseases like tuberculosis, leprosy, cholera, diarrhoeal diseases, viral hepatitis, leptospirosis, vector borne diseases like malaria, kala-azar, dengue fever, chikungunya, filariasis, Japanese encephalitis and other viral infections are endemic in the country and pose serious public health problems. In India, Dengue cases in the country were an all-time high, cases and deaths were reported from almost all parts of the country during July to November 2015. With the emergence of Zika virus, the country faced a challenge of Zika virus infiltration. The world is once again reminded of the vulnerability to emerging diseases after events of pandemic avian influenza A (H7N9), Middle East respiratory Syndrome (MERS) in Asia Pacific Region and Ebola virus in West Africa. ICMR's research efforts in the area of communicable diseases were made by 16 institutes/centres including Regional Medical Research Centres (RMRCs) and their field stations located in different parts of the country as well by granting ad hoc projects in extramural mode in universities/medical colleges and other organizations. The research activities carried out by different ICMR Institutes and their outcome are presented.

Intramural Research

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS, CHENNAI

Randomised Clinical Trial of daily vs intermittent therapy of Anti tuberculous Therapy TT in HIV-TB co-infected patients

Interim findings suggest a trend towards a better response to the daily regimen. Sputum smear and

culture conversion seem to be better with the daily regimen.

Randomised Clinical Trial to study the efficacy and tolerability of 3- and 4-month regimens containing Moxifloxacin in the treatment of patients with sputum positive pulmonary TB

Sputum conversion was more rapid in the moxifloxacin containing arms compared to the control arm. Interim findings of the clinical trial using moxifloxacin indicate promise for 4-month regimens for treatment of TB.

Fourth Prevalence survey of Pulmonary Tuberculosis in Tiruvallur district

A survey is underway to estimate current prevalence of bacteriologically positive TB among adults aged ≥ 15 years in Tiruvallur district and to determine the prevalence of TB infection among children aged < 15 years in TB affected households.

TB treatment outcome among TB-HIV co-infected patients under programme conditions in south India

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

Performance Accuracy between Classifiers of Disease Conversion for Clinical Trial Tuberculosis Data: Data Mining Approach

Multilayer perception achieves a better performance than naïve Bayes, and when the number of features to be classified, were reduced, naïve Bayes performed well.

Factors influencing first-line anti-TB drug concentrations and TB treatment outcome in adult TB patients

This study undertaken in adult TB patients treated in the RNTCP in Chennai, has shown that age, body mass index and blood glucose were important factors that impacted rifampicin, isoniazid and pyrazinamide concentrations. Low RMP, low body mass index, baseline INH resistance and patients treated with category II regimen were identified as risk factors for unfavourable treatment outcome.

SLCO1B1 gene polymorphisms and plasma rifampicin

This study for the first time in Indian patients showed that rs11045819, rs4149032 and rs4149033 polymorphisms in the *SLCO1B1* gene did not influence RMP concentrations.

Pharmacokinetics of thrice weekly rifampicin, isoniazid and pyrazinamide in adult TB patients in India

Several risk factors for drug concentration variations were identified. Two-hour post-dosing drug concentrations mimicked peak concentration. A high proportion of TB patients had peak concentration of rifampicin below the expected range (<8µg/ml).

Impact of food on anti-TB drug concentrations

The study showed that anti-TB drugs taken after consumption of food significantly reduced plasma concentrations of rifampicin, isoniazid and pyrazinamide and also delayed drug absorption.

NAT2 gene polymorphisms and plasma isoniazid

Genotyping of TB patients from south India for *NAT2* gene polymorphism revealed that 58% of the study population consisted of slow acetylators. Two-hour plasma isoniazid concentrations differed significantly among the slow, intermediate and fast acetylators.

Immune responses in tuberculosis

This study demonstrated that serum vascular endothelial growth factors and their receptors are both markers of vascular dysfunction in tuberculosis

and biomarkers of disease and therapeutic outcome. It was shown that coexistent malnutrition in latent tuberculosis is associated with perturbations in systemic and antigen-specific cytokine responses. Further, coincident diabetes mellitus modulates CD4+ Th1, Th2 and Th17 cell responses in latent tuberculosis in an IL-10 and TGFβ dependent manner.

Immune responses in helminth infections

This study describes the parasite antigen-specific regulation of Th1, Th2 and Th17 responses in *Strongyloides stercoralis* infection. It elucidated the systemic cytokine profiles in *Strongyloides stercoralis* infection and alterations following treatment. The study also described the modulation of CD4+ and CD8+ T cell function in filarial infections by IL-19 and IL-24. The study also demonstrated that coincident helminth infection modulates systemic inflammation and immune activation in active pulmonary TB.

Development of a novel method to improve sensitivity of tuberculosis diagnosis by culture using Resuscitation Promoting Factor

Interim findings from 284 samples showed a higher positivity for growth where culture filtrate supernatant was used in comparison with conventional culture.

Re-evaluation of the critical concentration of Isoniazid and Rifampicin among M. tuberculosis strains isolated from patients using wild-type MIC distribution

Evaluation of 266 strains showed the critical concentration for determining drug

susceptibility to be 0.2µg/ml and 1 µg/ml for Isoniazid and Rifampicin respectively using solid media.

Multicentric Evaluation of Drug Susceptibility Testing by Luciferase Reporter Phage Assay

Drug Susceptibility testing by Luciferase Reporter Assay for the detection of drug resistance using solid cultures yielded good sensitivity and specificity in comparison with MGIT960 for Rifampicin and Isoniazid.

An experimental study to enhance treatment adherence in TB patients with Alcohol Use Dependence (AUD)

Alcohol use intervention among TB patients is effective in ensuring favourable treatment outcomes and treatment adherence. This study suggests the need for trained counsellors in TB care settings to ensure effective alcohol intervention strategies in order to promote TB treatment compliance.

Estimation of the burden of TB among tribal population and development of an innovative health system model to strengthen TB control in the tribal areas

A multi-centric study has been initiated to estimate the burden of TB amongst tribal groups in various states of the country. A tribal health system model will also be developed with feasible interventions to improve case finding and compliance for TB treatment through a community based approach.

Identification of biomarkers for active TB among HIV-TB co infected persons

The study concluded that TB antigen specific IL-1 β can be recommended as an additional biomarker for active TB diagnosis among HIV positive subjects.

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

Antigen-specific immune response evaluated in 39 pulmonary TB patients and 35 healthy household contacts showed Rv2204c and Rv0753c specific IFN- γ and IFN- γ /TNF- α responses to have promising accuracy in identifying LTBI

Vitamin D receptor gene polymorphisms and treatment outcome in pulmonary tuberculosis

The study results revealed the association of VDR genotypes such as BSMI “bb”, ApaI “aa”, TaqI “tt”, FokI “ff”, Cdx-2 “GA” and A-1012G “GG” with time to sputum conversion, but the results were found to be not significant.

Identification and characterization of broadly cross clade neutralizing antibodies from HIV-1 subtype C infected individuals

Plasma samples of 101 individuals recently infected with HIV-1 subtype C were screened and 12 samples were identified that were capable of broad

and potent cross clade neutralization. Interestingly, these samples had antibodies of multiple epitope specificities.

Molecular characterization of the envelope of HIV-1 isolates transmitted through the horizontal and vertical routes

The vertically transmitted viral variants were unique with respect to the length of V1V2 loops in the HIV-1 *env* gene and the number and pattern of PNGs in contrast to HIV-1 strains transmitted recently through the horizontal route.

A study on the pattern of HIV drug resistance seen in children exposed to HIV prophylaxis in the PPTCT program

This study was designed to assess the proportion of young children with drug-resistant HIV in a representative population of infants (from three southern states of India *viz.* Tamilnadu, Kerala and Pondicherry), aged <18 months, tested for HIV infection in the Early Infant Diagnosis (EID) Program of the National AIDS Control Organization (NACO), and to determine the drug resistance pattern in infants who test positive for HIV infection.

Banking of biological specimens from large cohorts of TB patients and their house hold contacts for the C-TRIUMPH (Cohort for TB Research under the Indo-US Medical Partnership) study

The C-TRIUMPH study is a multicentric Indo-US partnership study that aims to measure host and microbial factors associated with TB treatment outcomes in Indian adults and children (Active TB cohort), investigate the host and microbial factors associated with progression from latent infection to active TB disease in adults and children (Household Contacts) and explore the host and microbial factors associated with TB transmission (Household Contacts and Control Cohorts). In the year 2015-16, 257 baseline samples and 902 follow-up samples have been received and processed for immunological parameters and long term storage for biomarker studies.

Product patent obtained during the year

- **Title of the invention:** A composition for selectively eliminating a microorganism or

a group of microorganisms from biological sample

- **Inventors:** Vanaja Kumar, S. Balaji and N. Selvakumar
- **Patentee:** Indian Council of Medical Research
- **Patent No:** 269659, **Date of filing:** 12/12/2008, **Date of Grant:** 30/10/2015

NATIONAL JALMA INSTITUTE FOR LEPROSY AND OTHER MYCOBACTERIAL DISEASES, AGRA

DNA fingerprinting of *Mycobacterium tuberculosis* isolates from defined population by using IS-6110 probe

During this period, a total of 70 clinical isolates of *M. tuberculosis* recovered from paediatric patients of Agra region were included for analysis. Drug susceptibility testing of these isolates was done by proportion method. All the isolates were subjected for IS6110 RFLP method. There is no correlation observed with drug sensitivity profile and RFLP pattern of *M. tuberculosis*.

Whole proteome analysis of aminoglycoside resistant isolates of *Mycobacterium tuberculosis*

Seventeen protein spots were to be consistently over expressed in all resistant strains as compared to sensitive isolates. However, five spots matched with already identified proteins and therefore total twelve proteins were found to be upregulated. Interacting residues were almost common for both drugs, which suggests similar binding site for both. Docking with Rv2744c showed that both drugs interact at the similar interacting residue of conserved PspA domain of hypothetical protein. Cloning and expression studies have been standardized and initiated with hypothetical protein

Therapeutic efficacy of Moxifloxacin and Econazole based drug regimen against MDR-Tuberculosis

Based on the study it can be concluded that chemotherapy with 8 doses of nanoformulations iPLGA- NP- ECZ+PLGA-NP-MOX+PLGA-NP-ETH) given to mice infected with MDR-TB strain (JAL-11050 resistant to INH and RIF), the

clearance of bacilli from lungs and the spleen was similar to that observed with daily dose of free drugs (ECZ+ MOX+ ETH (100 doses + 50 doses + 50 doses). This is a first report signifying the efficacy of three-drug combination ECZ, MOX and ETH against MDR-TB.

Modelling of murine CNS-TB model

The present study demonstrated that intravenous inoculation by C3 strain from TBM patient's leads to of progressive dissemination and development of TBM disease in mice. Such models can be further used to identify and study host- pathogen associated markers involved in microbial invasion of CNS and pathogenesis of disease. The identified markers can be used in development of improved vaccines targeting CNS disease using existing model of TBM infection in future studies to come.

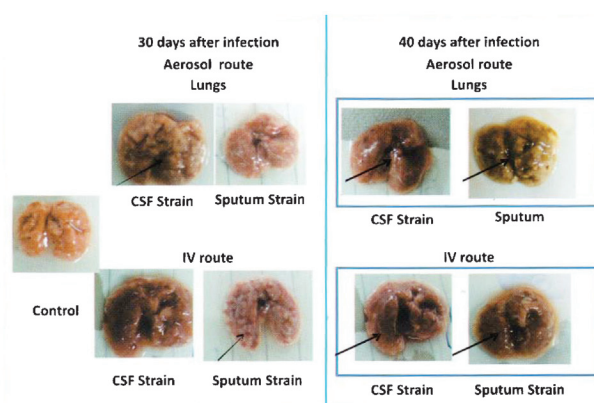


Fig 1. Representative lung morphology in mice infected with CSF and sputum strain by aerosol and IV route at 30 and 40 days post infection.

Studying the immunomodulatory effect of immune complexes in the tissue correlates of guinea pigs infected with TB

It was observed that the animals from Group I (antigen excess immune complex), showed considerable increase in body weight while those from Group II (antibody excess immune complex) showed slight decline in weight after infection, whereas the control group (group III) also showed decline in body weight.

Evaluation of different treatment regimens being used at Model Rural Health Research Unit, Ghatampur, Kanpur Nagar, India (Phase I & II)

Follow up of patients on various regimens of TB - Category II regimen along with immunomodulator Mw - a total of 156 patients were recruited for the study of which 107 patients have completed 24 months follow up. Category I regimen along with immunomodulator — a total of 296 patients were recruited for the study of which 206 patients have completed 24 months follow up. TB patients put on treatment under DOTS detected outside the Mw trial through the TB prevalence survey and also through the OPD — a total of 1223 category I patients and 926 category II patients of which 504 and 308 respectively have completed a 24 months follow up.

Follow up of patients on various regimens of Leprosy - a total of 3933 cases have been enrolled in the study. Of the 3933 cases enrolled for the study 1740 (44.2%) were multibacillary (MB) cases and 2193 (55.8%) paucibacillary (PB) cases. 60 months follow up has been completed for 49% of the patients on clofazimine regimen; 46% of the patients on oflox-mينو regimen; 29% of the patients on standard MB regimen; 29% of the patients on standard PB regimen and 91% of the patients on UMDT regimen. A total of 1644 patients (41%) have completed a 60 months follow up among the 3933 cases enrolled in the study. Other patients are in various stages of follow up.

Elucidating the strain differentiation and transmission dynamics of *M. leprae* through Inter Simple Sequence Repeats (ISSR-PCR) marker

A total of 117 samples were collected from 66 villages of Ghtampur Tehsil. Results have demonstrated that ISSR-PCR is more sensitive tool than RLEP-PCR or AFB microscopy in leprosy case detection.

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

The Department of Health Research 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.

Research projects underway at MRHRU, Haroli, Una, Himachal Pradesh:

1. 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.
2. Data triangulation: Record based analysis to assess the health status of Haroli, Una, Himachal Pradesh, India.

PUBLIC HEALTH IMPORTANCE

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

NJIL&OMD has undertaken monitoring and accreditation activities of different laboratories for Line Probe assay, viz., JNU Medical College, AMU, Aligarh; and IRL, KGMU, Lucknow; IRL, Guwahati Assam.

Strengthening of Mycobacterial repository for translational studies (Phase II)

Presently, 5341 isolates from different parts of country are being maintained and are available to support the investigators from different of the country. Isolates of *Mycobacterium avium* subspecies *paratuberculosis* were included in the repository. Forty one centres are presently collaborating with repository centre.

NRL (National Reference Laboratory) For Tuberculosis Activities

On Site Evaluation visits and panel testing to various IRLs under NJIL&OMD were under taken. Central Internal Evaluation of Chhatisgarh, Punjab, New Delhi and Madhya Pradesh state was attended.

Patient Care and Related Activities

A total of 29697 patients having symptoms suggestive of leprosy and skin diseases attended the OPD in 2015-16. Out of the above 3110 new leprosy cases were diagnosed and put on treatment. 19130 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.

Activities of HIV/AIDS Unit

Routine HIV screening and Counseling - a total of 1295 clients were screened and 89 (6.87%) were found to be sero positive for HIV 1 or HIV2.

Out of 221 TB patients referred by RNTCP, 3 were found to be HIV seropositive. Out of 40 HIV sero positive subjects 3 patients were tested positive for TB. Out of four HIV negative patients screened for TB, 2 tested positive for TB. Altogether, six out of 261 (2.29 %) TB patients were found to be HIV seropositive.

Model Rural Health Research Unit, Ghatampur

The MRHRU Ghatampur has been providing outdoor patient care services limited to tuberculosis, leprosy, filariasis and a few skin diseases. The whole population of Ghatampur Tehsil accesses these services. The patients are diagnosed and treated as per the National Policies and standard diagnostic algorithms and treatment modalities. The Unit continues to function as a Designated Microscopy Centre and also as a DOTS centre under the RNTCP being implemented by the State of Uttar Pradesh in Kanpur Nagar. Services under NLEP are also being provided through the unit.

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

Baseline data collection as per guidelines issued has been completed from all villages.

Patent filed

1. Indu Verma, U.D. Gupta, Pushpa Gupta, Javed Sheikh. (2014). Indian Patent Application No.: 1050/DEL/2014. Title : "Compositions and Vaccines for Prevention of Mycobacterial Infections" our Ref : P56861N00.
2. VM Katoch, K Venketasan, DS Chauhan, Hera Wati, Kiran Katoch, VD Sharma, Rahul Sharma. (2014). Patent applied on 21 July 2014 about "Probes and primers for identification of mycobacterial proteins useful as potential

drug target". File no. 884/Del/ 2007.

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES, KOLKATA

NICED continued as WHO collaborating centre for research and training on diarrhoeal diseases and WHO collaborating centre for *Vibrio* phage typing and research during 2015-2016.

Determination of Diarrhoeal disease etiologies, prediction of outbreaks, treatment and prevention by disease surveillance

Major enteric pathogens were isolated and identified at NICED since last 20 years in hospital based enteric disease surveillance. Rotavirus was the major pathogen (48.3%) among children below 5 yr. while *Vibrio cholerae* O1 is the commonest among adult population (13%) in 2015. Emergence of multidrug resistant (MDR) bacteria led to the use of ofloxacin or azithromycin with caution for empirical treatment of hospitalized cholera and shigellosis cases.

NICED is the referral centre for National Rotavirus Surveillance Network (NRSN) in eastern-India. Rotavirus was detected in 45% of the stool samples collected from hospitalized under-five diarrhoeal children. Genotyping showed circulation of G1P[8]/P[6], G2P[4]/P[8], G9P[8]/P[4] serotypes and other uncommon strains of zoonotic origin.

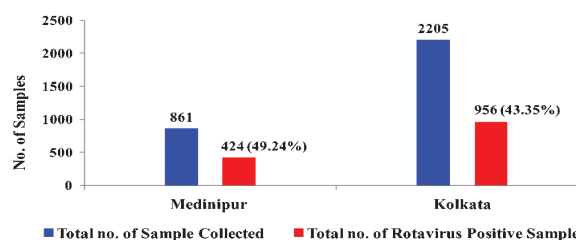


Fig. 2. Rota virus in stool samples.

Upsurge of polymyxin B sensitivity among recent El Tor variant *Vibrio cholerae* strains in Kolkata: Progressing towards the classical biotype?

NICED report the emergence of El Tor strains that have lost an important El Tor biotype marker and acquired a vital classical biotype characteristic probably altering the regulatory mechanisms of lipid A modification machinery in *V. cholerae*. This

shift is an important event in the history of cholera after 1961 when El Tor vibrios first appeared.

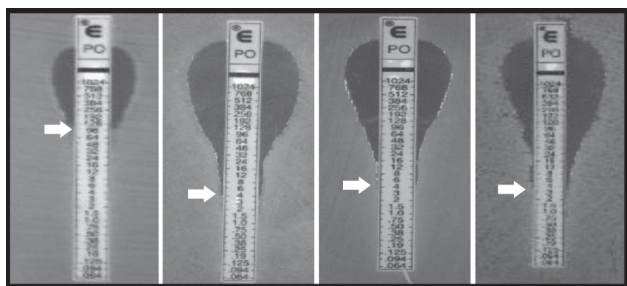


Fig. 3. This change in *V. cholerae* O1 strains need to be carefully monitored with respect to their clinical and epidemiological implications.

NICED extended diagnostic laboratory support during the state/national emergency of H1N1 outbreak in 2015, which helped the M/o Health and Family Welfare, Govt. West Bengal in successfully managing the cases needing antiviral treatment.

Emerging increased antimicrobial resistance in neonatal septicaemia cases

Emphasis was laid on isolation of cephalosporin and carbapenem resistant organisms from neonatal septicaemia cases. High cephalosporin and carbapenem resistance was observed in *Klebsiella*, *Escherichia coli* and in *Acinetobacter spp.* Carbapenem resistance has emerged due to the presence of plasmid mediated NDM-1 gene; which can spread very easily causing difficulty in treatment. Again, presence of NDM-I complicates detection of ESBL enzymes that hydrolyze cephalosporins, leading to delayed intervention and spread of such β lactamases. A method has been developed where inclusion of a metal ion chelator (dipicolinic acid) facilitates detection of ESBLs in the presence of NDM-1.

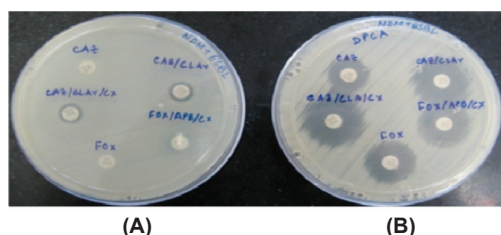


Fig. 4A. NDM+ESBL on MHA plate. **(B)** NDM+ESBL on MHA/DPCA plate.

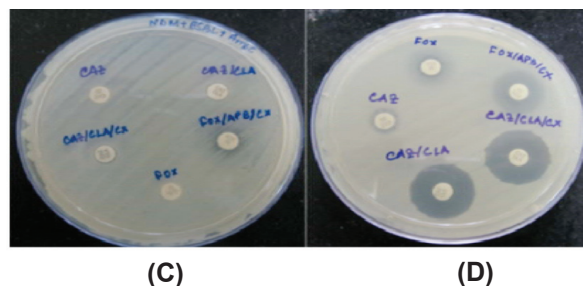


Fig. 5. NDM+ESBL+ AmpC on MHA plate. **(D)** NDM+ESBL+ AmpCon MHA/DPCA plate.

Environmental: Determinants of cholera

The Environmental Bacteriology Laboratory of NICED conducts regular field expeditions in different perennial, rain fed and sea fed rivers in the deltaic West Bengal with the aim to assess and monitor the role of environment, climate and anthropogenic factors on the enteropathogens, which affects human health. NICED investigated and identified the seasonal abundance pattern and distribution of toxigenic *Vibrios* and established that Gangetic riverine-estuarine aquatic ecosystem regulates the survival, distribution and transmission of diarrheogenic *Vibrios*. A biomonitoring tool of physicochemical stimulants, tidal and climatic variants collating *Vibrios* and phage dynamics has been identified that can forewarn impending diarrhoeal outbreak.

Development of a cost effective Outer membrane Vesicles (OMVs) based Shigella vaccine

Efficacy and immunogenicity of OMVs based *Shigella* vaccine has been established and documented. The *tolA* gene, necessary to maintain outer membrane integrity, was disrupted from *Sh. boydii* type 4 (Sample Id: BCH612) strain. It was observed that in the *tolA* mutant, 60% increase in vesicle secretion was noticed than that of the wild type strain. The mutant $\Delta tolA$ -OMVs could encapsulate more OmpA protein, showed better stimulation of macrophages and epithelial cells leading to more secretion of pro-inflammatory and anti-inflammatory cytokines. This study showed the potency of $\Delta tolA$ -OMVs as candidate for next generation vaccine against shigellosis.

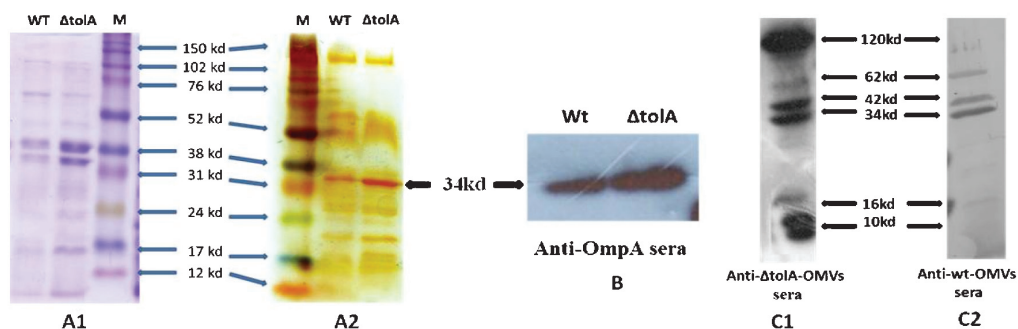


Fig. 6. SDS-PAGE of OMVs, stained with coomassie blue (A1) and silver stain (A2) respectively. Lane M, WT and Δ tolA are the molecular weight marker, Wt-OMVs and Δ tolA-OMVs respectively. (B) Western blot of wt-OMVs (Lane Wt) and Δ tolA-OMVs (Lane Δ tolA) with anti-OmpA sera. (C1) and (C2) are the western blots of the whole cell lysates of BCH612 wild type strain against anti- Δ tolA-OMVs sera and anti-wt-OMVs sera respectively.

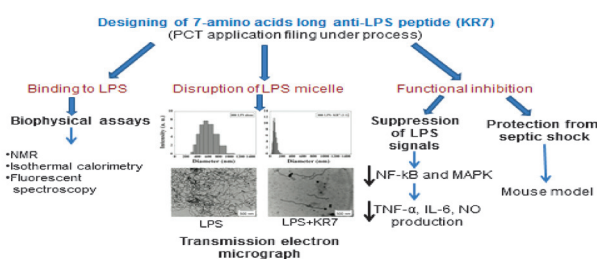


Fig. 7. Designing of a new peptide which binds and degrades lipo-polysaccharides: may be a potential therapeutic molecule against gram negative sepsis.

Mapping of STH prevalence in India

Modified Kato Katz technique for rapid identification of soil transmitted helminths (STH) has been developed, which is low cost and effective. Govt. of India (GOI) has taken the initiative under National Health Mission to assess the prevalence of STH in India. NICED has been chosen as an expert member of this core committee and nodal centre for Eastern and North Eastern states for providing training on Kato Katz technique followed by nationwide mapping of STH prevalence. NICED has completed prevalence mapping in Rajasthan, Madhya Pradesh, Uttar Pradesh, Chattisgarh, Telengana, Sikkim, West Bengal and Tripura.



Fig. 8. Mapping of STH prevalence.



Fig. 9. STH prevalence mapping. Dengue and Viral hepatitis.

In 2015, Kolkata and its suburbs witnessed a dengue outbreak. On request of state health Department, dengue serotype prevalence study was conducted by ICMR Virus Unit-NICED. Co-circulation of all four dengue serotypes was found with DENV3 being the major circulating strain (~64%) followed by DENV2 (~19%), DENV1 and DENV4 respectively.

Molecular evolutionary analysis of hepatitis B virus sequences at ICMR Virus Unit, Kolkata identified emergence of newer HBV strains (HBV/D3 ayw2 & HBV/D2) in Eastern India.

HIV/HBV co-infected patients experiencing long-term Lamivudine therapy as a part of ART, demonstrates progressive increase in viral failure associated with the drug resistance, along with high frequency increase in vaccine-escape mutants. This necessitates use of another anti-viral active against HBV in the ART regimen, which has been communicated to the State Health Authorities. Phylogenetic analysis shows new hepatitis C virus subtypes (1c, 3e, 3k, 6h, 6k) emerging in Eastern part of India.

Laboratory Containment of Wild Polio Viruses in India

NICED extended full support to the National Task Force (NTF) of Govt. of India for laboratory containment of wild polio virus. Compliances to guidelines set by the NTF are maintained in all laboratories of NICED. A meeting was organized on August 14, 2015 to sensitize researchers from various Institutes of eastern India on the importance of the compliance with Phase-II containment programme guidelines as derived from WHO Global Action Plan III. This meeting was important for an effective implementation of “Global Polio Eradication and the Endgame Strategic Plan (PEESP) (2013-2018), which refers to the strategies on sequential global withdrawal of OPV2 from trivalent live attenuated OPV (tOPV) to make bivalent live attenuated OPV (bOPV). The OPV2 is scheduled to be withdrawn globally in April 2016.

Capacity building, Training and public outreach programs

Field training for human resource development at remote hilly areas of North-East India

NICED being a NACO Regional Institute for HIV Surveillance, provides support to the East and North-Eastern states to fight against HIV/AIDS. Scientists imparted training to the state health officials, high risk individuals and field research agency investigators for Integrated Biological & Behavioural Surveillance (IBBS) to capture Real-time, Computer Assisted Information and Biological Samples from targeted groups at Kohima, Nagaland.

District-wise assessment of nutrition status among school children was carried out by NICED, epidemiology division. On request of Secretary, Dept. of School Education, Govt. of West Bengal, a series of ‘Dissemination Workshops on Malnutrition’ was organized in every district to sensitize the district authorities and other personnel involved in school meal program to improve the nutritional status of school children.

On request from West Bengal State Health Department, NICED Influenza Lab provided

“Hands on training on Molecular diagnosis of Pandemic Influenza A/ H1N1 viruses” to State Health Virologists and Technicians from School of Tropical Medicine Kolkata for establishing additional H1N1 testing facility in the State.

NATIONAL INSTITUTE OF MALARIA RESEARCH, NEW DELHI

Therapeutic efficacy of antimalarial drugs

The joint NVBDCP-NIMR surveillance system for monitoring the therapeutic efficacy of antimalarials used in the national programme with several innovations has been developed since 2009. Under this system, fifty three studies have been conducted where 3391 number of uncomplicated falciparum malaria patients have been recruited for the efficacy study of Artesunate plus sulphadoxine-pyremethamine (AS+SP), while 448 number of *P. falciparum* patients have been recruited for Artemether-Lumefantrine efficacy studies at six sites in North-eastern region of the country. In addition, 696 number of *P. vivax* patients have been enrolled under nine efficacy studies of chloroquine conducted in vivax endemic sites.. The results of these studies guided the national policy for malaria and based on observed high treatment failure of recommended ACT (AS+SP), the National drug policy on malaria for Northeastern states has been changed to Artemether+ lumefantrine (AL) for treatment of uncomplicated *P. falciparum* malaria since 30th April 2013. However, artesunate plus sulphadoxine-pyremethamine (AS+SP) remains the first line of treatment for uncomplicated *falciparum* malaria in rest of the country. The studies conducted till 2015, showed >95% PCR corrected efficacy at 48 sites after 28 days follow up and between 90-95% at 2 sites. Only 3 sites in NE region showed efficacy below 90% during 2012. The efficacy of AL remains >95% at all the six sites, so far. The efficacy of chloroquine in *P. vivax* malaria has been observed as 100% at 9 sites. Molecular markers of partner drug sulphadoxine-pyrimethamine (single nucleotide polymorphisms in *dhfr* and *dhps* genes) are also showing increasing trend over the years. Overall the study indicates high efficacy of AL (100%) at all the three sites in NE region, while the efficacy of AS+SP at 7 sites ranges between

94.4-100% during 28 days follow during 2015-16. The monitoring of clinical efficacy of anti malarials and mapping of artemisinin resistance will provide information to the programme to adopt evidence based decisions on drug policy in the country.

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 morbidity 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. The outcome will be available for planning effective malaria control strategies by the NVBDCP and for designing appropriate National Malaria Control Policy.

Establishment of a WHO-recognized Laboratory for Quality Assurance of Malaria RDTs

NIMR is carrying out lot testing of malaria RDTs procured by NVBDCP since 2009, following the WHO FIND SOPs. Panels of parasitaemia of 2000 and 200 parasites/micro liter are prepared from endemic areas. The laboratory passed the proficiency testing for microscopy, PCR and lot testing and also the assessments, based on which WHO recognized the laboratory as a lot testing lab. Now this facility at NIMR is one of the four global referral labs. The labs provide quality assurance of RDTs used in the country by the programme. The laboratory was inaugurated on 7th March 2016 by Regional Director, WHO-SEARO, New Delhi.



Fig. 10. Director NIMR & scientists involved are receiving WHO recognition certificate from Regional Director WHO, Dr. Poonam Ketrpal Singh.

Comprehensive Case Management of Malaria

This operational research study is being carried out in collaboration with Government of Odisha 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.

The following additional services are provided in the intervention block namely the introduction of microscopy at the PHCs, use of patient cards for follow up, buffer stocks of rapid diagnostic tests and ACTs at the CHC level to avoid stock outs and training of ASHAs to detect primaquine-related adverse events. In areas with poor presence of ASHAs, services of alternative service providers are being used.

CCM 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 prevented complications. The time from onset of fever to treatment has decreased with the larger proportion receiving treatment within 24 hours of onset of symptoms. The study provides important information to the programme to improve the implementation of interventions and pharmacovigilance and planned elimination activity more effectively.

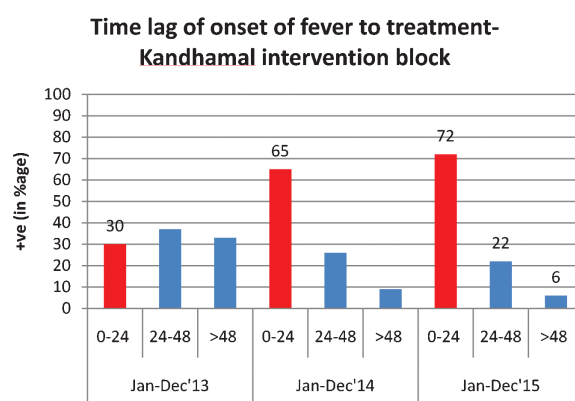


Fig. 11. Time lag of onset of fever to treatment Kandhamal Intervention block.

Sentinel site for dengue

NIMR Delhi is one of the sentinel sites for diagnosis of dengue. In the year 2015, total of

3534 samples were tested for dengue, of which, 935 were confirmed dengue cases. About 70% of the total dengue cases reported in the month of September. Serotyping performed in 18; serotype 2 was detected in 17; while as one sample had serotypes 2 and 3.

Health Impact Assessment

Health Impact Assessment of Narmada Basin Dams and Resettlement & Rehabilitation colonies in Madhya Pradesh initially started in 2004 in 3 major dam areas in MP, was extended further for 5 years in 2010 to cover entire Narmada Basin. Under this, at three study centres viz. Jabalpur, Bhopal and Narmada Nagar, entomological, parasitological and microbiological (water quality) studies were undertaken to identify problems related to vector borne diseases in the affected area of Narmada Basin. Mitigation measures were suggested to NVDA and State Health Department i.e. de-weeding, introduction of larvivorous fishes, channelization of pools in main river and larvicidal spray to control the breeding. Health camps were organized involving Health Department in Narmada Basin area for the awareness of vector borne diseases and their possible controls. Due to proper implementation of interventions by NVDA, State health department and other stake holders, in the study areas, 29 dams reduction in malaria cases was observed and no outbreaks of malaria and other vector borne diseases were reported.

Follow up of dengue cases was also carried out. Of the 580 cases contacted, 42 required hospitalization. No death was reported. Vector surveillance also carried out in houses of 101 dengue cases. *Aedes* breeding was observed in 14 houses in solid waste, plastic containers, OHTs, bird pots, coolers etc. The observations provided vital information on the disease prevalence and transmission.

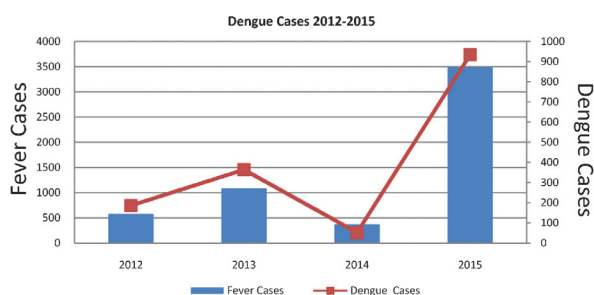


Fig. 12. Dengue cases 2012-2015.

Surveillance for dengue

An epidemiological study on Dengue was undertaken in Delhi which included active surveillance and vector surveillance. Serological tests (NS1, IgM&IgG Rapid Diagnostic Test) were conducted on family members for Household Index and Neighborhood Index case study (around 50 index cases) from 18 localities among High, Medium and Low income group of Delhi. Proportion of asymptomatic cases estimated to be 63% among individuals of all 18 localities of Delhi. With respect to three population groups, proportion of asymptomatic infections in localities of HIG, MIG and LIG were 60%, 56% and 70% respectively.

Field validation of bottle assay for monitoring insecticide resistance in adult mosquito vector species

The bottle assay method developed by NIMR was validated for routine insecticide resistance monitoring of lab and field mosquito vector species. The study was validated at VCRC, Puducherry and the NIMR field units at Raipur, Bengaluru and Chennai using laboratory/field collected mosquitoes. Insecticide solution and bottles (without impregnation) for the assay were provided to the investigators by NIMR. Validation was done for the determined diagnostic dosages of 10 µg/bottle for anopheline species and 2 µg/bottle for *Aedes* species. The assay will be standardized for other insecticides and synergists and validated in field and adapted for large scale use.



Fig. 13. Monitoring insecticide resistance.

Immunology supplement studies under Centre for the study of complex malaria in India

The primary aim of this study is to describe the serological profile of naturally acquired immune responses in malaria positive individuals collected at three eco-epidemiologically diverse sites of the Centre for the Study of Complex Malaria in India (CSCMi) using protein microarray technology. NIMR probed protein microarrays covering 500 unique *P. falciparum* and *P. vivax* protein features with 96 malaria positive plasma samples (79 from symptomatic and 17 from asymptomatic individuals; 68 *P. vivax* samples, 25 *P. falciparum* and 3 mixed species samples) and 69 age and gender matched malaria negative samples from the clinic and cross-sectional studies at the Nadiad and Chennai CSCMi field sites. Results show: (1) overall sero-reactivity to *P. vivax* antigens is higher than reactivity to *P. falciparum* antigens; (2) asymptomatic individuals show higher reactivity to both *P. vivax* and *P. falciparum* antigens compared to symptomatic individuals and (3) individuals with mixed species infections show minimal reactivity to both sets of antigens.

Dynamics of gametocytogenesis among *Plasmodium falciparum* isolates from areas of seasonal malaria transmission: correlation with antimalarial drug resistance

A total of 91 field samples have been collected and analysed for the study. 45 isolates were successfully adapted and further processed for various *in vitro* studies and the molecular analysis for the other non-adapted samples were also carried out. Gametocyte production was studied in these 45 isolates and successful production was seen in 19 isolates. The samples were successfully analyzed for *msp1msp2* and for *glurp* loci for classification of the allelic families prevalent in the isolates. The samples were initially screened for *Pfs25* gametocyte gene and then also further analysed for *Pfg377* gene another important gametocyte gene. The expression levels ranged from 0.11-9.12 and 0.08-30.37 fold for *Pfs25* and *Pfg377* gene on comparison with the reference isolate NF54 by RT-PCR. The allele typing of *Pfg377* gene in the isolates revealed four different genotypes viz Type A, B, C and D varying in sizes, between 280 and 400 bp with the most common

allele being 350bp. The cryo preserved and adapted samples of the same isolates showed difference in their genotypes on allele typing indicating the presence of multiple clones in the same sample on sequence comparisons. The isolates were also analysed for the presence of mutant alleles in drug resistance genes. Several mutant alleles were observed viz: *crt-K76*, *mdr-1 N86* and *Y86*, *dhfr-C59*, *S108* and *dhps-K540* in these isolates. A trend in the parasite population indicative of emerging resistance for the antimalarial drugs in use currently in ACT, also multiclonality prevalent by typing of the gametocyte gene, family grouping markers was observed. It is important to analyse the functional role of *Pfg377* gene to understand its role in transmission. NIMR was able to detect gametocytes in most blood samples isolated from patients during *P. falciparum* infection by a sensitive PCR detection method.

Proteases as drug targets for malarial parasite, and structural-functional analysis of falstatin, an endogenous inhibitor of malarial cysteine proteases

Metacaspases (MCAs) are important enzymes in malaria parasite, which are absent in humans and differing significantly from the orthologous human caspases. Therefore MCA offer a new potential drug target for anti-parasitic chemotherapeutics, which needs biochemical characterization to support the discovery of innovative drug candidates. Metacaspases (MCAs) are cysteine proteases with structural similarity to caspases and a catalytic cysteine and histidine dyad. In caspases cleavage site is aspartic, but in MCA it cleaves Arg/Lys. In case of *Trypanosoma*, using RNAi technology, it has been showed that silencing metacaspase gene caused severe growth retardation. In Malaria parasite three types of MCAs; MCA-1, MCA-2 and MCA-3. Previous report suggested that MCA-1 has role in DNA fragmentation in *P. falciparum* and KO of PbMCA-1 suggested that absence of loss of function in Pb MCA-1 deficient parasite. The functional activity of recombinant enzyme has been tested using different known fluorogenic substrates, Z-GGR-AMC and Z-GRR-AMC. MCA-2 cleave both the substrates efficiently further suggests that the purified recombinant enzyme is active.

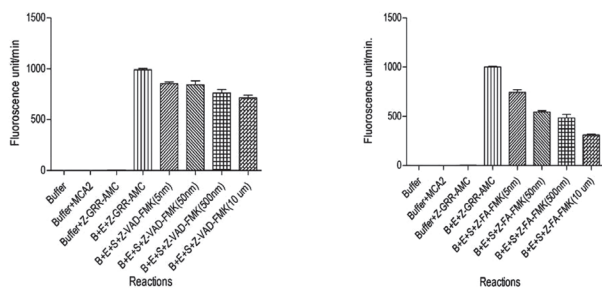


Fig. 17. MCA2 cleaved fluorescent substrate Z-GRR-AMC, and activity was inhibited Z-FA-FMK (Caspase -3 Inhibitor).

Capacity Building Activities

- Under Re-orientation training programme on strengthening of entomological surveillance in MCD training was imparted to 11 participants on 5th June 2015 (funded by MCD)
- WHO sponsored workshop on Malaria Vector Insecticide Resistance Monitoring and Management in India, in collaboration with WHO – SEARO, WHO- at National Institute of Malaria Research (ICMR), New Delhi from 7-11 Dec 2015 was organised 18 entomologists from different Indian states, 4 Institutional participants from India and 2 participants from Bhutan attended the workshop.



Fig. 18. NIMR Capacity building activities.

- Training to Master trainers to control vector borne diseases in Simhastha Mela during April-May, 2016 in Ujjain, Madhya Pradesh was imparted to 20 participants from 11 – 15 Jan, 2016 (funded by MP Govt.)
- Training on basic entomological techniques with reference to malaria elimination in Bhutan to Mr. Tenzin Wangdi, Senior Entomologist, Vector Borne Disease Control Programme - Bhutan as APMEN Thematic Fellowship on

Insecticide Resistance, NIMR, Delhi 11 Jan to 19 Feb 2016

- Training on basic entomological techniques to participants from Tamil Nadu, Thiruvavur, Tamil Nadu at NIMR, Delhi from 23-24 Feb 2016.
- Five days hands on training to Laboratory technicians from 9 states for conducting antimalarial efficacy studies during 23-27th May 2015 at NIMR, New Delhi.

VECTOR CONTROL RESEARCH CENTRE, PUDUCHERRY

LYMPHATIC FILARIASIS

Development of electrochemical based biosensor for detection of lymphatic filarial parasite, *Wuchereria bancrofti*, in vectors

A miniaturized version of Electro Chemical-biosensor, comprising an assembly of DNA probe sensitized screen printed electrode, a miniaturized potentiometer and signal detector, was developed as a tool for the detection of filarial parasite DNA in vector mosquitoes for the first time. Real samples were tested in six experiments using the device and it was found that impedance signals of the real samples were highly reproducible. Further improvisation will lead to a portable miniature detector.

Comparative evaluation of a new test strip against the currently available ICT for the detection of filarial antigenemia in humans

Immuno Chromatographic Card Test (ICT) is being used in TAS for detecting circulating filarial antigenemia in children. Compared to ICT, the new test namely Filariasis Strip Test (FST) is reported to have a longer shelf life and be cheaper. However, its diagnostic ability needs to be assessed under Indian situation to arrive at a critical threshold for stopping MDA before being recommended for use in TAS in the national programme. This study aims at comparing the stability, sensitivity and specificity of the test strip against ICT under laboratory conditions and making a comparative assessment of the new test strip against the currently available ICT for TAS in two EUs. The new FST showed high rates of sensitivity and specificity and could detect about

67.3% more positives compared to ICT. The agreement between the tests was more than 99% with strong tests lines, but with weak positives, the agreement was only 33%. The positive results were stable. Test readings after 10 minutes showed 28.7% more positives by FST while it was 40% by ICT as the negative test results at 10 minutes turned to be positives afterwards. The higher levels of detectability by FST compared to ICT will have implications in taking decision for continuing/stopping MDA in the EUs.

Morbidity Management and Disability Prevention Programme (MMDP) for filarial lymphoedema: Assessment of impact and impediments

MMDP continues to be a challenge in ELFP in India in terms of assessing the clinical impact of limb hygiene and sustaining the MMDP in the community. Interim observations on a cohort of lymphoedema patients showed that there are perceptible improvement in colour (17.2%) and texture (20.8%) of the lymphoedema legs in patients practising limb hygiene regularly. In addition, more than 10% reduction in intertrigo prevalence was observed on follow-up.

Development and validation of sampling strategies for xenomonitoring of infection in *Culex* vector by PCR as a surveillance tool for assessing post-MDA situation of lymphatic filariasis elimination programme

Transmission Assessment Survey (TAS) is the recommended strategy for stopping / continuing the mass drug administration (MDA) and for monitoring post-MDA period of programme for LF elimination. VCRC has developed and validated a rapid and cost-effective xenomonitoring protocol (gravid trap for vector collection, PCR assay for detecting vector infection, and sampling strategies) based on the survey in one of the primary health centres (PHC) in Thanjavur district, Tamil Nadu. This could identify the low level of infection following eight annual rounds of MDA. The district level validation of this tool as an alternative/supplement to TAS for detecting 'hotspots' and for tracking long term changes in vector infection with human infection after stopping MDA is in progress.

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

The Simulation model 'LYMFASIM' for LF transmission and control was used to estimate the required duration of MDA and the residual infection levels one-year after the minimum required rounds of MDA for different transmission settings (baseline levels) and MDA coverage. The minimum required rounds of MDA increased with higher baseline endemicity and lower MDA coverage (varying between 2 to 12 rounds). Residual infection levels one-year after the required rounds of MDA, though independent of coverage, declines with transmission intensity. The simulation results indicate that to achieve elimination in high transmission settings, MDA must be continued for at least 12 years and levels of Mf and Ag-prevalence must be reduced to 0.1% (Mf) and 2.0% in children (6-7 years).

Prediction and evaluation of antigenic determinants of proteins of *Wuchereria bancrofti*

Prediction of antigenic determinants of the cuticular collagen 2 protein of *Wuchereria bancrofti* resulted in the identification of three epitopes of 37-72 aa. Four peptides of 11-20 aa length were synthesized and evaluated for their immunogenicity for the specific diagnosis of *Wuchereria bancrofti* infected individuals with an aim of developing an antigen assay. Among the four peptides, peptide CCP2 and CCP4 showed higher immunogenicity at a concentration of 3.0 ug. Improved specificity of the assay could be obtained when secondary antibody-peroxidase labelled anti-human IgG (IgG4) was used in place of anti-human IgG (whole molecule) in the assay protocol. Further evaluation is in progress.

Development and demonstration of strategies to enhance community compliance for MDA in Palakkad district of Kerala state (EM 1511)

Palakkad district with 29.8 lakhs population showed persistence of infection above 1% in six out of 8 sentinel/spot check sites even after 9 rounds of mass drug administration (MDA) and a gap of 30% in compliance was observed in the 9th round. An intervention study with 2 arms having

2 different inputs with involvement of VCRC and one arm with only programme activities without involvement of VCRC has been carried out. Baseline microfilaria surveys were conducted jointly with the Health Dept of Kerala, in 9 randomly selected sites which showed at least 2 PHCs each in each of the arms with >1% Mf prevalence. Participant observation during MDA showed that there were lacunae in both programme delivery as well as social mobilization. The results of in-depth surveys in 29 PHC/municipalities indicated that among 3806 individuals interviewed, coverage of drug distribution and consumption were below the target level of 85% and 65% respectively. The post intervention assessment is in progress and the preliminary survey of MDA assessment showed that the optimum level of 80% of drug coverage and 65% of drug compliance has been achieved in the hot spot areas and need to be sustained. A marked increase of above 25% was observed in hot spot as well as non endemic areas in coverage of drug distribution, consumption and compliance respectively, from the pre intervention round of MDA supporting the effectiveness of intervention. Qualitative and quantitative studies are ongoing in 21 PHC areas to understand the effect of intervention. Impact assessment will be carried out 6 months post MDA.

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

The VCRC, Puducherry is collaborating with RMRC, Port Blair in an implementation research study to assess effectiveness and operational feasibility of mass DEC fortified salt as a supplementary intervention to MDA towards elimination of the lone foci of diurnally sub-periodic *W. bancrofti* in Noncowry Islands of Andaman & Nicobar Islands. Baseline data collection on mf prevalence, antigenemia prevalence among children and salt usage pattern of the community has been completed. The Tamil Nadu Salt Corporation has supplied the DEC fortified salt and the DEC-salt distribution is in progress since November 2015.

MALARIA/LEISHMANIASIS/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

Comparative assessment of the impact of combo vector control LLINs, plus indoor residual spraying (IRS)] versus single measure (only LLIN or IRS) on malaria transmission was carried out in Koraput district of Odisha State, where the state NVBDCP has already been implementing intervention measures. The study consists of three Arms viz., Arm 1, 2, and 3. In Arm 1 and Arm 2, LLINs were distributed and in Arm 1 and 3, yearly two rounds of indoor residual spraying with DDT has been carried out; thus Arm 1 is with two intervention measures. Vector parameters and incidence of malaria did not differ significantly between the three arms over the study period of two years. Coverage and compliance of the two intervention measures were not adequate in the three arms. The insecticidal effect of the LLINs reduced from 97% to 77% in Arm 1 and Arm 2. Therefore, in order to achieve the desired impact on vector parameters and malaria incidence, the coverage and compliance are enhanced through further strengthening of information, education and communication (IEC) activities. The final outcome of the study would clarify whether the combo vector control provides added benefit over the measure implemented singly.

Scrub Typhus: Establishment of disease and vector surveillance to assess the extent of disease occurrence and vector prevalence

To generate evidence for the endemicity of scrub typhus infection in Gorakhpur District, studies were initiated on the prevalence of scrub typhus vectors/rodent hosts and the pathogen, *Orientia tsutsugamushi* in areas reported for human cases in Gorakhpur. VCRC carried out two entomological surveys in 13 villages (6 blocks) of Gorakhpur District. A total of 2282 chigger mites (*Leptotrombidium deliense*) were collected. The overall chigger index was 13.8, which was well above the critical level of chigger index (0.69

per rodent) indicating the receptivity of the area. Out of 50 rodents screened, 20 were positive for antibodies against the scrub typhus pathogen (*O. tsutsugamushi*) in Weil Felix test using OXK antigen. One of the shrew mice (*Suncus murinus*) was positive for Weil Felix test as well as by PCR assay, indicative of presence of reservoirs and potential for transmission to human. However, the possible association of the human cases and vector abundance is to be established by more number of entomological surveys, especially in areas reporting human cases of scrub typhus.

DENGUE/KFD/JE

Research-cum-intervention project on JE/AES - Vector control to minimize the risk of transmission of JE in Gorakhpur District

In the research cum intervention study on JE in Gorakhpur District, initiated in 2013, baseline data on vector density, blood meal index and minimum infection rate were generated. Blood meal analysis has shown that the JE vector *Cx. tritaeniorhynchus* predominantly feeds on cattle. For JE virus detection in mosquitoes, a total of 3480 mosquitoes (in 176 pools) from Campierganj and Belghat intervention blocks of Gorakhpur district and Majhgawa control block in Deoria were collected and analyzed. A total of 8 pools in the intervention blocks and 7 pools in the control block were positive for JEV. MIR for intervention and control blocks was 4.59 and 4.03 respectively. Indoor Residual Spray (IRS) with Lambda cyhalothrin 10% WP and use of Long Lasting Insecticidal Net (LLIN) are the two intervention methods planned. Materials required for these measures are procured and is in the implementation stage.

Preliminary studies on Kyasanur Forest Disease virus in ticks and antibodies in rodents in potential risk areas of adjoining States to Karnataka

A total of 4403 tick specimens belonging to 8 species of genus *Haemaphysalis*, two species each of genus *Amblyomma*, *Boophilus*, and *Rhipicephalus* and one species of genus *Ixodes* was collected. In all the districts surveyed *Haemaphysalis spinigera*, the primary vector of KFD virus was the predominant tick species collected. *Haemaphysalis turturis*, the other major vectors of KFD virus

was also recorded in all the districts surveyed and constituted 7.7% of the total tick specimen collected. Other *Haemaphysalis* species collected were *H. bispinosa*, *H. intermedia*, *H. cupsidata*, *H. wellingtoni*, *H. aculeata* and *H. pupua kinnaeri*. *Amblyomma* species formed 5.6% of the total ticks collected. All the tick samples (pooled in 213 vials) were labeled and coded and sent to the NIV, Pune for laboratory testing for evidence of KFD virus infection. High abundance of *H. spinigera* and *H. turturis*, the major vectors of KFDV in the above forest ranges indicate that the areas are highly receptive for KFD outbreak in future.

Faunistic studies on the diversity and distribution of mosquitoes of the high altitude Himalayan regions of Himachal Pradesh and Jammu & Kashmir

Mosquito faunistic survey was carried out in Jammu & Kashmir to document the species diversity. A total of 1238 mosquitoes comprising of 34 species in 10 genera were recognized. Thirteen species were collected in Ladakh division, which included 7 species of *Culex*, 1 species of *Aedes*, 3 species of *Ochlerotatus* and 2 species of *Culiseta*. Of the 13 species, 7 species namely, *Aedes stenoetrus*, *Cx. modestus*, *Cx. pusillus*, *Cx. quinquefasciatus*, *Oc. caspius*, *Oc. pulchriventer* and *Cs. indica* were first time records from Ladakh. In Kashmir division, 22 species were collected, of which 5 species, namely, *Cx. bitaeniorhynchus*, *Cs. niveitaeniata*, *Oc. oreophilus*, *Lutzia halifaxi* and *Coquillettidia perturbans* were first time records. The record of *Cq. perturbans* is significant as it is a new country record for India. In Jammu division, 11 species were collected of which *Cx. minutissimus* and *Malaya genurostris* were first time records. Voucher specimens of all the species collected have been deposited in the mosquito museum at VCRC.

MICROBIAL/CHEMICAL AGENTS FOR VECTOR/PARASITE CONTROL

Development of monoterpenes extracted from the seeds of *Trachyspermum ammi* as macrofilaricidal composition

Two macrofilaricidal monoterpene combinations from *Trachyspermum ammi* MCT-6 and MCT-7 were formulated as self-emulsifying drug delivery systems (SEDDS) which could improve

the oral bioavailability of poorly soluble drugs by improving the solubility and maintaining the drug in a dissolved state, in small droplets of oil, all over its transit through the gastrointestinal tract. The preliminary experimental results showed that the formulation was effective in killing the adult female filarial worms in infected *M. unguiculatus* when the drug (MCT-6 SEDDS) was given orally at 100mg/Kg body wt. However, at higher concentrations of 300mg/Kg body wt and 500 mg/Kg body wt the drug was not showing activity. Further *in vivo* animal experiments will be carried out in CDRI, Lucknow, in view of the limited facilities at VCRC.

Development of naphthoquinone analogues as macrofilaricidal agents

Out of eleven naphthoquinone (NPQ) analogues found promising as macrofilaricidal agents all the targeted 11 compounds have been synthesized, purified by column chromatography, purity checked by TLC and HPLC, analysed the chemical structures by FT-IR, ¹HNMR and ¹³CNMR spectra, screened for *in vitro* macrofilaricidal activity and studied the *in vitro* ADME properties such as chemical stability, aqueous solubility, permeability, plasma protein binding and metabolic stability in comparison with standard drugs. Based on the ADME results six compounds *viz.*, TR-NPQ 1, 2, 4-7 needs to be evaluated for *in vivo* macrofilaricidal activity. These compounds have to be sent to CDRI, Lucknow to get it evaluated because of the limited facilities at VCRC.

Isolation and development of newer microbial agents for vector control

Bacillus cereus VCRC-B540 isolated for the first time from the gut contents of marine fish (*Lutjanus sanguineus*) for the control of larval stages of mosquito vectors. The molecular characterization of mosquitocidal toxin i.e. surface layer protein (SLP) and sequencing, amplification, cloning and expression of toxic encoding genes were completed. However, safety aspects of the strain have to be studied before developing this strain further.

Optimization of upstream and downstream process for the production of mosquitocidal metabolite(s) by an indigenous bacterium

Bacillus amyloliquefaciens and assessment of its anti-microbial activity

A mosquitocidal bacterial strain, *Bacillus amyloliquefaciens* has been isolated and its metabolite is relatively more active against *An. stephensi* followed by *Cx. quinquefasciatus* and *Ae. aegypti*. The metabolites of the bacterium were found to exhibit anti-microbial activity. Methicillin resistant *Staphylococcus aureus* (MRSA) & Vancomycin resistant Enterococcus (VRE) were found to be sensitive to the crude metabolites of *B. amyloliquefaciens*. PCR assay was standardized for all antimicrobial genes reported from *B. amyloliquefaciens* (VCRC B483) namely, Iturin, Bacilysin, Macrolactin, Bacillaene and Difficidin. The gene responsible for antimicrobial activity is yet to be determined.

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 developed for an Aqueous Suspension mosquito larvicidal formulation, using the indigenously isolated bacterium, Bti (VCRC B17) has been transferred to 12 commercial firms till date. Hence, it is essential to generate the DNA sequence data for this strain which would be useful for monitoring its presence in the applied sites. Primers were designed based on the chromosomal nucleotide sequences of the taxonomical marker genes, 16s rDNA, 23s rDNA and *rpoB* and amplification of complete genes with fragment size of 1680 bp, 3191 bp, 3585 bp achieved. Sequencing of these gene fragments is in progress.

Phase III evaluation to compare insecticidal efficacy and household acceptability of Icon Maxx, a long-lasting treatment for nets, with conventional insecticide treated nets in India

- The National Vector Borne Disease Control Programme has been distributing long lasting insecticidal nets (LLINs) in malaria endemic areas since 2009. In this context, information on net availability, durability of insecticidal efficacy of the available nets and physical integrity of LLINs and community

usage at different time intervals of their use were generated facilitating the programme to plan net replacements to maintain universal coverage for an effective and sustainable malaria control strategy. Adequate IEC activities are necessary to increase the net use rate and avoid incorrect washing practices.

- Four to five years of field use of synthetic pyrethroid treated LLINs in the southern districts, viz. Rayagada, Nowrangpur, Kalahandi, Malkangiri and Koraput of Odisha State has resulted in the development of triple resistance in *An. culicifacies* to DDT, malathion and deltamethrin. Hence, there is a need for developing resistance management strategy by appropriate rotation of different groups of insecticides and incorporating a synergist with synthetic pyrethroids for treating mosquito nets for the control of malaria vectors in these areas.

FACILITIES/NEW INFRASTRUCTURE

Preparation and supply of insecticide impregnated papers (IIP) for determining susceptibility of vector mosquitoes to insecticides

Procedures for preparation of insecticide impregnated papers has been standardized at VCRC to establish National facility for supplying insecticide impregnated papers (IIPs) conforming to WHO standard for monitoring resistance/susceptibility in malaria vectors to different insecticides used in the National vector control programme. The papers were evaluated at NIMR New Delhi and Institute of Vector Control and Zoonoses, Hosur.

Biomedical Informatics Centre of ICMR

The Biomedical Informatics Centre was established in VCRC in 2014 with multiple objectives covering multidisciplinary research areas. The VectorInfo database has been covered with comprehensive genomic and proteomic data resources for arthropod vectors of public health importance in India. The role of three *kdr* mutations of Voltage Gated Sodium Channels such as L1014S, L1014H and L101F were studied in conferring insecticide resistance using computational approaches. Potential antigenic epitope regions were predicted from the cuticular collagen protein of *Wuchereria*

bancrofti, synthesized and are being evaluated *in vitro*. Presently the Vectorinfo website is ready to be launched in the public portal to make it available to the public.

PATENTS/PRODUCT PATENTS OBTAINED/FILED DURING THE YEAR

- Process patent on 'Fly ash based mosquito larvicidal formulations of *Bacillus thuringiensis* var. *israelensis*' filed in Bhutan, Myanmar and Nepal.
- Process patent (# WO 2014141304 A1) granted for 'A process for the production of Cyclosporin-A using the fungus *Tolypocladium* sp. strain NRRL no. 18950'.

NATIONAL INSTITUTE OF VIROLOGY, PUNE

Influenza surveillance and diagnostic services

An upsurge of influenza activity was observed in January-March 2015 when 830/2402 (34.55%) referred samples were tested positive. Pandemic A (H1N1) contributed to 98.31% while influenza type B and H3N2 contributed to 1.45% and 0.24% respectively. Analysis of isolates (n=31) and clinical samples (n=816) for neuraminidase inhibitor drug susceptibility by real time PCR, NA gene sequencing and phenotypic assay revealed that two clinical samples had reduced sensitivity to Oseltamavir. Overall, 1399 (29.44%) of 4752 clinical samples referred to NIV for diagnosis were positive for influenza. Of the positives, 28% were influenza A H1N1pdm09, 0.3% influenza A/H3N2 and 1.4% influenza B respectively. Forty five influenza A/H1N1pdm09 and 7 influenza type B isolates were obtained. Sixty six of the 670 throat/nasal swabs collected for ILI surveillance were tested positive; 55 (8.2%) for H1N1pdm09 and 11 (1.6%) for influenza type B.

Clinically diagnosed paediatric pneumonia patients were sampled and tested for different respiratory viruses. Out of 340 samples, 201 (59.11%) samples were found positive for different respiratory viruses. RSV-A was detected in 32.5% samples, Rhinovirus in 12.5%, Adenovirus in 6.7%, PIV 1,2,3 in 6.1%, H1N1pdm09 in 5.8% and hMPV in 3.5% samples.

Study of T regulatory cells in dengue infection

Profiling of T regulatory cells (Tregs), CD4+ and CD+8 T cells in dengue cases revealed significantly higher frequencies of Tregs in mild cases compared to moderate case, more evident in secondary infections; especially during post-defervescence. The levels of IL-6, IL-7, IL-8, TNF- α and IL-10 were significantly higher in moderate cases. IL-6 and IL-8 levels correlated negatively with Treg frequencies during post defervescence and in secondary infections. The results suggested that higher Treg frequencies may favour a beneficial outcome in dengue. Higher cytokine levels may contribute to disease severity by exerting an inhibitory influence on Tregs.

Potential of anti-capsid protein monoclonal antibodies to Chikungunya virus (CHIKV) to detect recent CHIKV infection

Monoclonal antibodies against Chikungunya virus (CHIKV) were developed and assessed for their use in the development of MAb based IgM capture ELISA (MAC ELISA) for detection recent CHIKV infection. Twenty nine MAbs were obtained; 11 had IgG isotype and recognized conserved Capsid protein of CHIKV. Using these MAbs, an IgM capture ELISA was developed. The new C MAb ELISA yielded 87.01% sensitivity, 100% specificity, 100% positive predictive value (PPV) and 94.47% negative predictive value (NPV) in comparison to the existing CHIKV MAC ELISA. The results indicate that anti C-MAbs has strong potential for use in CHIKV MAC ELISA and can replace the existing antibodies.

Immunological memory in mice immunized with hepatitis E virus (HEV) candidate vaccine

An indigenously developed hepatitis E candidate vaccine which showed immunogenicity in mice and non-human primates was characterized for memory further. Longevity of vaccine induced memory response in mice was assessed and found that anti-HEV antibody titers remained high till 60 weeks after immunization with the vaccine candidate (rNEp+ liposome based adjuvant). Trend analysis estimated the antibodies to persist for the entire life of the mice. Twenty-four wks post immunization, higher levels of memory B cells and detection of

HEV specific IgG secreting functional memory B cells established the pertinence of immunological memory in mice in response to indigenously developed hepatitis E vaccine candidate.

Assessment of diversity in group A rotaviruses and non-rotavirus enteric viruses in patients with acute gastroenteritis

During an attempt to isolate common and unusual group A rotavirus strains in cell culture and to determine their genogroups, isolation of a set of three rotavirus strains with unusual G-P combinations (G1P[4], G9P[4] and G12P[6]) was succeeded and in adapting the G9P[4] strain in MA-104 cell line at passage level 4 giving 2+CPE. Sapoviruses (SaVs) were detected at a frequency of 3.5% (9/266) in acute gastroenteritis cases. Circulation of GGI, GGII and GGIV that shared nucleotide identities between 92.8-98.5%, 95.5-96.3% and 94.8-95.2% respectively with prototype strains were detected. Thirty two out of 184 (17.4%) and 26 (12.5%) fecal specimens from acute gastroenteritis cases tested positive for EV and HPeV. Phylogenetic analysis of the strains revealed the presence of fifteen genotypes belonging to three different species (EV-A:2 [8.3%], EV-B:9 [37.5%] and EV-C:4 [16.7%]). E14 was the predominant type observed. Six different genotypes (HPeV1, HPeV2, HPeV5, HPeV7, HPeV8 and HPeV14) were identified while HPeV-1 (61.1%) was found to be the predominant type.

Detection and characterization of Adenoviruses associated with Kerato-conjunctivitis

Cases of conjunctivitis were reported from Pune (Maharashtra) India, and were clinically diagnosed as kerato-conjunctivitis. Fourteen of the 23 (60.8%) eye swab specimens tested positive for Adenovirus (AdV) by PCR using Hexon and penton genes. AdV8 (n=11), AdV3 (n=1), AdV4 (n=1) and AdV37 (n=1) were detected and reported for the first time among conjunctivitis cases from Maharashtra, India.

Measles

Laboratory support was provided to Maharashtra State 'measles outbreak- based surveillance' and 187 suspected measles outbreaks were investigated. Altogether, 916 serum samples were referred for

lab diagnosis of which 574 (62.6%) samples were confirmed as measles and 57 (16.6%) as rubella. Of the 387 serum samples referred from 19 local hospitals, 276 (71.3%) samples tested positive for measles and 15 for rubella. In addition, support was provided to the WHO Measles LabNet for genotyping of measles strains circulating in different parts of the country.

Community based Surveillance of viral diseases/syndrome in Janata Vasahat in Pune city, Maharashtra

Community based surveillance was continued in a slum area in Pune city with a population of 31489. The highest community incidence rate of ILI was 0.5 per 1000 persons per week in week number 42 (year 2015). Throat swabs were collected from 120 (27.1%) ILI cases. Viral or bacterial etiology could be detected in 31 (25.8%) cases. Influenza virus etiology could be detected in 23 (19.2%) cases [InfA (H1N1) in 8, Type B in 9, Inf (H3N2) in one]. Bacterial etiology of ILI was detected in 8 (6.5%) cases [Streptococcus Pyogens (1), Group G Streptococci (4), Group A streptococci (1) and B Haemolytic Streptococci (2)].

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

The project aims to undertake surveillance of AES hospitalizations among children <15 years for detection of viral etiologies; especially JE, Chandipura and enteroviruses. CSF, acute sera and convalescent sera were collected from AES cases and stool/rectal swabs were collected from enrolled AES cases along with controls. During 2015-16, screening of 634 patients was done. Among them, 133 were AES cases, of which 110 were eligible. Sixty-four provided consent for enrollment. Anti JEV IgM antibodies were detected in 4/60 CSF, 14/101 acute sera and 2/7 convalescent sera. All specimens were negative for anti CHPV IgM. CHPV RNA was negative for all CSF, but was positive in one acute serum by RT-PCR. All CSF and stool/rectal swabs were negative for enteroviruses by RT-PCR. Most of the AES cases and JE confirmations occurred during July-October 2015.

Avian influenza viruses

A simple, sensitive, user-friendly and cost-effective procedure has been developed which has potential application for detection of AI viruses from different water samples during outbreak investigations or surveillance studies.

Surveillance of viruses in bats

Circulation of Nipah virus, a highly pathogenic paramyxoviridae, in *Pteropus* bat population in Dhubri district, Assam and Cooch Behar district, West Bengal was detected during a survey in the North-East region of India. Tioman virus (TioV), a new member of the Paramyxoviridae family was also isolated from tissues of *Pteropus giganteus* bats collected from Dhubri district, Assam, for the first time in India. The virus was identified and confirmed by RT-PCR, electron microscopy and sequence analysis.

Core facility activities

Bioinformatics, Phylogeography studies based on envelope gene sequences of genotype III and genotype I of JEV, representing different states of India and other countries was carried out, to study the spatiotemporal transmission histories of these two JEV genotypes separately. The study revealed at least two independent introductions for both genotypes to India from Japan and China. Genotype III was found to have been successively introduced in the 1930s, 1950s and in 1960s, followed by genotype I twice around 2003-2006. The population dynamics of both genotypes studied through the Bayesian skyline plot indicates that though GI has currently emerged as the dominant genotype in Asia, the genetic diversity of GIII is showing a stable growth phase, rather than an expected decrease. Further, a relational database consisting of gene sequence information for Influenza A virus subtypes, queryable with respect to drug resistance mutations and secondary mutations is being developed. Protein structure modelling was done to understand the molecular basis of enhanced viral fitness induced by secondary mutations in the evolution of oseltamavir-resistant influenza strains, and co-evolutionary mutations in polymerase genes.

Electron Microscopy, As a part of ongoing extramurally funded research work on dengue 2

virus infection on vascular endothelial cells, the role of viral NS1 protein in affecting cellular adhesion and transcriptome physiology was studied. Initial studies suggest that exogenous exposure of this protein can affect cellular signaling systems important for vascular haemostatic functions. Ultrastructural studies on *Mycobacterium Tuberculosis* showed a novel cytoplasmic existence between avirulent and MDR/ XDR strains. High resolution EM-application base was provided to NIV and other researchers from national laboratories.

Experimental animal facilities - Breeding, Maintenance and supply of laboratory animals

During the year, a total of 2130 mice belonging to different strains were supplied under 29 IAEC approved projects to researchers at NIV. In addition, 910 ml of blood from different species of laboratory animals were supplied for in house diagnosis and research. The laboratory animals (mice) were randomly sampled and tested for microbial and genetic contamination at ACTREC, Navi Mumbai. Similarly, randomly collected fecal samples from the colony were tested for parasites. All the tests provided negative results. Testing of consumables (bedding material, feed, and drinking water samples) for microbial and chemical contamination revealed the results within permissible limits. Procurement, quarantine and conditioning of 10 juvenile rhesus macaques was accomplished and husbandry and veterinary care was provided to 41 rhesus monkeys at the primate enclosure of the institute.

RESEARCH STUDIES AT FIELD UNITS

GORAKHPUR UNIT

Diagnosis of Japanese encephalitis infection in AES patients from Eastern Uttar Pradesh:

During 2015, 1719 clinically confirmed AES cases were hospitalized in BRD Medical College and Nehru Hospital, Gorakhpur with 425 (24.7%) deaths. JE IgM ELISA based diagnosis was provided to clinical specimens referred from 1606 patients, of which 129 (8.03%) patients tested JE positive. A total of 39 deaths (30.2%) recorded among the 129 JEV positive cases. JE infection was documented in all age groups but the 5-15 year age group was mostly affected with 97/129

(75.19%) JEV associated deaths. Among the total JE positive cases, 78.29% cases were from the pediatric age group (up to 15 years of age) while 21.71% cases belonged to adult age group. Most of the AES cases were reported from Gorakhpur, Kushinagar, Maharajganj, Deoria, Siddharthanagar, Sant Kabirnagar, Basti and other districts of eastern UP along with bordering areas of Bihar. Among the JE positive cases hospitalized in BRDMC, 81.4% cases were from different regions of UP while 18.6% cases were from different districts of Bihar situated in the border region.

Investigations of clinical specimens collected from hospitalized Dengue fever cases

Human clinical specimens (whole blood / PBMC) collected from 155 hospitalized cases diagnosed as Dengue patients by commercially available Dengue NS1 Antigen Micro-ELISA were referred for confirmation. Dengue virus serotype 1-4 specific diagnostic RT-PCR amplified dengue genome in 110 sera. Further investigations confirmed amplification of Dengue 2 genome in most of the cases clearly indicating it as the primarily associated serotype in Gorakhpur region.

KERALA UNIT

Development of NS1 protein capture ELISA for detection of West Nile virus infection

Recombinant West Nile virus envelop protein based indirect ELISA has been standardised. The ELISA cross reacts with Japanese Encephalitis and Dengue virus specific IgG in the samples. Thus this ELISA can be used as a diagnostics for detection of flavivirus IgG in the sample.

Vector surveillance

Tick survey carried out in different dairy farms in Alappuzha during summer season showed that *Haemaphysalis* sp was the predominant species in the surveyed areas. Morphological identification of mosquito larvae collected from coastal areas in Alappuzha revealed the presence of 5 species: *Ae. albopictus*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus*, *Cx. bitaeniorhynchus* and *Anopheles subpictus*. These larvae tolerate a wide range of temperature, pH and salinity, which showed the adaptation of these mosquitoes in a wide variety of habitats.

NIV, Bengaluru

Outbreaks investigated under the WHO measles and rubella programme showed some outbreaks independent of measles, rubella and mix, serologically. Genotyping of measles viruses showed mostly D4 and D8 genotypes in circulation. Under NVBDCP, in some parts of Bangalore urban and rural areas both Dengue (50%) and Chikungunya (10%) were serologically confirmed. Sporadic cases of Japanese encephalitis virus were also laboratory confirmed. Routine program activity under WHO surveillance network for polio and acute flaccid paralysis was also carried out.

Human resource development

VRDL: Training imparted to 99 participants of 21 VRDLs on 05 different modules, including one training for 26 participants on Zika virus preparedness

Training program on Biosafety for India and SEAR countries

- WHO sponsored training program on “Biosafety preparedness to handle infectious materials in laboratory settings” was conducted for staff of Medical Research Centre, Sri Lanka at NIV, Pune, during March 2015.
- WHO sponsored training program on “Engineering aspect of Biosafety level 3 laboratory” was conducted for staff of Public Health Laboratory, Bhutan at NIV, Pune, during April 2015.
- Onsite training program on “Biosafety preparedness to handle infectious materials in laboratory and hospital settings” was conducted at Krishna Institute of Medical Sciences, Karad, Maharashtra in May 2015.
- Regional workshop on quality management system (QMS), biosafety and biosecurity practices in laboratories in the WHO South-East Asia Region was conducted for SEARO countries from 24-28th August 2015.
- Onsite training support was provided to PHL, Bhutan and NPHL, Nepal by Dr. Mourya and Dr. PD Yadav during WHO consultancy assignments.

- Biosafety preparedness to handle potential bio-hazardous material in laboratory setting, NDTL, New Delhi 19 -20th March 2016

Besides conducting the routine M.Sc. virology course (21 students graduated in 2015 and 21 were enrolled), PhD programs and scientific workshops, were also carried out successfully.



Fig. 19. Biosafety training at Karad Medical College Maharashtra ICMR.

PUBLIC HEALTH IMPORTANCE

Outbreak investigations and support

- **Kyasanur Forest disease virus:** Diagnosis of referred human and monkey necropsy samples and tick pools received from Karnataka and Kerala for Kyasanur forest disease (KFD) was carried out. Kyasanur Forest Disease outbreak was investigated in Sindhudurg, Maharashtra, in March 2016. Detection of anti KFD IgG antibodies in population suggested that the infection was old but KFD was confirmed for the first time in Maharashtra.
- **Crimean Congo haemorrhagic fever, Rajasthan:** Timely diagnosis led to the confirmation of a nosocomial outbreak of Crimean Congo hemorrhagic fever (CCHF) in a private hospital in Jodhpur, Rajasthan during January 2015. Genetic analysis revealed the introduction of a new CCHFV strain which has the highest nucleotide sequence identity with Afghanistan strains. This provided evidence for the disease prevalence in this state that needs to be under surveillance.
- **EBOLA threat:** During the Ebola threat, NIV served as apex laboratory. Individuals coming from Ebola affected countries were screened

at airports and suspected individual samples were referred to NIV for Ebola diagnosis. All the samples were tested at NIV and found negative except for a semen sample.

- **Dengue outbreaks:** During 2015, 3223 samples were screened for dengue (DENV) and chikungunya (CHIKV) by MAC-ELISA. Of the 2637 suspected DEN cases, 1260 (47.8%) samples tested positive for DENV specific IgM antibodies while 252 of 586 (44.4%) samples tested positive for CHIKV specific IgM antibodies. The study shows the resurgence of CHIKV during the year with positive cases reporting from Maharashtra, Gujarat and Delhi (57/170). Among the DENV serotypes in circulation, Pune and Delhi had DENV-2&4; rest of Maharashtra had DENV-2&3 and Gujarat had DENV-3&4. Circulation of DENV-4 was detected in Pune, Delhi and Gujarat during 2015.
- **Diagnosis of referred samples from AES cases:** Diagnosis of AES cases was carried out by testing CSF and serum samples for presence of anti IgM antibodies and neutralizing antibodies against JEV and CHPV. During 2015, a total of 220 samples were referred to NIV (encephalitis group) from Maharashtra, Karnataka, Tamil Nadu, Madhya Pradesh and Gujarat. Anti-JEV IgM antibody was detected in 11/218 cases. CHPV diagnosis was mainly established by RT-PCR detection. CHPV activity was confined to Gujarat with 11 RT-PCR positives and one virus isolation out of 38 CSF samples screened. Screening of AES samples referred by Department of Health Services, Epidemiology and Disease Control Division of Nepal demonstrated JEV IgM antibody positivity in 8/25 samples and anti CHPV IgM in 1/11 samples. Anti CHPV neutralizing antibodies were detected in five of ten serum samples tested.
- **Hepatitis outbreak in Shimla, Himachal Pradesh:** There was a massive outbreak of Jaundice in Shimla during Dec 2015-Feb. 2016. NIV team collected blood samples from Jaundice patients and water samples from

different points. IgM anti-HEV antibodies were detected in patients indicating HEV to be the etiological agent. All water samples were positive for HEV. Comparative analysis of virus sequences from patients and water samples showed 100% identity suggesting mixing of effluent water and lapses in the chlorination treatment to be the major cause of this outbreak.

TECHNOLOGY DEVELOPMENT/ TRANSFER:

Six ELISA based technologies transferred to M/S Zydus Cadila, Ahmedabad (2015)

- Development of recombinant protein-based assay for diagnosis of hepatitis E
- Development of IgM assay for detection of anti KFDV antibodies
- Development of IgG assay for detection of anti CCHFV antibodies in Sheep and Goat
- Development of IgG assay for detection of anti CCHFV antibodies in Cattle
- Development of IgM assay for detection of anti CHPV antibodies
- Development of Mab based antigen capture ELISA for detection of Japanese encephalitis virus from mosquito.

Technology transfer to HBL Biotech, Chennai for development of inactivated vaccine against Japanese encephalitis virus

- Candidate Japanese encephalitis virus vaccine (JEV) strain was transferred and MoU signed with HBL Biotech for inactivated vaccine development against JEV vaccine.

Diagnostic services rendered to the country: 42,766 samples were tested for 33 viral diseases.

JEV/DENV/ CHIKV MAC ELISA kits supplied under the National program: Total 6969

- JE: 515 (412 supplied to NVBDCP and 103 supplied to WHO-SEAR)
- DEN: 5408 (5397 supplied to NVBDCP and 11 supplied to WHO-SEAR)

- CHIK: 1046 (1035 supplied to NVBDCP and 11 supplied to WHO-SEAR)

product patent filed/ obtained during the year:

- “Composition for virus precipitation from dilute samples and a method thereof” Indian patent application no. 160/MUM/2015 dated July 09, 2015 by Director, NIV, Pune
- “Composition for precipitation of avian viruses and a method thereof” Indian patent application no. 201623016520 dated May 12, 2016 by Director, NIV, Pune (Patent of addition for main patent 160/MUM/2015 dated July 09, 2015)
- Patent application (PCT application no. PCT/IN2014/000441) “RNAi agent for inhibition of Chikungunya virus” has been filed in Australia (date Dec. 22, 2015), China (date July 2, 2014), USA (Dec. 17, 2015) and France (Feb. 2, 2016).



Fig. 20. Visit of Hon. Union Minister of Health & Family Welfare, Shri. JP Nadda to NIV Pune, Feb. 2015.



Fig. 21. Visit of Dr. S Swaminathan, Secretary DHR & DG ICMR to NIV BSL4 Lab, December 28, 2015.



Fig. 22. Training program being carried out at the RCVDRL

CENTRE FOR RESEARCH IN MEDICAL ENTOMOLOGY, MADURAI

Barcoding of mosquito fauna of public health importance in Tribal area of Tamil Nadu and creation of a vector DNA sequence database

A total of 15 species of mosquitoes belonging to the genera *Culex Anopheles*, *Armigeres*, *Toxorhynchites*, etc., which are vectors and non-vector to different infectious diseases were collected from the tribal areas in the Nilgiris, Western Ghats, Tamil Nadu. Each individual mosquito was identified by conventional taxonomical methods and processed for DNA extraction. The mitochondrial CO I gene region was amplified by PCR and the PCR product was visualized and sequenced. The SNPs were identified with multiple DNA sequence analyses. The analysis provides first-hand information on the DNA barcode of Indian mosquito vectors.

Development of recombinant NS1 antigen based diagnostic kit for acute dengue virus infection

Dengue virus was propagated in cell lines and RNA extraction and RT-PCR assays were carried out. The amplified PCR products were utilized in the cloning of dengue virus NS1 gene in the expression of virus protein for the development of indigenous diagnostic kit. The recombinant protein was used

for the development of monoclonal antibodies. The clones were successfully developed by fusion of recombinant viral protein immunised mouse spleen and mouse myeloma cells.

Molecular characterization of Chikungunya viruses circulating in vectors in Tamil Nadu

This study was undertaken to unravel the persistence pattern of CHIKV/DEN viruses in vector mosquitoes. Vector mosquitoes were collected from Nagercoil and Chennai and processed for virus isolation by inoculating in C6/36 cell lines. The inoculated cells were screened for CHIKV and DEN virus antigen by IFA using virus specific monoclonal antibodies. Further, RT-PCR reactions were carried out for virus gene amplification, sequencing and sequence analysis.

Estimation of endemicity of dengue in Nilgiris district, Tamil Nadu

Dengue prevalence study was undertaken in the Nilgiris district to find out the occurrence of dengue in the tribal pockets. A total of 111 samples were collected from Kotagiri, Coonoor, Ooty and Gudalur blocks of Nilgiris district and 6.3% were found to be dengue positive. Dengue positive cases reported from Nilgiris district showed slightly higher percentage in females (57%).

Studies on the occurrence and resurgence of dengue and malaria vectors in Nilgiris and adjoining hill areas of Western Ghats

Ae. albopictus was predominantly recorded in the breeding habitat in and around tribal areas. In pre-DDT era, *An. fluviatilis* was the dominant species but recent surveys show predominance of *An. culicifacies*.

Field bio-efficacy evaluation of DRDO Defender Net against mosquitoes

The field bio-efficacy of insecticide impregnated DRDO nets were evaluated for their efficacy against mosquito vectors for JE and filariais. It was found that the insecticide impregnated nets were highly effective against the vector mosquitoes, such as *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* compared with non-treated nets.

Research-cum-intervention project on JE / AES in Gorakhpur

In Gorakhpur district, 15 villages from three blocks (Khorabar, Bhathat and Chargawan) were selected for entomological studies including dusk collection, outdoor and indoor resting collections *etc.* to secure samples for vector incrimination and blood-meal identification. In dusk collections, *Cx. quinquefasciatus* was the predominant species followed by *Ar. subalbatus* and *Cx. tritaeniorhynchus*. There was an increase in the abundance of vector mosquitoes from the month of July which remained high up to November. Indoor residual spray using Lambda Cyhalothrin 10% a.i. WDP (Packet in Sachet 125 gm) was carried out in Bhathat Block.

Role of *Anopheles subpictus* along with other Japanese encephalitis vectors in JE virus transmission in Tirunelveli District

JE virus transmission in Tirunelveli was studied. There were no JE cases in recent years after the outbreak in 1973, though JE virus circulation in mosquitoes was noted. Besides the characteristic feature of 'silent transmission', another important trait emerged is the involvement of *An. subpictus*. This fact however needs more thorough investigation.

Biological control of dengue/chikungunya vector *Ae. aegypti* larvae by using *Bradinopyga geminata* (Anisoptera: Libellulidae) larvae in a limited urban area, Madurai

The 12th instar larvae of *Bradinopyga geminata* were used to control larvae of *Ae. aegypti* in limited urban areas in Madurai. The predation experiments revealed that *B. geminata* is a good predator of *Aedes* larvae. The predator potential and consumption rate was high in cement containers than plastic containers.

Understanding the biology of Chikungunya virus infection in permissive cell lines and mosquito vectors

Oral infection of Chikungunya virus due to *Ae. aegypti* and *Ae. albopictus* has been standardized followed by real time RT-PCR assay to confirm

infection. Virus overlay protein binding assay (VOPBA) was carried out first with *Ae. aegypti* midguts and then with the whole mosquitoes. CHIKV interacting protein bands from both species of mosquitoes (~140kDa) were excised from the gel and submitted for MALDI-TOF/MASCOT analysis at C-CAMP, Bangalore.

NATIONAL AIDS RESEARCH INSTITUTE, PUNE

EPIDEMIOLOGY

Estimation of Hepatitis C virus disease burden in India

Hepatitis C virus (HCV) infection differs from other chronic viral infections; it can be cured by treatment. Newer HCV therapeutics are reported to cure > 90% HCV infections. A study was undertaken to obtain national and regional seroprevalence estimates of HCV infection in general population and high-risk group populations with the support from the World Health Organisation and National AIDS Control Organisation. A total of 450000 samples [dried blood spots (DBS) samples from high-risk populations from IBBS and 300000 serum samples from ante-natal clinic (ANC) attendees] collected for HIV sero-surveillance are being tested for anti-HCV immunoglobulin G antibodies using 3rd generation ELISA kits at State and National Reference laboratories across the country. NARI was entrusted the task of testing all HIV infected samples from IBBS and HIV negative samples sent to it for quality control. At NARI 6582 DBS samples [3900 HIV-positive and 2682 HIV-negative] samples were tested. The preliminary analysis of this data showed HCV positivity among HIV infected individuals belonging to at risk sub-population in India is as 42%. HCV positivity of 2.7% in HIV-negative samples from high-risk populations was observed. In the 2170 serum samples tested from ANC attendees (general population) HCV positivity of 0.1% was observed. The regional and group-specific analysis is ongoing. These findings were utilised by the National AIDS Control Program to develop a new free anti-HCV treatment strategy for HIV infected individuals in India which would be implemented after procurement of medicines.

MOLECULAR EPIDEMIOLOGY

Near Full-Length Genomic Characterization of a Novel CRF 01_AE/C Recombinant from Western India

The HIV epidemic in India is primarily driven by subtype C, although sporadic circulating and unique recombinant forms are also reported. We report a novel CRF01_AE/C recombinant from an HIV infected 32-year old heterosexual female from Pune, India who was on antiretroviral therapy for 12 months. This circulating recombinant form (CRF) has been reported from Thailand and Cambodia. This CRF is known to be associated with rapid emergence of immunologic failure. Interestingly, the husband of the index case was also HIV infected and had died earlier. Both had never travelled outside the country. The emergence of a novel recombinant of CRF01_AE/C is indicative of the increasing genetic diversity of the HIV epidemic in India.

Non-vertical, Non-sexual Transmission of HIV in Children

Two paediatric cases of HIV infection whose mothers were uninfected were subjected to investigations. *Case 1: HIV transmission from Grandmother to grandson, child of a HIV seronegative couple:* A 3.5 years old child was detected HIV positive on 07th April 2015 and confirmed with Western blot on 09th April 2015. The CD4 count was 388 cells/mm³ and viral load was 191,688 copies/ml on 18th April 2015. Both parents were working and were HIV negative while his grandmother who was the primary caregiver at home was found to be HIV positive. *Case 2: Father to child HIV transmission:* A 4 year old male child, born on 7 April 2007 was found HIV positive. Mother of the child was tested and found HIV negative whereas father of the child was HIV infected. In both the cases, investigations were done to rule out alternative modes of HIV transmission. Blood samples from both, the grandmother and the child were genotyped for HIV-1 pol gene and phylogenetic analysis was done for possible transmission linkages. Phylogenetic analysis of pol sequences from both cases (grandmother-grandson and father-son) showed phylogenetic clustering with posterior probability of 1.0 which confirmed the possible transmission linkage from the caregiver in case 1 and father in case 2.

SEXUALLY TRANSMITTED INFECTIONS

Relation between Genetic Markers of Drug Resistance and Susceptibility Profile of *Neisseria gonorrhoeae* strains

This ongoing study aims to study the association of specific gene mutations in *N. gonorrhoeae* strains with resistance to different antibiotics i.e. tetracycline, penicillin and fluoroquinolones. Forty Five *Neisseria gonorrhoeae* clinical isolates from patients from different parts of India [23-Delhi, 20-Hyderabad and 2-Mumbai] were collected in the reporting year, making a total of 111. The findings of this study suggest that no change in National treatment guidelines for *N. gonorrhoea* is required.

HIV BIOLOGY

Identification of HIV modulated cell signalling pathways in context with persistence of HIV after activation

In vitro model of HIV latency using memory CD4 cells infected with HIV-1 Indian subtype C primary isolate was established to study gene expression pattern by Real Time PCR after TCR (T cell receptor) stimulation to determine mechanisms contributing to persistence of these cells after HIV reactivation. The results showed that the reactivated infected cells of the model expressed lower levels of cytokines as compared to uninfected cells suggesting their compromised functionality. The reactivated cells showed increased CD27 (long-term memory marker), CD28 and ADA (responsible for induction of telomerase activity) while decreased FAS expression indicating their enhanced survival capabilities by preventing apoptosis and senescence. Phenotypic characterization of P24+CD4 cells from HIV infected patients by flow cytometry confirmed higher CD27 and telomerase expression on P24+ CD4 cells compared to HIV uninfected (P24-ve) CD4 cells before and after TCR stimulation indicating their possible role in their long-term survival of these cells.

Antibody-Dependant Cellular Cytotoxicity (ADCC): a new frontier in vaccine research

Anti-HIV antibody mediating Antibody Dependant Cell Cytotoxicity (ADCC) are known to be important in governing the protection and slowing

down the disease progression. Little is known about the ADCC responses across the HIV clades and the frequently recognized antigenic regions by ADCC antibodies that could be associated with slow or no disease progression. Recognition of such region would have implication in HIV cure as well as prevention. Hence the present study is carried out to characterize the ADCC responses in HIV-1 subtype C infected Indian LTNP and identify the dominant antigenic regions recognized by these antibodies along with their ability to lyse reactivated latent cells. The ADCC responses were measured in 34 LTNP and 58 progressors against overlapping HIV-1 peptides encompassing HIV-1 C and B Env, Gag, Pol and Rev, Tat, Nef and Vpu antigens using NK cell activation assay. Twenty of 34 LTNP (58.8%) and 20/58 progressors (34.4%) showed ADCC responses against at least one of the HIV antigens. The LTNP showed significantly higher ADCC responses against HIV-1 C Env and Gag as compared to the responses as well as in its breadth observed in the progressors. Twenty one percent of LTNP showed responses against more than 3 antigens as compared to 7% in progressors. Among LTNP showing ADCC responses, the percentage of female LTNP showing ADCC responses were significantly higher as compared to the males. Indian LTNP identified epitopes from the conserved (C1, C2, C4 and C5) region, from the variable (V3, V4 and V5) region and also from few CD4 binding sites. The Env C V3 region (a.a.288-330) was preferentially identified by Indian LTNP (5/12 Env C responders). None of the progressors identified this epitope ($p=0.0002$) indicating probable role of ADCC antibodies against this epitope in virus control. Three 'Tat' regions were also frequently recognized by Indian LTNP; The amino acid stretches ; a.a. 29-43, a.a.45-63 and a.a.77-101 were recognized by 3 (8.82%) , 4(11.6%) and 5 (14.7%) of LTNP. None of the progressors identified these regions. The reactivated cells showed increased CD27 (long-term memory marker), CD28 and ADA (responsible for induction of telomerase activity) while decreased FAS expression indicating their enhanced survival capabilities by preventing apoptosis and senescence. Recognition of these early proteins by ADCC antibodies from LTNP might have implication in vaccine design.

Role of SAMHD1 in HIV-1 infection of human Dendritic Cells

Among various host restriction factors, SAMHD1 ('Sterile Alfa Motif' domain and "Histidine-Aspartate" domain containing protein 1) is one host protein that has been identified to block the HIV infection of cells myeloid origin. A study was undertaken to show down regulation of intracellular SAMHD1 in the human monocyte derived DCs (MDDCs). The findings suggest down regulation of SAMHD1 followed by HIV-1 infection of the MDDCs. The CD4 as well as CD8 T cells stimulated in vitro using these MDDCs also showed HIV-specific Cytotoxicity by secretion of CD107a. The findings from this study might be useful in developing more effective interventions to improve HIV-specific T cell response.

ANTIRETROVIRAL THERAPY

Antiretroviral Therapy (ART) for HIV prevention among Heterosexual Couples: is durable and highly efficacious [Final results from the HPTN 052 study]

This decade long multicentric HPTN 052 study, in which 175 HIV-1 serodiscordant couples were enrolled at NARI, was completed in the reporting year 2015-16. ART reduced HIV acquisition among HIV negative partners of persons living with HIV (PLHIV), by 93% at the end of the entire ten year study, confirming the durability of the interim results and are similar to the 96% reduction announced in 2011. These findings have been adopted by the World Health Organisation in their antiretroviral therapy guidelines for adults.

HIV drug resistance genotyping in patients failing on second line treatment

A cohort of 170 patients on second line antiretroviral therapy was followed in a collaborative study between NARI and Banaras Hindu University. It was found that the mortality rate was higher at the end of first year (13.7 per 100 PY) as compared to that between first and fifth year (3.88 per 100 PY) after initiation of second line ART. Drug resistance genotyping was successfully done on 16 samples where 8/16 of samples showed mutations associated with high level drug resistance to protease inhibitors (PI) and 10/16 had minor PI

mutations. Among PI mutations, M46I (n = 5) was the commonest mutation, followed by N88S (n = 3), I50L (n = 2), I84V (n = 2), V 82A (n = 1). M184V was the commonest NRTI mutation and was present in 13 out of 16 patients. 10/16 patients had ≥ 3 TAM. 13/16 had more than 1 NNRTI mutation. Y181C and G190A was the most common NNRTI mutation present. However, 87.5% of those who failed continued to be harbouring virus susceptible to Darunavir. The proposed National third line regimen of Raltegravir and boosted Darunavir does not need any modification.

HIV Pretreatment Drug Resistance Among Adults

The success of first-line ART is influenced by prevalence of HIV pre-treatment drug resistance (PDR) in the population. We determined the prevalence of PDR among adults attending the state-sponsored free ART clinic in Pune in western India. Fifty-two individuals eligible for ART as per National guidelines were enrolled. Population-based sequencing of partial pol gene from plasma specimen revealed prevalence of PDR of 3.8% which is well within the WHO threshold limit of <5% and indicates successful implementation of free ART program. There were two individuals who had resistant mutations – V106M and K103N against NNRTI drugs. It may be a reflection of receipt of NNRTI drugs prior to seeking treatment from public free ART centre.

PEARLS Trial

Analysis from this complete multinational, multi-centric clinical trial revealed that presence of pre-treatment drug resistant mutations among 4.2% study participants. HIV-1 subtype C was associated with higher risk of early treatment failure (HR: 1.57; 95% CI: 1.04-2.37) as compared to HIV-1 subtype B (HR: 0.47; 95%CI 0.22-0.98). These findings are of public health significance in India as they indicate that efforts to prevent emergence of drug resistant HIV epidemic is critical.

IMPLEMENTATION SCIENCE RESEARCH

Validation of the GeneXpert HIV-1 Quant Assay for HIV-1 RNA estimation

GeneXpert® HIV-1 Quant assay was validated in comparison with the Abbott m2000rt Real Time

HIV-1. A total of 219 patient's plasma specimens falling in different viral load ranges (< 40 to > 5L cp/ml), 20 seronegative; 16 stored specimens (1, 2, 3 months storage) and 10 spiked copy controls (20 to 40 L cp/ml) were tested by both assays. The statistical analysis indicated that the GeneXpert® HIV-1 Quant assay compared well with the Abbott m2000rt Real Time HIV-1 with 91.3% concordance, good reproducibility, 97% sensitivity and specificity. With the ease of performance and rapidity, the POC GeneXpert® platform that is routinely used for detection of *M. tuberculosis* DNA, can be used for HIV-1 quantification to facilitate an integrated HIV and TB management. The assay can also be an important tool for scaling up the UNAIDS 90:90:90 initiative in resource limited settings.

Community Consultation for Newer Methods for Pre-exposure Prophylaxis (PrEP) and Participation in PrEP Clinical Trials

A consultation meeting to understand the awareness, knowledge and attitudes towards pre-exposure prophylaxis (PrEP) as a prevention intervention among TG community and community willingness for participation in clinical trials for PrEP. 18 Participants included 10 community representatives from Mumbai, Thane and Pune, persons working with transgender (TG) and men who have sex with men (MSM) community. Though 12 of these community leaders had heard of PrEP, detailed knowledge was less pervasive and some misconceptions existed. While 11/17 could state that it was used for HIV prevention, only one person could name the drug used for PrEP correctly. About four could correctly identify currently approved dosage schedule and two felt that there was no need for regular HIV testing while on PrEP. The group felt that feasibility studies would be important before programmatic implementation. It was suggested that the member of key population should not be required to go to ART OPD for PrEP. PrEP programming should be TI- centric (targeted intervention-centric). Many community leaders expressed a concern that their endorsement of PrEP may pose challenges for condom programming which is being done for a long time. In general, they felt that adherence to daily PrEP may be challenging but long acting injectable preparations will be more acceptable. In general the community was willing

to participate in trials. These findings are useful for research on PrEP and its programming.

Assessing willingness for sharing Aadhar number with health care delivery system as an identifier

This was a qualitative study carried out to study the community (normal individual vs patients with stigmatizing /non-stigmatizing diseases) perspectives on linkage of Aadhar number with universal health care service delivery. In this study, 36 in-depth interviews, 6 key informant interviews and 2 focused group discussions were conducted. There was no major resistance to use of Aadhar card in health care delivery system as an identifier, provided that the confidentiality regarding the information collected was assured. Key subpopulations of female sex workers and men having sex with men faced many difficulties in obtaining Aadhar. However, they were willing to share their Aadhar number with health care.

New Drug Discoveries

The acetone and methanol extracts of *Terminalia paniculata* (TP) (NCL-51 and NCL-52) and *Polygonum glabrum* (PG) (NCL-53 and NCL-54) that had shown anti-HIV1 activity previously were further studied for determining the mechanism of action using cell based (HIV-1 entry and fusion inhibition) and enzymatic assays (protease and reverse transcriptase inhibition). Three extracts (NCL-51, NCL-52 and NCL-53) showed inhibition of HIV-1 entry but did not inhibit HIV-1 fusion. In the enzymatic assays, two TP extracts (NCL-51 and NCL-52) inhibited enzymes reverse transcriptase and protease (at 25µg/ml). Subsequently, three leads identified (compounds B, C and D). Confirmation of the obtained leads is in progress.

JE/AES PREVENTION & CONTROL

Community Engagement for JE and AES Prevention and Control

Using the principles of community empowerment, this study aims at developing strategies to engage community in JES/ AES control in Gorahkpur district of Uttar Pradesh. A cross section survey among 1976 households were conducted. Only 47% reported first dose and 13% reported second dose of JE vaccination of the youngest child respectively.

Mothers of children who reported fever/ diarrhea/ convulsions, were less likely to access appropriate government facility as compared to village doctor [OR 0.86 (0.62 – 1.21)]. Mothers were more likely to access treatment for their sick children at government facility after considerable delay [OR 1.34 (0.95 – 1.9)]. Evidence based community engagement module to control JES/ AES in the endemic areas of Gorakhpur has been developed which is being tested. The intervention utilizes participatory and health promotion approaches. The module is developed in Hindi language. The modules aims at engaging community especially the marginalized community, empowering them, identifying their own hygiene needs, helping them access government resource, empowering them to take care of their own health.

SUPPORT ACTIVITIES FOR THE NATIONAL AIDS CONTROL PROGRAM

Integrated Biological and Behavioral Surveillance (IBBS) and HIV Sentinel Surveillance

Population	State/Region	No. tested	HIV Prevalence (%)
FSW	Gujarat, Goa	1982	1.1
	Karnataka	1534	5.8
	Maharashtra	1349	7.4
	India	27007	2.2
MSM	Gujarat, Goa	1954	6.8
	Karnataka	1950	4.1
	Maharashtra	2075	4.9
	India	23081	4.3
IDU	Gujarat, Goa, Maharashtra	1157	1.5
	India	19902	9.9

Source: IBBS 2014-15, NACO, Feb 2016

NARI as a Regional Institute (RI) supervised IBBS in four states Goa, Gujarat, Karnataka and Maharashtra, which included 54 survey domains for different typologies - 16 female sex workers (FSW), 16 men having sex with men (MSM), four each from transgender (TG) and intravenous drug users (IDU) and 14 for migrants. NARI trained the manpower and supervised the surveys in the states of Maharashtra, Gujarat, Karnataka and Goa. The

NARI team extensively reviewed both SFD and main survey data in real time using the online IIMS and provided timely feedback for corrective actions to the field research agency to ensure adherence to study protocol and generation of good quality data. IBBS database was reviewed and frozen in the month of December 2015, which was then used by NACO in preparing IBBS report. The results were disseminated by NACO in February 2016.

Role as a National Apex Laboratory

NARI as an Apex laboratory carries out number of activities approved in NACP IV, which include providing External quality Assessment for HIV serology & CD4 count estimation, monitoring quality control for HIV diagnosis and CD4 count estimation, training, analysis of EQA data, development and distribution of SOPs for HIV testing and CD4 count estimation and mentoring of SRLs in case of discordant results.

HIV serology: The network of laboratories consist of Apex lab (NARI), 12 NRLs, 117 SRLs and around 5000 ICTCs. HIV serology EQAS is conducted twice a year wherein proficiency panels are distributed from Apex lab to NRLs, NRLs to SRLs and SRLs to ICTCs. Two EQAS rounds (R1 & R 2) were conducted for the year 2015-2016. All 117 SRLs participated in R1 & R2. Two SRLs showed discordance in R1 and all SRLs showed 100% performance in R2. Around 4463 ICTCs participated in R1 and 4317 ICTCs participated in R2. In R1 16 ICTCs showed discordant results and in R2 6 ICTCs showed discordant results.

Confirmation of the HIV-2 diagnosis: Identification of types of HIV (HIV-1 or HIV-2 or both) is important because it has implications on the treatment regimen to be provided at ART centres. Data on 1825 samples (HIV 1+2 positive, HIV 2 positive only by one differentiating kits) received at HIV 2 referral laboratories during the period February 2013 to September 2015 was analyzed. Out of 1825 samples, 303 were found to be HIV-1, 1136 HIV-2 and 386 were HIV1+2.

Proficiency Testing Unit: NARI is NABL accredited as Proficiency Testing Provider as per standard ISO 17043:2010 for HIV Serology and CD4 count estimation. The PT provider unit of NARI provides EQAS to all CD4 estimating

laboratories linked to NACO ART centres. 257 centres were enrolled under CD4 EQAS program till the reporting year. Three rounds were conducted in the reporting year.

Consortium of NRL’s for Kit Quality Testing:

Consortium of four National Reference Laboratories (NRLs) namely National AIDS Research Institute (NARI), Pune; National Centre for Disease Control (NCDC), New Delhi; National Institute of Cholera and Enteric diseases (NICED), Kolkata and National Institute of Mental Health and Neurosciences (NIMHANS), Bengaluru provided evaluation of the diagnostic kits for the National Program. In the reporting year a total of 151 kits were received for evaluation. Out of these 151 kits, 115 kits were for HIV, 11 for HBV and 25 for HCV diagnosis and nine kits could not fulfill the criteria.

Patent

A Triaminotriazine piconitrile derivative developed by Dr. D.V. Mane, Shri Chhatrapati Shivaji College, Omega was tested at NARI. Two of these molecules had shown significantly higher anti-HIV1 activity (10 folds) than the control drug AZT. The molecules have been patented (PCT/IN2015/000386) and the patent “Triaminotriazine Picolinitrile Derivatives as Potent Reverse Transcriptase Inhibitor of HIV-1” has been published (WO 2016059647 A2) in April 2016.

HIV awareness and voluntary testing camp: A workplace intervention

NARI conducted an HIV awareness and voluntary counselling and testing camp in collaboration with Inner Wheel club Nigdi, for factory workers in Navalkhumbre, Tal. Maval, Dist Pune on 20th October 2015. This activity was conducted on the occasion of NARI foundation day (19 October).



Fig. 23. HIV awareness camp.

Sensitization cum training workshop on Hepatitis C testing for National Reference Laboratories

Under the survey for Estimation of *Hepatitis C* virus disease burden in India, a two day orientation cum training workshop on Hepatitis-C testing with dried blood spot samples for National Reference Laboratories (NRLs) was conducted at the National AIDS Research Institute, Pune on 23rd and 24th November 2015. Representatives (Laboratory in-charges and laboratory technicians) from all National Reference Laboratories (NRLs) attended the training and officials from National AIDS Control Organization and World Health Organization (WHO) attended the workshop.



Fig. 24. Dr. Nicole Seguy, Technical officer, STI/ HIV/ Hepatitis, WHO and Dr. Neeraj Dhingra, DDG for TI, NACO delivering talk on HCV infection.



Fig. 25. Dr. Soumya Swaminathan, Director General, ICMR & Secretary, DHR visit to NARI on 21st December 2015

Other activities

Institute celebrated Intranational Yoga Day on 18th June 2015. A talk by Swami Pragyapadji, Art of living, Bengaluru was organised on Yoga Day.



Fig. 26. International YOGA day 18th June 2015.

ENTEROVIRUS RESEARCH CENTRE, MUMBAI

Poliomyelitis Surveillance: 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. Polioviruses isolated from AFP cases from National Polio Laboratories from India as well as from Bangladesh, Myanmar and Sri Lanka are received at ERC for genomic sequencing for poliovirus identification as wild, Sabin-like or Vaccine Derived Polioviruses (VDPV).

In 2015, a total of 47243 cases of AFP were reported through AFP surveillance from India out of which 4800 AFP cases were reported from Maharashtra, Madhya Pradesh, Chhattisgarh and Goa. A total of 9621 stool samples from 4800 AFP cases were tested at ERC for the presence of polioviruses and other Enteroviruses. Polioviruses were isolated from 192 (4%) and Non-Polio Enteroviruses (NPEV) from 1331 (27.7%) AFP cases. Type 2 VDPV were detected from 2 AFP cases from Madhya Pradesh and Delhi. All poliovirus isolates were Sabin-like. However, no VDPV circulation was observed in Indian population. India continues to be free from wild poliovirus for the 4th consecutive year.

Environmental surveillance for detection of wild poliovirus: Environmental surveillance involving testing of sewage samples to supplement AFP surveillance provides information on wild and VDPV circulation in the absence of AFP cases. ERC has been actively involved in environmental surveillance in the three high risk blocks in Mumbai since 2001. The surveillance activity was expanded in 2015 with three additional sites in Mumbai and also in other parts of the country i.e. Delhi (2010), Patna (2011), Kolkata (2011) and Punjab/ Ahmedabad (2013).

In 2015, a total of 240 sewage samples (160 sewage samples in Mumbai and 80 samples in Patna) were tested for the presence of polioviruses. 11 type

2 VDPVs were detected from sewage samples collected from various sites in India (Mumbai: 3, Patna: 1, Delhi: 2 and Punjab: 5). Environmental surveillance, along with AFP surveillance confirmed the absence of wild poliovirus circulation in India during 2015.

Genomic analysis of iVDPV3 by classical and next-generation sequencing methods: Vaccine derived polioviruses (VDPVs) in the gut of immunodeficient patients have been found to exist as mixtures of genetic variants (quasispecies) due to persistent poliovirus replication. A VDPV type 3 isolate from an immunodeficient patient identified with Severe Combined Immunodeficiency (SCID) was studied using Next Generation Sequencing (NGS) and Sanger sequencing methods. 31 plaques purified from the isolate were tested by both methods. Mutations with frequency more than 90% in NGS were also detected by Sanger sequencing, whereas mutations with frequency less than 20% were least detected by the Sanger's method. NGS provided quantitative mutation analysis within the quasispecies and provided advantage over Sanger's sequencing resolving the variant sequences existing as mixtures.

Cross-sectional serologic assessment of immunity to poliomyelitis and measles in differential risk areas of India (India Seroprevalence Survey, 2014): Seroprevalence studies in high risk areas of Uttar Pradesh and Bihar have been regularly conducted at ERC in collaboration with WHO since 2007. Although wild poliovirus has been eradicated, seroprevalence studies are necessary to monitor the sustenance of immunity of children in those areas. 1110 serum samples from children belonging to age group of 6-11 months living in risk areas for polio transmission in India (Bihar, Madhya Pradesh and Mumbai) were collected to determine the neutralizing antibody titres against all three poliovirus serotypes. The study indicated remarkable increase in population immunity over the years.

PROVIDE study: Exploration of biological basis for underperformance of OPV and Rotavirus vaccines in India: A study in collaboration with International Vaccine Institute (IVI), Korea and NICED, Kolkata was undertaken at ERC to determine relationship between tropical enteropathy and immune response to oral polio and rotavirus

vaccines in children. A total of 372 children were enrolled from hospitals in Kolkata. The study included neutralization assays determining anti-polio antibodies in children before and after vaccination. The maternal serum samples were also tested for anti-polio antibodies. Faecal excretion of polioviruses was also detected for evaluating the vaccine efficacy. A total of 3312 stool and 1761 sera samples were tested at ERC out of which 324 samples were found positive for poliovirus.

A Phase III, randomized, double Blind Placebo controlled trial to evaluate the non-interference in immune response of three doses of ORV 116E to antigens contained in childhood vaccines and to assess the clinical lot consistency of three production lots: A study was undertaken at ERC to determine efficacy of co-administration of ORV116E developed by Bharat Biotech India Limited (BBIL) with other childhood vaccines (OPV, DPT, Hib and HepB) during routine immunization without interfering with the immune response developed by each vaccines. Neutralization assay of 1157 sera samples was performed to determine anti-polio antibody titres.

A Phase III, multicentric, double-blind, randomized study of Bovine Rotavirus Pentavalent Vaccine (BRV-PV) to evaluate lot-to-lot consistency and to investigate potential interference with routine UIP vaccinations in healthy infants in India: There is an urgent need to introduce an affordable efficacious rotavirus vaccine in the childhood immunization program in India. Serum Institute India Limited (SIIL) has developed a live attenuated bovine-human (UK) reassortant pentavalent rotavirus vaccine (BRVPV) for oral vaccination against human rotavirus gastroenteritis in infants. The potential interference of BRV-PV with UIP vaccines administered concurrently (OPV, DPT, Hib and HepB vaccines) was assessed for the immune responses to those vaccines when administered with/ without the study vaccine. ERC participated in the study to perform neutralization assays to detect anti-polio antibody titres against all three serotypes of poliovirus from a total of 440 sera samples.

Host virus interaction and the significance of apoptosis in infections by viruses causing Hand, Foot and Mouth disease (HFMD): A study has

been conducted to find out differential immune responses to three HFMD causing Enteroviruses using real time PCR, confocal microscopy and flow cytometry based apoptosis assays in human cell lines. The results of part of the study confirmed that CVA16 infects human neuronal cells and induces apoptosis by both extrinsic and intrinsic mechanism. Investigations undertaken with two strains of CVA6 isolated from AFP and HFMD cases respectively showed differential immune responses and apoptosis in human cell lines. The virus isolated from HFMD case was found to induce higher TLR-7, TLR-9 and Interferons as compared to the virus isolated from AFP case in human muscle cell lines. The results indicated that higher TLR-7 and TLR-9 activated during the infection induced release of Interferons to protect the host towards neuronal complications.

Studies on poliovirus infections in children with immune-deficiency: In the ongoing study to identify long term poliovirus and Enterovirus excretors among children with immunodeficiency, from 58 stool samples (n=29 children) tested for polio and other enteroviruses, one child with SCID was found to be positive for P3 VDPV and another child with HLH (chronic granulomatous disease) was found to be positive for P1SL. 5 years old Male child, a case of Leaky SCID was found excreting P3 VDPV since December 2014 and P3 VDPV has undergone 64 nucleotides changes which pose a grave threat to our society.

Enterovirus infections in healthy children from AES affected area in Gorakhpur district, Uttar Pradesh: Acute Encephalitis Syndrome (AES) has been a serious health problem in certain districts in eastern parts of Uttar Pradesh since last decade. Laboratory investigations suggested Enteroviruses (EVs) as one of the causes of AES cases. EV infections in healthy children in AES affected areas of Gorakhpur district, UP was studied to identify prevalence of EVs and their seasonal variation. The genetic diversity of circulating EVs was determined by isolating EVs from stool samples collected from 6274 healthy children below 5 years of age for 13 months. A very high virus isolation rate (49%; 3078/6274) was observed, out of which 72% children were below 3 years of age. EVs were isolated from majority of children (98.8%; 3042/3078), however Adenovirus and Human Parechoviruses were also

isolated from the remaining 36 children. The study revealed EV circulation with high diversity (87 out of 108 EV serotypes) in Gorakhpur area reflecting very poor sanitation in the AES affected areas.

Major Achievements having Public Health Importance

- No wild poliovirus was isolated from Acute Flaccid Paralysis (AFP) cases from AFP surveillance in India.
- No wild poliovirus was isolated from sewage samples collected in Mumbai, Delhi, Kolkata, Patna, Punjab and Ahmedabad from Environmental surveillance.
- Detection of Vaccine Derived Poliovirus 2 (VDPV2) from two AFP cases and 11 sewage samples from India.
- High levels of immunity in infants from high risk areas as well as areas with low routine immunization indicate success of the programme in sustaining population immunity.
- Containment of wild poliovirus 2 has been achieved successfully.
- Detection of long term excretion of type 3 VDPV in a Severe Combined Immuno Deficient (SCID) child in a collaborative study with NIIH and Wadia Children's Hospital.
- High diversity of non polio Enteroviruses in AES affected areas in Gorakhpur district, UP.

RAJENDRA MEMORIAL RESEARCH INSTITUTE OF MEDICAL SCIENCES, PATNA

The research outcomes of RMRIMS, Patna has empowered the Kala-azar elimination programme with improved intervention tools in terms of early detection, effective treatment, vector management, supervision & monitoring, capacity building and strengthening partnership apart from high-end basic research.

SURVEILLANCE AND EPIDEMIOLOGY

Role of private practitioners in Kala-azar case management

A sampled survey of private practitioners to assess their potential role in kala-azar treatment revealed

that under current scenario, only negligible proportion of patients (about 4%) preferred treatment from private practitioners, but on the other hand delayed treatment seeking (ranged from 35-110 days) at public set up warrants improved strategies for early detection and referral.

Development of PKDL in Kala-azar cases treated with single dose AmBisome

Post-treatment follow-up of 300 VL cases treated with SDA (10 mg/kg bw) revealed 3.3% (n 10) conversion into PKDL during 13-27 months post-treatment. The finding suggests that at least 2-years follow up of VL cases specially treated with SDA, the 1st line drug under programme mode, is needed for PKDL conversion.

DIAGNOSTICS

Novel noninvasive method for diagnosis of Kala-azar by rK39 test using oral fluid samples

As suggested by SAC, this test was performed on oral fluid samples collected from other diseased individuals viz. malaria, tuberculosis, leprosy and typhoid as well as healthy controls to confirm its specificity. Specificity was found to be 100%. The report has been submitted to NVBDCP for further line of action. Moreover, it has been identified as one of the innovations for exhibition at Rastrapati Bhawan on 16th March 2016. Communication in regards to IPR issue with rK39 manufacturing company (InBios) is under process.

Noninvasive PCR-based diagnosis of Kala-azar using urine samples.

The nested-PCR (minicircle of Kinetoplast-DNA) of urine samples of confirmed kala-azar cases and healthy controls revealed sensitivity and specificity of 90% and 95% respectively. After successful third party validation, the test performed on urine samples of other diseased patients viz. malaria, tuberculosis, leprosy and typhoid confirmed its high specificity.

THERAPEUTICS

Efficacy and safety of Amphotericin B in two different doses in treatment of PKDL

Altogether 50 fresh cases of PKDL were enrolled in the study, 25 in Arm-A (Amphotericin-B, dose

0.5 mg/kg) and 25 in Arm-B (Amphotericin-B, dose 1 mg/kg). After 3rd course of treatment, all patients were found parasitologically cured in both arms, however complete disappearance of lesion was observed in 89% in Arm A and 80% in Arm B. Post-treatment follow-up completed so far revealed complete cure after 3-months in both arms. Major cause of withdrawal (N=8) from study was raised blood urea and serum creatinine. Vomiting, abdominal pain, increased blood urea, serum creatinine, bilirubin, SGPT and SGOT were the main side effects. Follow-up of the cases till one year is in progress.

Efficacy and safety of Paromomycin and Miltefosine combination (two Vs three courses) in treatment of PKDL

A total of 30 fresh cases of PKDL were treated with Paromomycin (11mg/kg b.w.) for 10 days, followed by miltefosine (2.5 mg/kg b.w.) for next 10 days. Patients receiving two courses did not improve, hence all patients were treated with three courses. After 3rd course, all were found negative for L.d. parasite, though diminished macular lesions were observed in few cases. Post-treatment follow up revealed 2 relapse at 3-months follow-up and 2 at 6-months follow-up. All the 4 relapsed cases were treated with injection Amphotericin-B (1 mg/Kg, 15 injections alternated day) for three courses. Mild increase in serum creatinine was observed as the main side effect of the combination therapy.

BASIC RESEARCH

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

The cloned and expressed 15kDa protein (*LdPDI*) from *L. donovani* was found directly correlated with parasite growth. Stimulation of PBMCs of VL patients with *r-LdPDI* for 72 hours revealed gross impairment in trafficking of leukocytes (CD 62L shedding and CD11b up regulation), down-regulation of MIP 1- α , IP-10, IL-8, IL-12, CCR5, IFN- γ , and up-regulation of IL-10, which reverted after stimulation with cocktail of *LdPDI* (10 μ g/ml), Hsp70 (5 μ g/ml) and neutralizing IL-10(2 μ g/ml). *In-vivo* experiment in BALB/c mice demonstrated enhanced proliferation of spleenocytes and higher expression of IFN- γ in PDI DNA construct

immunized mice as compared to infected and vector SLA immunized mice. ERK1/2 phosphorylation, which is associated with IL-10 production, was lower in the macrophage in PDI DNA construct vaccinated animals. Immunization with the PDI DNA increased p38 MAPkinase. The finding suggests immunomodulatory role of r-LdPDI in VL infection. The PDI DNA immunization can evoke the protective immune response by suppressing disease promoting cytokines.

Screening of ornithine decarboxylase inhibitor along with immunomodulator for their role in immunity and therapeutic implication in visceral leishmaniasis

The 77Kda recombinant ornithine decarboxylase from *L. donovani* (r-Ld-ODC) induces parasite growth (both promastigotes and amastigotes). The r-Ld ODC stimulation induces CD4+Tcells cytokines (IL-10) whereas its inhibitor (DFMO) decreases IL-10 production & induces IFN. Experiments on anti-leishmanial activity of macrophages indicated down-regulated IL-12 pro-inflammatory response triggered by r-Ld ODC, with marked impact on the free radical (super-oxide and NO) generation in macrophages during VL infection. A defective IFN- response with elevated IL-10 secretion against r-Ld ODC, suggests that r-Ld ODC leads to the indispensability of IL-12, which may interfere in the induction of protective immunity and anti-leishmania defense during VL. Study is in progress to assess its role in pathogenesis and use of its inhibitor, DFMO, as therapeutic option in animal model.

Immunogenic and protective role of dendritic cell exposed KMP-11 antigen in Visceral Leishmaniasis

In-vitro study on host cells (*L. donovani* infected macrophage) revealed that treatment with

amphotericin-B (0.5µg/ml) either alone or in combination with KMP-11 (10µg/ml) reduced viability of monocytes by 10-15%. BALB/c mice were immunized with DC-primed KMP and challenged for leishmania infection. Splenic parasite burden reduced to 90.6% with enhanced T-cell proliferation in DC primed KMP-11 immunized mice. DC-KMP-11 immunization in response to r-KMP-11 protein showed about 6.38 fold more IFN-γ, 4.40 fold IL-12 and 3.26 fold TNF-α as compared to controls. Further, shift of immune response towards Th1 and 3.54 fold increased ROS production in splenic macrophage suggests role of ROS in KMP-11 mediated Leishmania killing. It was also observed that DC-KMP immunization enhances the protective responses by modulating the P³⁸MAPK phosphorylation.

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

There was higher accumulation of CD4+NKT cells in comparison to CD8+NKT cells towards infection site. Increased level of CCR3 in CD4+NKT cells (1.59 fold) shows survival of CD4+NKT cells and decreased CCR3 receptor (1.97 fold in CCR3+CD8+NKT cells) suggests suppressive activity of CCR3+CD8+NKT cells in VL. However, there was reduced expression of CCR5 in both CD4+ and CD8+ NKT cells. It was observed that *L. donovani* promotes MIP1β mediated migration of CCR3+ve cells which is more pathogenic in nature.

Involvement of Sir2 of *Lishmania donovani* in DNA damage and repair.

LdSir2 has potential role in regulation of cell viability possibly by repairing the damaged DNA (caused by MMS) and its ribosyltransferase activity. The level of ribosylation was found higher by Sir2

Table 2: Treatment and follow up details of the study subjects

Arm	Dose	Patients (N)	1 st course completed	2 nd Course completed	3 rd Course completed	Excluded from the study	Follow Up Visit	
							3 Month	6 month
A	0.5 mg/ kg/b.w	25	25	22	18	3	15	10
B	1.0 mg/ kg/wt	25	25	20	15	5	12	8
Total		50	50	42	33	8	27	18

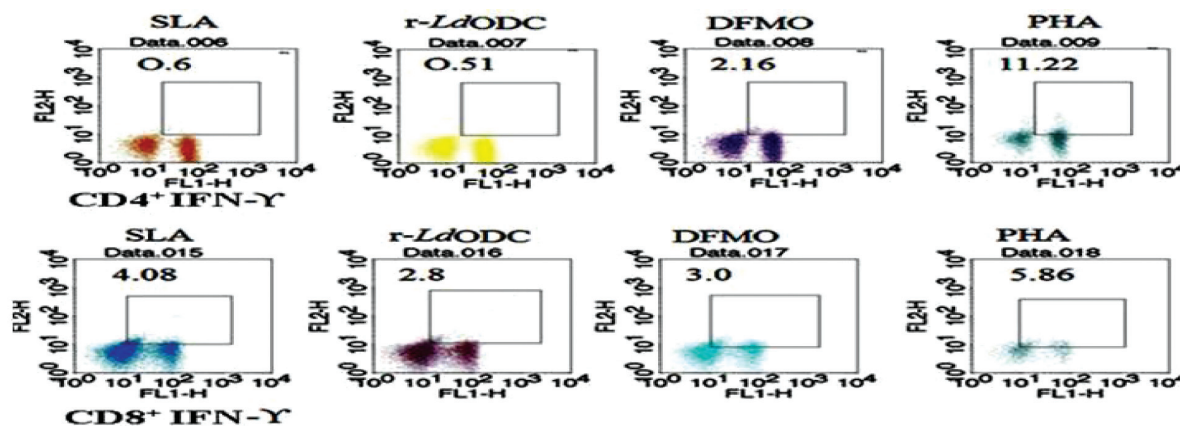


Fig. 27. NKT cells in visceral leishmaniasis.

in response to MMS (oxidative stress). Further, in presence of DNase, the digestion of chromatin was higher in MMS treated Sir2-over-expressed parasites and similar digestion pattern in the MMS pretreated Sir2-knockout cells as compared to the wild type cells. Thus Chromatin is more open when cells over-express Sir2. Therefore, it can be conferred that Sir2-over-expression induces more relaxed and open state of Chromatin which is necessary for DNA repair.

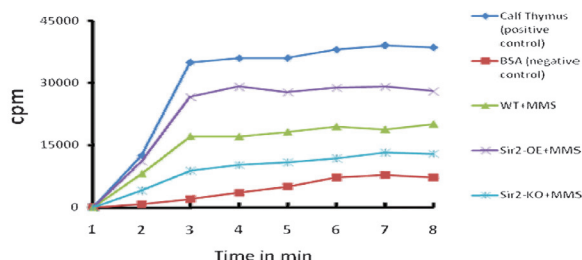


Fig. 28. LdSir2 dependent Ribosylation of histones in response to MMS In WT, Sir2-over-expressed, Sir2-deleted and sirtinol pretreated.

Role of L-arginine in immuno-modulation of host response in VL

Prolonged L-arginine deprivation for *Leishmania* parasite leads to generation of oxidative stress and caspase independent apoptotic-like death involving ROS mediated DNA damage by increasing intracellular calcium pool followed by loss of $\Delta\psi_m$. Increased level of host arginase and decreased iNOS was observed at the mRNA and protein level. PI-3 kinase activation is associated with up regulation of host arginase activity. Activation of PI-3 kinase and induction of host arginase in *Leishmania* pathogenesis was found to be correlated with

increased level of Th2 cytokines (IL-4 and IL-10) and decreased level of Th1 cytokines (IL-12 and TNF- α).

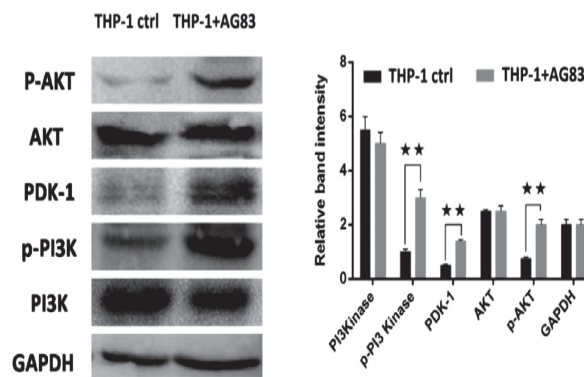


Fig. 29. Effect of Leishmania infection on activation of PI-3 Kinase pathway.

Identification and characterization of secreted/excreted proteins of Amphotericin B resistant *L. donovani* parasite

The western blot analysis for expression pattern validation of two secretory proteins P54 (54 kDa) and P17 (17 kDa) in Amph-B sensitive and resistant parasites revealed higher concentration in resistant isolates as compared to sensitive ones. The ES proteins might have potential role in parasite virulence and host protective immunity.

Role of translation initiation factor 2-alpha (LdeIF2 α) of *L. donovani* in parasite survival under stress condition

It was found that phosphorylation of LdeIF2 α under acidic pH and elevated temperature showed ROS generation that activates PERK-LdeIF2 α

stress responsive pathway enabling parasite to thrive under harsh conditions. Furthermore, the anti-leishmanial drugs viz. SAG and Amphotericin B treatment also activates this pathway, indicating its possible involvement in drug unresponsiveness.

Pentose phosphate pathway of *L. donovani* in oxidative stress regulation.

The rate limiting enzymes of Pentose phosphate pathway, Glucose-6-phosphate dehydrogenase (G6PDH, ~5 folds) and Transaldolase (TALD, ~4 folds), when episomally over-expressed in *L. donovani* were found to resist exogenous oxidative assault generated by H₂O₂, menadione and diamide. Further, over-expressions of these enzymes lead to decreased formation of cellular protein carbonyls and lipid peroxidised products in the parasite. Interestingly, these over-expressing parasites showed higher LD₅₀ towards SAG, Amphotericin-B and Miltefosine, all known to generate intracellular ROS for parasite killing.

Pathophysiology of lipoproteins in *Leishmania donovani* infection

After demonstration of suppressed MIP1 α expression in VL expression, chemokine and cytokine expression by human monocytes, stimulated with different lipoprotein fraction and cholesterol concentration, all were assessed using the flow cytometry. There was increased expression of MCP-1, IP-10, CD8+IFN γ and MIP1 α and decreased expression of IL-10 by Cholesterol + SLA stimulation as compared to SLA alone. Further, it was observed that cholesterol and 4th fraction of lipoprotein (HDL-c) may have a potential in pro-inflammatory role against VL infection.

VECTOR BIOLOGY & CONTROL

Evaluation of active molecule of plant extract having insecticidal effect on *P. argentipes*

Three plant extracts (PK₁, PK₈₁, and PK₉₀) were found having insecticidal effect on *P. argentipes*. The bio-assay guided isolation of active molecule from PK₉₀ leaves (hexane extract) through column chromatography, followed by thin layer chromatography for detection of its fractions, one fraction (a compound of class diterpenoid)

having insecticidal effect was detected. UV-Vis Spectroscopy of fraction showed a peak at 213 nm in the range of λ_{max} of diterpenoid class. About 61% mortality of *P. argentipes* with this fraction (bio-assay test) and nil mortality against HPLC washout of this fraction indicate insecticidal effect of this active molecule. GC-TLC chromatogram showed prominent peak of this compound present in significant quantity. Mass spectra revealed this compound of diterpenoid group but GC-FID response revealed it as a nitrogen containing compound. Purification of this fraction for further analysis is in progress.

Semiochemical mediated response in oviposition behaviour of *P. argentipes*

The gravid female *P. argentipes* were found more responsive towards the pre-existing colony containing highly rich and fertile organic material for egg laying as compared to new (control) ones. Aqueous extract of frass and hexane extract of conspecific eggs from the pre-existing colony significantly attracted gravid flies for egg deposition. Comparatively, odor emanating from rabbit faeces (hot manure) served as better attractant for gravid flies for egg laying but had reduced emergence of adult ones than cow dung (cold manure).

Predicting sand fly density and its correlation with the kala-azar transmission

Prediction accuracy of the GIS, geo-statistics and remote sensing based software model was evaluated. Explanatory variables such as LULC factors (11 categories), NDVI (mean, minimum and maximum) and climate data were generated at GIS platform for endemic site. A significant linear relationship was observed between field abundance and predicted density of *P. argentipes* ($R^2 = 0.72$, $P < 0.001$).

BIOINFORMATICS

Fabrication of novel drug delivery technology and nanomedicine to improve the current options of VL treatment

Characterization of nanoparticle done by transmission electron microscope (TEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectra technique revealed that GINPs

encapsulated amphoterecin B and miltefosine has optimum size (15-25nm) for drug delivery system with uniform distribution, high drug loading affinity (90%) and high release rate at lower pH. *In-vitro* and *in-vivo* study demonstrated significantly higher efficacy of GINP encapsulated AmB and miltefosine as compared to un-encapsulated form.

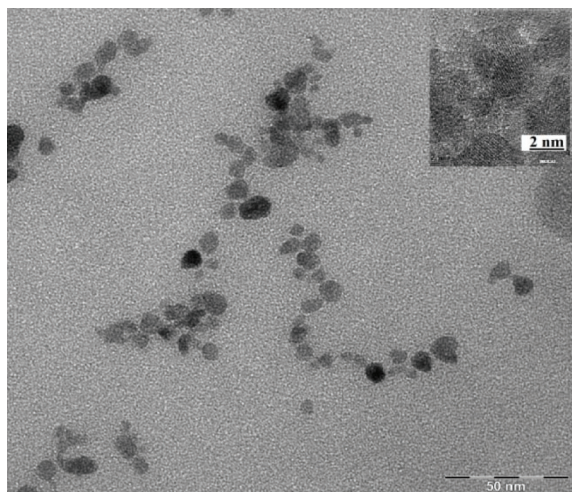


Fig. 30. Tem result showing size of nanoparticles (10-15 nm)

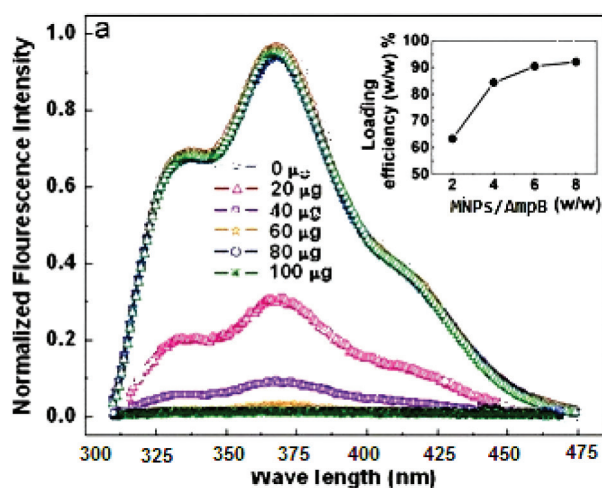


Fig. 31. Drug loading efficiency GINPs for AmB

VIROLOGY

Out of 757 stool samples tested for human calcivirus, 45 were found positive for rotavirus (5.94%), 34 for adenovirus (4.5%), 25 for astrovirus (3.30%) and 1 for norovirus (0.13%). Two samples had mixed infection of astrovirus and adenovirus. Astrovirus and adenovirus were confirmed by sequencing and phylogenetic analyses. Apart from Rotavirus, Adenovirus and Astrovirus was found as

the most frequent cause of viral gastroenteritis in both hospitalized and non-hospitalized children in Bihar. Maximum positivity of the viruses was seen in children less than two years of age.

Twenty four viruses are being diagnosed routinely at VDRL facility. A total of 2,251 samples (14.5%) were found positive for one or other types of viruses. This is the only centre in the state for diagnosis of H1N1 by RT-PCR and diagnosis of dengue through NS1, IgM and IgG ELISA tests. H1N1 was found as major problem in the state in March 2015. DEN1 was observed as predominant serotype in Bihar. Investigational support was provided during various outbreaks of disease viz. Swine flu, chicken pos, AES/JE.

Model project for Kala-azar elimination

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 (1st round) & Synthetic pyrethroid (2nd round) etc were the key component. The concerted effort lead to remarkable reduction in kala-azar incidence at block level in Vaishali district. It has been suggested by VBDSF to replicate this model in other districts.

Extramural Research

Dengue, the world's most rapidly spreading mosquito-borne viral disease, is taking a far bigger human toll than was believed to be the case. Since October 2013, large outbreaks due to Zika have been recorded in the Pacific Islands. Zika virus disease has the potential for further International spread given the wide geographical distribution of the mosquito vector and the lack of immunity among population in newly affected areas. As of now, the disease is not reported in India. However, the mosquito that transmits Zika virus, *Aedes aegypti*, also transmits dengue virus, is present in India. ICMR institutes have been asked to build capacity for Zika diagnosis in the country and also undertake entomological surveillance to detect Zika virus transmission.

NIV provided support to MoH&FW in the National preparedness plan by providing diagnostic support and also capacity building and training of 10 additional labs for Zika diagnosis. Resource centre for virus research and diagnostic laboratories (RCVRDL) imparted training to 160 participants of VRDL's network. Besides this NIV also tracked the spread of Crimean Congo Hemorrhagic fever (CCHF) and Kyasanur Forest Diseases (KFD) in different parts of the country, during the year.

India witnessed a major outbreak of influenza H1N1 from Jan to March 2015. ICMR through NIV, Pune helped the MoH&FW to strengthen the diagnostic platform of the country. A total of 31 ICMR Institutes and VRDLs were trained and supported with reagents and kits. All these labs were actively involved in testing Influenza cases in their respective States.

The National Task Force on laboratory Containment of wild Polioviruses completed the containment activities for wild Polio virus type 2 as per the WHO timelines. Poliovirus type -2 has been declared eradicated from India, a few selected wild type strains and vaccine strains have been securely contained in BSL-4 facility.

Increasing bacterial resistance is also an emerging threat due to widespread misuse of broad-spectrum antibiotics. In view of above, the ICMR initiated Antimicrobial Resistance surveillance network in 2013 and the data is currently being collected from four hospitals across the country on the six pathogenic bacteria groups. The data obtained so far shows very high resistance to 3rd and 4th generation drugs including carbapenems in *E. coli*, *Klebsiella*, *Acinetobacter* and *Pseudomonas*. This is worrisome and highlights a need for in- depth understanding of the transmission dynamics of drug resistant bugs and also for strong infection control practices across hospitals to reduce the drug resistant infections. Emerging genomic technologies, systems biology approaches and bioinformatics capabilities provide opportunities to generate novel data sets that identify changes in molecular composition of the pathogen, alterations in the pathogen-host pathways and other factors such as strain fitness as the pathogen develops resistance and selective advantage over other strains. ICMR has signed a letter of Intent with NIAID, NIH,

USA to collaborate on research on antimicrobial resistance in June 2015. This collaboration will focus on systems biology approach to understand the transmission dynamics of drug resistant infections and improve our understanding of the molecular mechanisms that occur in pathogens and evolution of strains resistant to develop improved therapeutics and rapid, accurate and easy to use diagnostics for treatment of patients.

Under Global Health Security Agenda (GHSA), ICMR is collaborating with AIIMS and the U.S. Centres for Disease Control and Prevention (CDC) to implement appropriate infection control procedures, enhance antimicrobial stewardship practices, and strengthen routine hospital-based surveillance for HAIs. These activities serve the need for reliable AMR data to support successful patient care, and the public health need to measure, track and report the magnitude of types of AMR threats affecting India.

The national rotavirus surveillance network conducted during 2012-2015, revealed that rotavirus accounted for 39.3% hospitalization due to diarrhoea among under-five children and thereby provided evidence base for introduction of Rotavirus vaccine in the immunization program. NIE and CMC, Vellore with ICMR have initiated studies to measure impact of vaccination in Haryana, Himachal Pradesh, Andhra Pradesh and Odisha where 'Rotavac' – the indigenously developed rotavirus vaccine is being introduced as part of the Universal Immunization Program (UIP). Additional studies also showed the devastating economic impact of rotavirus gastroenteritis among poor families, for whom a child's hospitalization resulted in catastrophic expenses.

Under the Indo- US Joint Statement on 'Prevention of Sexually Transmitted Diseases and HIV/AIDS' which is continuing for the 3rd time a workshop was held to mentor the young and middle level scientists who participated the workshop by the faculty from US and India and were given suggestions to improve the quality of their application which will be helpful to them to submit proposals well.

Besides dealing with threat of above emerging infections in India there are still hot spots in four eastern States of India namely Bihar, Jharkhand,

Uttar Pradesh and West Bengal where leprosy is still endemic. In an effort to intensify the fight against leprosy, a research cum intervention project using Mw (MIP) vaccination as an adjunct of the cases of leprosy as well as its household contacts in high endemic areas of leprosy and also comparing with single dose of rifampicin chemoprophylaxis and both in other arms would be undertaken in collaboration of the NLEP and the NJIL&OMD, Agra. The project is proposed to be initiated.

The fight against TB is getting progressively tougher as drug-resistant TB increasingly makes its presence felt. India accounts for the largest TB burden of the world and also the highest mortality in middle age group. The International community has adopted new 'End TB Strategy' to end TB by 2030. Therefore ICMR had launched "India -TB research consortium" wherein various Ministries and International Organizations have agreed to support this initiative. The consortium would be established for concerted, multidisciplinary effort to look at a complex problem like TB in mission mode. It has a national network of researchers to address high priority research questions in TB, capacity building to train scientists, doctors etc, develop GCP/GST guidelines etc. There will be multiple national and international stakeholders who would also pool in the funding for the various activities as approved by the Committees. CEO, Tata trust has offered to initially provide an initial commitment of funds for the consortium. The priority areas will be developed through a consultative process.

Recently CTD, MoH&FW have sanctioned a Global fund project on 'Targeted Intervention to Expand and Strengthen TB Control in Tribal Populations under the Revised National Tuberculosis Control Programme, India' to be co-ordinated by ICMR Hqrs. In collaboration with CTD and to be carried out at 4 ICMR Institutes (NIRT, Chennai, NJIL&OMD, Agra, RMRCT, Jabalpur, RMRC, Bhubaneswar). The project aims to provide evidence to RNTCP for improving health of tribal population using clear intervention strategies through outreach to community using mobile vans to provide diagnostic services, engage the community, involve traditional healers, improve awareness of community on TB and availability of treatment, each of which have been identified

to be continually deficient & foremost reasons for vulnerability of tribal population to various health problems, particularly TB.

Under the chairpersonship of Secretary DHR & DG, ICMR a GBD India Tuberculosis an Expert group has been formulated with objective to have State level disease burden estimates for India of tuberculosis. The data sources available for GBD India with the states will be used. Additional data sources will be identified and various modelling strategies will be used to assess the disease burden.

The Tribal Health Research Forum is ICMR's flagship programme of ICMR/DHR. NIRTH is the nodal centre for the Tribal forum. Under the Tribal Sub-Plan multi-centric studies on haemoglobinopathies (sickle-cell and thalassemia) and on developing an innovative model to strengthen the RNTCP are being carried out. On the request of Ministry of Tribal Affairs, NIRTH, Jabalpur & NIIH, Mumbai trained health personnel and tribal research officials of 14 states in screening and diagnosis of sickle cell anemia and conducted master's trainer's training in 9 states.

Stratification of malaria in Tribal regions by the NIRTH, Jabalpur revealed that tribal population constitutes about 8 % of India's total population and accounts for 70% *P. falciparum* infection and 47% death due to malaria. Based on these findings and voluminous work carried by the institute on Tribal Malaria, NIRTH has revised the Tribal Malaria Action Plan.

There is a strong political will by GoI to effectively implement strategies to eliminate malaria, kala azar and lymphatic filariasis so that target of elimination is achieved well within the possible time frame. Vector Borne Disease Science Forum (VBDSF) established with the aim to identify neglected aspects/gap areas of vector and vector-borne diseases research in public health and to address the challenges of the national programme/ policies to help develop better control strategies and enhance capacity building in public health aspects of vectors and vector-borne diseases. The Forum is a strong network of ten ICMR institutes, educational and research institutes/ colleges/ universities/ departments engaged in work on VBDs.

ICMR supported VCRC; Puducherry to prepare Insecticide impregnated papers (IIPs) for monitoring vector resistance / susceptibility to insecticides to the Dt of NVBDCP. Currently, they are being imported from Malaysia. The indigenously prepared papers are being independently validated at NIMR, Delhi and Institute of Vector Control and Zoonoses, Hosur.

In view of the request made by Dt of NVBDCP to ICMR a study was carried out by VCRC, Puducherry in the Koraput district of Odisha to assess the impact of DDT 75% and DDT 50% on malaria vectors, relevant entomological parameters of *An. fluviatilis* and *An. culicifacies* were monitored and the cone-bioassay tests conducted indicated that DDT 75% had significantly higher residual activity than DDT 50%, even on the mud plastered-sprayed surfaces.

Under the Research Cum Intervention project on AES/JE, a study was carried out at NIE, Chennai to assess the etiological role of *Orientiatsutsugamushi* in AES cases. The multicentric study is ongoing at NIE, Chennai in collaboration with BRD Medical College, Gorakhpur, CMC, Vellore and GMC, Puducherry. In addition to human case detection, to generate evidence for the endemicity of scrub typhus in Gorakhpur district a study was initiated with aim to assess the prevalence of scrub typhus vectors/rodent hosts and the pathogen *Oriental tsutsugamushi* in areas reported for human cases. Out of 50 rodents screened 20 were positive for weil Felix test using OXK antigen. One of the shrew mice (*Sancus murinus*) was positive for Weil Felix test as well as by PCR assay. The PCR product was also sequenced. This result is indicative of presence of reservoir and potential for transmission to human. However the possible association between of the human cases and vector abundance needs to be established.

Under the Joint-ICMR-ICAR initiative on zoonoses and as a prelude to the proposed 'National Institute of Zoonoses' to be established at, Nagpur, two studies in Task Force mode were initiated on surveillance of select zoonotic diseases and to identify their novel biomarkers at MGIMS, Wardha and Central Institute of Medical Sciences, Nagpur respectively. ICAR has launched a study under Niche Area of Excellence in on selected zoonotic diseases at Nagpur Veterinary College. The interim results

suggest that Rickettsial diseases, Leptospirosis, Tuberculosis, Listeriosis and Brucellosis are major zoonotic diseases prevalent in the area.

ANTI-MICROBIAL RESISTANCE

Pulmonary delivery of the enzymes can provide effective delivery of the therapeutic agent to the diseased regions and thereby inhibit bacterial infection via their anti-biofilm property. A study was planned to develop and determine *in vivo* efficacy of a co-spray dried liposomal Fluoroquinolone antibiotic complemented with antimicrobial enzyme, to overcome the biofilm resistance in respiratory infection where enzyme acts as a nano-driller to disrupt the biofilm and paving the way for the antibacterial agent to elicit its action. *In-vivo* microbiological studies showed that Serratiopeptidase (SRP) and Lysozyme (LYS) in combination with LFX and CPX showed promising results in eradication *S. aureus* biofilm. LFX at sub-MIC concentration in combination with SRP and LYS successfully eradicated >90% of preformed biofilm *in vitro*. Entrapment efficiency of Levofloxacin (LFX) in liposomes was >80%. Pharmacokinetic study in *S. aureus* infected rats confirmed higher concentration of LFX in lungs and bronchoalveolar lavage fluid when administered intra-tracheally using Microsprayer Aerosolizer. Acute toxicity study in rats revealed that LFX liposomes in combination with SRP as well in combination with LYS were safe and non-toxic. Pulmonary delivery of this combination could be a promising approach to overcome the serious problem of biofilm resistance in respiratory infection.

A study isolated antimicrobial peptides from heart and lung tissue of *Capra hircus* (Goat) and to check its antibacterial activity against antibiotic resistant and antibiotic sensitive bacterial strains i.e. MRSA, MSSA, ESBL, *E. hormaechei*, *E. asburiae* and *K. pneumoniae*. Two Crude proteins: Crude protein heart (CPH) and crude protein lung (CPL) were isolated from heart and lung tissue. The study led to the isolation of six antimicrobial peptides from heart and lung tissue of goat (*Capra hircus*) which showed antimicrobial activity against Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Sensitive *Staphylococcus aureus* (MSSA), Extended Spectrum β -lactams (ESBL),

Enterobacterhormaechei, *Enterobacterasburiae* and *KlebsiellaPneumoniae*. Mass spectroscopy analysis of active fractions revealed that their molecular weights were 6.5kD and partial amino acid sequence showed homology to β -defensin of *Capra hircus* which are the components of innate immune defense system and are present in oligomeric form.

A study conducted by a research group from IIT Roorkee developed and demonstrated an effective strategy of finding out efflux pump inhibitor against a particular family of efflux pump protein. The study targeted AbeM, a multidrug efflux pump in *Acinetobacterbaumannii*, a dangerous “ESCAPE” pathogen responsible for highest number of hospital acquired infections worldwide in humans, especially in immune- compromised individuals. The study led to the identification of a series of new chemical entities which can potentiate the activity of fluoroquinolone against multidrug resistant *Acinetobacterbaumannii*. IITR08027, a small molecule was found to be most effective among the others and has been characterized as an efflux pump inhibitor of AbeM. Cytotoxicity of IITR08027 was determined by MTT assay against mammalian HeLa cells and showed that IITR08027 has very low cytotoxicity. The molecule is not inhibitory on its own but is capable of potentiating the activity of ineffective concentration of ciprofloxacin against the fluoroquinolone resistant bacteria. In presence of the molecule a remarkable (16-128 fold) decrease in MIC were observed both in case of ciprofloxacin and norfloxacin. The molecule is also found to improve the pharmacodynamic potential of the antibiotics by enhancing the killing efficiency of the drug, by extending its PAE (post antibiotic effect) and reducing the mutant selection frequency. This molecule can be used along with antibiotics for therapeutic purpose to treat infections caused by multiple drug resistant *A. Baumannii*.

A prospective comparative trial was conducted in Pediatric Intensive care units (PICUs) of Advanced Pediatric Centre PGIMER Chandigarh over a period of 2 years, which compared two strategies of antibiotic administration strategies: cycling vs. mixing using a Latin square design. A total of 778 children were enrolled in the study during 2 years, more than half 56 acquired new resistance during

the PICU stay. The acquisition of resistance in the baseline phase was significantly higher than both mixing (22.6%) and cycling (18.5%) strategies; the baseline phase rate was significantly higher than even the washout periods: 32.8% for washout 1 (after mixing) and 20.4% for washout 2 (after cycling). However on combining the mixing and cycling phases with their respective washout periods 77 out of 411 (18.7%) in cycling as compared to 73 out of 268 (27.2%) in mixing phase developed acquisition of resistance. The study indicated that the two antibiotic administration strategies resulted in reduced acquisition of resistance in pediatric ICUs. After adjusting all confounders, it was observed that cycling the antibiotics was better than mixing strategy in terms of acquisition of resistance rates, time to acquisition of resistance, incidence of health care associated infections and mortality.

Under a study, a total of 138-antibiotic resistant (AR) lactic acid bacteria (LAB) were isolated from 124- poultry and 32- meat samples. Irrespective of the genus and species, several isolates were found to be resistant to clinically important antibiotics like erythromycin, tetracycline, gentamycin, streptomycin, kanamycin, vancomycin, and ampicillin. Genotypic characterization of AR-LAB revealed the presence of antibiotic resistance determinants to nine aminoglycoside modifying genes. The results obtained showed the prevalence of multiple antibiotic resistant genes in native isolates, associated with conjugative transposon Tn916 and non-composite transposon Tn917. These determinants were also found to be functional and transferable by *in vitro* and *in vivo* HGT, which reveals the commensal organisms from poultry and meat source as common reservoirs of antibiotic resistance plasmids, transposons and genes. The project highlighted the presence of transferable antibiotic resistance genes in commercial/probiotics Lactic acid bacteria, which are used in large number of fermented products highlighting the need for strict regulation in the usage of antibiotics in poultry and animal husbandry to avoid development and transfer of resistance in from animals to humans.

In a study carried out in Mangalore, four hundred samples from fishery products (100), poultry (50), fish farm (50), sediments (50), processing

industry effluent (50), piggery (50), and livestock waste (50) were collected from different sampling stations in Mangalore and adjacent areas. A total of 1122 Gram negative isolates were obtained from 400 samples. From the collected samples, isolates of *Escherichia coli*, *Enterobacter* and *Salmonella* spp. each, *Klebsiella* spp., *Pseudomonas* spp., *Vibrio parahaemolyticus* documented high percentage of resistance to ampicillin followed by nitrofurantoin. The isolates were most sensitive to chloramphenicol. Isolates obtained from seafood showed high prevalence of ampicillin resistance (53%) while the resistance in aquaculture in Farms isolates was the least (7%). Resistance towards gentamicin was very low from livestock and seafood sources, aquaculture farms, processing plant effluent and piggery while there was a noted increase in resistance in poultry isolates (33%). Resistance towards Nalidixic acid was significantly higher in isolates from poultry (44.4%) and piggery (32%) compared to other sources (~7% resistance). Tetracycline resistance was the highest in isolates from the processing plant effluent (42%) followed by poultry (33%), piggery (18%) while isolates from seafood and livestock showed a resistance of 8%. Tetracycline resistance was lower in isolates from aquaculture farm (4%). The research group also developed a multiplex PCR method for simultaneous detection of antibiotic resistance genes (floR, sul1 and tetG), SPI-2 genes (ssaT and sseF) and *Salmonella* specific gene (invA).

DIARRHOEAL DISEASES

A study on “**Molecular epidemiology of rotavirus diarrhoea among the children under five years of age**” carried out to estimate the prevalence and deaths along with seasonal distribution and genotyping of rotavirus strains in Southwest India at the Manipal University, Madhav Nagar, Manipal. Among the total 315 diarrhoeal cases recruited, a laboratory confirmed etiology was established in 212 (67.3%) cases. Rotavirus was the most common etiological agents and it accounted for 39.4% of cases. The most common genotype of Rotavirus was G1P[8] and G1P[6], G1P[4], G2P[4], G2P[8], G3P[6], G3P[8], G4P[4], G4P[6], G4P[8], G9P[4], G9P[6], G9P[8], G12[P6]. Predominant rotavirus G- genotypes are G1 and G2 whereas for P-genotypes it's P4 and P8. This study documented

the presence of G9 and G12 genotypes, for the first time in southwest India.

A project on “**Apoptosis in cultured human intestinal epithelial cells of different origin (INT-407, HCT-15) infected separately with enteroaggregative *Escherichia coli* (EAEC-T8, EAEC-042) and identification of the bacterial protein(s)**” involved in this process was completed at PGIMER, Chandigarh. Maximum extent of apoptosis was noted in case of EAEC-T8 as compared to EAEC-042 and EAEC-pT8 in both the cell lines, indicating the importance of invasion as well as the plasmid of this organism in this process. EAEC induced upregulation of Bax & Bak, downregulation / unalteration of Bcl-2 & Bcl-XL, reduction in the mitochondrial transmembrane potential, release of cytochrome c from mitochondria into cytosol and activation of pro-caspase-9 & pro-caspase-3 resulting in DNA fragmentation in the intestinal epithelial cells were also observed. An increased expression of Fas receptor, activation of pro-caspase-8 and up regulation of Bid in these cells clearly indicated the involvement of extrinsic pathway in EAEC induced apoptosis. Moreover, the plasmid-borne membrane proteins of EAEC were found to have maximum involvement to this process and the adhesion of the organism was found to be a possible contributor. The wild type invasive strain of EAEC could also arrest maximum number of cells at the S phase and G2-M phase of the cell cycle in both the intestinal epithelial cell lines and the plasmid-borne membrane proteins of EAEC were found to have maximum contribution to this process. The outcome of this study has led to an improved understanding of the basic mechanism underlying the pathogenesis caused by EAEC.

A project on “**Diversity Profile of *Salmonella* specific bacteriophages and screening potent phages to treat Salmonellosis**” carried out at Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu. Six *Salmonella typhi* specific phages and six *Salmonella typhimurium* were isolated and purified as per standard protocol. Among the all phages, phages of *Salmonella typhi* were highly stable under different physiological conditions and were stable up to pH 4, whereas the phages of *Salmonella typhimurium* were stable only up to pH 5. The study shows that the phages were

similar in its molecular makeup, but diversified in their physiological properties. A novel, simplified SHIME system was designed and validated. The phages were stable in the SHIME system after the addition of antacid into the system. Hence, these isolated phages can be used as a therapeutic agent to treat Salmonellosis as these phages were highly stable under extreme physiological conditions. The efficiency of the phages during the phage therapy can be increased on oral administration after intake of the antacid.

MYCOBACTERIAL DISEASES

Leprosy

A study on the “**Endemicity of leprosy and utilization of health services in selected areas of UP**”: Role of RLEP-PCR in reducing endemicity was completed at NJIL&OMD, Agra. This study found that the molecular technique (*M. leprae* RLEP-PCR) in SSS helped in definitive diagnosis of the disease could establish early diagnosis, more sensitive than AFB detection in SSS, was simple and could be undertaken in the field conditions. This could be used in the programme for value addition to the definitive diagnosis of leprosy.

A study entitled “**Strengthening of Mycobacterial repository for translational studies**” was completed at Agra. The study aimed to collect, characterize and maintain Mycobacterial strains from defined geographical areas, to provide well characterized strains from the Mycobacterial repository for translational programmes aimed at diagnosis (detection and determination of drug resistance) and molecular epidemiology of TB and Leprosy which provide well characterized strains to other researchers. The first phase of study was completed achieving the goals and have established more linkages with different research organizations/medical collages from different parts of the country, presently collaborating with 46 centres. Computersation of data has been done. Well characterized isolates available in the repository are being maintained by regular passages and are available to support the investigators. The facility provides reference as well as characterized Mycobacterial isolates/DNAs/RNAs/Culture Filtrates/Lysates to 9 Scientists/students from different Institutions/Universities.

As a part of Repository formal/informal training in identification, handling of pathogens under Bio-safety conditions, preservation of isolates, sensitivity screening and newer molecular DNA fingerprinting methods for characterization of mycobacteria is being provided continuously.

A study entitled “**Evaluation of different treatment regimens being used at MRHRU for Tuberculosis and Leprosy, Ghatampur, Kanpur Nagar, India**” was completed at Agra. The study aimed to Follow-up of patients treated for leprosy and TB using Standard as well as alternate regimens for leprosy/ tuberculosis in the various leprosy and TB surveys undertaken at MRHRU, Ghatampur, Kanpur Nagar. Digitalization of records and implementation of data management system at MRHRU, Ghatampur for analysis of impact of different interventions. Data analyzed, has demonstrated that the relapse that the rates are high at 6,12,18&24 months of follow up in both Cat I & II TB patients. The higher relapses were observed to have occurred during early periods after treatment completion and the number of relapses fall with passage of time. There is hardly any reliable data available through long term longitudinal follow up of TB patients which have developed evidence on the incidence of relapses. Completion of the full complement of follow ups in phase II will provide strong and valid data on the incidences of relapses among the new and retreatment cases. A phase II of the project wherein the long term follow up of all TB patients enrolled under the phase I need to be completed has been initiated.

Tuberculosis

A study entitled “**Whole proteome analysis of aminoglycoside resistant isolates of mycobacterium tuberculosis**” was completed at NJIL&OMD, Agra. The study analyzed the membrane protein profiles of aminoglycosides resistant and total sensitive isolates. Seventeen protein spots were found to be consistently overexpressed in all resistant as compared to sensitive isolates. However, five spots matched with already identified protified proteins and therefore total twelve proteins were found to be upregulated. Identified proteins were further characterized by MALDI-TOF-MS/MS taking at least three peptides

to be matched significantly for each protein. Results of MALDI-TOF-MS/MS further validated earlier data (MALDI-TOF-MS) regarding the identity of the protein spots. Molecular docking analysis of selected 3D models of hypothetical proteins showed the interaction of both drugs into the central cavity of conserved motif of SDR domain and Usp domain of hypothetical proteins respectively. Interacting residues were almost common for both drugs, which suggest similar interacting residue of conserved PspA domain of hypothetical protein. By utilizing the default threshold (medium), GPS-PUP predicted six pupylation sites at positions K7, K71, K94, K120, K134, and K135 in Rv2744c. Rv2005c and Rv0148 showed two pupylation sites at positions K80, K248 and K280, K285 respectively.

A study entitled “**Role of early diagnosis of drug resistant tuberculosis in guiding therapeutic strategies in treatment of Extra-pulmonary tuberculosis**” was completed at AIIMS, Delhi. Current study reports observations in a patient care setting in a high burden region, from a large collection of extra-pulmonary samples and puts to rest questions regarding sensitivity, specificity, detection of infrequent mutations and mutations responsible for low-level Rif resistance by GeneXpert. Advances in the assay could offer further improvement in sensitivity of detection in different patient samples; nevertheless it may be difficult to improve sensitivity of Rif resistance detection if only one gene is targeted. Assay specificity was high both for TB detection and Rif resistance detection. Despite a few misses, the assay offers major boost to early diagnosis of TB and MDR-TB, in difficult to diagnose paucibacillary TB.

A project entitled “**Impact of HLA-G on host immune response in human tuberculosis**” has been completed at AIIMS, Delhi. The study findings show promising results to use HLA-G as a potential biomarker to distinguish different states of TB disease but further validation is required in a longitudinal study. In addition, HLA-G expression could serve as a correlating biomarker for assessing the host immune response during TB progression and vaccine trials with new generation vaccines.

OTHER MICROBIAL INFECTIONS

ICMR funded diverse projects which dealt with identification of potential drug leads and vaccine candidates, characterization of mechanisms of action of drug candidates against pathogens causing nosocomial infections, application of molecular techniques for diagnostics, and some projects which conducted basic science research. The projects focused on several pathogens (bacterial and fungal) which are important from the perspective of nosocomial and/or community settings.

Pseudomonas aeruginosa and *Klebsiellapneumoniae* are one of the most frequently isolated bacterial pathogens from the cases of nosocomial burn wound infections and they cause most serious complications in such infections. A study was conducted using the standard strains of *K. pneumoniae* B-5055 and *Pseudomonas aeruginosa* PAOI, to apply both quorum sensing and O-polysaccharide (OPS) to develop potential candidate vaccine against burn wound infections caused by the two pathogens. The *in vivo* protection studies of the conjugates in burn wound BALB/c model indicated that the OPS conjugates showed 3 log reduction in bacterial counts as compared to control. The results highlight that the OPS conjugates may further be characterized and optimized for further research as potential vaccine candidates against *Pseudomonas aeruginosa* and *Klebsiellapneumoniae* infections in patients with burn injuries.

A study was conducted to identify the natural reservoir of *H. capsulatum* var. *capsulatum* through PCR based identification and the prevalence of histoplasmosis in HIV positive patients and other immune-compromised patients along with the determination of the susceptibility of isolates obtained from environment and clinical sources. Only 4 bat guano samples were found to be positive by the PCR technique. *In vitro* susceptibility testing of both the mold and yeast forms of *H. capsulatum* along with the reference strains (CDC B-5324 and ATCC 66368) of *H. capsulatum* found that both the forms were susceptible to itraconazole, posaconazole, voriconazole, amphotericin B and the new drug isavuconazole. The correct diagnosis of histoplasmosis enabled timely administration of amphotericin B and itraconazole, leading to

successful therapeutic outcome in 50% of the cases. The results of the study directly impact the early diagnosis and successful therapy of patients suffering from potentially fatal histoplasmosis, which is often misdiagnosed as tuberculosis. The application of direct PCR on clinical specimens suspected of histoplasmosis will help in rapid diagnosis of this potentially fatal disease.

Another study determined the utility of various polymerase chain reaction (PCR) methods (uniplex, nested, semi-nested, multiplex and nested multiplex), in terms of sensitivity and specificity, in intra-ocular fluids for detecting the infectious genomes in the diagnosis of endophthalmitis infection. This prospective and consecutive analysis included a total 326 intra-ocular fluids and four other ocular specimens obtained from 330 clinically diagnosed endophthalmitis patients that were submitted for the microbiological evaluation. Of the 326 intra-ocular fluids, 3 showed positive results in 10% KOH, 46 in gram stain, 76 in cultures and 156 showed positive results in PCRs. Among the 156 positively amplified intra-ocular fluid samples, 155 were identified as eubacterial genome and 1% revealed pan-fungal genome. Nested PCRs are superior to uniplex and multiplex PCR. The study established that PCR can be used as a powerful tool in the diagnosis of endophthalmitis, especially for detection uncultured microbes.

A research group from AIIMS, New Delhi studied the prevalence, antibiotic resistance patterns, virulence determinants and molecular characterization of *Enterococcus faecium* and *E. faecalis* causing blood stream, skin and soft tissue and urinary tract infections in hospitalized patients. A total of 500 patients with nosocomial *E. faecium* (73.2%) and *E. faecalis* (26.8%) infections were enrolled. *E. faecium* was predominant in blood (82.4% vs. 17.6%) and skin and soft tissue (73.8% vs. 26.2%) culture isolate, whereas in case of urine samples, *E. faecium* (51.6%) and *E. faecalis* (48.4%) were isolated in similar proportion. Resistance rates to ampicillin, ciprofloxacin, rifampicin, erythromycin, teicoplanin and vancomycin in *E. faecium* isolates were statistically higher than *E. faecalis*. Thirty eight percent *E. faecium* isolates were resistant to Vancomycin and teicoplanin and harbored *vanA* gene while *E. faecalis* isolates

were susceptible. All isolates were susceptible to linezolid, tigecycline and fosfomycin. The *gelE*, *asa1*, *ace*, *cylA*, *esp* and *hyl* genes were detected in *E. faecalis* isolates. The study highlighted drug resistant *E. faecalis* isolates in hospital setting. It is evident from various reports that persistent use of antimicrobial agents in hospitals, in the community and in animal husbandry will certainly further promote the selection and spread of MDR enterococcal clones. Therefore, regular surveillance and improved infection control procedures at hospitals are obligatory to curb further spread of the high risk clones.

A study investigated the mechanism of action and the structural activity relationship of the five fragments of Alpha-Melanocyte Stimulating Hormone (α -MSH) against *S. aureus*. Alpha-MSH is an endogenous neuro, tridecapeptide, known for its anti-inflammatory and antipyretic activity which has been shown to possess strong staphylocidal activity and bacterial membrane damaging potency. The results of the study suggested that C-terminal containing peptides α -MSH (6-13), α -MSH (11-13) efficiently killed *Staphylococcus aureus* cells even in the presence of salt and their efficiency were as comparable as entire α -MSH whereas N-terminal region α -MSH (1-5) was found to be ineffective against *S. aureus*. Similar to the parent peptide α -MSH, α -MSH (6-13), α -MSH (11-13) depolarized and permeabilized *Staphylococcus* cells. Scanning and transmission electron microscopy showed remarkable morphological changes on *S. aureus* cell surface due to exposure of α -MSH and its C-terminal fragment [α -MSH (6-13)] and the fragment showed negligible cytotoxic effects. The fragment also showed synergistic effect with gentamicin, ciprofloxacin and tetracycline. The synergistic activity of α -MSH with antibiotics is encouraging, and promises to restore the lost potency of discarded antibiotics. These observations are important and critical in rational designing of α -MSH based therapeutics with optimal efficacy.

PROJECTS IN NORTH EAST REGION

A recently completed 3 year multisite surveillance of viral hepatitis from 2012-15, across seven hospital based centres in six north eastern states of India showed 9.9% cases of hepatitis to be positive

for Hepatitis B surface antigen (HBsAg). Among acute viral hepatitis cases, the prevalence of HBsAg was found to be 56.1%, while in chronic hepatitis cases and hepatitis B related cirrhosis cases prevalence of HBsAg was found to be 16.7% and 10.9% respectively. However, it was found 52.6% in hepatitis B related hepatocellular carcinoma (HCC). Among blood donors and health care workers, the prevalence of HBsAg was found to be 1.0% and 1.2% respectively. The most common genotype found in all the cases was genotype D. Apart from the surveillance, surface, precore and core gene mutants were also studied, which showed mutation in upto 39% cases. Furthermore, phylogenetic analysis revealed the prevalence of multiple genotypes of HBV from the region within a radius of ~200km (genotypes A, C, D, & I) which are diverse from each other by over 8% in entire genome sequences except for genotype I which has a separation between 6-7% from genotype C.

In view of difficulties in diagnosis, symptomatic similarities with tuberculosis and endemicity of the paragonimiasis in North Eastern states, a 2 year study was carried out to identify immunodiagnostic antigenic fractions of adult lung flukes, which could be used for diagnosis of pulmonary paragonimiasis cases. A simple and reliable western blot assay using soluble *P. westermani* adult worm antigen for serological diagnosis of paragonimiasis was developed. Three antigenic fractions at ~34, ~32 and ~25 KDa of adult *P. westermani* type 1, which reacted with pooled sera of confirmed pulmonary paragonimiasis human patients were identified. The study revealed that there is a differential pattern of immunoreactivity depending upon the maturation stage of the experimental lung flukes. The results of the project may prove useful for diagnosis of early and chronic paragonimiasis and development of dipstick based immunodiagnostic tests.

In a hospital based cross-sectional study, aimed to study the pattern of microorganisms isolated from CSF, their sensitivity pattern as well as the associated risk factors in clinically suspected cases of neonatal meningitis cases admitted to a tertiary care hospital of Dibrugarh, Assam was completed. *Acinetobacter*, *Enterococcus* and coagulase negative *Staphylococcus* were found to be the predominant bacterial pathogens in CSF

of neonates with meningitis. A wide spectrum of antibiotic resistance was found in isolates. Genetic analysis showed that beta lactamase gene *bla* OXA 51 was the most common gene detected among those tested and *mec* Agene was detected in 80% of methicillin resistant *Staphylococcus* isolates. Birth at an outborn setting and maternal risk like prolonged labour was found to be a risk factor for neonatal meningitis.

In another study aimed to determine (“**Drug resistance in ‘new’ and ‘previously treated’ smear positive pulmonary tuberculosis patients**”) was completed. The drug susceptibility testing revealed that 5.6% isolates were multidrug-resistant (MDR-TB) among new TB cases in Assam, while in case of previously treated TB cases in Assam, 15% isolates were found to be multi drug resistant TB. Further, direct sequencing of the genes *katG* and *rpoB* PCR fragments of *M. tuberculosis* complex (MTBC) identified the most frequently mutated codon in *katG* and *rpoB* gene. More over, 189 MTBC isolates were spoligotyped, revealing 89 distinct spoligo patterns. The most dominant MTBC strain belonged to Beijing lineage and was represented by 35.45% of total isolates. Phylogenetic analysis of orphan (23.28%) MTBC spoligotypes revealed that majority of these orphan isolates from Assam represent two new sub-clades Assam/EAI and Assam/CAS. Overall, the study showed higher MDR in previously treated cases, suggesting need for better TB management / drug regimen

TRIBAL HEALTH RESEARCH

A study on “**Clinical and Molecular Surveillance for monitoring the emerging resistance to antimalarials drugs in *Plasmodium falciparum* in Central India**” carried out by NIRTH, Jabalpur to determine the therapeutic efficacy of ACT and the novel DHFR and DHPS mutations associated with treatment failure in the Balaghat and Dindori district where vector mosquito are highly prevalent. Among the 237 patients completed therapeutic efficacy study, (99.6%) patients showed adequate clinical and parasitological response. Molecular study revealed 72% parasite were mutant genotype (27.2% single mutant, 43.5% double mutant and 1.3% triple mutant) for *pf**dhfr* while *pf**dhps* showed

78.2% wild type alleles and 21.8% mutants (18.1% single mutants and 3.7% double mutants).

A study on “**Wild mushrooms used by different tribal population in West Bengal**” to search the new source of antileishmanial agents with specific challenge against drug-resistant strain was carried out by West Bengal State University, Kolkata. A novel triterpene, astrakurkurone, has been isolated from Indian mushroom, *Astraeushygroetricus*, which established as significant anti-leishmanial lead. In this study it was found that active extracts of *Astraeushygroetricus* could efficiently inhibit the proliferation of *L. donovani* promastigotes and intracellular amastigotes *in vitro*. It was shown that astrakurkurone, a triterpene, isolated from Indian mushroom *Astraeushygroetricus* (Pers.) Morgan induced reactive oxygen species leading to caspasein dependent apoptosis in *Leishmaniadonovani* promastigotes and also effective in inhibiting intracellular amastigotes at the IC₅₀ concentration of 2.5 mg/ml. Finally, characterized the associated immunomodulatory potentials (induce CD4+IFN- γ +T cells *in vivo*) and cellular activation provided by astrakurkurone leading to effective antileishmanial activity, *in vitro* and *in vivo*. Astrakurkurone caused a significant increase in TLR9 expression of *L. donovani*-infected macrophages along with the activation of proinflammatory responses.

A Case Control study was carried out on “**Human Genetic Polymorphisms and Malaria in the Tribal Population of Maharashtra by NIIH, Mumbai**”. A total of 1327 samples were collected from malaria endemic area and 962 samples were from malaria non endemic area to screen 11 genetic markers. A total of 26 cases of beta thalassemia trait and five G6PD deficient samples were found. The detailed data showed that ABO and Rh blood group system and two bio chemical markers like G6PD and pyruvate kinase deficiency have no role in malarial pathophysiology in the Chipluntaluka. CR1 polymorphism among the malaria positive cases showed 48% A/A genotype and 37% A/G genotype in comparison to their relatives, A/A, 25% and A/G, 56.6%, which showed statistically significant difference. This suggests that A/A genotype may be susceptible while A/G genotype is resistant to malarial infection. Hence, α – thalassemia and CR1 polymorphism seem to

play some role in pathophysiology of malaria in chipluntaluka.

A study on “**Entomological and Epidemiological investigations on Leishmaniasis among the Kani Forest Tribes of Thruvananthapuram Dt., Kerala**”) carried out by VCRC to determine the prevalence and incidence of cutaneous leishmaniasis and incriminate vector species of sandfly (s) involved in its transmission. Data showed that the prevalence of CL/ suspected cases was 0.00027/1,00,000 population. Infection with *Leishmaniadonovani* was confirmed in five patients by PCR and sequencing. Entomological investigation revealed that among the occurrence of 19 species of sandflies, *P. argentipes* was prevalent throughout the year, with a peak of abundance during northwest monsoon (October). However, *P. argentipes* (18.64%) was the second predominant species indoors, next to *S. Baghdadis* (36.52%). Distribution of sandflies was significantly higher ($p < 0.0001$) indoors (87.6%) than outdoors (12.4%). The density of *P. argentipes* ranged from 0.5 to 11.1 female / MHR. *P. argentipes* population was found to be susceptible to DDT as well as deltamethrin. The females were found to rest predominantly on the lower part of the walls during rainy months and on upper surface including the ceiling in summer. Based on the result, an intervention measure has been planned to control the CL transmission.

A study on “**Epidemiology of Lymphatic filariasis in tribal areas of Jharkhand**” was carried out by NIMR Field unit, Ranchi to understand the epidemiology and determine the prevalence of filarial infection due to *W. bancrofti* through determining parasitological and entomological indices. Microfilaria rate found highest in Saraikela (10.86%), followed by Godda ((8.63%) and west Singhbhum (6.0%) with infection rate 15%, 10.3% and 13.34% respectively. Another important finding of the present survey was the presence of microfilaria *Brugiamalayi*. The result showed that the species variation in different geographical and environmental conditions in terms of vector specificity, periodicity and infectivity to domestic and wild animals, causing control measures very difficult.

A study carried out on “**Intervention to improve KAP related to tuberculosis and its impact on**

risk factors and TB disease burden amongst Saharia, primitive tribe of Madhya Pradesh". A total of 9225 individuals were covered under the study. It was found that prevalence of TB disease in the study area 3003 per 100,000. The Knowledge, attitude and practices (KAP) survey indicated, poor knowledge about various aspects related to TB. Only 47.7% heard about TB disease, among them 23.6% thought TB is not a curable disease. Overall prevalence of tobacco smoking was 47.3% and higher among males (76.4%). Prevalence of alcohol consumption was 28.9%, higher amongst male (49.7%). Malnutrition was highly prevalent (47.0%). The fuel used for cooking (95.8%) was wood/crop residual leading to indoor air pollution, another risk factor for TB. IEC intervention carried out in study villages with training of volunteers (ASHA, ANM etc), community meeting, street plays, and distribution of pamphlets, health camps, rallies, school children involvement, patient's visit, DOT provider to create awareness among them.

A study on **"Role of rapid diagnostic kit (rk39) and oral Miltefosin to control Leishmaniasis in tribal population of South Bengal"** to evaluate the sensitivity of kit (rk39) in field set up. A total of 189 individuals were found to be positive for anti-leishmanial antibody, among them only 23 were VL cases and 19 were PKDL cases. Sero-conversion [rk39 positive to negative] from positive anti-leishmanial antibody to negative was noted among 47 (~50%) of the detected asymptomatic cases. At the end of the study, 34 individuals (~38%) remained positive for anti-leishmanial antibody as asymptomatic carriers. Both Miltefosine and SSG were found equally effective in VL. In PKDL, these drugs were equally effective in terms of regression of lesions but reappearance of lesions was recorded in 2, (21%) cases after 2 years of treatment with Miltefosine. Therefore, Stysy showed that RDK (rk-39) is a useful tool for screening of the population at community level.

Burden of Hepatitis B and Hepatitis C virus infection was estimated in Irula Tribes by SRM Medical College, Chennai. Also, genotypes of HBV and HCV in Irula tribes were identified and correlation of the above with risk and behavioral aspects was worked out. Among the collected samples 5.10% was positive for HBsAg and 5.10%

was positive for anti HCV. Only 0.26% was positive for both HBsAg and anti HCV. Hepatitis B viral DNA was positive in four HBsAg positive samples & Viral RNA was extracted from anti HCV positive samples. HBV genotyping done on four DNA positive samples and they were HBV genotype 'D'.

VECTOR BORNE DISEASE SCIENCE FORUM

A study entitled **"Evaluation of diagnostic potential of loop-mediated isothermal amplification (LAMP) for rapid diagnosis of malaria"** was carried out by the National Institute of Malaria Research, New Delhi with the main objective to evaluate was to evaluate LAMP in the diagnosis of *Plasmodium falciparum*, *P. vivax* and mixed infections in the hyper endemic tribal areas of Chhattisgarh and Jharkhand. Molecular diagnosis for *Plasmodium* parasite detection was carried out by standard 18S nested PCR assay and compared with the visual species specific 18s rRNA based LAMP assay. LAMP showed 100% sensitivity and specificity for *P. falciparum* malaria cases, whereas it detected *P. vivax* parasites with sensitivity of 95.16% and specificity of 96.7%. LAMP assay for the detection of *P. vivax* and *P. falciparum* in salivasamples showed 76% sensitivity in parasite detection, and this was lower compared to blood

A study entitled **"Understanding the molecular genetics of mosquito vector competence in Indian malarial vectors"** was carried out at the National Institute of Malaria Research, New Delhi with the objective to understand the physiological and immunological responses of the mosquito tissues that affect the vector competency i.e. ability to support *Plasmodium* development and transmission. These studies were carried out in *An. culicifacies* species A. Comparative salivary transcriptomic study showed that adult female salivary glands are specialized organ to manage meal specific choices and decision, through 'gene switching' mechanism. Analysis of sugar fed mosquito mid gut transcriptome, not only revealed the unique dataset to understand the molecular complexity of the mid gut biology, but also led to identify large scale putative immune genes.

A study entitled **"Structural proteomics of plasmodium vivax: development of a three**

dimensional structural database of 500 soluble proteins in malarial parasite” was carried out by the Indian Institute of Technology, New Delhi with main objectives of to develop a metasever to assess tertiary structural prediction accuracy of proteins of *P. vivax* and develop a comprehensive three dimensional structural database of soluble proteins encoded by *P. vivax* genome. Research work resulted into a structural database of predicted protein structures of *P. vivax* along with their functional annotations which can be a potential aid for identifying novel protein targets. A metasever named ProTSAV for a better assessment of tertiary structure prediction accuracy was developed. Web-interface of 3D structural database (named PvaxDB) consisting of predicted models for 615 soluble proteins with their predicted functions also was developed. These predicted proteins models are for proteins that are mostly interacting with RBC and endothelial cells. PvaxDB, is expected to eventually help in determining novel drug targets.

A study entitled **“Expression profiling of host immune responsive genes activated by IFN- γ during *Plasmodium* liver stage development”** was carried out at the University of Hyderabad. The study was planned to unravel the IFN gamma responsive pathway manipulated by the *Plasmodium* liver stages during their attempt to sabotage the host defense mechanisms with an objective to identify robust intervention strategies that can help eliminate liver stage infection and thereby malaria. The results pointed out to a very early takeover of the host cellular machinery by *Plasmodium* sporozoites following establishment of infection in the hepatocytes. Post exposure of IFN gamma treatment after sporozoite infection had minimal effect in limiting the growth of the EEFs. Microarray experiments showed dramatic alterations in the gene expressions across control (sporozoite infected), pre and post IFN gamma treatment samples. The study suggested that pre treatment of HepG2 cells with IFN gamma appears to be activating an arsenal of defense responses that can effectively curb the growth of EEFs. There is a potential for the HepG2 system developed for screening new molecule/new compound against hepatic stages of human malaria parasites

A study entitled **“Studies on macrophage: T cells Interaction in mouse model of malaria”**

was conducted by National Institute of Malaria Research, New Delhi to examine the differences in immune responses i.e., regulation of pro-and anti-inflammatory cytokines secreted by splenic macrophages, dynamics of Th1, Th2, and Th17 subsets of immune responses and determination of extrathymic IL-17 producing innate cells against the lethal (*Plasmodium berghei*) and non lethal (*Plasmodium chabaudi*) rodent malaria parasite. Expression of ICOS on CD4 T cells was observed to increase with disease progression and maximum expression was noticed on 10-day post infection in case of lethal infection while during non-lethal course of infection, the expression of ICOS on CD4 T cells increases as the parasite increased and goes down as the parasite got cleared. The study suggested that malaria parasite evades host protective immune responses by modulating cytokine productions in APCs and thereby altered T cell differentiation. The study was on basic immunological responses during lethal parasite infection in mice.

A study entitled **“Evaluation of a blood-based antigen detection assay by quantitating unique sialoglycoprotein specifically induced on erythrocytes for early diagnosis and monitoring patients with Indian Visceral Leishmaniasis in two referral centres”** was carried out by the Indian Institute of Chemical Biology, Kolkata, West Bengal. The main objective of the project was to evaluate a novel blood based antigen assay (RBC ELISA) for the early diagnosis of VL by quantitating induced *O*-acetylated sialic acid on erythrocyte for diagnosing and monitoring VL patients for relapse and drug unresponsiveness, PKDL patients, and endemic and non endemic controls as well as checking its cross reactivity in malaria, tuberculosis and HIV etc. The results showed that cross reactive diseases like Malaria, Leprosy, Vitiligo and even PKDL had very low titers of *O*-acetylated sialoglycans, establishing that RBC ELISA assay had no cross reactivity and RBC ELISA was VL specific and even capable of diagnosing asymptomatic VL and PKDL patients.

A study entitled **“Induced Apoptic L. Donovanii S Prophylactic/ Therapeutic Agent Against Experimental Visceral Leishmaniasis In Combination With M.W: Searching The Low**

Cost Torrent For Switching The Th2 Response Towards Th¹, being carried out at West Bengal State University, Kolkata following the treatment with pulsed DC, *L. donovani*-infected mice splenocytes showed a 2.5 & 4.5-fold increase in the release of TNF- α and IL-12p70 respectively in comparison to *L. donovani*-infected mice splenocyte cells alone. This significant increase in TNF- α release might play a pivotal role in triggering the signal for enhanced NO generation. On the other hand, the release of TGF- β and IL-10 was significantly abrogated upon treatment with the Mw+HIP pulsed DC in *L. donovani*-infected murine splenocytes

A study entitled “**Evaluation of molecular diagnostic markers for detection of Post Kala-Azar Dermal Leishmaniasis (PKDL) in West Bengal**” was carried out at the Institute of Post Graduate Medical Education and Research, Kolkata. The magnitude of PKDL was estimated in VL affected districts of West Bengal using rk39 strip test, ELISA and DNA based markers (ITS-1 PCR) following either passive case detection (cases of PKDL that reported to the district hospitals) or active case detection, wherein patients were identified during a house to house survey. The maximum number of cases of PKDL were reported from Malda (n = 65) followed by Darjeeling (n=33) and South Dinajpur (n=20), active case detection substantially increased identification of cases of PKDL and PCR was found more sensitive than RK39 and ELISA in detecting PKDL cases

A study entitled “**Environmental management based on bio-ecology of *Phlebotomus argentipes***” was carried out by RMRIMS, Patna with the objective to find out the efficacy of ‘environmental management’ based intervention methods to control the disease transmitting sand fly vector, *Phlebotomus argentipes* by destroying their indoor breeding sites that will be alternative, sustainable, cost effective and eco friendly. The study revealed that Rabish (Fly ash from brick chimney) is cheaper (Rs.320/- per room), locally available at very low cost and skirting by Rabish + Lime is mostly acceptable to the people and people are willing to do this strategy at their own level. The strategy can be implemented in Programme as one of the tools under Integrated Vector Management to control sand flies, vector of Kala-azar.

A study entitled “**Bio-manipulation of *Aedes* mosquitoes to reduce adult mosquitoes life span and inhibit viral competence through *Wolbachia* transfection**” was carried out by Bangalore University, Bangalore. The current project was taken up to study prevalence, diversity and density of *Wolbachia* infections in various *Aedes* mosquito populations and trans-infect them through micro injection into *Ae. aegypti*. *Aedes albopictus* were treated with tetracycline to establish *Wolbachia* free colonies for bio-assay crossing experiments. The main objective was to develop an *Ae. aegypti* strain infected with *Wolbachia* that inhibits dengue virus development in it. Eleven species including *Aedes* and *Anopheles* were collected and examined for the presence of *Wolbachia*, an endosymbiont. Except in *Anopheles* species and *Aedes aegypti* all other species examined were found with *Wolbachia*. Experiments were successful in infecting adult *Ae. aegypti* with *Wolbachia* isolated from *Ae. albopictus*. The *Wolbachia* infected strain could be maintained in the laboratory for 12 generations (later there was mortality and the strain was lost), and this strain has exhibited incompatibility properties when crossed with other strains.

A study entitled “**Mapping of mosquito breeding habitats and location of vertebrate hosts in north and southern parts of virus using space technology (RS & GIS)**” was carried out by DMRC, Jodhpur. Several field visits were made to five districts of Rajasthan state viz., Udaipur, Dungarpur, Banswara, Sri-Ganganagar & Hanumangarhin order to collect ground data in respect of mosquitoes, vegetation type, location of water bodies & places of vertebrate hosts which play important role in JE epidemiology. Thematic layers of Normalized Difference Vegetation Index (NDVI) and Land Use/Land Cover (LU/LC) comprising agricultural land, waste land, forest, surface water bodies and other cultural features of the study sites have been prepared using remote sensing and GIS techniques. Different classes of Normalized Difference Vegetation Index (NDVI) of all study sites have been determined. Roosting places of paddy birds and location of pig sties in Udaipur, Dungarpur, Banswara, Sri-Ganganagar & Hanumangarh have been determined and documented along with their geo-coordinate data. Data collected strongly suggests that there is a

possibility of emergence of JE virus in North & Southern parts of Rajasthan state

A study entitled “**Bioassay guided isolation of mosquito larvicidal principles from western ghat plants**” was undertaken at the KLEU college of Pharmacy, Bangalore. The objective of the study is to determine the efficacy of different plant extracts and essential oils containing drugs on different species of mosquitoes like *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. The whole plant of *Leucas aspera* (Willd.) Link. (Lamiaceae), leaves of *Artocarpus lakoocha* Roxb (Moraceae), aerial parts of *Cleome viscosa* (Capparaceae) were collected from Western Ghats for this study. Among the extracts, petroleum ether extract of *L. aspera* and chloroform extract of *C. viscosa* showing potential larvicidal and juvenile hormone mimicking activity were selected for isolation. Among the 4 fractions isolated from *L. aspera*, LA/FC1-2/C2/F3-5/02 and LA/C1/F08/03 exhibited larvicidal activity. Two fractions from *C. viscosa*, CV/C1/F6-8/C2/F4/03 and CV/C1/F16-17/04 exhibited larvicidal activity.

A study entitled “**Development of new plant based insecticide for larval and adult mosquito control**” was carried out at the Periyar University, Tamil Nadu. Acetone leaf extracts of *Clausen adenata*, *Cipadessa baccifera* and *Glycosmis pentophylla* were evaluated for their potential as larvicides, adult mortality and smoke toxicity against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Bioassay-guided fractionation of acetone leaf extracts of the three plants led to the separation and identification of bioactive compounds with the potential against larvae and adult mosquitoes of the three mosquito species.

A study entitled “**Evaluation of indigenous plant extracts for insecticidal and repellent activities against mosquitoes of medical importance**” was carried by Mohan Lal Sukhadia University, Udaipur. The investigation dealt with four important potential plants i.e; *Moringa olifera* (Lam), *Catharanthus roseus* (L), *Lantana camara* (L) and *Thyme vulgaris* (L) against the third and fourth instars of these important vectors for malaria, dengue, filarial and chikungunya. Leaf and flower. The study revealed that among all the treatments, leaves and flower extracts of *Moringa oleifera* had

maximum effect in repelling *Ae. aegypti*. Within a minimum time of 15 minutes exposure, maximum repellency was observed. In general flowers were found more effective than leaves.

A study entitled “**Preparation and supply of insecticide impregnated papers (IIPs) to National Vector Borne Disease Control Programme (NVBDCP) for monitoring susceptibility/ resistance in vector mosquitoes in the country**” was taken up by VCRC, Puducherry. This project was initiated to establish a national facility to prepare the IIPs and supply to NVBDCP for monitoring vector resistance/susceptibility to insecticides in the country. VCRC has standardized the procedure for the preparation of IIPs and established the facility for the preparation of IIPs. DDT (4%), malathion (5%), deltamethrin (0.05%), Permethrin (0.75%), Alpha cypermethrin (0.05%) and Lambda cyhalothrin (0.05%) were prepared. Both in bioassay tests and in chemical analysis, papers were found comparable to WHO papers.

VIRAL DISEASES

HIV/AIDS

A study entitled “**Long Term HVB treatment outcome among cohort of HIV/HBV co-infections patients on antiretroviral regimens having tenofovir plus lamivudine and adefovir plus lamivudine as HBV active agents**” was completed at Kolkata. The study aimed to comparatively evaluate the long-term (48 months) outcome among treatment experienced (18 months) HIV/HBV co infected patients on antiretroviral regimens of Tenofovir (TDF) + Lamivudine (3TC) + Efavirenz and Zidovudine/ Stavudine + Lamivudine (3TC) + Efavirenz with Adefovir (ADV). The results indicated that the overall effectiveness of both the treatment regimens (TDF +3TC+EFV+ADV and AZT/d4T+3TC+EFV+ADV) was high and similar. After 30 months of treatment there were no statistically significant difference in bio-chemical, serological virological parameters among both the arms.

A study entitled “**To evaluate the role of negative regulatory factors in the functional impairment of dendritic cells during HIV-1 infection**” was completed at Chandigarh. HIV-1 infection is

characterized by several immune abnormalities including functionally impaired dendritic cells. Till date, there is insufficient knowledge about the regulatory mechanisms in DCs that control the magnitude and activation of T cell responses. The study reveals that the DCs get functionally defective during the late stage of HIV disease due to the virus mediated modulation of some regulatory genes. The PCR array results show some signature genes involved in negative regulation of DC functions getting upregulated. These findings may have important clinical implications and suggest the design of new generation preventive or therapeutic agents based on silencing of one or more of these negative regulators after studying the precise contribution of each of these factors at transcriptional as well as protein level. Further elucidation of more genes involved in cytokine signaling could help in revealing pathways used by HIV to drive its spread and to identify therapeutic strategies to bias DC biology towards immunity.

A study entitled **“To evaluate the role of negative regulatory factors in the functional impairment of dendritic cells during HIV-1 infection”** was completed at Chandigarh. HIV-1 infection is characterized by several immune abnormalities including functionally impaired dendritic cells. Till date, there is insufficient knowledge about the regulatory mechanisms in DCs that control the magnitude and activation of T cell responses. The study reveals that the DCs get functionally defective during the late stage of HIV disease due to the virus mediated modulation of some regulatory genes. The PCR array results show some signature genes involved in negative regulation of DC functions getting upregulated. These findings may have important clinical implications and suggest the design of new generation preventive or therapeutic agents based on silencing of one or more of these negative regulators after studying the precise contribution of each of these factors at transcriptional as well as protein level. Further elucidation of more genes involved in cytokine signaling could help in revealing pathways used by HIV to drive its spread and to identify therapeutic strategies to bias DC biology towards immunity.

A study entitled **“HIV Drug Resistance Monitoring In Chennai, India”** was completed at Chennai. The study aimed on transmitted drug

and acquired drug resistance mutations selected TB patients who are failing and experiencing success with TDF based regimen. The transmitted drug resistance observed was low among the ART naïve patients among ART experienced M1984V, V106M and K103N mutations were predominantly selected. ASPCR assay was developed by the investigators and is highly sensitive and could detect even 1% mutation as against 30% mutation by conventional genotyping assay. This assay will enhance the clinical management of the patients in resource limited settings and more antiretroviral drugs can be preserved for future treatment.

A study entitled **“Improving HIV Testing among wives of Men who Have Sex with Men (MSM) in India”** was completed at Chennai. The study aimed to characterize the context of disclosure of same-sex behavior among married MSM in India and its impact on HCT for their wives. The results showed that most men are still hesitant to divulge their sexual preferences (Specific to Same sex) due to conjugal disharmony and stigma issues. Also there is very less conjugal communication in this regard though wives of MSM seem to be aware of their spouses’ sexual activities and preferences. Very low HIV testing is occurring among Wives of MSM and substantial risks seem to be prevalent for hidden partners of MSM who are married and may have multiple partners. Current HIV prevention interventions targeting MSM population seem to offer basic drop in centre services and linkages to Government settings for HIV testing and or other related health care needs. However there is very negligible level of access to wives of MSM as there are multiple issues- women are not willing to visit Drop in centres, perceptions among MSM about risks for these women if they accompany MSM to drop in centres, there is no focused service delivery for MSM wives resulting in higher possibilities of them ignoring health care.

OTHER VIRAL DISEASES

A study on **“Identification of host cellular proteins interacting with influenza A viral nucleoprotein (NP) and their role in the regulation of viral replication”** was completed at School of

Biotechnology, JawaharLal Nehru University, New Delhi, wherein they tried to clone and express nucleoprotein (NP) of human Influenza A viral strains (H1N1 seasonal and Pandemic strains, H3N2 seasonal strains). By co-immunoprecipitation (Co-IP) and Immunofluorescence Assay (IFA) staining studies it was observed that POTEE family member and nucleolin (NCL) co-precipitate and co-localize with recombinant viral NP. These interactions were further confirmed in A549 cells infected with influenza A virus of H1N1 pandemic and seasonal H3N2 strains. Depletions of host NCL by siRNA targeted NCL not only confirmed the disruption of viral NP interaction with host NCL, surprisingly increased levels of late viral structural genes such as Matrix and HA genes expression at transcript level was noted. On contrary, over expression of NCL using pEGFP-NCL construct lowered the late gene expression to significant level and reduced the viral induced cytopathic effect as compared to cells with siRNA targeted NCL and regular viral infections.

A study on **“Evaluation of herbal extract for anti-rabies activity”** was completed at Haffkine Institute for Training, Research & Testing, Mumbai. The study was aimed at *in-vivo* evaluation of Herbal Hydro-alcoholic extract against Rabies virus in mice model. They succeeded in post exposure Animal Trials with both Hydro-alcoholic extract and the Finished Product (Oral Liquid suspension) in Swiss Albino Mice – 4 weeks with Avg Weight 24 ± 4 gm with the following observations: 80% Survival in Test group with 2.5 LD 50 Virus dose. 60% Survival in Test group with 25 LD 50 Virus dose. 30% Survival in Test group with 50 LD 50 Virus dose. It was observed that when Vaccine was given in combination with extract by oral route (Extract) & Vaccine by Intramuscular route, the rabies Titre was found to be 2 fold higher on 5th and 10th day as compared to Vaccine Control group.

A multi-centric study on epidemiology of Hepatitis B & C Viral Infections in Ladakh Region was completed at SKIMS, Srinagar & SGPGI, Lucknow, to determine the prevalence of serological markers of HBV and HCV infection in the Ladakh region, and to identify risk factors associated with the transmission of these viruses. Of the 2674 subjects screened, 141 (5.3%) were tested positive for

HBsAg, i.e. had current HBV infection and 339 (12.7%) tested positive for either HBsAg or anti HBc, i.e. had either current or past infection with HBV. Anti-HCV antibody was detected in 22 of 2674 subjects, with a prevalence rate of 0.8%. None of the risk factors studied was significantly related to the evidence of HBV or HCV infection. Of the 134 and 12 specimens that contained HBsAg and anti-HCV (one had both), 79 and none tested positive for HBV DNA and HCV RNA, respectively. Of the 29 specimens that had sufficient HBV DNA for genotyping, 21, 7 and 1 specimens had HBV genotypes D, C and A, respectively.

A study on **Markers of dengue disease severity** was completed at G.S.V.M Medical College, Kanpur to identify biomarkers associated with severe dengue disease and correlation of plasma viral load and complicated dengue fever was studied. During the study period blood samples from 6000 clinically suspected dengue patients were tested for presence of Dengue NS1 antigen and anti-dengue IgM by MAC ELISA and 2307 (38.5%) patients were positive. Of these; 550 patients were enrolled in study based on inclusion & exclusion criteria. DF was seen in maximum (54%) patients followed by DHF (40%) and DSS (6%). Majority of patients were in age group of 20-40 years, however complicated cases (DHF and DSS) were predominantly seen in young (< 20 years) male patients. The PI concluded that, Dengue plasma viral load did not correlated with dengue disease severity; however majority of complicated cases were seen in secondary dengue infection.

A project on **“Association of Host IL28B Genetic Polymorphism with sustained virological response to interferon-ribavirin combination treatment for chronic HCV infection in Indian patients”** was completed at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. The study was aimed to determine the relationship of host genome single-nucleotide polymorphisms in the IL-28B gene region with sustained virological response to interferon (standard or pegylated) and ribavirin combination treatment in Indian patients with chronic HCV infection. They found out that alleles known to be associated with better response to treatment were more common in the Indian population (nucleotide T at rs12979860:

75.0%; nucleotide G at rs8099917: 83.5%) than the frequencies reported in other population groups (Europe, North America, Japan, etc). For the 120 patients for whom follow-up data were available, either alleles or genotypes at both these sites had no relationship with sustained virological response following pegylated-interferon ribavirin combination therapy. The PI concluded that, determination of IL-28 polymorphism does not have value in Indian patients with HCV infection who may be treated with interferon-ribavirin therapy.

A study of **“Hepatitis B vaccine effectiveness depending on vaccination schedule”** was completed at St. Stephen’s Hospital, Delhi. The study was aimed at evaluating efficacy of Hepatitis B vaccine in those who were vaccinated at birth (within 48 hours of delivery) and those who were vaccinated thereafter. The PI concluded that Hepatitis B carrier rate is not improved by birth dose. Protective antibody (HBsAb) in unvaccinated babies was seen in nearly half the children at 1 year of age. This could be as a result of passage of antibodies from mother to child during the initial days when the chance of developing long term complications like development of Hepatocellular carcinoma (HCC) is highest. Universal immunization may reduce transfer of passive protection to newborns and has potential of paradoxically increasing hepatocellular carcinoma in the population.

A study on **“Prevalence of human parvovirus B19 and other genotypes of erythroviruses in relevant population”** was completed at King George’s Medical University, Lucknow. They have designed Real Time PCR based assays for detection of Parvovirus 4 and B19V. Total 406 patients and 350 controls (healthy blood donors) were enrolled in the study. Of the 406 patients, 233 had hematological malignancies, 96 had solid tumors and 77 had aplastic anemia.

A study was completed with the purpose to study the factors that are responsible for stability of transmission dynamics of the H1N1 epidemic. Epidemic spread modeling based on the specific property of population growth, the spread rules of infectious diseases, and the related social factors, for

Vellore was carried out. By applying the structural similarity algorithm, 4 cluster locations were found to have high mobility of the infected host. The parameters that are significant for the transmission of H1N1 from the densely populated locations was predicted to be age, temperature, wheezing and smoking using geographically weighted regression (GWR) method. Multi-criteria decision-making method predicted that people described as Age < 9 & Age > 65, those affected with asthma, diabetes, heart disease, liver disorders, blood disorders, endocrine disorders, kidney disorders and pregnant women, people who are morbidly obese, people who live with or care for children younger than 6 months of age are best suited targets for efficient vaccination.

ZOONOSES

A study entitled **“Identification of Plant Based Molecules (s) and its Mechanism of Functionality against Mycobacteria with species specification of Tuberculosis in Man and Animal”** was carried out at College of Veterinary Science, Assam. The study included two candidate plants i.e. *Alstoniascholaris* and *Mucuna imbricate* against mycobacterium H37Rv in mice model, which have traditional uses in the North East Region. Results showed that the plant extracts protect murine peritoneal macrophage from *mycobacteria* by decreasing free radical generation, lipid damage and also increasing antioxidant status. It was also observed that activity of the synergistic group i.e. rifampicin (10mg/Kg) and *Alstoniascholaris* (100mg/Kg) displayed powerful and maximum response against TB infection with lower CFU counts.

A study entitled **“Clinical Geographical Epidemiology and Molecular Characterization of Scrub Typhus in South Karnataka”** has been completed at Manipal Medical College, Manipal. Among the various serological and molecular methods of scrub typhus diagnosis, it was observed that the Micro-immuno-fluorescence Assay (MIFA) was able to detect maximum number of scrub typhus cases 310 (24.9%) cases. Number of cases positive by specific tests were, IgM ELISA (22.7%), Weil Felix test (OX K \geq 1:80)(31.0%) and Nested PCR (17.8 %). Nested PCR showed a sensitivity and specificity of 66.4% and 100% respectively.

Sensitivity and specificity of IgM ELISA for scrub typhus were 77.5% and 97.3% respectively. It was observed that sensitivity of Weil Felix test decreased and specificity increased with when the cut off titer was raised from $\geq 1:80$ to $\geq 1:160$ for OX K. Therefore, among the all diagnostic tests for the detection of scrub typhus, M IFA detected large number of cases.

A study entitled “**Clinical and Sero Epidemiology Study of Scrub Typhus, Spotted Fever and Q Fever in Pudduchery**” was carried out at MGMCRI, Pondicherry. It was found that IgG, Seroprevalence among normal population for Scrub Typhus was 19.63%, Spotted fever 10.54%

and Q fever 6.18%. Sheep, goat, cattle and buffaloes showed very low prevalence for Q fever 0.97%, 1.06%, 1.85% and 5.64% respectively. True prevalence of acute Q fever in humans could not be estimated, since all three commercial *C. burnetii* phase II IgM ELISA kits (Novatec, Serion/Virion and Vircell) were found unreliable due to their poor sensitivity and/or specificity. ‘Gold standard’ IFA was positive in 22 patients (28.95%). The study also proved the unreliability of three commercial ELISA kits for *C. burnetii* Phase II IgM. Hence use of these kits should be discouraged and to be replaced by IFA and to be preferably confirmed by PCR.

REPRODUCTIVE HEALTH

The ICMR is undertaking research in the field of reproductive health through two of its research institutions viz, (i) National Institute of Research in Reproductive Health, Mumbai and (ii) Genetic Research Centre, Mumbai as well as extramural research projects. These studies are aimed to protect and enhance the reproductive health of people through research and development of technologies and programmes for field applications which can be incorporated into National Programmes.

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

Intervention to Enhance Acceptance of Contraceptive Use among Couples by Reducing Domestic Violence from Husband

The objective of the study was to understand the effectiveness of an intervention to increase the contraceptive use among couples where women reported unmet need for contraception. The study was conducted in two slum communities Tunga Village and Kajupada in Mumbai where 1136 women aged 18-39 reported unmet need for family planning. Counselling on contraceptive methods was provided to all women. Additionally counselling on maternal communication was provided to women who reported of domestic violence during the baseline survey. Endline survey showed significant decrease in incidence of domestic violence in the past 12 months preceding the survey and increase in contraceptive use may be because of counselling. The study is completed.

Exploring Use of Emergency Contraceptive Pills and other Temporary Methods of Contraception among Unmarried Youth in Mumbai

Use of emergency contraception among unmarried youth in Mumbai was explored using web based technology method which provided more confidentiality for youth to reveal their sexual practices in an anonymous manner. Among those who responded, 23% boys and 18% girls revealed that they were sexually active. This disclosure was found to be much higher than previously reported in literature. Among those who indulged in sexual activity, 46% used Emergency Contraceptive pills. Study highlights the need for imparting information and counselling on sex and sexuality issues to young people.

INFERTILITY

Female Infertility: A Genetic Analysis of Polycystic Ovary Syndrome (PCOS) with Special Emphasis on Genes involved in Insulin Resistance

PCOS is a complex endocrine disorder in women of childbearing age characterized by presence of anovulation, insulin resistance, hyperandrogenism and polycystic ovaries. In the current reporting year, we have investigated the association of PON1 and INSL3 genes with PCOS risk and its related phenotypes. We report for the first time significant association of the rs6523 polymorphism and AGAG haplotype of INSL3 with PCOS susceptibility. We have also shown that the L55M but not Q192R polymorphism of PON1 is associated with decreased PCOS susceptibility in lean women only. Further, it improves both metabolic and hyperandrogenemia related traits in lean women with PCOS only. Among six known polymorphisms

screened in the promoter region of PON1, only the -907G/C polymorphism showed significantly different frequency distribution between control and PCOS women.

Molecular Signature of Human Follicular Fluid in PCOS by Proteomics Approach

Differential Proteome Analysis of Follicular Fluid (FF) from PCOS women indicate defect in Cumulus Oocyte Complex (COC) matrix. The growth factors such as Amphiregulin (AREG) and Growth Differentiation Factor-9 (GDF-9), which induces COC matrix gene expression were significantly low in PCOS FF. We analyzed expression of several Genes in Granulosa Cells (CGCs) of PCOS and control women and found TNFAIP6 and HAS2 gene expressions were significantly altered in CGCs from PCOS, indicating a problem in COC expansion and mucification. The gene expressions of BMPRII, EGFR1 and NRIP140 were comparable between control and PCOS.

Deciphering the Putative Epigenetic Mechanisms Pertaining to Polycystic Ovary Syndrome

Epigenetic studies indicate that complex interactions between environmental components and genetic determinants play a role in etiopathology of PCOS. This study aims to investigate the prevalence of epigenetic modifications in PCOS women with special emphasis on DNA methylation. We compared the global DNA methylation levels between cases and control. The global methylation levels remained unaltered in peripheral blood lymphocytes (PBLs) and granulosa cells (CGCs) between control and PCOS women. L1 CpG site analysis revealed hypermethylation of CpG22 in all PBLs and CGCs of PCOS women which can be used a candidate methylation marker for PCOS. Additionally we found ovary specific L1 methylation in both control as well as PCOS women.

Autoimmune Markers for Diagnosis of Endometriosis

A multi-centre study on endometriosis is on-going. In the reporting year, we carried out the clinical phenotype analysis of 500 Indian women suffering from endometriosis. Seventy seven percent women had dysmenorrhea, 34% women had chronic

pelvic pain, 23% had dyspareunia and 14% had dyschezia. Dysmenorrhea was the chief complaint associated with endometriosis and there was a correlation between severity of dysmenorrhea and severity of disease. Fifteen percent women were classified as peritoneal endometriosis, 58% as ovarian endometriosis and 24% were having Deep Infiltrating Endometriosis (DIE/RV). History of abortion was reported in 33% women who ever conceived. Family history of endometriosis was observed in 13% of women with endometriosis either in mother, sister or both suggesting the need to investigate the role of genetic factors.

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. We have earlier shown that HOXA10 regulates endometrial decidualization and trophoblast invasion.

In the reporting year, the institute studied the roles of HOXA10 in epithelium. It was observed that HOXA10 is required to maintain an epithelial phenotype and loss of HOXA10 leads to epithelial to mesenchymal transition (EMT). This EMT was associated with switch in expression proteins and transcription factors like SNAIL and SLUG.

To understand the roles of HOXA10 in endometriosis, a transgenic mice was generated that overexpresses a shRNA against HOXA10 leading to loss of HOXA10 functions. The mice are being characterized for their copy number and transgene expression.

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

Endometriosis affects approximately 10% of women of reproductive age and is associated with extensive morbidity. It is also a leading cause of female infertility. Presently, endometriosis is diagnosed by laparoscopy which is invasive. There is a need to identify non-invasive diagnostic and prognostic markers of endometriosis.

A mouse model was developed for endometriosis and characterized it for its similarity to the human

conditions. It was observed that endometriosis can be induced in mice and morphologically it shows similarities to the human condition. The mice developed lesions that had glands and stroma which were proliferating and minimal cell death. In the endometriosis tissues, there were alterations in mRNA and protein expression of steroid receptors. The expression of estrogen receptor alpha and progesterone receptors was reduced whereas the estrogen receptor beta isoform is increased. These observations suggest that in endometriosis there is altered sensitivity to steroid receptors. This information will be useful while designing steroid hormone based drug strategies for treatment of endometriosis. We are utilizing this model for biomarker discovery.

The Factors of Relevance in Endometrial Adhesiveness to Embryonic Cells

Endometrial receptivity is defined as the unique temporal sequence of surface expression of Cell Adhesion Molecules (CAMs) on the endometrial cell to facilitate blastocyst attachment. Studies were undertaken to establish the role of Rab11A (a marker of recycling endosome) in the recycling of Integrin $\alpha V\beta 3$ and E-cadherin in endometrial epithelial cells. We previously demonstrated a decrease in the cell surface localization of Integrin αV in Rab11A knockdown Ishikawa cell. This in turn affected the adhesion of these cells towards JAr trophoblastic spheroids indicating role of Rab11A in the trafficking of Integrin αV to the cell surface in endometrial epithelial cells. In the reporting year, the localization of E-cadherin, another CAM important for embryo-endometrial interactions, was assessed. A decrease in the expression of E-cadherin was observed in Rab11A knockdown clones compared with Rab11A intact clones. This correlated with increased migration of Rab11A knockdown clones, indicating a loss in epithelial properties of these cells as compared intact Rab11A expressing cells. Hence, Rab11A is associated with maintenance of epithelial properties of Ishikawa cells.

Investigations on the Role of SP-A, SP-D and MBL in Fertility and Embryo Implantation

Surfactant protein D (SP-D), an innate immunity protein, is secreted by uterine glandular and

luminal epithelial cells under positive regulation of estrogen. SP-D knockout female mice exhibited a prolonged metestrous and diestrous phase, significantly lower litter size concomitant with increased pre-implantation loss. Estrus phase of these mice showed higher levels of serum progesterone, estrogen & progesterone receptors, hormone responsive genes and elevated levels of pro-inflammatory cytokines and chemokines. In early pregnancy, a pro-inflammatory immune environment was evident with elevated levels of TNF- α positive uterine macrophages with a skewed M1/M2 phenotype and down regulation of Foxp3 mRNA. Studies suggest that altered level of SP-D during early pregnancy results in dysregulated immune response and contributes to pre-implantation loss. The project is completed.

Identification and Development of a Potential Stem Cell Growth Factor from the Peri-Vitelline Fluid of the Fertilized Eggs of the Indian Horseshoe Crab for its Application in Stem Cell Research

Peri-vitelline fluid (PVF) was isolated under sterile conditions from fertilized eggs of horse shoe crabs and was fractionated. Fraction VII showed angiogenic differentiation and tube formation in MSCs isolated from Umbilical Cord tissue and enhanced expression of MMP-2, 3 and 9.

Development of a Non – Human Primate Model for Endometrial Hyperplasia

Endometrial hyperplasia is proliferative disorder of endometrium, characterized by an increase in the gland to stroma ratio. We developed a non-human primate model of endometrial hyperplasia. Ovariectomized marmosets treated with estrogen demonstrated increased proliferation of glandular epithelial cells. Histological characterization of endometrial tissue from marmosets with endometrial hyperplasia showed a similarity to endometrial samples from women with simple endometrial hyperplasia. Immunohistochemical studies demonstrated dysregulated expression of ER- α , PTEN, pAKT and COX-2 in hyperplastic endometrial samples from marmosets. Microarray analyses of hyperplastic endometrial samples from human and marmosets showed similar expression pattern for 251 genes, when compared with their respective normal counterparts.

Understanding the Role of Receptor for Advanced Glycation End Product (RAGE) and its Ligand HMGB1 in Endometrial Physiology and Pathophysiology

Receptor for Advanced Glycation End Product (RAGE) is a multiligand immunoglobulin cell surface receptor. High Mobility Group B1 (HMGB1) is a nuclear protein released from damaged or necrotic cells. Exogenous HMGB1 interacts with RAGE and induces inflammation to support the regeneration of injured tissue. Studies have been undertaken to investigate the role of endometrial expression of HMGB1 and RAGE. These investigations demonstrated a cycle dependent expression of endometrial RAGE and HMGB1 and also presence of HMGB1 in the uterine fluid of rats. Our study also shows that HMGB1 is released from endometrial cells via exosomes.

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

Metformin, an anti-diabetic drug improves ovulatory rates, menstrual cyclicity and pregnancy outcomes in women with poly cystic ovarian syndrome. In vivo studies have shown that metformin treatment leads to increased expression of surrogate markers of endometrial receptivity. However, the exact mechanism by which metformin improves reproductive outcomes is not known. Our in vitro studies have shown that metformin at 20 - 100 μ M concentration induces endometrial cell proliferation. Further flow cytometry analysis shows increased expression of $\alpha\beta3$ in endometrial cells treated with 50 μ M metformin compared to untreated cells. These warrant further investigations in the direct effect of metformin in human endometrium.

MALE INFERTILITY

Proteomic Influences on Sperm Function with Reference to Motility

Histone Deacetylase 6 (HDAC6) is an alpha (α)-tubulin deacetylase and its over-expression has been demonstrated to promote chemotactic cell movement. We hypothesized that HDAC6, by virtue of being α -tubulin deacetylase, may modulate sperm motility. However the presence of HDAC6

on sperm has hitherto not been reported. We have reported for the first time the presence of HDAC6 transcript and protein in testicular- and caudal-sperm of rat. We show that 1) an active HDAC6 enzyme is present in sperm, 2) HDAC6 in sperm is able to deacetylate α -tubulin, 3) inhibition of HDAC6 increases acetyl α -tubulin expression, and that 4) HDAC6 inhibition affects sperm motility. These evidences suggest that HDAC6 may be involved in modulating sperm movement.

Taking cues from our proteomic observations which show that phosphorylated but not total GRP78 expression is reduced in sperm with poor motility, we determined phosphorylation changes in GRP78 during epididymal maturation. Ours is the first report indicating GRP78 in sperm to be phosphorylated at serine, threonine and tyrosine residues contrary to published literature reporting GRP78 not to be tyrosine phosphorylated. We report the presence of GRP78 phosphoforms in rat- and human- sperm and our data suggest that GRP78 phosphorylation in sperm undergoes spatial reorganization during epididymal maturation. Significant differences observed in 2 out of 3 phosphoforms in asthenozoosperm suggest that GRP78 phosphorylation may have functional relevance in sperm with consequent clinical implications.

Genetics of Male Infertility: Y Chromosome Deletions

Male infertility is usually manifested as azoospermia or oligozoospermia due to defects in spermatogenesis. One of the causes of male infertility is deletions of specific regions of Y chromosome. This genetic defect is essential to diagnose as men with Y chromosome deletions have a severe phenotype, poor success rate in assisted reproduction and these men will pass on the genetic defect in all their male offspring born after assisted reproduction. We have identified markers that would be useful to detect Y chromosome deletions in Indian population. We have shown that beyond the major deletions minor deletions like gr/gr, b1/b3 and b2/b3 are also associated with infertility. In addition we have identified the CDY1 gene and GOLGA2LY genes as a major risk factor for male infertility.

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

Congenital bilateral absence of vas deferens (CBAVD) occurs in 2-6% of infertile but otherwise healthy men and is associated with mutations in cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. During the reporting year we recruited 19 CBAVD men and their female partners. So far a total of 85 CBAVD men, their female partners and 50 proven fertile men have been recruited in this study. Out of these 85 CBAVD men, 10 patients (11.76%) were found to have Unilateral Renal Anomalies (URA). Direct DNA sequencing of the *CFTR* gene in 5 CBAVD-URA men revealed IVS8-5T mutation in 4 men with an allelic frequency of 40%. Three potential regulatory *CFTR* gene variants (c.1540A>G, c.2694T>G and c.4521G>A) were detected in association with IVS8-5T mutation in 3 CBAVD-URA men. Twelve couples had risk of transmitting *CFTR* gene mutations to the progeny. Genetic counseling was provided to the couples before undergoing ICSI.

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

Expression of immunoregulatory proteins SP-A, SP-D and MBL in the testis is under testosterone regulation. Germ cells and myoid cells are a predominant source of these proteins in murine testis. Testis of SP-D gene deficient mice show increased TGF- β , IL-10 and serpin3n and F4/80⁺ macrophages while a decrease in the percentage of CD11c⁺ dendritic cells. Ongoing studies with the mice model of LPS induced male infertility will reveal role of SP-D in regulating the immune response and sustenance of spermatogenesis in testis.

Studies to Elucidate the Molecular Mechanism of Estrogen Action in Spermatogenesis

Estrogen, through its receptors (ER) α and β regulates spermatogenesis. The individual role of the two ERs in spermatogenesis and fertility was elucidated by *in vivo* treatment with selective ER agonist to adult male rats. Our results suggests two

ERs affect fertility through different mechanism. ER α acts through the hypothalamus pituitary axis whereas ER β acts predominantly at the testicular level. Both ERs regulate distinct aspects of spermatogenesis leading to low sperm count. ER α is mainly involved with regulation of spermiogenesis while ER β regulates spermatocyte apoptosis and spermiation. Since many environmental estrogens can bind to the two ERs with different affinity, these observations can be useful in understanding their potential effects on spermatogenesis.

RTI/ STIs/HIV/ MICROBICIDES

Association of Host Immunogenetic Factors with HIV Infection

HLA-G is an important immunomodulatory molecule, and it plays a fundamental role in maternal-fetal tolerance. This immunosuppressive capacity of HLA-G is exploited by many viruses, which have developed multiple strategies to escape host immune defences. Few studies have reported association of variation in HLA-G gene between mother and infants with perinatal HIV transmission. Our analysis on HLA-G in HIV positive mothers and their newborns highlighted; (i)HLA-G*01:01 and HLA-G*01:04 were the only two alleles present in the studied population (n=210), (ii)There was no difference in the frequencies of HLA-G alleles between mothers and their respective children in both transmitting/non-transmitting groups and hence did not play any significant role in HIV transmission.

Characterization of Antimicrobial Peptide (AMP), SsALF-24 Isolated from the Hemocytes of Indian mud crab, *Scylla serrata*

The peptide, *Scylla serrata* antilipopopolysaccharide-24(SsALF-24) isolated from Indian Mud crab has been characterized. The gel formulation of SsALF-24 is active against several STI causing pathogens. The formulation did not cause toxicity to rabbit vaginal epithelium *in vivo*.

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

We designed a peptide, named HbAHP-25, from human haemoglobin- α (Hb- α) against CD4 binding

site of gp120 of HIV-1. This peptide showed significant anti-HIV activity against various strains of HIV-1 and its activity is specific to gp120. HbAHP-25 retained its anti-HIV activity in the presence of seminal plasma and vaginal fluid. The peptide is non-toxic to vaginal cells. HbAHP-25 is not active against STI causing pathogens.

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

The miRNA, Let-7f was identified to be involved in Toll-like receptor (TLR) tolerance in human cervico-vaginal epithelial cells (End1/E6E7). During the reporting period, we studied the possible targets for let-7f and identified C/EBP and ELK-1 as targets of Let-7f. These targets likely to modulate vaginal cellular immune responses.

Approaches for Controlling Biofilm formation by *Gardnerella vaginalis*

Bacterial vaginosis (BV), a common genital tract infection is found to recur very often in affected women despite antibiotic treatment. Polymicrobial biofilm formation with the predominance of the anaerobe, *G. vaginalis* is associated with recurrence of BV. In view of the lack of effective measures to resolve this issue, approaches towards either prevention or clearance of *G. vaginalis* biofilms are being developed. During the reporting period, two peptides namely, 1018 and 1037, reported to be effective against other pathogens, were investigated. These were found to effectively prevent biofilm formation by *G. vaginalis* ATCC 14019 *in vitro*, in a dose dependent manner. Further, *in silico* analysis of predicted surface proteins of *G. vaginalis* led to the rational design of three Specifically Targeting Anti-Microbial Peptides (STAMPs).

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

The role of the gut homing receptor, integrin $\alpha_4\beta_7$ in the pathogenesis of HIV-1 infection is not completely understood despite its recognition as an additional receptor for the HIV-1 envelope glycoprotein, gp120. Our previous work suggests that integrin $\alpha_4\beta_7$ mediated signaling may influence cell-cell transmission of HIV-1. During this

reporting period we show that attenuation of HIV-1 gp120 mediated integrin $\alpha_4\beta_7$ signalling either by inhibition of HIV-1 gp120-integrin $\alpha_4\beta_7$ interaction through siRNA mediated downregulation of integrin β_7 or inhibition of intracellular signaling components such as ERK 1/2, p38 MAPK, PI3K and RAP-1, result in a significant reduction of HIV-1 replication. These results thus highlight the potential of inhibiting HIV-1 gp120 mediated integrin $\alpha_4\beta_7$ signalling as a strategy for the prevention and control of HIV-1 infection.

Evolution and Diversity Analysis of Env gp41 based BNAb Epitopes of HIV-1 Clade C

NGS based frequency and variation in Broadly Neutralizing Antibody (BNAb) epitopes carried out will significantly instruct vaccine design for the Indian population. It was observed that all the epitope sequences analysed were found to be resistant for 2F5, have moderate predicted sensitivity to Z13e1 and high sensitivity to 10E8 BNABs. Assessment of *in silico* neutralization efficiency through docking analysis of 17 unreported epitopes for 10E8 showed 7 of epitopes to be sensitive, 8 to be resistant and 2 to have low binding affinity (Potentially resistant haplotypes) and need to be validated experimentally.

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

Our study shows that dysregulation occurs in CD25 (IL-2R α) and CD127 (IL-7R) expression following both HIV1 and HIV2 infection. This dysregulation was found to be associated with HIV disease progression. 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.

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.

Osteocalcin a Biochemical Marker of Bone Formation for Use in the Diagnosis and Management of Osteoporosis

Second party validation of the developed Sandwich ELISA showed a good correlation of the Osteocalcin levels with BMD and commercial ELISA.

Preparation of a Multi-analyte ELISA Array of Bone Markers for the Assessment of Bone Health

ELISAs for the prominent markers Osteocalcin (OC), Procollagen peptide (PINP), C terminal telopeptide (CTX) and Deoxy-pyridinoline (DPD) were developed with the generated key reagents. The levels of these bone markers correlated well with the commercial kits.

Point of Care (POC) Device for Osteoporosis (In collaboration with IITB under Health Consortium)

Key reagents generated for OC were used for optimisation of microfluidic based assay for OC on the device.

Osteoprotegerin (OPG), Receptor Activator of Nuclear Factor Kappa B (RANK) and Receptor Activator of Nuclear Factor kappa B Ligand (RANKL) as Biomarkers for Osteoporosis

OPG, RANK and RANKL, belonging to the tumor necrosis factor (TNF) superfamily are molecular links between bone remodelling and their ratio is critical in pathogenesis of bone loss. OPG and RANKL levels increased with age and were high in postmenopausal women reflecting increased rate of bone loss in these women. OPG and RANKL levels inversely related to BMD and positively with bone markers reflecting increase bone turnover during menopause. The study suggests RANKL: OPG ratio may aid in identifying women at risk for osteoporosis. The T950C polymorphism of OPG gene was studied in a cohort of 84 women by restriction fragment length polymorphism (RFLP) using the restriction enzyme HpaI. The TT genotype of T950C polymorphism is associated with low bone mass in post-menopausal women.

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

Increased differentiation of Monocytes (MO) into osteoclast, the bone resorbing cell is one of the consequences of increased bone loss leading to osteoporosis. Hence, MO are potential candidates for identifying protein signatures in early stages of osteoclastogenesis. Therefore, protein alterations in MOs from premenopausal and postmenopausal women with low and high BMD were studied which revealed that total HSP27 and phosphorylated HSP27 (pHSP27) were upregulated in low BMD condition in premenopausal and postmenopausal women. BMD showed a strong correlation with pHSP27 irrespective of menopausal status. Additionally exogenous pHSP27 increased MO migration and transendothelial migration towards RANKL indicating that pHSP27 may boost MO migration towards the bone microenvironment. Thus, a novel link was shown between HSP27 and low BMD, and HSP27 may thus emerge as potent biomarker of low bone mass irrespective of menopausal status.

A Study of Lifestyle and Genetic Factors in a Tribal Population and its Association with Bone health and Cardiovascular Status

The latest data support the correlation of atherosclerosis and osteoporosis, indicating the parallel progression of two tissue destruction processes with increased fatal and non-fatal coronary events, as well as higher fracture risk. A study has been initiated in northeast tribal populations known to be at increased risk of developing both the diseases. Lipid profile and homocysteine levels were relatively abnormal in women with low BMD in both pre and postmenopausal women.

MATERNAL AND CHILD HEALTH

Study of Preterm Birth and Neonatal Outcome among Women Conceived by Assisted Reproduction Techniques (ART) in Mumbai

There is lack of data on preterm births in pregnancies conceived by ART in India. This clinic based cohort study was undertaken to assess the magnitude of preterm birth among pregnancies conceived by ART, its neonatal outcome and associated risk factors through 8 ART clinics across Mumbai. Study showed very high incidence of preterm birth

(76.2%) among pregnancies conceived by ART. Multiple gestations were observed in 45.1%.

Incidence of heterotrophic pregnancy was high i.e 3% which could be due to multiembryo transfers. Incidence of gestational diabetes was high i.e 11% in this group. 96.9% participants underwent LSCS, The incidence of live babies was 98 % and the study showed just 2 still births. 62 babies required NICU stay. All babies were discharged after delivery from hospital in healthy condition. Multiple pregnancy was the risk factor significantly associated with preterm birth. There is increasing trend in the number of pregnancies conceived by ART and hence interventions to prevent preterm birth in this focussed group like preventing multiple pregnancy by restricting number of embryos transferred, need to be addressed.

Review of Traditional Childbirth Practices Among Various Tribal Communities in Maharashtra

The objective of the study is to review of traditional practices during childbirth among various tribal communities in Maharashtra. The published articles, reports from years 1990 onwards collected using search engine (bibliographic databases and online resources, reference list citations), NFHS and DLHS reports were included for review. A total of 33 manuscripts/reports were included for desk review. It was found that though the institutional deliveries increased over the time period, the ANC registration, 2 doses of TT and IFA consumptions, were low. The reasons documented were a) losses in households work as well as wage earning, and b) consumption of IFA would increase the size of the baby hence increase labour complications. The study is ongoing.

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

Vitamin D deficiency is the commonest nutritional deficiency worldwide. Despite ample sunshine, vitamin D deficiency prevails in 50 to 90% of Indian subcontinent. Apart from its skeletal benefits, vitamin D is associated as significant risk factor for severe lower respiratory tract infections in children and also has other extra skeletal benefits.

Therefore assessing vitamin D status in children and maintaining optimal levels of vitamin D may aid in reducing the child health burden.

The study aims to assess magnitude and factors responsible for Vitamin D deficiency in 1-5 year age group. Recruitment and screening of the participants is ongoing.

GENETIC RESEARCH CENTER

Genetic Research Centre has been successful in identifying molecular basis for Indian patients with Sandhoff disease, a fatal lysosomal storage disorder. Mutation p.R284X in HEXB gene was identified as a hotspot, accounting for 21% of Indian cases. Molecular and clinical characterization of Indian patients with glutaric aciduria type I showed allelic heterogeneity in GCDH gene. Exons 8, 9 and 11 of GCDH gene was identified as mutation hotspots for Indian cases. Spectrum of mutations were identified in FOXP1 gene in cases of Rett syndrome, one of the common causes of mental retardation in females. Novel mutation has been identified in Androgen Receptor (AR) gene in patients with complete androgen insensitivity syndrome. Clinical and molecular characterization of Lenz Majewski syndrome and a novel mutation identified in PTDSS1 gene, involved in nucleic acid salvage pathway. The centre has also identified the molecular and karyotype abnormalities in cases of autism spectrum disorder.

STRUCTURAL BIOLOGY AND BIOINFORMATICS

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

Common marmoset (*Callithrix jacchus*) is a New World primate monkey, used as animal model in the area of biomedical research. Many reports in marmoset, like multiple ovulation per cycle and presence of a mid-follicular phase follicle stimulating hormone (FSH) peak in the ovarian cycle, indicate that FSH requirement of marmoset in terms of concentration and secretion pattern is different than in other primates. Understanding the regulatory mechanism of FSH β - subunit expression is required to decipher differential synthesis and secretion of FSH in these species. During the current

reporting period polyclonal antibody raised against marmoset FSH β -subunit was characterised for detection of parent protein. Three serially deleted regions of marmoset FSH β promoter was cloned in luciferase reporter vector and the reporter activity was analysed in gonadotrope cells. The basal and GnRH induced activity of the promoter regions were found to be significantly different than that of the corresponding human FSH β promoter regions.

Creating a Gene Based Repository for Infertility

The manifestation of infertility is dependent on several confounding factors like genes, environment, lifestyle etc. Studies have found association of a number of genes independently or in combination with several reproductive conditions leading to infertility. A manually curated knowledgebase with information on these genes will aid researchers in deciphering the causes of infertility. The work on this database is in progress and it currently holds manually curated information for 250 genes.

Analysis of the Structures of Known Antimicrobial Peptides Using Machine Learning Algorithms and Molecular Dynamics Simulations

Antimicrobial peptides (AMPs) are evolutionarily conserved integral components of the host defence mechanism. AMPs exhibit family-specific sequence conservation which can be used for identification of novel AMPs. For facilitating this study, a manually curated database (CAMP_{R3}), that holds information on families and family-specific signatures of AMPs was created. The database currently contains information on 10247 sequences, 757 structures and 114 family-specific signatures represented by patterns and hidden Markov models. CAMP_{R3} is freely available online at www.camp3.bicnirrh.res.in. The signatures present in CAMP_{R3} have been used to create an online webserver for AMP identification (CAMPSign). CAMPSign available online at www.campsign.bicnirrh.res.in can aid in high-throughput identification of AMPs.

Developing a Gene Based Knowledgebase for Poly-Cystic Ovarian Syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is one of the most common causes for female subfertility in India. A comprehensive knowledgebase (PCOSKB) that

is freely accessible online at <http://www.pcoskb.bicnirrh.res.in> has been developed. PCOSKB has manually curated information on 241 genes, 107 SNPs, 500 disorders and 175 pathways associated with PCOS. Users can browse as well as analyse the data available in PCOSKB. The tools section in the database can be exploited to analyse common genes, pathways and gene ontologies in various comorbid conditions associated with PCOS.

Identification of Genes Involved in Controlled Ovarian Hyperstimulation

Predicting the ovarian response to Controlled Ovarian Hyperstimulation (COH) during in-vitro fertilization protocol is difficult as it varies with individual. So far several predictive markers have been proposed, such as age, ovarian reserve (ovarian volume and early antral follicle count), hormonal status (FSH, LH, estradiol, inhibin-B, Anti-Mullerian Hormone (AMH), and cigarette smoking. The effect of SNPs in genes on the outcome of COH is also being investigated. Work on constructing a database of the genes involved in FSH signalling network has been initiated with an aim to identify key factors associated with ovarian response. Data collection has been completed for 102 genes.

STEM CELL BIOLOGY

Pre-clinical Studies Using (a) hES cells Derived Cardiovascular Progenitors (b) hES Cells Derived Islet progenitors

In-house derived human embryonic stem cells (KIND-1) were differentiated into pancreatic progenitors and were used to test their ability to alleviate diabetic symptoms in mice. Pancreatic progenitors packed in immuno-isolatory device were transplanted, matured *in vivo* into beta islets as evident by detection of human C-peptide and successfully lowered blood glucose levels.

Pluripotent Stem Cells (VSELs): Newer Dimensions

In-depth studies were undertaken to characterize and study functional potential of a novel population of pluripotent VSELs in umbilical cord blood and mouse bone marrow. They are distinctly spherical cells with high nucleo-cytoplasmic ratio, smaller

than RBCs, 3-5 μm in size and with a surface phenotype of LIN-/CD45-/CD133+ in human cord blood and LIN-/CD45-/SCA-1+ in mouse bone marrow. VSELs survive 5-fluorouracil treatment (150 mg/Kg) and were involved in re-colonization of chemoablated mouse bone marrow. Besides expressing pluripotent markers, VSELs were found to differentiate into 3 embryonic germ layers, hematopoietic stem cells and germ cells *in vitro* (Fig. 3). The results clearly show that a sub-population of cells in bone marrow is pluripotent and has the ability to differentiate into 3 germ layers. Hematopoietic stem cells are committed progenitors that can only differentiate into blood cells. Thus the true regenerative ability in bone marrow/cord blood lies with CD34- VSELs and not with CD34+ HSCs.

Role of FSH in Mammalian Gonads from Stem Cell Perspective

Follicle Stimulating Hormone Receptors (FSHR) expression was observed on mouse testicular, ovarian and bone marrow VSELs and progenitors. The results challenge existing paradigm in reproductive biology that FSHR are expressed only on Sertoli cells in testis and Granulosa cells in ovary.

MicroRNA Regulation in Prostate and Ovary upon Exposure to Endocrine Disruptors

Bisphenol A (BPA) a weak estrogenic endocrine disruptor is detected in biological fluids/tissues, indicating ubiquitous exposure to humans. *In vitro* and animal studies have shown that BPA exposure can increase the risk of mammary gland, brain, and prostate cancers. Recent studies have demonstrated that aberrant expressions of miRNAs are closely associated with the development, invasion, metastasis and prognosis of various cancers including prostate cancer (PCa) and ovarian cancer. The present study was undertaken to investigate the effect of exposure of BPA on miRNA expression in prostate and ovary using both *in-vitro* and *in-vivo* approach.

The expression of miRNA 221, 125b and let7c was significantly down-regulated in BPA treated groups both in PC3 (prostate adenocarcinoma derived) and RWPE (Normal prostate epithelial) cells

For *in-vivo* exposure of BPA, perinatal model was used and 25 $\mu\text{g}/\text{kg}$ bw dose was given as oral gavage from gestation day 6 to Post natal day (PND) 21 to the mother. The F1 offspring were weaned, and then sacrificed on PND 75 and prostate and ovary along with other vital organs were collected. The microRNA expression of miRNA221, 125b and let7c was significantly down regulated in treatment group as compared to control.

Preliminary results revealed that BPA exerts effect on expression of selected miRNA, however, more miRNAs will be screened using tissue specific miRNA array followed by validation.

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

Cypermethrin is a broad-spectrum insecticide and known endocrine disruptor. We observed that maternal exposure to 25 mg/kg bw/day cypermethrin has adverse effect on reproductive development and growth of F1 male and female progeny. At this concentration cypermethrin affects the body and reproductive organ weights. The F1 generation showed delay in puberty. We observed decrease in sperm count and motility in male and irregular estrous cyclicity in female offspring. The testosterone levels did not change significantly at PND 22 but reduced significantly at PND 75. Histopathological, and biochemical changes were also observed in F1 progeny. The study revealed that, the perinatal exposure of cypermethrin may adversely affect the reproductive development and function of F1 progeny.

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

Parabens are a group of alkyl esters of p-hydroxybenzoic acid, namely methyl-, ethyl-, propyl-, butyl-paraben, used as preservatives. Butylparaben has been reported as estrogenic in nature. Perinatal exposure to butylparaben has been shown to affect male reproductive system, and has impact on steroidogenesis and spermatogenesis

In our study investigating the effect on F1 generation, perinatal exposure (Gestation Day 6- Postnatal Day 21) of Butyl paraben in corn oil injected SC at a

dose range of 10 - 1000 mg/kg BW did not show any treatment related alteration in the body weight of dams (F0) during gestation and lactation period as compared with the vehicle control. The male rats from the F1 generation showed a significant delay in testicular descent at 100 and 1000 mg dose as compared to control group). The female rats showed delay in vaginal opening at 100 and 1000 mg/kg bw dose.

The time taken for copulation was significantly increased in the F1 generation female rats; whereas normal females copulated with, F1 generation male rats did not show any difference. The normal cycling female rats sired by F1 generation male rats showed increase in pre-implantation loss and post implantation loss at 100 and 1000 mg/kg bw dose respectively. In case of F1 generation female rats increase in pre-implantation loss at 100 and 1000 mg dose; was observed whereas, increase in post implantation loss was observed only at 1000 mg/kg bw dose. A significant decrease in litter size was observed in F1 generation female rats and female rats sired by F1 generation male rats at 100 and 1000 mg/kg Bw dose. These results demonstrated that perinatal exposure of rats to butyl paraben via subcutaneous route disturbed the fertility potential of F1 generations suggesting long lasting effects of butyl paraben on reproductive function.

REPRODUCTIVE CANCERS

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

To develop better therapeutics for prostate cancer, it is essential to gain comprehensive knowledge of estrogen signaling in prostate cancer cells. While there exist some data on dysregulated expression of nuclear estrogen receptors in prostate cancer cells, there are no reports indicating the presence of specific estrogen binding proteins/ receptors on the cell surface of prostate cancer cells. Our studies identified estrogen binding proteins on the cell surface of LNCaP (androgen- dependent cells), PC3, DU145 (androgen-independent cells) and also in non-tumorigenic RWPE1 cells. Further our studies demonstrated that non-genomic activation of membrane estrogen receptors in cancerous cells

leads to Epithelial to Mesenchymal Transition (EMT), unlike that of non-tumorigenic RWPE1 cells. This indicated that the activation of cell surface localized estrogen receptors elicits different pathways in non-tumorigenic and tumorigenic prostate epithelial cells.

Analyzing the effect and mechanism of CRISP-3 upregulation during prostate tumorigenesis

The objective of the study is to characterize human CRISP-3 promoter to understand the mechanism of its upregulation in prostate cancer. CRISP-3 promoter fragments of various lengths were cloned in a luciferase reporter vector and transfected in CRISP-3 expressing and non-expressing prostate cancer cell lines. Basic minimal promoter was identified which covered the 5'UTR region. This region imparted promoter activity in both the cell lines suggesting additional regulatory regions were present elsewhere. Stable clones generated using -1142 bp region showed more than 150 fold higher activity in the CRISP-3 expressing cell line as compared to the control vector clones whereas CRISP-3 negative cell line showed only 2-10 fold higher activity suggesting epigenetic regulation of the CRISP-3 promoter. Epigenetic silencing or activation of *Crisp-3* gene is probably part of two independent global transcriptional pathways active in two different subtypes of prostate cancers. Further understanding of these pathways and the factors involved in it may help in subtype-specific therapeutic strategies

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

Update data analysis on cytokine polymorphism in 61 cervical cancer cases, 61 HPV positive women with normal cervix and 61 women without any infections revealed significant association of IL-1beta -511 CT and IL-12 at -1188 CA with cervical cancer.

Elucidating the Role of Surfactant Protein D (SP-D) in Prostate Cancer

SP-D is involved in immune-surveillance of various tumors. Results showed that SP-D is expressed by prostate tumorigenic cell lines PC3, DU145

and LnCAP. Treatment with Dihydrotestosterone enhanced SP-D expression in prostate tumorigenic cells. Exogenous rhSP-D reduced viability of prostate tumor cells in a dose responsive and time dependent manner. rhSP-D treated prostate tumorigenic cells showed increased expression of Annexin V and BAX, markers of apoptosis.

Extramural Research

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

The Phase-III Clinical Trial is going on at six centres in the Country and four more centres are in process of inclusion under the Trial. Total around 280 subjects have received RISUG injection and all these subjects have been followed for their efficacy and safety. An interim data analysis of all these subjects was undertaken. Data indicate that people from all major religion (i.e. Hindu 84.1%, Muslim 8.5% and Sikh 7.0%) have accepted the RISUG injection. People from all major caste (SC 41.4%, General 34.0%, OBC 17.6% and ST 7.0%) have accepted the RISUG injection. Majority of the RISUG subjects injected were having either primary education (43%) or Graduation (29%). Even few subjects were having post graduate degree and were professional. Even the wives of these subjects were also having the similar trend so far their education is concern. The data clearly indicate that testicular size of the subjects enrolled under the study varied from State to State. No adverse side effect was reported and observed on clinical evaluation of these subjects even up to 6 years of post RISUG injection. No adverse trend were observed in any parameter related to haemogram, Liver Function Test (LFT), Kidney Function Test (KFT), blood sugar, urine examination of the subjects up to 6 years post RISUG injection. Over all data indicates that 92.6% subjects achieved azoosperma at 2½ month post injection and it reached to highest level (96.7%) at 6th month post RISUG injection. 1.2% method failure was observed. Over all failure of the drug RISUG was 2.1%. Hence over all contraceptive efficacy of the drug RISUG was 97.9%. The dropout rate of the subjects observed in the trial was 4.6% (12 subjects). The reasons of dropout were:- personal reasons, subjects got transferred, family problem, residence shifted away from the participating (center) and subjects could not be located.

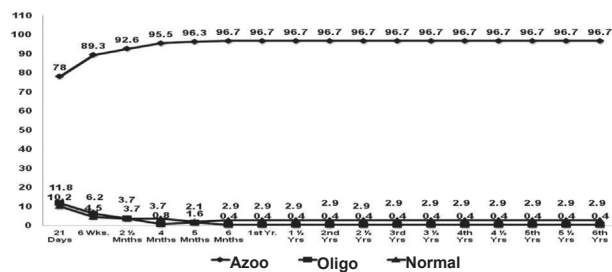


Fig. 1. Overall Efficacy of RISUG Injection.

The interim 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)

A vaccine was developed against hCG, which induced anti-hCG antibodies in women³. The response was reversible and free from side effects, as confirmed by trials in Finland, Sweden, Chile and Brazil under the International Committee on Contraception Research of the Population Council New York⁴. An improved vaccine underwent both Phase-I safety^{5, 6} and Phase-II efficacy trials in India, demonstrating for the first time in the world that sexually active women of proven fertility can be protected against pregnancy at and above antibody titers of 50ng/ml⁷. These studies on the hCG vaccines based on hCGβ established their safety and reversibility. The oLHα-hCGβ-TT/DT vaccine was highly effective in preventing pregnancies at and above antibody titers of 50ng/ml (1 pregnancy in 1224 cycles)⁸. Women kept ovulating and had regular cycles. But the limitation of this vaccine was its moderate immunogenicity, only 60-80% women made antibodies above protective threshold of 50ng/ml. Therefore, a recombinant hCG β-LTB vaccine was developed which was very highly immunogenic in animals. This vaccine then subjected to preclinical toxicity studies. The data of pre-clinical toxicity studies conducted on rodent and marmoset monkey was submitted to RCGM. RCGM accepted the data and recommended for clinical testing of Revived Recombinant hCG β-LTB vaccine. Therefore ICMR developed a clinical trial protocol entitled “Phase I-II Clinical Trial on Safety, Immunogenicity and

probing efficacy of the Revived Recombinant Vaccine against Human Chorionic Gonadotropin (hCG)". The protocol has been approved by the ICMR Experts Committee and has been submitted to DCG(I) for approval.

National Registry of ART Clinics and Banks in India

Around 1684 ART Clinics and Banks have been identified and out of that, 1035 ART Clinics and 181 ART Banks have confirmed that either they will be acting as ART Clinic or ART Bank. As the registration under the National Registry is a three step process therefore based on the prescribed performa, the complete information from 736 ART Clinics have been received on the minimum infrastructure facility, trained manpower and procedures being under taken at the respective ART Clinic. Out of 736 ART Clinics, 385 ART clinics were already enrolled under the National Registry and the information of 11 new ART clinics was found in compliance with the provision of the National ART Guidelines and hence these ART Clinics have been approved. Based on the direction of the DHR, the enrollment no. has not been issued to these 11 ART clinics. Remaining 340 ART Clinics have submitted their performa but have not full-filled all the provisions of the National ART Guidelines. On the other hand 468 ART clinics have not yet responded.

The state and city wise distribution of ART Clinics and Banks in India clearly indicated that the ART Clinics are present in 27 States/UTs of the country. More than 100 ART clinics are present in each of the following States i.e. Maharashtra, Tamil Nadu, Delhi, Karnataka, Uttar Pradesh, Gujarat whereas State of Kerala, Punjab, Haryana, Telangana, Rajasthan are having between 50 to 100 ART clinics in each. Remaining states and UTs are having less than 50 ART clinics in each. The zone wise distribution of ART clinics indicated that Eastern Zone of the country was having the least number of ART clinics i.e. 81 (4.8%). It was also noticed that in majority of North Indian States ART clinics are either at capitals of the States or at District Hqrs. whereas in majority of South Indian States ART clinics are present even at Tehsil level.

This clearly indicates that this difference may be because of the following possible reasons which

need to be explored. 1. Geographical position 2. Population density 3. Per capita income of the people 4. Prevalence of infertility 5. Socio-cultural /life style factors 6. Environmental factors.

The interim data analysis of the registry indicated that out of 396 approved ART clinics, only 4 ART clinics were IUI clinic whereas out of 340 under process ART clinics, 32 ART clinics were performing IUI. Only two ART clinics were found in Govt. sector of approved clinics as compared to seven ART clinics in under process category. All ART clinics were allopathic in approved category whereas 12 ART clinics did not provide any information about their nature of clinic in 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 340 under process ART clinics, 319 ART clinics were registered under PCPNDT Act. Whereas 3 ART clinics did not provide any information about their registration and 18 ART clinics were not registered under PCPNDT Act because of the following reasons (i) 9 ART clinics reported that they are IUI clinics and not having Ultrasound machine (ii) 6 ART clinics submitted their application for PCPNDT Act registration (iii) 2 ART clinics could not provide proper reason (iv) Remaining one ART clinic was basically serving as an ART Bank but claimed as ART clinic. Out of 396 approved ART clinics, 33 ART clinics were sub-clinics of main ART clinic. In approved ART clinics, all gynaecologist were specialized in obst. & gyn. whereas in under process category, 6 ART clinics did not have the qualified gynecologist and 39 ART clinics did not provide any information about their qualification. Similarly all approved ART clinics were having andrologist and 148 were on regular basis whereas in under process category, 41 ART clinics did not have any andrologist. In approved ART clinics, 392 ART clinics were having clinical embryologist and 72% were on regular basis whereas in under process category, 32 ART clinics did not have qualified embryologist. All the approved ART clinics were having regular counselor whereas in under process category, 18 ART clinics did not have counselor and 79 ART clinics did not have regular counselor. All the approved ART clinics were having sterile area whereas 5 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, 15 ART clinics did not have Operation Theater for carrying out surgical endoscopy and vaginal ovum pickup. Information regarding ART procedures being undertaken by the ART clinics, 31 ART clinics did not provide any information in under process category. In approved ART clinics, 384 ART clinics were doing IVF-ET whereas in under process ART clinics, 270 ART clinics were doing IVF-ET. In approved ART clinics, 313 ART clinics were doing surrogacy whereas in under process ART clinics, 176 ART clinics were doing surrogacy. In approved ART clinics, 210 ART clinics were not doing cryo-preservation of ovarian tissue.

Draft National Guidelines for Accreditation, Supervision and Regulation of ART Banks in India have been developed which are under process of finalization.

Effect of Non-ionizing Electro Magnetic Field (EMF) on Human Health

This is a multidisciplinary prospective cohort study going on to find out the possible health hazards if any because of radio frequency radiation emitted from cell phone. An interim review of the study was undertaken. After fulfilling the inclusion and exclusion criteria's, 2516 male and female subjects have been enrolled under highly exposed (726), moderate exposed (1255) and control group (535). Out of 2516 enrolled subjects the data of 2122 subjects have been analyzed. The result indicated that majority of the subjects were of 24 – 35 age-groups, having either graduate or post-graduate qualification and having Rs.5-10 lakh annual income. The interim data analysis of various disciplines is as follows.

i). Otorhinolaryngology (ENT) Observations

Slight hearing loss was noticed more in highly exposed group in comparison to moderately exposed and control group both at first and second visit. The symptoms of vertigo were increased during the second visit in both female and male subjects and the percentage of vertigo symptoms is higher in female subjects than male. Air conduction at lower frequency (0.5 KHz) is increasing significantly in highly

exposed group during the second visit in male subjects whereas Air Conduction at lower (0.5 KHz) and moderate (2 KHz) frequencies is increasing significantly in highly exposed female subjects in comparison to control group both at first and second visit. Speech Recognition Threshold (SRT) in both ears was increasing significantly both in male and female subjects in comparison to control group both at first and second visit. Speech Discrimination Score (SDS) is decreasing in female significantly in second follow up visit in comparison to control group.

ii). Neurological Observations

Attention, memory, fluency, visual spatial and Addrenbrooke's Cognitive Examinations-Revised (ACE-R) total were reduced significantly in highly exposed and moderately exposed male and female groups in comparison to control group both at first and second visit. Epworth Sleep Score & Diagnostic and Statistical Manual of Mental Disorders, IV Edition (DSM-IV) criteria in highly exposed male subjects increased significantly in comparison to control group in second visit. Whereas in highly exposed female subjects, Mini-mental examination (MMES) and Epworth score is increasing significantly in comparison to control group both at first and second visit.

iii). Cardiological Observations

No significant difference was found in pulse rate, BP and basic ECG parameters like PR interval (except in high exposed male subject), QRS duration, QT interval. In highly exposed and moderately exposed male and female groups, the percentage and frequency of snoring was significantly high in comparison to control group both at first and second visit.

iv). Reproductive Health Observations

Male - Desire and frequency of sexual activity were decreased significantly both in highly and moderately exposed male subjects in comparison to control group both at first and second visit. Problem in getting erection and problem in ejaculation before the partner is

ready were increasing significantly both in highly and moderately exposed group both at first and second visit in comparison to control group. In the male subjects of highly and moderately exposed group, the following semen parameters were altered significantly both at first and second visit: Semen Volume decreased, Liquefaction time increased, % of Linear motility decreased, % of non progressive sperm increased, % of abnormal sperm increased, % of dead sperm increased, % of pyriform, amorphous and tapering heads increased, % of mid piece defects increased. The levels of testosterone were decreased in highly exposed male subjects at second visit. The levels of Cortisol and Prolactin were significantly decreased both in highly and moderately exposed male group comparative to control group both at first and second visit.

Female - An increase in number of miscarriage was noticed in highly exposed female subjects in comparison to control female subjects at second visit. Number of preterm children born is higher in highly exposed female group at second visit in comparison to moderately exposed and control groups and in comparison to first visit. Desire and frequency of sexual activity were decreased both in highly and moderately exposed female subjects both at first and second visit in comparison to control group. Percentage of women reporting irregular menstrual cycle is higher in highly exposed group in comparison to moderately exposed and control group both at first and second visit. The levels of Cortisol were significantly decreased both in highly and moderately exposed female group in comparison to control group both at first and second visit. The levels of T4 were increased significantly in highly exposed female group at second visit in comparison to moderate and control group.

Other Observations - The percentage of male and female subjects reporting sores in mouth were significantly higher both in highly and moderately exposed groups in comparison to control group both at first and second visit. The percentage of male subjects reporting white patches in mouth was significantly higher both in highly and moderately exposed groups in

comparison to control group both at first and second visit.

v). **Biochemical Observations**

Percentage of Apoptosis in lymphocyte of both highly and moderately exposed male and female subjects was increased significantly both at first and second visit. Levels of Malondialdehyde in both highly and moderately exposed male and female subjects were increased significantly both at first and second visit. Levels of Superoxide Dismutase (SOD) were significantly decreased both in highly and moderately exposed male and female subjects both at first and second visit. The levels of total cholesterol, LDL, Triglyceride were significantly increased in highly and moderately exposed male subjects in comparison to control group both at first and second visit. But in female subjects of highly and moderately exposed group only the levels of LDL were increased significantly in comparison to control group both at first and second visit.

vi). **Hematological Observations**

In hematological observation, Total Leukocytes Count (TLC) were decreased in highly exposed male and female subjects in comparison to moderate exposed and control subjects both at first and second visit. Monocytes counts were increased both in highly and moderately exposed male and female subjects in comparison to control subjects both at first and second visit.

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 511 subjects of 18-45 age groups including 334 male and 177 female 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. The 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 both male and female subjects residing at 10m of distance from

cell phone tower and the percentage of people reporting these symptoms decreased as the distance increased i.e. Mental stress, Headache, Depression, Nausea, Vomiting, Slurred Speech, Fever, Fatigue, Heart Diseases etc.

NATIONAL ANIMAL RESOURCE FACILITY (NARF) FOR BIOMEDICAL RESEARCH, HYDERABAD

The Cabinet has approved the proposal for establishment of National Animal Resource Facility for Biomedical Research (NARF-BR) at Hyderabad with a budgetary provision of Rs.338.58 Crores. Based on the notification issued by the Department of Health Research, Ministry of Health and Family Welfare, Govt. of India, ICMR created its 32nd permanent institute of ICMR on 01/01/2016. Following this notification all the required Committees i.e. Project Management Committee and Project Execution Committee, to monitor the activities of the facility have been established with their Terms of References. The CPWD has been appointed as Project Management Consultant and ICMR is in process of signing MoU with CPWD. Simultaneously the layout plan and draft architectural designs have been prepared.

A study of maternal nutrition in hepatitis E during Pregnancy and its role in vertical transmission

This study aimed (a) to evaluate the maternal nutritional status in viral hepatitis during pregnancy and correlate it with severity of hepatitis E in pregnancy; (b). study the HEV viral load and genotype in mother and new born with Hepatitis E virus infection; (c). to correlate the maternal nutrition with maternal & fetal outcomes as well as with vertical transmission of HEV; and (d). to study association of maternal nutritional status with the viral load and genotype of HEV in the mother as well as in the newborn and to correlate it with the severity of infection.

Results indicated that HEV was found to be the most common hepatotropic virus in pregnancy 144/267 (53.93%) and responsible for 41/46 (89.13%) of cases of the acute liver failure (ALF) in pregnant

women. High mortality rate (43.9%) was observed in HEV infected cases due to fulminant hepatic failure (FHF). Maternal nutritional status (anthropometric parameters and biochemical nutritional parameters) were significantly lower in pregnant women with viral hepatitis compared to pregnant controls. Low MUAC and lower levels of serum protein such as globulin, prealbumin and folate observed in HEV pregnant women compared to non-HEV pregnant women. This reflects that malnutrition might confer risk of HEV infection during pregnancy. Low BMI, MUAC, TSFT and lower levels of serum albumin, globulin, prealbumin and folate were observed in FHF group compared to AVH group

which reflects that lower nutritional status in pregnancy predispose towards severe form of disease fulminant hepatic failure during HEV infection. Serum prealbumin was the only independent factor which could reduce risk of mortality during HEV infection. Maternal serum folate was the only independent risk factor associated with baby mortality in FHF group in pregnant women with HEV infection.

Viral load of HEV in FHF pregnant women were comparatively higher than AVH pregnant women, which may be related to the severity of the diseases in pregnant women. Acute and fulminant viral hepatitis E pregnant patients belonged to subtype 1a of genotype 1. Thus this shows that vertical transmission occurs during HEV infection. Vertical transmission was observed in 46.09% of cases. All the HEV-RNA positive babies belonged to genotype 1. Thus this shows that vertical transmission occurs during HEV infection. Low maternal BMI was observed in HEV positive mothers of HEV-IgM positive babies and HEV-RNA positive babies compared to that of HEV-IgM negative babies and HEV-RNA negative babies. Cut-off value of maternal BMI <15.4 kg/m², was found to predict vertical transmission of HEV infection (Table 1). This indicates that low maternal BMI could be a risk factor which predisposes to vertical transmission of HEV infection.

Effect of maternal oxygen inhalation on maternal and foetal free radical activity during caesarean section

The aim of this study was to compare oxidant and Total Antioxidant Status (TAS) of mother and foetus

Table 1. Comparison of maternal nutritional parameters between HEV-RNA positive babies and HEV-RNA negative babies.

Maternal Nutritional parameters	HEV-IgM positive babies = 59		p value
	HEV-RNA positive babies N= 15 (25.43%)	HEV-RNA negative babies N= 44 (74.57%)	
	(1)	(2)	1&2
BMI (kg/m ²)	14.41 ± 1.63	16.37 ± 1.42	<0.001*
MUAC (m)	0.22 ± 0.01	0.22 ± 0.01	0.74
TSFT (mm)	11.69 ± 0.82	11.45 ± 1.89	0.41
ASN (m)	1.53 ± 0.01	1.53 ± 0.05	0.78
Total protein (gm/dl)	5.80 ± 0.84	5.87 ± 0.73	0.75
Serum albumin (gm/dl)	2.42 ± 0.90	2.18 ± 0.78	0.31
Serum globulin (gm/dl)	1.97 ± 0.70	1.86 ± 0.64	0.57
Serum prealbumin (mg/dl)	27.77 ± 0.71	27.35 ± 2.03	0.47
Serum folate (ng/ml)	6.00 ± 0.75	5.47 ± 1.15	0.12

Abbreviations: Body mass index (BMI), mid upper arm circumference (MUAC), tricep skin fold thickness (TSFT), arm span (ASN)
Data are given as Mean ± SD; *Significant p value <0.05

during elective and emergency CS (60 women in each group) under subarachnoid block (SAB) with (50% oxygen) or without supplemental oxygen inhalation (room air). Arterial blood samples of mothers were collected at baseline and then every 5 minutes until delivery. Umbilical artery and vein samples were collected at delivery. All the blood samples were evaluated for arterial blood gases, Malondialdehyde (MDA) and TAS.

50% oxygen inhalation to mothers during ELCS or EMCS under SAB did not result in significant difference in foetal MDA and TAS as compared to air. At delivery, mothers undergoing ELCS receiving 50% oxygen had significantly higher maternal PaO₂ as compared to the group receiving air [136.83±24.11 vs 181.93±36.00 mmHg, (P=0.00)]. In EMCS, mothers receiving 50% oxygen had also significantly higher maternal PaO₂ as compared to air [136.88±24.46 vs 171.06±33.76 mmHg, (P=0.00)] at the time of delivery. During ELCS, mothers inhaling 50% oxygen had higher MDA levels at delivery as compared to baseline [5.42±2.63 vs 6.25±3.43 µmol l⁻¹, (P=0.04)]. In EMCS, mothers inhaling 50% oxygen had lower TAS levels at 10 min as compared to air, [0.98±0.13 vs 1.40±0.20 m mol l⁻¹, (P=0.01)].

Maternal hyperoxia did not concomitantly change foetal MDA and TAS levels following ELCS or EMCS under SAB. Rationalization of oxygen inhalation for increased duration is required during CS under SAB.

Impact of the two training strategies on the status of maternity preparedness among primigravida women attending antenatal clinic in PGIMER, Chandigarh

In overcrowded Obs. & Gynae OPDs of hospitals in India most of the time of nurses/ obstetricians is spent in clinical care. They have little time to counsel pregnant women especially primigravida and living in the nuclear family set up, regarding their minor and day-to-day needs. The objectives of the study were to ascertain the status of maternity care preparedness among primigravida women in the study population and to compare the impact of the two training strategies (5 contacts) on the status of maternity care preparedness among primigravida women in the study population. The feasibility of operating a maternity care room in Gyne/Obst OPD of a tertiary care hospital of north India was also ascertained.

Women (n=200) attending antenatal clinic were registered and after examination by the doctor were enrolled in the study after obtaining informed consent. They were randomized into 2 groups on the basis of the training strategy used. Group A received a self-instruction manual, a compact disc and individual counseling; while Group B women received self-instruction manual, a compact disc and telephonic contact. Demographic details of the participants were recorded and the training package administered.

Socio-demographic profile of women in two groups was similar at baseline. Status of maternity preparedness among primigravida women was average. Both the training packages helped in improving their maternity preparedness. All the primigravida women & their family care givers were highly satisfied with interventions provided by the project team. There were administrative and operational challenges for creating a separate space for counseling which were resolved through repeated meetings and providing feedback from patients. Convincing other family members about the optimal care of the pregnant women was a very big challenge and therefore spending time with the family in the counselling room was adopted in such cases. The IEC material developed during the study and counselling protocol can be used for dissemination and adoption for the state governments and institutions.



Fig. 2. Husband's participation



Fig. 3. Video Based Counseling.

Conclusion: It is quite feasible to introduce the concept of a separate maternity care room in OBG department of medical college.

Impact assessment of an intervention package to improve maternal and child health services among primitive Baiga tribe of Dindori District in M. P

There is low level of awareness and underutilization of maternal health services among the primitive Baiga tribe of Dindori District in M. P. A study was carried out with the aim of developing and implementing an IEC based intervention and to evaluate its usefulness using a case control study design. The study was carried out in two phases; IEC intervention in 12 villages (phase-I) and impact evaluation survey in 24 villages (phase-II). The IEC materials were developed based on existing tools and consultation with the state authority. These

Table 2. IEC events in intervention villages

S. No	IEC Activities	1 st Round IEC (in number)	2 nd Round IEC (in number)	3 rd Round IEC (in number)	Total
1.	Village level Committee formulated	12	-	-	12
2.	Health Education camp conducted	12	12	12	36
3.	Number of women participated in the camps	206	242	253	-
4.	Slogans written on wall	120	110	60	290
5.	Banner displayed in village	60	12	03	75
6.	Pamphlet pasted & distributed	240	280	440	960
7.	Group Discussion	12	12	12	36
8.	Interpersonal Discussion	60	80	84	-

Table 3. Distribution of improvement from baseline findings

MCH services	Baseline findings (X)	Outcome of result in Intervention group (Y)	Improvement (Y- X)	Outcome of results in Control group (Z)	Improvement (Z- X)
Antenatal checkups	72.0%	88.0%	+16.0%	76.1%	+4.1%
T.T vaccination	68.0%	93.7%	+25.7%	91.5%	+23.5%
Consumption of IFA tablet	76.0%	93.7%	+17.7%	85.5%	+9.5%
Institutional delivery	08.0%	49.7%	+41.7%	40.0%	+32.0%
Awareness of MCH services	33.0%	85.2	+52.2%	42.7%	+9.7%

included banners containing different messages about registration for ANC, regular ANC checkups, promoting institutional deliveries, postnatal checkups and child immunization; Pamphlets contains eleven point messages on importance of maternal and child health services; slogans painted on walls. Group communication and interpersonal communication were used to administer the intervention (Table 1). The implementation of IEC was done with the support of Block Medical Officer, Health worker, Anganwari worker, ANM, ASHA, etc.

A total of 556 ever married women were interviewed, of them 367 women (191 from intervention and 176 from control group) had given birth during the last three years. Data was collected regarding their socioeconomic condition, antenatal care, natal, postnatal care, child immunization status and awareness on MCH issues.

Results indicate that there was improvement of the utilization of MCH services and awareness of women in intervention group. This indicates that IEC intervention was useful and is shown in the table 3.



Fig. 4. Training to Anganwari worker & ASHA in Anganwari Kendra.



Fig. 5. Displaying banners in village.

The experiences from the current study show that IEC intervention improved utilization of maternal and child health care services and knowledge among Baiga primitive tribe in the district Dindori. Similar comprehensive studies in other tribal communities can be carried out.



Fig. 6. Conducting Health Education Camp.

Prevalence and Physiotherapy intervention for Pelvic Floor Dysfunction in women of Udupi Taluk

The present study was conducted to assess the prevalence of PFD, identify the risk factors and to study the impact of pelvic floor muscle strength training in patients with urinary incontinence. A Cross sectional study among 1256 married women of Udupi taluk (>18 years age) were screened using the Manipal Pelvic Floor Dysfunction screening questionnaire and Manipal Risk factor stratification questionnaire. Women with symptoms suggestive of PFD, were interviewed with Manipal Modified pelvic floor dysfunction questionnaire. The prevalence of PFD was 21.01% (n=264). Distribution of women with different types of PFD is shown in the figure below:

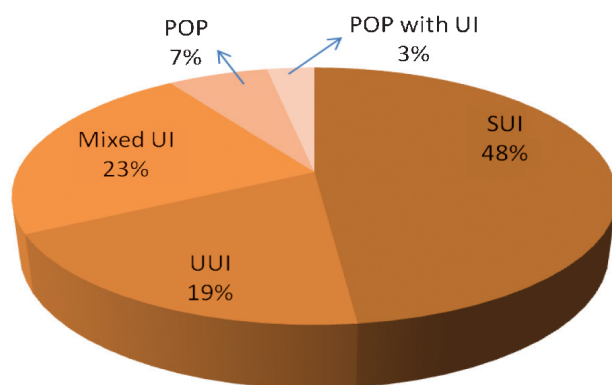


Fig. 7. *SUI – Stress urinary Incontinence, UUI – Urge Urinary Incontinence, Mixed UI – Mixed type of Urinary Incontinence, POP – Pelvic Organ Prolapse, POP with UI – Pelvic Organ Prolapse with Urinary Incontinence.

Multivariate logistic regression analysis showed that occupation, abortions, menopause, straining while passing stools, history of UTI, family history of PFD and diabetes are the significant independent risk factors associated with PFD.

Women with PFD were randomized to either Pelvic floor muscle exercise Vs Pelvic floor muscle exercise along with postural education and specific exercise programme. At the end of 12 weeks the improvement in symptoms and muscle strength was assessed. Clinically, a greater improvement was observed on the perineometer readings in the interventional arm (who received Pelvic floor muscle exercise along with postural education and specific exercise programme) as compared to that of the control group (who received Pelvic floor

muscle exercise only), but the improvement was not statistically significant. However, the women who visited the centre more than 7 visits, indicating compliance to intervention in the intervention arm showed a greater improvement in the perineometer readings as compared to the women visiting the centre less than 7 visits. Some women reported that they were able to do the exercise once a day only as they had to take care of the household and tend to the farm animals also. One hundred and one women reported of doing the exercise twice a day whereas 135 women did the exercise only once a day. Conclusion: PFD is a common problem among women. Teaching pelvic floor exercises and improving patient compliance help in ameliorating symptoms.

Microbicide for prevention of HIV transmission and other sexually transmitted infections: *In vitro* efficacy and pre-clinical safety evaluation

Three aqueous gel formulations comprising 50% ethanolic extracts of 3, 4 and 5 plants have been formulated based on anti-HIV-1 activity of the individual plant. These herbal formulations showed potent *in vitro* anti-HIV-1 activity. In addition, these formulations showed anti-HSV-2 activity, which is present as co-infection in HIV infected human subjects. These formulations also inhibited HIV-1 reverse transcriptase, integrase and protease activities suggesting that these may be acting at multiple steps of HIV life cycle. Further, preclinical safety evaluation revealed that these formulations have no adverse effect on the growth of lactobacilli associated with female reproductive tract, do not possess hemolytic activity, have no adverse effect on the integrity of monolayer formed by Caco-2 cells, and do not affect the viability of human cervico-vaginal cells. Interestingly, treatment of human cervico-vaginal cells with these herbal formulations does not lead to increase in the production of pro-inflammatory cytokines, further suggesting their safety. Mutagenesis studies further strengthened the safety aspects of the herbal formulations. In addition to the above herbal formulations, various synthetic compounds made by 4 different universities/scientific establishments have been evaluated which have yielded promising results.

Table 4. Effect of two types of interventions on muscle strength

	Manual muscle testing (MMT)		Perineometer	
	Pre intervention {Median (IQR)}	Post intervention {Median (IQR)}	Pre intervention {Median (IQR)}	Post intervention {Median (IQR)}
Control arm (n=123)	2 (2,2)	3 (3,3)	10 (8,13)	16 (13,21)
interventional arm (n=123)	2 (2,2)	3 (2,3)	9 (7,12)	19 (14,24)
P value	0.38		0.37	

Table 5. Effect of compliance to intervention on the muscle strength

Type of intervention		Number of visits (n)	Mean ±SD
Control arm	Perineometer Pre-intervention	1 (8)	9 ± 5.9
		2 (115)	10.6 ± 4.7
		Total (123)	10.5 ± 4.8
Control arm	Perineometer Post-intervention	1 (8)	0
		1(115)	17.5 ± 5.5
		Total (123)	16.4 ± 6.9
Intervention arm	Perineometer Pre-intervention	1(12)	8.3 ± 3.2
		2-7 (62)	9.08 ± 4.9
		7-12 (49)	10.4 ± 4.7
		Total (123)	9.5 ± 4.7
	Perineometer Post-intervention	2(12)	5.6 ± 8.4
2-7 (62)		17.6 ± 7.6	
7-12 (49)		21.4 ± 5.4	
Total (123)		17.9 ± 8.2	

Table 6. *In vitro* anti-HSV-2 activity of herbal Formulation-1

Experimental conditions	Inhibition of HSV-2 Infection in Vero cells (IC ₅₀ µg/ml)	
	Acyclovir	Formulation-1
Direct anti-viral activity	27.12 ± 1.5	0.018 ± 0.009 (17.91 ± 8.83 ng/ml)
Attachment inhibition activity	Nil	0.30 ± 0.25
Penetration inhibition activity	Nil	1.93 ± 1.47

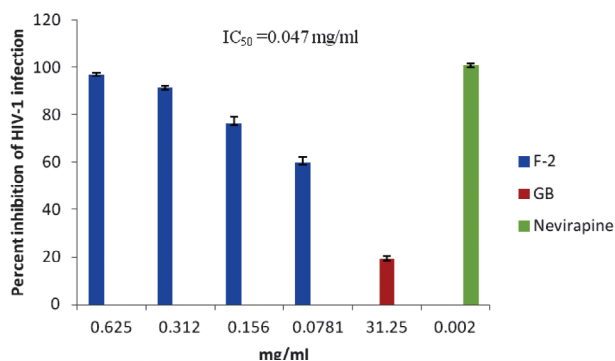


Fig. 8. Anti- HIV-1 activity of herbal Formulation-2. TZM-bl cells were infected with HIV-1NL4.3 (MOI, 0.05) pre-treated for 1 h with Formulation-2 at different concentrations and incubated for 48 h. Anti-HIV-1 activity at varying concentrations of herbal Formulation-2 and Nevirapine (0.002 mg/ml), used as a positive reference control, has been shown. Y-axis represents the percent inhibition of HIV-1 infection. Values are expressed as mean ± SE of 3 different experiments performed in duplicates.

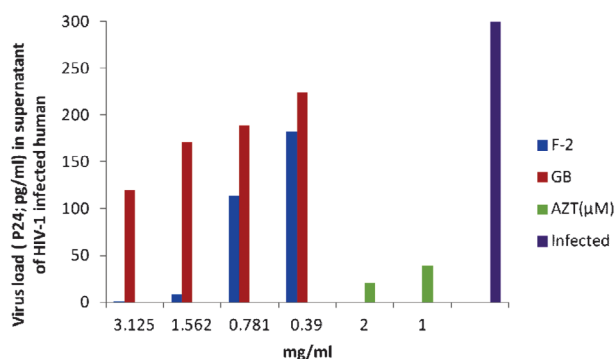


Fig. 9. Anti-HIV-1 activity of herbal Formulation-2 (F-2) using human peripheral blood lymphocytes (PBLs) against HIV-1NL4.3. PHA-P activated PBLs were infected with virus and were treated with different concentration of F-2 and p24 was estimated by ELISA on 5th day in the culture supernatant. Viral load is represented as the amount of p24 present in the culture supernatant.

Table 7. Anti-HIV-1/HSV-2 activity of Formulation-3

	Formulation-3 IC ₅₀ (mg/ml)	Gel Base IC ₅₀ (mg/ml)
Inhibition of HIV-1 activity in TZM-bl cells	0.047 ± 0.006	1.61 ± 0.22
Inhibition of HIV-1 RT activity	1.64 ± 0.09	No inhibition
Inhibition of HIV-1 integrase activity	8.56	No inhibition
Inhibition of HIV-1 protease activity	0.083	No inhibition
Direct anti-viral activity against HSV-2	0.018 ± 0.002	No inhibition

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. Project is completed recently. Blood culture was positive in 10.0% clinically suspected sepsis cases. Detail analysis is ongoing.

Vitamin D Supplementation for Treatment and Prevention of pneumonia in Under-five Children:

A Randomized Double-blind Placebo Controlled Trial. Pneumonia remains the leading cause of childhood mortality accounting for 15% of all deaths in children below 5 years of age. Few observational studies have shown an association between vitamin D deficiency and respiratory tract infections, probably due to its immune-enhancing properties. Efficacy of single oral mega-dose of Vitamin D3 for treatment and prevention of pneumonia in under-five children was evaluated in a tertiary care hospital in 324 children (of 980 assessed) between 6 mo-5 y age with WHO-defined severe pneumonia. Of these 39% were vitamin D deficient (serum 25(OH)D <12 ng/mL). 100,000 IU of oral cholecalciferol or placebo (n= 162) in single dose, administered at enrolment. Median (95% CI) time for resolution of severe pneumonia was 30 (29, 31) h in the vitamin D group as compared to 31 (29,33) h in the placebo group [adjusted hazard ratio (95% CI): 1.39 (1.11, 1.76); P=0.005]. The risk of recurrence of pneumonia in next 6 months was comparable in the two groups. The study concluded that there is no robust evidence of a definite biological benefit, either for therapy or prevention, to suggest a routine megadose supplement of vitamin D3 for under-five children with severe pneumonia.

Prevention of Pneumonia in children with HIV infection

Haemophilus influenzae type b (Hib) remains a major cause of morbidity in children with HIV

infection, especially in places where pediatric HIV remains a problem and the Hib conjugate vaccine (HibCV) is yet part of the universal immunization program. Objectives of this study was to look at the direct impact of Hib conjugate vaccine on nasopharyngeal carriage in HIV infected children and the indirect impact on carriage in their HIV infected parents from West Bengal, India. 125 HIV infected children (ages 2-15 yrs) received two doses of the HibCV, 44 of these children were between ages of 2-5 years. 44 HIV negative children ages 2-5 years received one dose of the HibCV. Baseline carriage in HIV infected children was 17/123, (13.8%-9% CI: 8.2%-21.2%). Baseline carriage in the HIV negative group was 2/44 (4.5%-95%CI: 0.5%-15.4%). Following two doses of HibCV the carriage rate in HIV infected children dropped to 4/121 (4.8%-95%CI: 1.8%-10.3%). A paired pre-post analysis of Hib carriage in individual HIV infected children showed an (OR=0.15 for the HIV infected child having Hib as compared to baseline, following two doses of HibCV (p=0.0045). Following immunization, carriage in the HIV negative group dropped to 2.2% (95%CI: 0.05%-12.2%).

In HIV infected parents the risk of Hib colonization was directly related to whether the child was colonized (RR=12.2; p=0.0079). None of the parents of HIV uninfected children had Hib colonization at any point. This study concluded that in high risk families with both adults and children with HIV infection, Hib conjugate vaccine decreases carriage with Hib in the HIV infected child and also has the indirect effect of decreasing carriage in parents.

Outcome and very low birth weight (VLBW) babies and Economic burden of intensive care of families of VLBW (<1250 Grams) babies during Hospital Stay

An observational descriptive study, all VLBW babies (≤ 1250 grams) born during one year period at PGIMER Chandigarh were subsequently followed up till CA 2 years for growth and development. Mortality, morbidities, growth, neurodevelopment, neurosensory behavioral and language outcomes of VLBW babies at 2 years and Economic burden of care of VLBW babies on families during hospital stay was studied. The study concluded that nearly 72% VLBW (≤ 1250 grams) babies survive in our

set up with the existing infrastructure. These babies remain significantly growth retarded even at 2 yrs CA. Abnormal NDI (composite outcome) was seen in 25% babies. The average expenditure by family to salvage a sick VLBW was moderate amount. This information can be provided to family while making a decision for caring of such babies.

Effect of Malnutrition and HIV Infection on the Pharmacokinetics of Anti-Tubercular drugs

Drugs in Children

Pharmacokinetics studies in children utilizing the revised doses of isoniazid, rifampicin, pyrazinamide and ethambutol are scarce; the effect of malnutrition and HIV infection on the levels of these drugs have also not been conclusively studied in children. A prospective drug estimation study was conducted in children, age 6 months to 15 years, with tuberculosis, receiving the current dosage of daily anti-tubercular therapy. Children were enrolled in 4 groups – with or without severe malnutrition and with or without HIV co-infection. All four drugs were simultaneously estimated by liquid chromatography-mass spectrometry (LC-MS/MS). No effect of malnutrition on pharmacokinetic parameters of the drugs studied was observed. Pyrazinamide seemed to have lower concentrations in HIV infected children while other drugs were not influenced by HIV sero status. Though a large percentage of children had sub therapeutic levels of one or other drugs, need for starting Cat-2 ATT due to non response/worsening showed no correlation to whether drug concentrations were considered therapeutic or not.

Role of innate immune cells in the pathogenesis of juvenile idiopathic arthritis/enthesitis related arthritis

Objectives of the study to compare the number of NK, NKT and γ T cells in patients with ERA-JIA as compared to healthy controls, compare the number of NK, NKT and γ T cells in patient with ERA-JIA with different categories of JIA, to assess the level of cytokines that primarily influence NK and NKY cells like IL-18 and IL-15 and IL-27; study the expression of HLA class I interacting receptors KIR3DL2 and KIR3DL1 on NK cells and to assess the cytotoxic function of NK and NKT cells in ERA-JIA.

50 children with JIA-ERA, 25 children with other category of JIA were enrolled and 25 healthy controls. After informed consent from parents/patients 5 ml blood was collected (2 ml in EDTA vial, 2 ml in Plair vial for serum separation and 1 ml in heparin vial) and analysed. It was observed that mean frequency of gamma-delta T Cells, total NK cells and CD56dim were higher in patients as compared to healthy controls; there was no difference in frequency NKT cells among different groups. NK cells in JIA-ERA had reduced expression of perforin, increased expression of KIR3DL and produced more IL-17 as compared to controls. Monocytes having CD16 expression (Intermediate and Non-classical) were higher in PB of patients as compared to controls. Serum level of IL-15, IL-18 and IL-17 were same in three groups but IL-27 levels were lower in patients. In paired samples, the frequency of gamma-delta T, total NK and NKT cells was similar, CD56 bright subset with low KIR3DL and perforin expression was more in SFMC. SFMC of patients is further enriched with Intermediate monocyte subset and CD1d expression was reduced in SFMC as compared to PB. Synovial levels of IL-15, IL-18 and IL-17 were more while that of IL-27 was low as compared to serum.

Evaluation of possible modalities to enhance the ocular kinetics of an anti cancer agent in rabbits for its utilization in the treatment of retinoblastoma

Presence of blood ocular barriers post a challenge to the drugs administered systemically for reaching effective concentration in the ocular tissues. This study was undertaken to evaluate the ocular disposition of drugs anticancer drugs such as etoposide, vincristine, carboplatin conventionally given as a triple regimen for the treatment of retinoblastoma and possibility of using advanced pharmaceutical drug delivery approaches to enhance the delivery by into the eye to increase the efficacy and decrease systematic exposure. The drugs from ocular tissues were analysed using liquid chromatography coupled tandem mass spectroscopy. For the first time this study showed the

ocular tissue levels of these drugs in ocular tissues. Although the presence of P-gp efflux transporters were recognized in the retina, for the first time this study showed enhanced drug penetration across blood ocular barriers by blocking them with GF 120918 (Elacridar- a specific approved blocker used for MDR cancer. This study has also attempted to use a nano-emulsion formulation of Etoposide for its delivery through subtenon route in rabbits. The non-emulsion of Etoposide was taken well into the ocular tissues indicating the possibility of developing them for clinical use after its animal toxicity studies. This study use topical iontophoretic drug delivery of Carboplatin using newly developed transcleral delivery device. However, the drawback was that the amount of drug transfer across sclera was much lower as compared to the systemically achieved levels.

Centre for Advanced Research

- Centre for Advance Research on Neonatal Health at AIIMS.
- ICMR Advanced Centre for Evidence Based Child Health (CAR on EBCH).
- Centre for Advanced Research on Community based Maternal Newborn and Child health at Sewagram Wardha.
- Centre for advanced research on Pediatric immune deficiency at PGIMER, Chandigarh.

Centre for Advance Research on neonatal health at AIIMS, New Delhi: A comprehensive database for neonatal sepsis through network of participating centres “Delhi Neonatal Infection Study (DeNIS) collaboration” (at AIIMS, New Delhi, Safdarjang Hospital, Maulana Aza d Medical College and Chacha Nehru Bal Chikitsalaya) was established to study epidemiology of neonatal sepsis and characterise pathogens causing sepsis. Among inborn cohort incidence of total and culture-positive sepsis was 14.3and 6.2%. *Acinetobacter* spp. (21.9%), was the major pathogen followed by *Klebsiella* spp. (16.6%), and *Escherichia coli* (13.7%). Majority of the pathogens exhibited multi-drug resistance. Nearly one-fifth of the deaths were attributable to sepsis. Among the *outborn cohort* 13.1%) had culture-positive sepsis. There was a predominance of Gram-negative isolates (52.9%), with common isolates being *Klebsiella pneumoniae*

(12.5%), *Acinetobacter baumannii* (11.5%) and *E. coli* (8.0%).

Studies were also carried out to assess the prevalence of vitamin D deficiency (VDD), and the optimal supplemental dose of vitamin D for prevention of VDD in LBW infants. In a study the prevalence of VDD ($25(\text{OH})\text{D}_3 < 20 \text{ ng/mL}$) following daily supplementation with 400 IU vs. 800 IU of oral vitamin D_3 at 40 weeks postmenstrual age in preterm infants born at 28 to 34 weeks of gestation was compared. It was observed that daily supplementation with 800 IU of vitamin D reduces the prevalence of vitamin D insufficiency at 40 weeks and at 3 months' corrected age in preterm infants without having any improvement in bone mineralization. In another study the prevalence of vitamin D deficiency (defined as $25(\text{OH})\text{D} < 15 \text{ ng/mL}$) at 12- 16 weeks in term healthy breastfed infants supplemented daily from birth with 400 IU of vitamin D was assessed. Results showed the prevalence of VDD and VDI continues to be high at 14 weeks of age in term healthy infants in India despite daily oral dose of 400 IU of vitamin D_3 , possibly indicating it as suboptimal dose. Then the prevalence of VDD ($25(\text{OH})\text{D} < 20 \text{ ng/mL}$) at 6 months in term healthy breastfed infants supplemented with 800 IU of vitamin D per day starting from birth (n=70). Daily supplementation of 800 IU of vitamin D resulted in vitamin D sufficiency in most term healthy infants at 6 months of age. However, in view of potential risk of toxicity in some infants, this regime cannot be universally recommended in all infants. Alternative approaches including correction of vitamin D deficiency at birth by appropriate mega dose followed by routine supplementation of a lower dose (400-600 IU) should be explored as a strategy to achieve vitamin D sufficiency in these infants.

Complementary feeding at four versus six months of age in preterm infants: a randomized, multicentre trial : The current guidelines for exclusive breastfeeding till 6 months followed by complementary feeding meant for term healthy infants cannot be extrapolated to preterm infants as their nutrient requirements are likely to be higher, and there is no evidence to suggest till what time breast or formula milk alone would be able to meet their nutrient requirements in early infancy.

This study concluded that in infants <34 weeks of gestation, initiation of CF at four compared to six months of CA does not result in any difference in WAZ12 at 12 months of CA. However, the risk of hospitalization increases among infants in the 4-month group. There is no difference in any other growth parameters, body composition, bone mineral content and density, lipid profile, insulin resistance measure (HOMA-IR) and BP between the two groups. Iron stores are significantly depleted in both groups despite iron supplementation. It therefore implies that in infants less than 34 weeks

of gestation, initiation of CF at six months of CA is preferable to four months of CA. Follow up studies on high risk babies for their neurodevelopmental and neurocognitive outcomes was also carried out. It was highlighted that in a tertiary care setting with adequate quality of care during NICU stay as well as post-discharge follow-up clinic, it is feasible to achieve low rates of disability even among high risk preterm infants. However postnatal growth of preterm and term SGA infants continues to remain an important concern, with few of the preterm infants showing catch-up growth.

NUTRITION

The ICMR continues to undertake the laboratory and hospital based research with community participation in the field of nutrition. The salient features of various research activities undertaken during the year under report are given below.

Intramural Research

NATIONAL INSTITUTE OF NUTRITION, HYDERABAD

STUDIES CONDUCTED IN VARIED FIELDS

- Manipulation of dietary fat to enhance carotenoid bioavailability and bioconversion to vitamin A: Development of mechanism based approaches.
- Effect of zinc supplementation prior to iron on iron absorption and status in rats.
- Isolation and Characterization of food derived iron binding peptides and their effect on iron bioavailability: A proteomic approach.
- Iron status during obesity and its association with metabolic syndrome and inflammation in Indian children
- Fish Egg Protein Hydrolysates (Peptides) as Nutraceutical/Health Food in Promotion of Immunomodulatory Activities
- Targeted Nutrition Communication for promoting Consumption of Micronutrient Rich Foods among Rural Households by Developing Dietary Diversity Scores
- Effect of pesticide exposure among the farm children and their mothers on the various biochemical parameters associated with reproduction, neurotoxic enzymes, oxidative stress and impact on the micronutrient status.
- Assessment of mycotoxin contamination in processed foods containing maize & groundnut
- Investigation of mycotoxin contamination in herbal and medicinal plants and products to formulate prevention & control strategies
- Dietary intake of aflatoxins from spices and risk assessment
- Evaluation of the role of molecular target/targets in HPV associated oral and cervical carcinoma is underway.

The potential of cinnamon extract as an anti-cancer agent was tested in two human prostate cancer cell lines. The results suggest that cinnamon extract decreases cell viability and alters growth kinetics of cancer cells in a dose-dependent manner. Further treatment with the extract led to apoptotic cell death in both cell lines tested. Cinnamon has proteasome-inhibitory potential and acts as an anti-cancer agent.

Molecular basis of maternal vitamin B12 restriction induced changes in the C57BL/6 mouse offspring were studied. Chronic dietary vitamin B12 restriction increased plasma Hcy and cortisol in weanling female C57BL/6 mice. Severe but not moderate deficiency resulted in anemia, leukopenia and affected reproductive performance. Vit B12 restricted mice had increased body fat %, altered lipid profile, higher oxidative stress and decreased antioxidant defense in liver. Offspring born to moderately B12 deficient mice had similar changes in body composition as their mothers suggesting a tendency towards obesity

Studies on obesity and obesity induced diabetes on accelerated ageing in WNIN/Ob and WNIN/Gr-Ob have shown that superimposing diabetes over obesity increased oxidative stress and impaired antioxidant status and may in general aggravate the existing changes in obese rats. Obese rats

fed high sucrose diet show high DNA damage in brain cells compared with the controls (*albeit* not significantly).

Capacity building activities to increase the NIN DFS (Double Fortified Salt) production in India have been taken up. Over 20 companies have signed MoU with NIN for manufacturing NIN-DFS, out of which 13 have completed the trial production successfully and are capable of producing NIN DFS.

A study showed that fluoride administration inhibits carbonic anhydrase activity of RBC and in kidney homogenate in fluoride intoxicated rats compared to control rats. Tamarind fruit extract (TFE) supplementation to fluoride intoxicated rats enhanced carbonic anhydrase activity with increased urinary fluoride excretion. The accumulation of fluoride in bone and teeth were lower in the tamarind fruit extract treated group compared to the fluoride group.

As part of integrated fluorosis mitigation activities in Nalgonda District, 2066 school children were screened for dental fluorosis in school children, 1183 students (57.2%) were affected by dental fluorosis. The prevalence of fluorosis in each category villages was 16% (Category I; water fluoride level 0.83 ppm), 47% (Category II; water fluoride level 2.06 ppm), 81% (Category III; water fluoride level 2.83ppm) and 82% (Category IV; water fluoride level 3.8 ppm).

Experimental lathyrism studies were conducted in guinea pigs. The animals were given lathyrus diet for 3 months, at the end of 2nd month, neck rigidity, extensor paralysis of legs, hyperextension of knee and ankle joints were observed in high Oxalyldiaminopropionic acid (ODAP) treated group as well as in low ODAP treated group. Oxidative stress studies revealed the increased oxidative damage in High ODAP treated group compared to the control group. Histopathological changes indicated extensive degeneration of motor neurons in high ODAP treated group compared to the low ODAP treated group.

Organophosphorus Pesticide Exposure of Urban Children through Bio-monitoring of different age group children with different socioeconomic status revealed that diethyl metabolites and dimethyl

metabolites levels were not different among the males and females in the 6- to 10-year-old group (means, 4.94 and 4.06 µg/L, respectively; (Mann Whitney U-test, $p = 0.25$). But the total diethyl DAP levels of girls aged 11-15 years were significantly higher than total diethyl DAP levels of boys (means, 2.372 and 1.433 µg/L, respectively; t test, $p < 0.009$).

Dietary exposure assessment of Polychlorinated biphenyls showed that the concentrations of Polychlorinated biphenyls (PCB) in fish have crossed the cancer bench mark of 0.0003 ng/kg/day.

Serotyping of RNA virus was carried out to study molecular epidemiology of dengue for supplementing emergency preparedness and capacity building in metro cities of Karnataka, India. Bioinformatic study showed that Adefovir dipivoxil is strongly bound with B2 receptor residues of THR10 and CYS14 when compared to existing drug of benzocaine. This molecule may act as a novel drug for the treatment of dengue fever.

Vitamin D status and regulatory T cell function in children with asthma were studied. Children with asthma had low serum vitamin D concentration and impaired Regulatory T cell function associated with decreased FOXP3 expression and increased IgE receptor (CD23/CD21) expression. The results suggest a vital role for vitamin D in immune regulation and asthma.

Effect of Fructo oligosaccharide and Probiotic LGG on fetal immune programming was studied. Prenatal probiotic or symbiotic supplementation led to a fourfold increase in antibody response to hepatitis B antigen (HBsAg) in the offsprings. This study on fetal immune programming, especially in the area of vaccine, shows encouraging results on immunoprogramming effect with probiotic and symbiotic and provides evidence that there is scope to modulate immune response to specific antigens with prenatal probiotics or prebiotics exposures.

An indigenously prepared probiotic curd containing *Lactobacillus bulgaricus* (*delbruki subsps* UBLB-38) and *Streptococcus thermophilus* (UBST-50) improved lipid profile in obese subjects, but had no effect on body weight. There was a significant decrease in the total cholesterol and serum

triglyceride and increase in the HDL in the probiotic group after 30 days when compared to their initial values.

Development of tools to identify and map IgE binding epitopes was another area of research. About 80 GB data was produced by Sequencing and transcriptome analysis of eggplant (*Solanum melongena L*) and Onion bulb (*Allium cepa*), and identified genes and proteins responsible for food allergy. Gene sequences are published on NCBI.

Vaginal lactobacilli profiling in pregnant women with normal and abnormal vaginal flora led to characterization of vaginal lactobacilli isolated from healthy women to formulate potential probiotic to prevent bacterial vaginosis (BV) and to improve reproductive health. *L. crispatus*, *L. jensinii*, and *L. helveticus* are predominant species in women with normal flora, whereas *L. iners*, *L. geserri*, and *L. vaginalis* are common in women with intermediate flora and (BV).

The scientists at the Institute have successfully engineered an oral vaccine for *Helicobacter pylori* using recombinant Lactobacilli expressing Heparan Sulfate binding protein. This system may be very useful as antigen delivery vehicle in oral vaccine formulation.

Studies have evaluated and identified lacunae in National Nutrition Programme in improving reproductive health and nutritional status of rural adolescent girls of Andhra Pradesh.

NIN's pre-clinical toxicology is the first public sector centre to submit reports on premarket safety (biosafety evaluation) of 'Transgenic Mustard' to GEAC for approval of field trial.

A report submitted to Tea Board of India on confirming the safety limits of Iron filing in Tea.

Preclinical toxicology centre has evaluated Pre Clinical Safety Evaluation of Liquid Pentavalent Vaccine (DTwP+HepB+Hib) for Local Tolerance.

A Taskforce program titled "Quantitative Detection of Heavy Metals and Phthalates in Toys" has been completed by multi-institute participation and report is submitted to Ministry of Health and Family Welfare, GOI.

Evaluated potential anti-atherosclerotic activity with combination of herbs and spices.

Studies were carried out to investigate the impact of specific fatty acids on the progression of nonalcoholic fatty liver disease in the setting of hepatic steatosis. The results showed that fructose: saturated/trans fatty acid combination induced hepatic steatosis of varying extent. Compared to fructose - saturated fatty acid combination, fructose - trans fatty acid combination induced nonalcoholic steatohepatitis as evidenced by increased inflammatory and fibrotic changes suggesting that fructose-trans fatty acids combination is detrimental to the liver. Substitution of n-6 PUFA with n-3 PUFA prevented high fructose, high cholesterol induced nonalcoholic steatohepatitis. The results reinforce the current recommendations of restricting the intake of trans fats, moderating the intake of n-6 PUFA and increase the intake of n-3 PUFA for the prevention of diet related chronic diseases.

Developed chemical (nSTZ) and Diet (High fat and high fructose) induced long term pre-diabetic animal models which developed retinal abnormalities to study biochemical and molecular mechanisms and intervention by dietary agents in pre-diabetes induced retinopathy.

A population based cross-sectional study assessed the subclinical micronutrients status and non-communicable diseases of urban geriatric population. There was a high prevalence of diabetes (51.9%), hypertension (67.8%), overweight (46.2%) and Obesity (31.6%), central obesity (61.8%) Dyslipidemia (76.8%) and metabolic syndrome (48.9%) in Urban free living elderly people in Hyderabad. A significant association was observed between Vitamin-D deficiency with Hypertension, BMI and Metabolic syndrome. In addition, a significant association was also observed between Zinc deficiency and Hypertension.

Demonstrated age dependent increase in the hypertrophy and hyperplasia of the adipocytes/pancreatic islet cells / Mesenchymal stem cells in WNIN/Gr-Ob and WNIN/Ob mutant rats with coprecipitation of Insulin Resistance/IR. IR forms an important predictor and predisposes to several metabolic alterations including T2D, CVD. These observations are akin to human scenario.

The in vitro micronucleus test is a well-known test for the screening of genotoxic compounds. However until now, most studies have been performed on either human peripheral lymphocytes or established cancer cell lines. We demonstrate for the first time the use of a normal diploid Umbilical Cord–Mesenchymal Stem cells as an alternative to the conventional micronucleus test.

A low cost, reliable and accurate assay was developed for a lipid-related enzyme called Lipoprotein associated Phospholipase A2 (Lp-PLA2). The assay will be used to identify asymptomatic individuals at high risk for the future development of coronary artery disease.

Achievements with Public Health significance

- Developed and launched a MobileApp on dietary guidelines for Indians. The App is based on the Recommended Dietary Allowances (RDAs) for Indians prescribed by NIN. It simplifies nutrient-centred RDAs into practicable, easy-to-follow guidelines and succinct explanation and rationale behind each of the 15 guidelines. The users can access this information from the App ‘Google Playstore’ as well as M-Gov AppStore. The APP was launched by the Director General, ICMR at NIN on 29th April 2016.
- Popular publications of NIN were launched in Braille format for the benefit of the visually challenged. Over 750 copies of three titles (books) were distributed free of cost to various blind schools, hostels, universities and visually challenged individuals.
- A novel iron binding peptide generated during gastro-intestinal digestion of egg white was isolated and characterized. The proof of concept of its activity was provided with synthetic peptide. The methodology developed is being used for isolation of similar peptides from other protein rich sources. The ultimate aim is to identify suitable peptides for application in nutraceuticals for improving iron absorption.
- Micellarization of dietary carotenoids from fruits and vegetables was demonstrated to be dependent on both quantity and type of dietary fat apart from microstructure carotenoid pigments in plant tissue. A minimum of 2.5% dietary fat rich in unsaturated fatty acids better promoted the micellarization and intestinal cell uptake of β -carotene. These results are expected to help in formulate dietary guidelines for optimizing the vitamin A nutrition from plant foods.
- It was demonstrated that Eicosapentaenoic Acid (EPA), a constituent of fish oil, inhibits intestinal carotene absorption via down regulation of lipid transporter expression in intestinal cells. These results further lend support to the receptor mediated intestinal absorption of dietary carotenoids, and helps in deciphering the intestinal physiology on vitamin A nutrition.
- The cell culture studies demonstrated that prior treatment of zinc enhances the iron absorption, ferritin content in intestinal cells via induction of divalent metal iron transporter-1 (DMT-1) expression. These results suggest that intestinal cell zinc status has a profound influence on iron absorption, and is expected to pave the way to for new intervention strategies of iron and zinc supplementation for improved efficacy.
- Developed potential anti-atherosclerotic agent for translating into promotion of nutraceuticals.
- Generated data on insulinemic and Glycemic index of six common Indian breakfast recipes of millets.
- Demonstrated the significant proteasome inhibitory activity and anti-cancer effects of aqueous cinnamon extracts.
- Installed capacity for NIN-DFS production in various salt manufacturers. As on date, there is a capacity of production of around 5 lakh metric tonnes per annum.
- Developed, validated and assessed the reproducibility of a Raw Food Based Quantitative Food Frequency questionnaire (RFQNFQ) to evaluate the dietary pattern and also for the estimation of their nutrient intake in chronic diseases.
- Provided the basis for the role of Advanced Glycation Endproducts (AGE) in tissue remodeling diseases such as diabetic nephropathy via a novel axis of NFkB-ZEB2 transcription.

- Demonstrated the role of growth hormone in podocyte loss leading to diabetic nephropathy
- Established that prenatal probiotics or prebiotics exposures during critical windows of gestation might have a significant influence on the infant's immune phenotype
- Characterized vaginal lactobacilli species from healthy women to formulate potential probiotic to prevent bacterial vaginosis (BV)
- Successfully developed recombinant Lactobacilli antigen expressing system to deliver desired antigen
- Established Model Rural Health Research unit (MRHRU) in Chandragiri village in Chittoor district to foster medical research
- Identified lacunae in National Nutrition Programme in improving reproductive health and nutritional status of rural adolescent girls of Andhra Pradesh
- Islet transplantation in non-human primates, paved way for its feasible application to try in human scenario. Based on these leads Asian Institute of Gastroenterology has initiated the pilot study on pancreatitis patients.

PRODUCT PATENT FILED

Antiglycating potential of procyanidin-B2 isolated from cinnamon bark: Prospects for prevention or treatment of diabetic complications (cataract, retinopathy & nephropathy) (GB Reddy, P. Muthenna, C. Akileshwari and G. Raghu). Patent Application Number: 1452/DEL/2012.

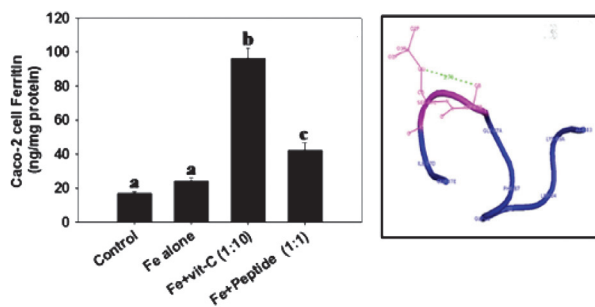


Fig. 1. Effect of iron binding egg peptide on iron induced ferritin expression and iron binding site.

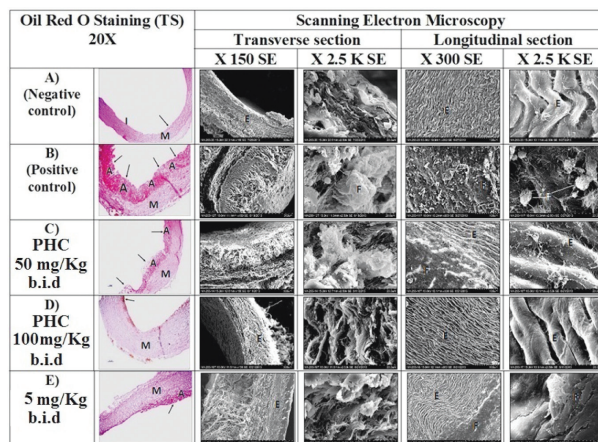
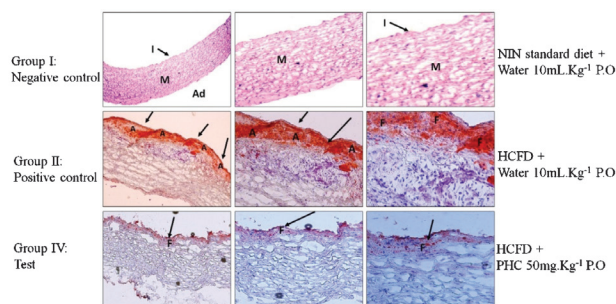


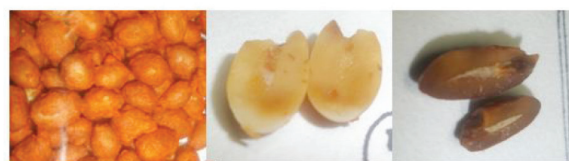
Fig. 2. Atherosclerotic morphological changes in rabbit aortic arch with Poly Herbal Formulation containing herbals (*Terminalia arjuna*, *Cyprus rotundus*) and nutraceutical (garlic, turmeric, ginger).



Source : . Vascular Pharmacology (2015) 72, 53-63.

Fig. 3. Source: . Vascular Pharmacology (2015) 72, 53-63.

Fried groundnut sample



Unsegregated sample

Segregated Discoloured nuts containing 76-1384ppm aflatoxin B1

Fig. 4. Fried groundnut sample.

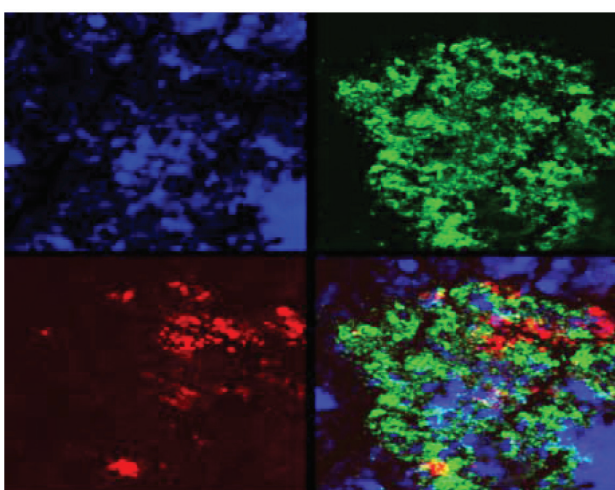
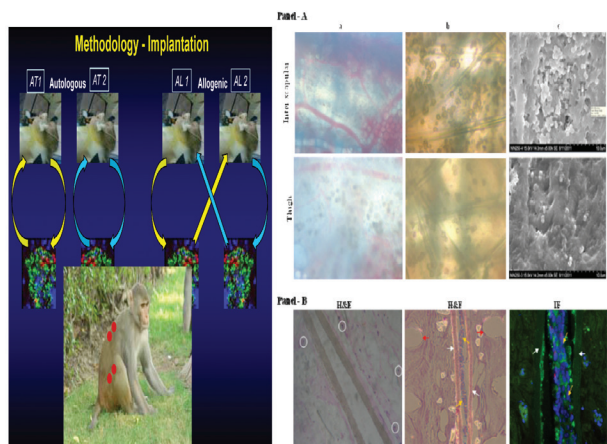


Fig. 5. Tx shown in Monkeys at thigh region and in hind limb region. The adjacent photographs show vascularization in the theraocyte region (device) post Tx. In addition insulin +ve cells were also demonstrated in the entrapped device even after one \ year of post Tx.



Fig. 6. Braille books brought out by NIN in 2015.

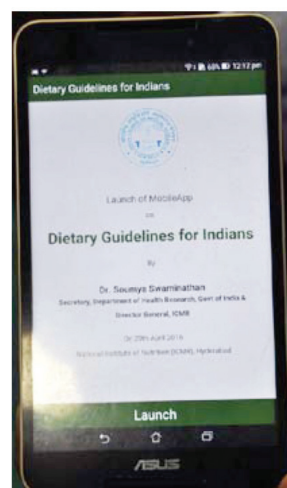


Fig. 7. Launch of MobileApp on Dietary Guidelines for Indians by Dr. Soumya Swaminathan, DG, ICMR.

Extramural Research

A recently completed study on 304 (156 Low Birth Weight (LBW) and 148 Normal Birth Weight (NBW) infants to assess the nutritional risk factors for growth and development of low birth weight infants in the first year of life revealed that LBW infants showed a significantly higher growth velocity compared to NBW infants as well as a high rate of catch up growth during first year of year. Although the nutritional status of LBWs improved significantly over the period of one year, yet, the differences remained significant between LBW and NBW infants as birth weight continued to remain a significant predictor of growth velocity even at 1 year of age.

Another study on rapid detection of probiotic lactic acid bacteria and assessing their suitability in vivo system revealed that isolates of lactic acid bacteria, i.e. *Lactobacillus*, *Pediococcus*, *Enterococcus* and *Staphylococcus* showed high tolerance to gastrointestinal conditions, cell surface hydrophobicity (0.55-77%), auto-aggregation (9-62%), bile salt hydrolase activity towards *micrococcusluteus*, *Listeria monocytogenes* Scott A, *Staphylococcus aureus* etc. Acute toxicity and sub-chronic toxicity studies of *L.salivarius* FIX and *L.fermentum* Cu05 revealed that they were safe for probiotic application.

A randomized clinical trial to study the effect of zinc supplementation on serum calprotectin level and acute inflammatory response in neonatal sepsis was carried out on 134 neonates (zinc group: 67; control group:67) in a hospital in Puducherry. The study revealed that after 10 days of zinc supplementation, there was a significant decrease in levels of serum calprotectin, IL-6 and TNF- α concentration ($p < 0.001$). further, after one month of follow up, there was 70% less chance of having abnormalities in the zinc group ($p < 0.02$), RR (95%CI)=0.28.

FELLOWSHIPS

Forty three fellowship research studies addressing diverse issues like effect of maternal vitamin B₁₂ supplementation on placental matrix in Wistar rat; identification and screening of food borne probiotic microbial strains with anti-colorectal cancer properties; effect of Long Chain Omega-3 Fatty Acid and Oleic Acid on dyslipidemia etc were ongoing during the year.

TASK FORCE STUDIES

An intervention Task Force study on 'Effectiveness of diet and lifestyle intervention through IEC tools with Angan Wadi Centres as the centre of knowledge dissemination for hypertension risk reduction' was ongoing at 10 locations [Chamba (Tanda, HP); Shimla; Dhar (Indore, MP); Puducherry; Junagarh (Gujarat); Adilabad (Andhra Pradesh); Udaipur (Rajasthan); Kalahandi (Odisha); Ranchi (Jharkhand); Dibrugarh (Assam)] across the country including 7 in tribal belt. During the year

2015-16; the first phase of the study (5 locations) was completed. Under the phase-II, intervention is ongoing. The preliminary analysis has indicated the prevalence of hypertension to be approx. 20% and that of diabetes to be approx. 9% in the study districts.



Fig. 8. Angan Wadi centre for knowledge discrimination.

Another Task Force study entitled "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" was initiated in 34 districts of the country. In the year 2015-16, the study was further expanded to 7 districts of north east. Hence, the study is currently ongoing at 41 districts in the country in 20 States. It is an intervention study with an aim to improve health and nutritional status of the vulnerable segment of population (pregnant women, adolescent girls, preschool children).



Fig. 9. Collection of baseline information under Nutrition Task Force study in Haryana).

CENTRE FOR PROMOTION OF NUTRITION RESEARCH AND TRAINING WITH SPECIAL FOCUS ON NORTH-EAST, TRIBAL AND INACCESSIBLE POPULATION

Approx. 12000 serum, plasma, urine samples and blood spots on filter papers were analyzed for various biochemical parameters like plasma glucose, haemoglobin, lipid profile, serum retinol, tocopherol, zinc, selenium, copper etc. These samples were collected either under Task Force study on hypertension or under small collaborative projects with various medical colleges, universities etc with the aim to facilitate researchers/ students for their degrees and providing assistance where adequate facilities for research are not available. Currently 6 Ph.D students are enrolled with the centre.

MAJOR ACHIEVEMENTS OF THE CENTRE

- The Expert Committee on Genetically Modified (GM) Foods addressed various issues related to GM food like Bt. Brinjal; Animal Feeding Studies with Bt Cotton Seed Meal etc.
- On the request of Food Safety and Standards Authority of India (FSSAI) to give expert views on HARMFUL EFFECTS OR SAFETY OF IRON FILLINGS upto 250 ppm in tea, the Division of Nutrition has constituted an Expert Committee on 'Issue of safety of presence of iron filings in tea'. Based on the available information, the Expert Committee recommended that iron filings in tea upto 250 ppm may not cause any ill effect on human health.
- On the request of the Planning Commission, ICMR constituted an Expert Committee consisting of various stakeholders (ICMR, ICAR, CSIR, FSSAI, Planning Commission etc.) to discuss the issue of safety of consumption of khesari dal. The Expert Committee in its meeting held in January, 2015 recommended to lift the restriction/ban on sale and storage of Khesari Dal in the country. The recommendation was communicated to FSSAI. Based on ICMR recommendation; FSSAI in its 19th Meeting of Food Authority held on 6th November, 2015, recommended that the ban on sale and storage of lathyrus sativus varieties with low ODAP content namely Ratan (Bio L 212), Prateek (LS 157-14), and Mahateora may be lifted and Ministry of Agriculture requested to promote their cultivation in traditional areas under cultivation of lathyrus to replace local varieties having high ODAP.
- The ongoing Task Force study on nutrition at 41 districts of the country has developed a model of intervention which if proved effective, may be adopted by the State Governments and could be scaled up to entire country.

ENVIRONMENTAL & OCCUPATIONAL HEALTH

The National Institute of Occupational Health, Ahmedabad and the National Institute for Research in Environmental Health, Bhopal are actively engaged in research in priority areas of occupational and environmental health relevant to national needs for various working groups. Major highlights of various programmes undertaken by the ICMR in the areas of occupational and environmental health during the year 2015-16 are given below.

Intramural Research

NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH, AHMEDABAD

BYSSINOSIS IN JUTE INDUSTRIES

Byssinosis is an acute respiratory illness that is caused following exposure to cotton and hemp dust. Regional Occupational Health Centre (E), Kolkata had earlier established the incidence of jute fiber related byssinosis and allied respiratory symptoms through studies conducted in jute handling operations. National Jute Board, Govt. of India has observed and promoted some mechanical improvements in some jute mills. This study was initiated to understand whether these changes can protect health of workers. The comparative epidemiological study was carried out in two jute mills (industry with old technology, industry-1 and industry with improved technology, industry-2) manufacturing jute cloth, jute bags etc. On comparing the subjects of the two industries, it was found that with subjects higher age group and higher job experience were more in the industry-2 with improved technology. While comparing morbidity, it was observed that chest tightness was significantly more in the industry-1. Breathlessness was also

more in this industry; however the difference was not statistically significant. On the contrary, irritation/watering in eye during working time was higher in industry-2. Musculoskeletal pain was relatively more in industry-1. Pulmonary function status revealed 5% cross shift changes of FEV₁ in industry-1 which was more than industry-2. Non significant Obstructive abnormalities, however, were more among the workers of industry-2.



Fig. 1. Byssinosis in Jute industries.

This comparative study showed that the acute symptoms and functional changes related to byssinosis were more associated with (not modernized) industry-1, whereas the chronic

changes (obstructive features) were more associated with industry-2 due to higher work experience in this industry. This study suggests that modernization of processes in jute industries may prove fruitful in lessening the respiratory problems of the workers.

Seroprevalence of Brucellosis among dairy farm workers of Gujarat

Yearly, half a million humans working in different occupations are affected by Brucellosis, which is the most wide-spread zoonosis throughout the world including India. Gujarat is one of the largest milk producing states in India with the contribution of 7.75% share in the total milk production of India. As per state census data, out of about 102 lakhs total household of Gujarat, 42.6 lakhs are engaged in Dairy and Animal Husbandry sectors as a primary or secondary source of their income. The study was carried out among dairy farm workers to find out the sero-prevalence of Brucellosis in three district of Gujarat state. 2586 milk samples were screened to establish the exposure of dairy farm workers to Brucella infection from 12 villages spread across 3 districts of Gujarat. Out of 2586 households (registered with Cooperative and Private Milk societies), 385 were found positive by milk ring test antigen. Total 400 blood samples of dairy farm workers screened by Rose Bengal Plate Agglutination Test (RBPT) showed 43 (10.75%) positive cases. Confirmatory ELISA test for human brucellosis showed 68 (17%) in IgM and 70 (17.5%) in IgG.



Fig. 2. Brucellosis in dairy farm worker.

The blood lead, serum iron and zinc levels among lead battery manufacturing workers

This study examined the relationship between Blood Lead Level (BLL) and serum Iron and

Zinc. A total 97 male workers aged 35.5 ± 5.1 years with working experience in the lead battery manufacturing plant for 12.4 ± 3.9 years were enrolled. BLL, serum iron, zinc and haematological parameters were estimated.



Fig. 3. Lead Battery manufacturing Plant.

The BMI of subjects was 25.8 ± 3.0 . The systolic blood pressure (SBP) was 127.8 ± 14.5 mm of Hg while diastolic BP was 77.8 ± 10.8 mm of Hg. The workers with $> 30 \mu\text{g/dL}$ BLL ($1745 \pm 723 \mu\text{g/L}$) had significantly lower serum Fe levels as compared to workers with $\leq 30 \mu\text{g/dL}$ BLL ($2063 \pm 784 \mu\text{g/L}$). The serum Fe showed significant negative correlation ($p=0.017$, $r=-0.242$) with BLL. Multivariate analysis between the four quartiles (Q1 to Q4) based on BLLs showed that the SBP ($p=0.004$, $F=3.483$ with 95% CI of 125.2 to 130.6 mm of Hg) and mean arterial pressure (MAP) ($p=0.027$, $F= 2.505$ with 95 % CI of 92.4 to 96.6 mm of Hg) significantly differed between four BLLs quartiles. The direction of difference was observed to be increasing in SBP and MAP with higher the BLLs. Study observed negative association between the BLLs and serum Fe levels even among the non-anaemic lead battery workers.

Identification, procurement and distribution of health and safety kits to the artisans of wood and iron crafts

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 were evaluated. The average value of respirable dust concentrations found was 3.92 mg/m^3 , which had exceeded the Recommended Exposure Level

(REL) of 1mg/m³ by NIOSH and Time Weighted Average-Threshold Limit Value (TWA-TLV) of 1 mg/m³ by ACGIH-2015 in wood-work handicraft industries and iron and steel handicraft industries. However, these were below Permissible Exposure Limit (PEL) of 5 mg/m³ by OSHA. The average respirable dust concentration in metal handicraft industry was 2.82 mg/m³ that were less than OSHA permissible exposure limit of 5 mg/m³. The noise generated was in the range of 80-130 dB(A).in wood and metal handicraft industries. The personal safety kits for wood and iron handicraft artisans were identified and suggested to the concerned agency for implementation.

A study of knowledge, attitude and perception of young Indian men towards females and about gender discrepancy at work

For centuries, role of women has been defined by men with apathy, far from liberal mindset. Inequality along with chauvinistic mindsets about work related gender specificity is prevalent, influencing the status of working women. The study undertaken to assess the knowledge, attitude and perception of young male adolescents (N=418) and young male workers (N=350) towards females and working women.

Overall attitude score of young workers depicted faintly negative attitude towards women whereas students' attitude was just on the edge of being positive. However, for some conventionally eccentric feminine gender specific roles like wearing of modern outfits, girls driving double seat were not supported by both the groups. On the contrary, leadership in women, higher education for women was supported by both groups. Gender stereotyping revealed that both workers and students enunciated minimal gender stereotyping for professions (pilot, soldier, doctor, nurse, scientist, etc.). However, muscular professions like rickshaw driving, fire fighting, wrestling had male gender preference. Questionnaire on perpetration of violence against females revealed that ~2/5th of workers didn't feel rape as a severest of crime. Other physical crimes (molestation, pinching, hitting) and psychological crimes (sending dirty SMS, eve teasing, whistling and emotional hurting) did get minimal to moderate crime rating among both the groups. Dowry demands were also not considered as a crime by 1/5th workers and 1/10th students. It is observed that

workers were more liberal to gender stereotype in occupations than the students who had some reservations about females around their age group. However, students more supported inequitable attitudes towards women, including condoning of violence against women.



Fig. 4. Attitude and perception towards women. Percentage distribution of workers (blue bars) and students (red bars) as responded for positive and negative gender attitude score.

Occupational exposure to Aflatoxins among oilseed mill workers

Oilseed mill workers are at risk for occupational exposure to airborne aflatoxins via inhalation of dust generated. This study was conducted at groundnut and cotton oilseed mills for environmental monitoring, isolation and identification of aflatoxigenic *Aspergillus* species. Maximum number of isolates belonged to genus *Aspergillus* followed by *Penicillium* from all the departments of groundnut oilseed mills. Maximum numbers of fungi were in processing department followed by storage and crushing departments of groundnut oilseed mills. Aflatoxigenic *Aspergillus* species were found maximum in groundnut oilseed mills as compared to cotton oilseed mills.



Fig. 5. Oil seed mill.

Most of the workers were working in processing, crushing and storage departments of both types oilseed mills. It was observed that maximum number of workers 24 (63.16%) were having 1-5 years of exposure followed by 11 (33.33%) having > 15 yrs of exposure in cotton and groundnut oilseed mills, respectively. According to questionnaire based findings most predominant symptoms were fever, chills, chest pain, body ache, abdominal pain, weight loss and weakness. It was found that 14 (42.42%) and 9 (24.32%) of groundnut and cotton oilseed mill exposed workers, respectively complained about chest pain as compared to 6 (13.33%) non exposed subjects. Exposed workers of groundnut oilseed mill had more complaints of abdominal pain as compared to cotton oil seed mill workers. Majority of the exposed subjects were not using any personal protective device. The findings showed that workers are exposed to airborne aflatoxigenic fungi and aflatoxins present in air of various departments of oilseed mill. These exposures might be responsible for the clinical symptoms reported by these workers. Special attention should be paid to use personal protective device by workers.

Evaluation of early biomarkers for the assessment of bone turnover and musculoskeletal disorders with long-term exposure to lead in workers from lead battery plant



Fig. 6. Long term exposure to lead in lead battery plant.

This study evaluated bone turnover biomarkers and musculoskeletal disorders (MSDs) in long-term Pb exposed workers from a lead battery plant. A total of 176 lead exposed workers and 80 matched controls were enrolled for the study.

All subjects were examined for blood lead levels (BLL), nutritional assessment and bone formation biomarkers (osteocalcin, alkaline phosphatase, and bone specific alkaline phosphatase), bone resorption biomarkers (pyridinoline, deoxypyridinoline, tartarate-resistant acid phosphatase -5b and urinary hydroxyproline) and MSD. Study noted significantly higher levels of BLL, C-reactive protein, bone turnover biomarkers (bone specific alkaline phosphatase, pyridinoline, tartarate-resistant acid phosphatase -5b and urinary hydroxyproline) and lower levels of serum total Ca^{2+} , corrected Ca^{2+} and ionized Ca^{2+} among exposed workers. A significant association was noted between bone resorption biomarkers- tartarate-resistant acid phosphatase -5b, urinary hydroxyproline with BLL. The highest prevalence of MSDs were observed at lower back (33%), followed by knee (26%), shoulders (16%), neck (14%), ankle/feet (11%), wrist/hand (10%), elbows (8%), upper back (7%) and hips/thighs (5%) among lead exposed workers. The workers who had MSDs of ankle/feet were associated with elevated levels of BLL, C-reactive protein, urinary hydroxyproline and low levels of osteocalcin. MSDs of upper and lower back were associated with higher BLL as well as urinary hydroxyproline. Long term Pb exposure was associated with elevated BLL, inflammatory activity, bone resorption biomarkers and musculoskeletal disorders with low mineralization.

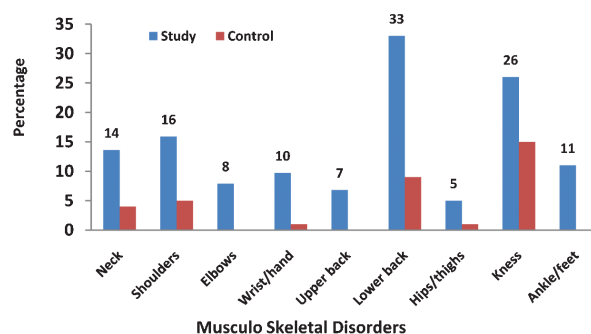


Fig. 7. Musculoskeletal disorders among exposed and control subjects.

Screening and brief intervention to reduce occupational harm from tobacco, alcohol and cannabis among coal mine workers in West Bengal

Tobacco (30%), alcohol (21.4%) and cannabis (3%) are three commonest substances of abuse prevalent in India. Compared to general population tobacco

and alcohol have higher prevalence rates among different occupational groups in India. Both tobacco and alcohol lead to significant occupational harm in terms of absenteeism, injuries, sickness and loss of productivity. This study proposes to examine the effectiveness of a single brief intervention (education / simple advice) appropriate for tobacco, alcohol and cannabis as secondary prevention based on patterns of substance use to reduce occupational harm in coal mine workers. Coal mine workers engaged in mining activities having ≥ 18 years of age of either sex were recruited from the Raniganj – Asansol coal mining areas of Eastern Coalfields (ECL). Brief intervention is cost-effective and can be delivered at primary care setting without specialist support. During random screening the following questions are asked.

- Have you consumed alcohol, cannabis in any form (eaten/beverage/smoked) and / or tobacco (smoked/smokeless) in any form during past one year?
- Have you consumed alcohol, cannabis in any form (eaten/beverage/smoked) and / or tobacco (smoked/smokeless) in any form during the past 30 days?

70 workers who answered “yes” to both the questions were included in the study. 70% workers perceived some degree of work stress. 73% (51) have both alcohol and tobacco problems. However, almost 55% participants are screen positives for either depression or anxiety. 2 subjects had lifetime use of inhalants and sleeping pills and 2 subjects were under DOTS treatment for pulmonary TB. Alcohol and tobacco are major substance use problems among coal mine workers. Approximately 80% subjects have tobacco consumption and almost 40% have alcohol consumption. This population is expected to benefit most from early intervention, which can be instituted at the primary care level. Almost 40% subjects possibly have alcohol dependence problems, which suggest significant medical, social and economic impact. Community based pharmacotherapy needs to be instituted for population with dependence problems.

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

The Indian population is continuously subjected to chronic environmental organophosphorus pesticide exposure, which is implicated in the aetiopathology of neurodegenerative diseases. There is a need to identify possible association between organophosphorus pesticide exposure and neurodegenerative diseases. For the pilot phase, screening of subjects was performed among directly exposed population in a high agricultural productivity as well as to pesticide exposure block (Galsi II) of district Bardhaman (W. Bengal). Samples were drawn from subjects of >50 years of age and of either sex, continuously living in the area for at least last five years. A set of general questionnaire based assessment of the subjects according to 1) memory, 2) mood and 3) motor problem observed over last 6 months as a primary screening for neurodegenerative disorders was applied. Out of 43 subjects screened, 51% (22) subjects have fulfilled criteria for cognitive impairment or possible depression or both. Eighty six percent among cases are involved in any agricultural activity compared to 67% among control participants. Among cases 73% reported direct exposure to pesticides as compared to controls (67%). Seventy nine percent cases with any cognitive impairment/depression reported use of combination of pesticides.



Fig. 8. Study on pesticide exposure.

A higher percentage of cases had less knowledge about safe pesticide use as well as high risk pesticide use practice. Most subjects among cases reported to be associated with agriculture, pesticide use and use of combination pesticides. Identification of

dementia and further clinical confirmation shall be carried out through clinical diagnostic procedures.

Personal and biological monitoring of workplace pollutants and their potential health effect indices assessment in fuel filling station workers

The personal monitoring of workplace pollutants and their potential health assessment in fuel filling station workers was carried out. A total of 59 petrol filling attendants with mean age of 27 yrs participated. Post-shift urine samples were collected. About 42% of workers do not use personal protective equipments. 51 % of workers reported 12 hrs of working hours. Smoking and alcohol intake habits were recorded among 32% and 44% of workers, respectively. Health complaints commonly reported among workers were respiratory problems (19%), itching (10%), skin irritation (5%), headache and eye irritation (27%). Although the Volatile Organic Compounds (VOCs) concentrations ranged between 0.1 to 0.7 ppm, the continuous exposure may lead to deleterious effects on worker’s health. The other biological parameters to assess the health effect indicators between exposure and levels of VOCs will be assessed to ascertain the relationship.



Fig. 9. Health studies on fuel filling station workers.

Assessment of relationship between levels of air pollutants and relevant childhood morbidity in selected urban areas

This study assessed the relationship between air pollution and childhood respiratory morbidity in various identified polluted areas. The relevant morbidity conditions of children in different living conditions were evaluated. The data collected from Public Health Centres (PHCs) are located near the highly polluted industrial areas. About 8074 and 3860 registered cases of children of ≤14 years were covered in two PHCs (I & II) from the period 2011 to 2015, respectively. The subjects were categorized into three groups, ≤5, 6-10 & 11- 14 years. From PHC-I it was observed that, out of 8074 cases

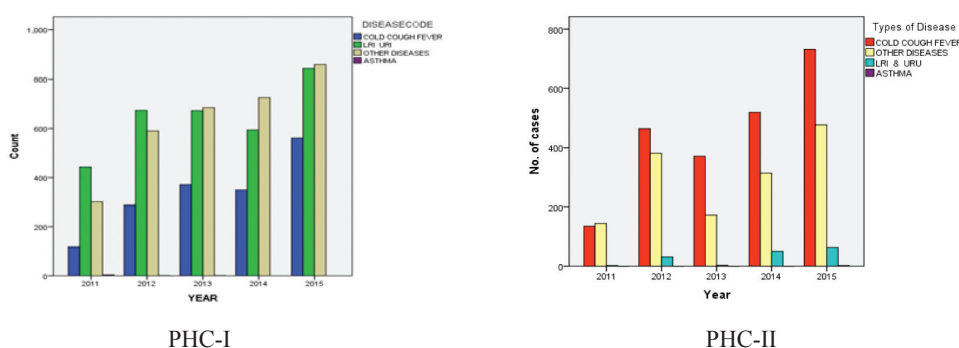


Fig. 10. PHC-I Distribution of types of diseases according to the period from 2011 to 2015. PHC-II distribution of types of diseases according to the period from 2011 to 2015.

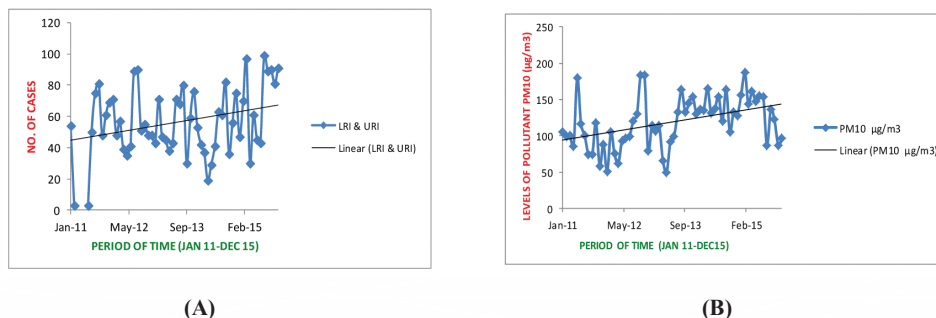


Fig. 11 (A). Trend analysis of morbidity conditions (LRI & URI) of the subjects (5 Years). (B). Trend analysis of levels of pollution (PM₁₀) µg/m³ recorded at selected industrial area.

3224 (39.9%) children suffered from respiratory illnesses viz. lower respiratory infection (LRI) & upper respiratory infection (URI). It was found that the prevalence of illnesses was more among the children aged ≤ 5 years than other age groups. From PHC-II it was observed that, out of 3860 cases 149 (3.9%) of children suffered with respiratory illnesses viz. LRI and URI and 2221 (57.5%) of children suffered with fever, cough and cold. Time series analysis showed that the trend of pollution levels and respiratory illnesses of the children of these areas increased. Further study is in progress to collect the data from the low level polluted areas for comparison.

Assessment of mercury exposure among coal mine workers and population on and around Coalfield area

Chronic low level mercury exposure may induce symptoms from the central nervous system (CNS) including tremors, delusions, memory loss and neurocognitive disorders. The mercury content of Indian coals ranges between 0.15 and 0.87 ppm. Mercury is highly volatile, hence it is released into the atmosphere during coal burning. Coal fired thermal power plants are the major contributor to atmospheric mercury levels.

The blood mercury analysis was done in 237 mining workers, 94 supervisory workers and 138 residents. According to USEPA, current Reference Dose (RfD) of mercury is 0.1 $\mu\text{g}/\text{kg}$ body weight/day which is equivalent to 5.8 ppb blood mercury level. When compared to the permissible level of mercury in blood i.e. $<5.8\mu\text{g}/\text{dl}$ it was found that 16 (6.8%) subjects in mining group and 11(8%) in the residents group had blood mercury levels higher than PEL. None of the subjects in supervisory group had higher than PEL levels of blood mercury. The nail mercury level estimation carried out in 109 subjects showed $33.91 \pm 25.17\mu\text{g}/\text{kg}$ (34 miners), $42.18 \pm 61.47\mu\text{g}/\text{kg}$ (49 supervisory workers) and $46.42 \pm 40.19\mu\text{g}/\text{kg}$ (26 residents.) of the nearby vicinity, respectively. The hair mercury level estimation was done in 118 subjects which included 40 miners, 53 supervisory workers and 25 residents of the nearby vicinity. The mean hair mercury levels of miners, supervisory workers and residents group was 95.78

$\pm 111.10\mu\text{g}/\text{kg}$, $90.96 \pm 124.67\mu\text{g}/\text{kg}$ and $135.8 \pm 111.76\mu\text{g}/\text{kg}$, respectively. According to USEPA, current Reference Dose (RfD) of hair mercury 1000 $\mu\text{g}/\text{kg}$ body weight/day. When compared to the permissible level of mercury in hair it was found that no subject had higher than PEL levels of hair mercury. The higher mercury level among control subjects might be due to the higher environmental exposure levels.

A study on quality of life among bank employees in Bangalore city

According to WHO, the measurement of health and the effects of health care must include not only an indication of changes in the frequency and severity of diseases but also an estimation of well-being and this can be assessed by measuring the improvement in the quality of life. This study was undertaken to assess the quality of life of Bank employees in Nationalised bank in Bangalore City. The study included a total of 120 subjects (66 men and 54 women with an average age of 36.5 years). The socio economic profile showed that 87 (72.5%) were graduates, the monthly average income was Rs. 57,542 with an average experience of 9.5 years and average working hours of 9 hours. 41.7% of them reported health complaints. The major complaints reported were musculo skeletal problems (29.2%), hypertension (18.75%), sleeplessness 15%, diabetes 9.2%, gastro intestinal problems 6.7% and migraine 5.8%. The major musculo-skeletal problem reported was lower back pain 22(18.3%) and upper back pain 15 (12.5%), neck pain 9 (7%) and shoulder pain 8 (6.7%). Quality of life scores showed a mean score of 82 in physical domain, 81 in psychological domain, 79.5 in environment domain and 79 in social relations domain. The health survey scores based on SF 36 questionnaire revealed highest mean score in role functioning/emotional (94), Role functioning/physical (93), physical functioning (88), general health (80), pain (81), emotional wellbeing (78), energy/fatigue (75) and social functioning (70). Among the 8 health concepts, social functioning showed the least. Their perception of health change score showed (63) as they expected their health to get worse. 57.5% of them did some form of physical exercise.

Studies of arsenic induced immunotoxicity in human

Arsenic is a natural metalloid toxicant that contaminates drinking water worldwide. Ground water in 79 blocks in several districts of West Bengal is under the risk of Arsenic contamination. Total risk population in the state of West Bengal is 286.54 lakh out of the total state 2001 population of 802.21 lakh which is about 36%. Our earlier study indicated decreased level of total anti oxidant capacity, as indicator of body defense, in a significant number of cases without signs and symptoms of arsenic exposure. The assessment of immune status has been a powerful tool in the in the hand of modern medicine for diagnostic and prognostic purpose.

Thus a case control study was undertaken in North 24 paraganas district, West Bengal. Evaluation of pro-inflammatory (TNF- α) and anti-inflammatory (IL-6) cytokines evaluated by ELISA method indicated elevation of both of cytokines in exposed subjects. Subjects with mild, moderate and severe arsenicosis showed more alteration of both of cytokines (TNF- α and IL-6) than the patients with pigmentation only. By exploiting the knowledge of arsenic induced immune-toxicity achieved, early biomarkers for assessing public health status of arsenic exposed area can be developed as outcome.

Environmental Information System (ENVIS) on Environmental and Occupational Health

Environment Information System (ENVIS) centre, at NIOH is a thematic centre of Ministry of Environment, Forest and Climate Change based on the theme of Environmental and Occupational Health. It is engaged in collection, collation, storage, retrieval and dissemination of information related to its theme. It is a resource centre for those engaged in research, policy making and implementation, students, working class and general population through its website and printed materials. During the year this centre brought out 4 Newsletters on Social Security Schemes for workers in India, Occupational Health issues of Sewage and Sanitary workers, Health Hazards of Mobile Phones and Towers and Manganese: Occupational and Environmental Health were

published. Bibliography is a compilation of Indian research carried out on select topic to facilitate a one point solution to the budding researchers for their literature search. Two issues namely Chromium Toxicity and Carbon Monoxide Toxicity were compiled using compiled using JabRef software and accessible through website.

To cater the workers and empower them on the occupational health, a journal “Shramik Swasthaye” was published on Restaurant workers’ job related problems and importance of cleanliness and distributed to the workers exposed to these hazards.

Swachh Bharat Abhiyaan: this centre also contributed through awareness programme, distribution of pamphlets and poster in regional language.



Fig. 12. Newsletter on social security scheme.

Workshops/ Training programme/ Conference / Seminar Organized

- Three days workshop on “Statistical Methods in Biomedical Research” in collaboration with National Institute of Medical Statistics, New

Delhi, organized at NIOH, Ahmedabad, 04-06 May, 2015.

- National Conference on Emerging Issues in Environment Occupational Health & safety its National Scenario and Regional Needs was organised by ROHC(S) during 22nd - 24th July 2015 at ROHC(S), Bengaluru.
- Workshop on Fundamentals of Biomedical Research Methods at ROHC, Kolkata from 7-9 October, 2015.
- Directorate of Industrial Safety and Health, Maharashtra sponsored Training to safety officer serving in the field of Industrial Safety and Health 27-29, January 2016.
- A Training cum Workshop on Occupational and Environmental Diseases” was conducted by ROHC(S) Bengaluru jointly with Directorate of Factories, Boilers, Industrial Safety & Health, Govt. of Karnataka, Directorate of Industrial Safety & Health, Govt. of Tamil Nadu, Directorate of Factories, Govt. of Andhra Pradesh & Telangana and Directorate of Factories & Boilers, Govt. of Kerala during 11-12 February 2016.
- International conference on Reproductive health with emphasis on Occupational, Environmental and Life Style Factors (to mark Golden Jubilee year of NIOH) and 26th Annual Meeting of the Indian Society for the study of Reproduction and Fertility, organized at NIOH, Ahmedabad, 18-20 February, 2016.



Fig. 13. International conference on Reproductive Health at NIOH.

Major Achievements having Public Health Importance

Seroprevalence of Brucellosis among dairy farm workers of Gujarat

Several lakh of households are engaged in Dairy and Animal Husbandry sectors as a primary or secondary source of their income. Screening of milk samples established the exposure of dairy farm workers to Brucella infection. The regular monitoring and educational programme for control of zoonotic disease especially Brucellosis should be organized regularly.

Poision Information Centre

Poisoning is a significant global public health problem. Established in 1993, Poison Information Centre at NIOH (PIC-NIOH) has been involved in addition to research work, the public health related activities like laboratory analysis, toxicovigilance, providing Information, teaching and training of health professionals related to poisoning cases. It collects the data about human exposures to chemicals, including information about the agents involved, the circumstances giving rise to exposure, and the health effects of exposure. The centre has been enrolled in the ‘World Directory of Poison Centres’ by WHO.

A total of 539 cases of poisoning were referred to the centre for the laboratory support or for getting the information on the toxicity. Thirteen cases of epidemic dropsy from Banaskantha district and thirty-seven cases of paralysis due to unknown etiology (Contaminated castor oil used for wheat storage) from different areas of Gujarat (mainly from Dehgam distict) were reported during the year. Analysis of toxic metals in urine and blood samples of patients referred from Civil hospital Ahmedabad was also carried out.

Studies of arsenic induced immunotoxicity in human

A large population in the country is under the risk of arsenic contamination through drinking water. Arsenic exposed population indicated decreased level of total anti oxidant capacity, as indicator of body defense, in a significant number of cases without signs and symptoms of arsenic exposure. By exploiting the knowledge of arsenic induced immunotoxicity achieved from this work and early biomarkers for assessing public health status of arsenic exposed area can be developed as an outcome.

Information System (ENVIS) on Environmental and Occupational Health

ENVIS Centre is involved in disseminating information on environmental and occupational health to promote and propagate the concept in the stakeholders and common man. ENVIS centre promotes its theme through different outreach programmes at school, villages, colleges and at various exhibitions. Social media is a very common platform for the public, for this centre has started a page on facebook to sensitise them on the theme. A flagship Programme of Government of India “Swachhh Bharat Abhiyaan” this centre has contributed through awareness programme at villages.

Four Newsletters on Social Security Schemes for workers in India, Occupational Health issues of Sewage and Sanitary workers, Health Hazards of Mobile Phones and Towers and Manganese: Occupational and Environmental Health were published. Swachhh Bharat Abhiyaan: A flagship Programme of Government of India and this centre also contributed through awareness programme, distribution of pamphlets in regional language to school children.

Assessment of relationship between levels of air pollutants and relevant childhood morbidity in selected urban areas

The health complaints of children living near the industrial sources were analyzed. The diseases recorded in the hospitals will help to plan suitable intervention programs for the whole community.

Personal and biological monitoring of workplace pollutants and their potential health effect indices assessment in fuel filling station workers

The workers employed in petrol filling station are continuously exposed to particulate matter and volatile organic compounds (VOCs) during their job. These workers are not covered under Factories Act, 1948. The adverse health impact caused due to exposure to low level toxic chemicals will lead to system impairment. Hence this study is important to estimate the burden of multiple toxic element exposure among large population.

Assessment of mercury exposure among coal mine workers and population around Coalfield area

Coal burning and coal fired thermal power plants are the major contributor to atmospheric mercury levels. General population residing nearby coal field are exposed to environmental mercury exposure. Study reported marginally higher level blood, nail and hair mercury levels in resident group than coal miners.

NATIONAL INSTITUTE FOR RESEARCH IN ENVIRONMENTAL HEALTH, BHOPAL

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 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 original cohort of exposed and unexposed persons, that was assembled in 1985, is being surveyed at six monthly intervals for morbidities and mortalities. During 2015-2016, 50th (January-June, 2015) and 51st (July-December, 2015) rounds of survey were completed covering a cohort of about 31,000 individuals (25,000 exposed and 6,000 unexposed) from severely exposed, moderately exposed, mildly exposed and control areas were followed up. During the year any morbidity recorded was 22.4% in severely exposed, 16.7-17.3% in moderately exposed and 16.9-17.1% in mildly exposed areas compared to 8.6-8.8% in the control areas. The respiratory morbidity rates remained high in the exposed areas (~7.8%) than control areas (1.8%). Overall, similar mortality rates were recorded in the exposed (2.46-3.93/1,000 population) and control (2.32-2.76/1,000 population) areas.

Study on the prevalence of morbidity of selected population/families with reference to the drinking water utilization

This study determined the prevalence and pattern of morbidities with reference to the drinking water

utilization among the population residing at variable distance(s) i.e. 0-1 km (stratum I) and 2.5-5.0 km (stratum II) radial distance from the Union Carbide India Limited factory with the underlying hypothesis that the population residing closer to the factory is more prone to adverse effects of consuming the allegedly contaminated underground water caused due to undisposed chemical waste stored in UCIL factory premises. A total of 2,184 families (10,827 individuals) following stratified random sampling method were surveyed during the study. In stratum I (0-1 km from UCIL boundary), covering 7 Bhopal municipal wards, data was collected for 5,467 individuals (1,092 families) whereas in stratum II (2.5-5.0 km from UCIL boundary) 5,360 individuals (1,092 families), residing in 32 municipal wards were surveyed and clinically examined. For each ward the target number of families was calculated proportionately to the population of that ward. The most prevalent diseases reported in the surveyed population were hypertension and diabetes mellitus. Correlation of morbidities, likely to be caused due to the consumption of water contaminated with biological or chemical contaminants with various risk factors, revealed no significant association between the morbidities and distance from UCIL factory. Also, no significant association was seen between prevalent morbidities and drinking water consumption pattern. It was concluded that the communities, irrespective of distance of their residences from UCIL factory or source of their drinking water, were equally vulnerable to various morbidities.

Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December, 1984

In this pilot study the cytogenetic status of 100 gas exposed and 100 unexposed individuals among those screened earlier under multi-centric genetic screening study of ICMR in Bhopal during 1986-1990 is being examined and compared with the previous genetic status to exclude the possibility of long term effects, if any, of MIC gas. Among the 800 retrieved pre-screened cases (543 exposed, 257 unexposed) only 174 (129 exposed, 45 unexposed) could be traced and contacted. Their 3-generation pedigree was prepared and biological samples from willing 141 subjects were collected. The

cytogenetic analysis of 53 subjects (19 exposed cases and 34 unexposed) has been completed. The study is in progress.

Cytogenetic analysis in Methyl Isocyanate (MIC) exposed population and their progeny

This study is investigating the cytogenetic status of 1,200 gas exposed people aged >28 years belonging to 6 sub-groups and their progeny. So far, a total of 1784 eligible individuals have been enrolled in the study from the JNCHRC registry; counseling and pedigree analysis has been completed for 123 cases; sample collection and lymphocyte culture has been completed for 320 subjects and slides for G-banding has been prepared from 1,280 subjects. The study is continuing.

To evaluate biochemical markers in cases of clinically stable stages of Chronic Obstructive Pulmonary Disease (COPD) in MIC affected population

This pilot study envisaged to evaluate six biochemical markers of potential prognostic value of Chronic Obstructive Pulmonary Disease (COPD) in serum of mild, moderate & severe COPD cases among gas exposed persons. A total of 38 subjects (30 with acute exacerbations of COPD and 8 healthy controls) fulfilling inclusion/ exclusion criteria were enrolled and biochemical markers evaluated for their expression and quantification in serum. Three markers *viz.* Monocyte Chemo-attractant Protein (MCP1), Matrix Metalloproteinase-9 (MMP9) and Chemokine Ligand 18 (CCL 18) have shown promising prognostic value. The pilot study has been completed.

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 new hospital based study was initiated in January, 2016 to compare the prevalence of congenital malformations in neonates born to gas exposed/first generation of gas exposed and non exposed mothers. So far, 2,445 neonates delivered in 2 major hospitals of Bhopal have been followed up in this study.

Public Health Interventions

NIREH has made available following community based services providing health benefits to the gas exposed persons –

- A special respiratory clinic has been functioning at NIREH with the help of Kamla Nehru Hospital and Bhopal Memorial Hospital and Research Centre. Under this programme patients with chronic respiratory symptoms, identified in the field during epidemiological surveys, are transported to NIREH for examination and advised treatment. Till March 2016, a total of 466 patients were examined and investigated, of which 120 were found to be suffering from Chronic Obstructive Pulmonary Disease (COPD) and 67 from bronchial asthma and other co-morbidities. Majority of COPD cases were in 40-70 years age group.
- Respiratory physiotherapy clinic is being run by NIREH at one of the Mini Units of BMHRC. COPD patients, identified after screening by NIREH doctors from the community, are referred for respiratory physiotherapy by a qualified physiotherapist. At present 75 patients are being provided physiotherapy regularly. The respiratory physio- therapy has been found to be very effective and has shown a definite improvement in the disability level of chronic respiratory patients.
- Under Community based health service programme, severely ill chronic patients having recurrent exacerbations of bronchial asthma and COPD, cardiac, gastrointestinal, neurological, ophthalmic morbidities, who need emergency care/specialized care, are availing the benefit of referral to BMHRC for investigations and door step ambulance service. Till March 2016, a total of 320 patients have been referred to BMHRC for treatment.

NON-COMMUNICABLE DISEASES

In the area of non-communicable diseases the ICMR's National 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, Bangalore focuses on the National Cancer Registry Programme and related activities like software module for cancer registration, patterns of cancer patient care and survival studies. Other studies included Population Based Urban Stroke Registry, Population Based Rural Stroke Registry, research studies on diabetes, obesity and metabolic syndrome, otorhinolaryngology, gastroenterology, ophthalmology and oral health. Major highlights of various programmes undertaken by the ICMR in the area of non-communicable diseases during the year 2015-16 are given below.

Intramural Research

NATIONAL INSTITUTE OF CANCER PREVENTION & RESEARCH, NOIDA

Prevention of cancer is one of the main mandates of the Institute. Main strategy to prevent cancer is to find cancer causative factors like environmental, behavioral, Genetic and their interaction and managing such factors to prevent cancers. Second is the early detection of cancers through screening and early detection. In order to achieve these goals for cervical cancer, NICPR adopted the following strategies: epidemiological studies and early cancer detection activities, genetic susceptibility studies and genetic markers.

NICPR has a unique approach of amalgamating strong basic, clinical and applied research, involving medical and community practice with an emphasis on early detection, primary and secondary prevention of cancer. The thrust areas of research include the cancer of the uterine cervix, breast and oral cavity.

Cancer Web Portal (India Battles Cancer)

NICPR designed an India-centric cancer portal, "India Battles Cancer" (www.cancerindia.org.in). This portal provides information on the leading cancers in India with a major focus on awareness, prevention and treatment of these cancers. The portal currently focuses on information for the general public and patients and will be upgraded in the near future to include relevant details for health care providers. This website has information base at two-tiers, firstly to make awareness and value added education amongst web-users in general population both in villages of India as well as cities and larger towns. The second tier will be to increase the level of knowledge to educate the primary level health providers, viz. ASHA, ANMs, paramedics, AYUSH practitioners etc. Main objective of this portal is to design an interactive web portal that provides information on the leading cancers in India with a major focus on awareness, prevention and treatment of these cancers and also to educate general public and patients regarding various cancer related information including risk factors, prevention, screening, treatment and palliative care.

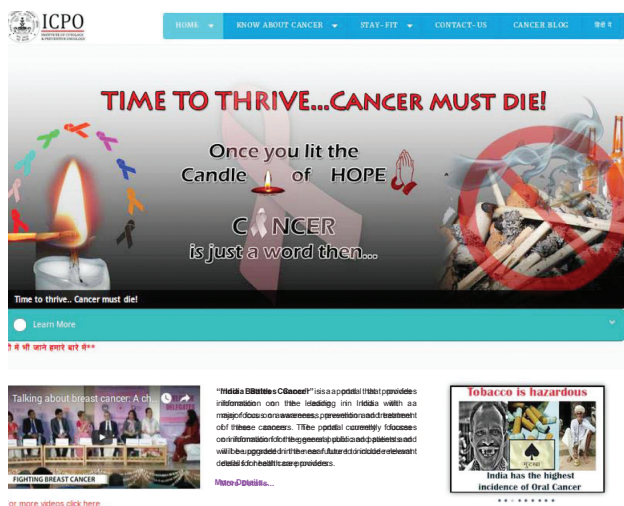


Fig. 1. Cancer Web Portal (English version).



Fig. 2. Cancer Web Portal (Hindi version).

Cancer Web Portal (Hindi version)

The institute has already designed and uploaded the portal at website “www.cancerindia.org.in”. The portal includes comprehensive knowledge on prevalent cancers in India viz breast, cervical, oral, lung, gall bladder, prostate. The site also provides links to various treatment centres across our country and financial aids available at various levels for cancer treatment. The site also provides information on common myths about cancers and answers to frequently asked questions about cancers. Additionally, there is a link for the public to ask their specific query regarding the content of the website. The institute has also created a cancer blog on the website which is being updated on a regular basis with recent news and articles related

to various aspects of cancer. The portal initially aims to focus on information for the general public and patients and will be upgraded in the near future to include relevant details for health care providers. This website has been developed as a mature pickle of a web-based education tool. This web portal is also being developed in different regional languages. Presently, the Hindi version of the website can be accessed from www.cancerindia.org.in/hindi. In second phase, this portal will be extended to the researchers.

Smokeless Tobacco Hub

World Health Organization Framework Convention on Tobacco Control (WHO-FCTC) has designated the Institute of Cytology and Preventive Oncology as Global Knowledge Hub on Smokeless Tobacco. The primary objective of this hub is to collect/create evidence for harmful effects of tobacco and develop a knowledge base for different stakeholders. The hub will also work with other agencies to increase awareness about the harmful effects of SLT and evaluate the effects of various programs.

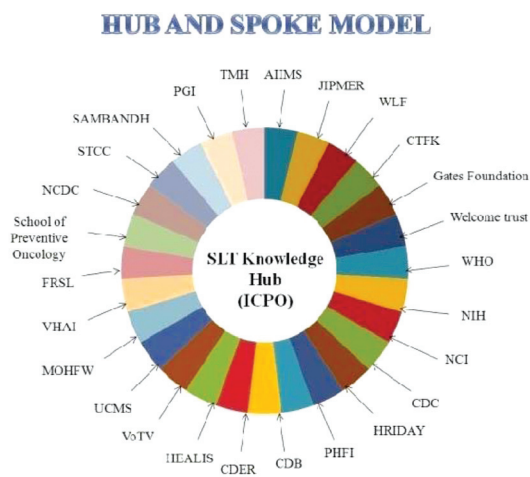


Fig. 3. Hub and spoke model.

Health Promotion Clinic

The health promotion clinic was started in Feb 2014 at NICPR. It functions in the OPD rooms situated in the clinical oncology wing, ground floor. The timing of the clinic is from 10:00 am to 4:00 pm from Monday to Friday. Individuals diagnosed with frank cancers are seen by faculty of Radiotherapy department, AIIMS.



Fig. 4. Health Clinic.

Cancer Screening Programs using low cost technology

NICPR in collaboration with Karuna Trust, is making efforts to incorporate cancer screening programs in the existing infrastructure and manpower in the country using the ECHO model to improve capacity and access to specialty care for rural and underserved populations. A pilot project has been started at the primary health centre (PHC) Gumballi, a tribal village in Karnataka. NICPR helps in empowering the community health workers (CHWs) at the PHC to conduct cancer screening of the population covered by the PHC.

Two modes of training health care providers are being used. Initial face-to-face training on cancer screening tests is there, allowing hands-on training and practice of skills. NICPR staff visited Gumballi in May 2015 and provided 4 days intensive training to the community health workers in screening of oral, breast and cervical cancer. Subsequently, fortnightly ECHO clinics are being conducted that included reiteration of the training, discussing patient cases, resource sharing and expert presentations. Currently, the community health workers have initiated cancer screening and are collecting data which is being sent to NICPR on monthly basis. The screen positives and suspicious cases are being evaluated and treated by a team of specialists from the Kidwai Memorial Institute of Oncology, Bangalore which is a tertiary care centre for cancer treatment in that region. Other medical establishments are being added as spokes to the NICPR Cancer Prevention hub in order to enhance outreach in the community and enable quality

cancer screening.



Fig. 5. Health Care Training.

A. Data Management Laboratory (DML)

NICPR has established a Data management Laboratory (DML) in collaboration with the Bioinformatics Centre, ICMR, New Delhi. The DML is a specialized unit for developing and providing professional data management solutions/applications for programs of ICMR and medical fraternity from medical college and research institutes.

B. Early detection of cancer by population screening initiated

NICPR has initiated programme of cancer prevention by directly approaching the population in Gautam Buddha Nagar District of Uttar Pradesh, with the aim to educate the population and screen them for early detection of Cervical Oral and Breast cancer. This approach has become very popular and is getting frequent requests from RWAs and gram panchayats for initiating screening programs in their sectors and villages. Following screening programmes were conducted during last year:-

SCREENING CAMPS ORGANIZED IN 2015-16

Table 1: Details of Screening Camps organised.				
S. No	Site of the camp	Date	Total individuals screened	Tests Done
1	CHC, Badalpur	18 th Jan 2015	57	CBE, PAP, VIA, VILI
2	CHC Bhangel	29 th Jan 2015	67	CBE, PAP, VIA, VILI

3	CGHS Noida	20 th Feb 2015	81	CBE, PAP, VIA, VILI
4	PHC, Bsrakh	19 th March 2015	17	CBE, PAP, VIA,
5	CHC, Bhangel	30 th March 2015	38	CBE, PAP, VIA, VILI
6	PHC, Dankaur	9 th April 2015	47	CBE, PAP, VIA
7	BSNL office, Delhi	30 th July 2015	153	CBE, PAP
8	IITF, Pragati Maidan Delhi	14-27 Nov 2015	446	PAP
9	Bhawani Shankar College, sector 46 NOIDA	11 th Dec 2015	52	CBE PAP
10	Aurbindo Bhawan Sector 62, NOIDA	5 th Feb 2016	42	Oral examination, CBE, PAP
Total			1000	

C. Digital Magnivisualizer development

After initial encouraging results and acceptance by the government of India as economical device that can be used for early detection of cancer and hence prevention, NICPR scientist have initiated the development of Magnivisualizer with attached digital photography system. It has been planned to make it possible to document and transmit the pictures through available mobile network to respective doctors like gynecologist, dentists or the pathologist for instant diagnosis or advice for future course of action.

Following Patents were applied for:-

- MAGNIVISUALIZER (Trademark Registration No. 1274543).
- MAGNIVISUALIZER (Design Application no. 236817)
- Magnivisualizer (Patent Application No. 1216/DEL/2010)
- Digital Magnivisualizer (Design application No. 283268, dated 9th May 2016)
- Digital Magnivisualizer (Provisional Patent

Application No.201611014610, dated 27th April 2016).

Technology transfer of AV Magnivisualizer to Industry. The technical dossier with instrument AV Magnivisualizer has been given to M/s Sooth Health Care Ltd, Greater NOIDA. They are looking forward for its production and marketing.



Fig. 6. Health Promotion Clinic.

NATIONAL CENTRE FOR DISEASE INFORMATICS AND RESEARCH, BENGALURU

Patterns of Care and Survival Studies - The primary purpose of hospital based cancer registries is assessing patient care. Clinical stage and treatment based survival is one of the key parameters for such assessment. Due to the challenges in obtaining follow-up parameters, a separate study on patterns of care and survival 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. The report for the year 2006-2008 is under progress. The main findings are:

- 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.

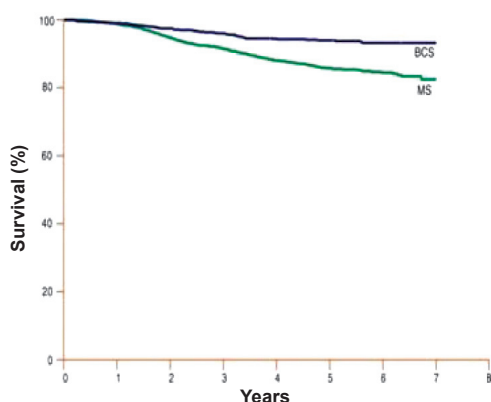


Fig. 7. Kaplan-Meier comparative survival curve for Cancer Breast patients who received BCS and MS for Stage II.

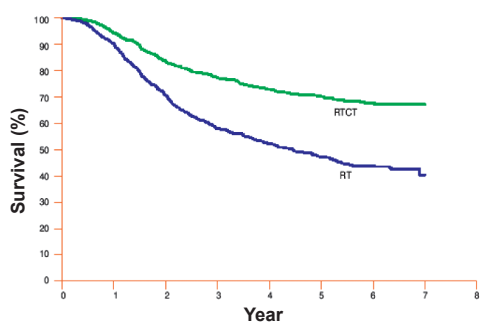


Fig. 8. Kaplan-Meier comparative survival graph for locally advanced Cancer Cervix patients who received Radiotherapy alone and those who received Radiotherapy and Chemotherapy.

- 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.
- The reasons for lower survival with Mastectomy (MS) and the biological or scientific rationale of the necessity of systemic therapy to achieve optimal survival in patients undergoing MS but not in those with BCS need further investigation.
- This study is a foremost example of cancer registration (through its national programme of cancer registries covering several cancer centres/medical institutions) evaluating clinical parameters and providing critical findings that could have an impact on patient

care. A network of cancer hospitals linked to a central coordinating centre (NCDIR) with a system to accrue good clinical data through modern electronic information technology is in place.

Karnataka State Cancer Notifiability –The Government of Karnataka has made the cancer as a notifiable disease in July 2015. Accordingly, all the government and private hospitals, medical colleges, pathology labs are registered to the NCRP for transmission of the cancer patients information. (51 centres registered from Karnataka and 6 centres from other than Karnataka)

Development of an Atlas of Cancer in Punjab State(PCA) – The purpose of the project is to have an idea of patterns of cancer in parts of the Punjab state not covered by the registries under the National Cancer Registry Programme (NCRP) of the Indian Council of Medical Research (ICMR). Wherever possible, it was also envisaged to calculate estimates of cancer incidence. Data had been collected for the project till the year 2015. Data analysis for the year 2014 and 2015 has to be done. Till date we have received 95961 cases from 60 registered collaborating centres.

Verification of Punjab Cancer Atlas (PCA) Cases -To verify the completeness of data obtained through PCA, a survey of almost 1,00,000 individuals (around 25000 families) in four districts Punjab, Muktsar, Batinda, Mansa, and Barnala have been done to estimate the magnitude of other three NCDs, i.e., diabetes, CVD, and stroke. The software application has been developed to capture the data in a systematic format. The analysis of the data is being done.

Development of an Atlas of Cancer in Haryana State(HCA) - The project on HCA was commenced with the objective of obtaining an overview of Cancer Patterns in Haryana state. This would also give important leads in undertaking aetiological research and would target cancer control measures. An inaugural workshop was conducted in Panchkula, Haryana to sensitize the collaborating centres about the project. Online access has been given to collaborating centres for data transmission to NCDIR for the project along with demonstration to participants regarding data entry. Access has

been given to chief coordinator to E-Monitor data capture by all the centres registered for this project.

E-MoR (Electronic Mortality Record) to register the actual cause of death, generate Death Notification Report, Medical Certification of Cause of Death(MCCD) and Death Report(Form 2) in Microsoft .Net platform and MS SQL Server is under development and has been pilot tested by Vydehi Institute of Medical Sciences, Bangalore. The same is being attempted in Open Source (Java) and My SQL.

The Three-year Report of Population Based Cancer Registries for the years 2012-14 has been printed. (This consolidated PBCR report for 2012 -2014 is based on the analysis of data from 27 PBCRs including three new ones (Naharlagun and Pasighat from Arunachal Pradesh and Patiala from Punjab). Reports of two other PBCRs – Hyderabad and Ahmedabad Rural have not been included here although some description of their work has been mentioned. The coverage is less than 10% of the population of India compared. Though this coverage appears very less in comparison to large population of India, even then it reflects the cancer profile of the country fairly well owing to representation of registries from different parts of the country.)

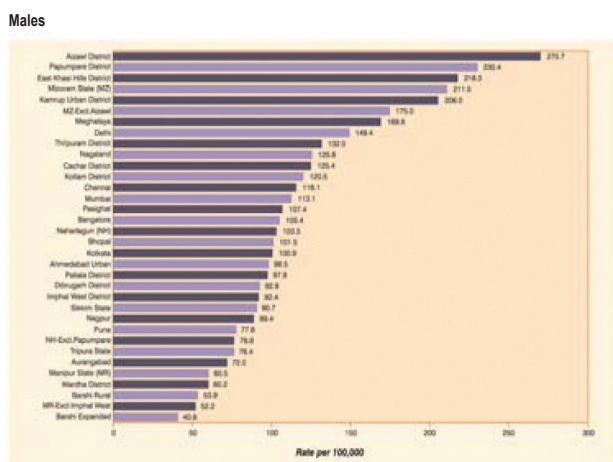


Fig. 9. Comparison of Age Adjusted Incidence Rates (AARs) of All PBCRs. All SITES (ICD-10: C00-C97).

Trends over time

The Consolidated Report of Hospital Based Cancer Registries for the years 2012-14 has been printed. The three year (2012-2014) report includes data from (i) Eight earlier HBCRs(i.e., in centres already in NCRP network) (ii) Nine newer HBCRs that have contributed data without financial assistance using HBCR-DM software)

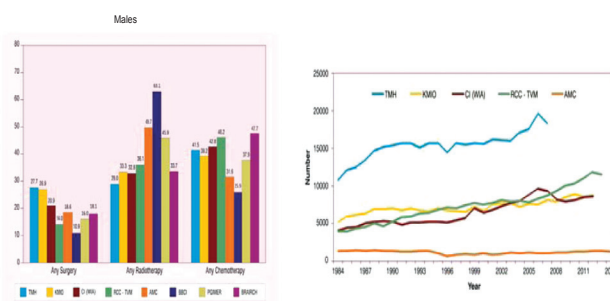


Fig. 10. Proportion of Type of Treatment (Patients Treated Only at Reporting Institution).

Network of National Centre for Disease Informatics and Research, Bengaluru

The following table provides numbers of registered centres.

COLLABORATING CENTRES

Table 2: Callaborating centres.		
Sl. No.	Network	Centres Registered
1	Population Based Cancer Registries	29
2	Hospital Based Cancer Registries (HBCR) including RCCs	29
3	Patterns of Care and Survival Studies (inclu. 5 HBCRs)	38
4	Cancer atlas NE	17
5	HBCR-Data Management Software	103
6	Punjab Cancer Atlas	154
7	Haryana Cancer Atlas	98
8	Pathology Software Module	137
9	Radiotherapy Software Module	17
Total		622

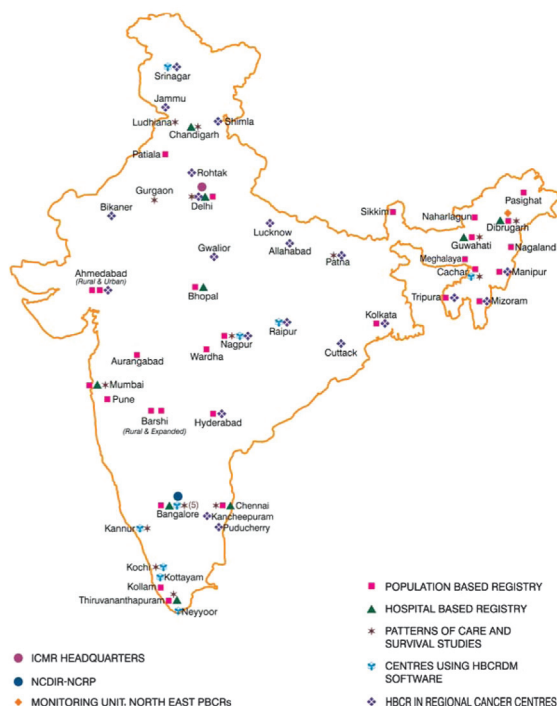


Fig. 11. National centre for disease informatics and research. National Cancer Registry Programme (Indian Council of Medical Research).

Extramural Research

ONCOLOGY

Review of Cancer Management Guidelines

The Task Force on Management of Cancers has been constituted to plan various research projects. Twenty three sub-committees were constituted to review the literature related to guidelines for management of various sites of cancers. The selected cancer sites are that of lung, breast, oesophagus, cervix, uterus, stomach, gall bladder, soft tissue sarcoma and osteo-sarcoma, tongue, acute myeloid leukaemia, ALL, CLL, NHL-high grade, NHL-low grade, HD, MM, MDS, and paediatric lymphoma. The consensus document for management of Non Hodgkin’s Lymphoma, Soft Tissue Sarcoma and Breast cancer are published during this period. A total of nine guidelines are printed and also available at ICMR website. The summary is also published in Indian Journal of Medical and Pediatric Oncology. These guidelines are expected to provide guidance to practicing clinicians and practitioners.

Molecular Basis of Genesis of Breast Cancer

Comparative Study of Genetic, Clinical and Epidemiological Factors of Breast Cancer in Rural and Urban Area of India. The preliminary findings show the role of XRCC genes, tobacco and alcohol usage and family history as risk factors. Around 85% of cases are with triple negative breast cancers in younger age group are identified.

Dissemination and Media Event of Cancer Registry Data (2012-2014)

The National Cancer Registry Programme has generated reliable data on magnitude and patterns of cancer from different regions of the country. The systematic collection of cancer data as per international norms is being done since 1982. 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 18th May 2016. The periodic publication of these reports has achieved the objective of dissemination of evidence based information on cancer in India. Most of the scientific data in the area of cancer in India has emerged using National Cancer Registry Programme data. Besides being a source of authentic information for the national programme, the reports have been cited by researchers /clinicians/Public Health Bodies/administrators on several occasions. 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). The event was well attended by cancer experts, investigators of cancer registries, members of Scientific Advisory Committee of NCDIR, scientists, representatives from Ministry of Health and Family Welfare, Directorate General Health Services, World Health Organization, Delhi State Cancer Institute and media persons. The highest age adjusted incidence rate (AAR) (271/100,000) of cancer of all anatomical sites among males is seen in Aizawl district in Mizoram State and among females in Papumpare district (AAR=249.0) of Arunachal Pradesh state. Among males; cancer of the mouth is the leading site in registry areas of western states of the country (Barshi Rural/expanded, Ahmadabad Urban, Nagpur, Pune and Wardha). Cancer of mouth is also the leading site in the registry of Bhopal, and the second leading

site in Mumbai and Aurangabad. Lung cancer is the leading site of cancer in 10 of 27 PBCRs and the second or third leading site in another 9 PBCRs. Among females, cancer of the breast is the leading site in 19 of 27 PBCRs and the second or third leading site in the remaining 8 PBCRs. Cancer of the cervix is the leading site of cancer in the rural areas in Barshi district of Maharashtra state, and in the states of Mizoram, Tripura and Nagaland. It is the second leading site in 16 PBCRs. Among females, East Khasi Hills in Meghalya state had the highest AAR (9.1) for cancer of the mouth.



Fig. 12. National centre for disease informatics and research. National Cancer Registry Programme (Indian Council of Medical Research).

Cancer projections are important and useful to plan and prioritize health care services (both diagnostic and treatment) in India. The estimated total burden (for the country as a whole) of cancer for the year 2016 is around 14.5 lakh new cases during 2016 and 17.3 lakh new cases in 2020. Cancer of the breast with estimated 1.5 lakh (over 10% of all cancers; 1.45 in females and 0.05 in males) new cases during 2016, is the number one cancer overall. Cancer of the lung is next with estimated 1.14 lakh (83,000 in males and 31,000 in females) new cases during 2016 and 1.4 lakhs in 2020. Cancer of the cervix is

the third most common cancer with estimated 1.0 lakh new cases in 2016 and about 1.04 lakhs during 2020. Cancers associated with use of tobacco account for about 30% of all cancers in males and females.



Fig. 13. Release of Cancer Registry Database.

For all sites of cancer, 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.

Indo Foreign Projects on Research in Oncology

Indo-German Collaboration

Nano carriers for intracutaneous targeting in management of skin cancer and skin diseases, under Dr. Nagarsenker, Bombay College of Pharmacy, Mumbai. The project was aimed at synthesis of a novel cationic lipid, designing and characterization of cationic and non-ionic charged nano carriers system namely liposomes, lecplex and invasomes. Drug delivery to the layers of the skin is the most sought after and successful approach in skin conditions like skin cancer, acne, psoriasis or keratinisation. Conventional dosage forms like ointments and gels, although easier to formulate, cannot release the drug in a controlled

manner and in most of the cases leads to non-desirable systemic side effects. The objective of this study is to design phospholipid based nanocarriers for targeting the skin layers. The nanocarriers were characterized for interaction of drugs with nanocarrier matrix followed by electron microscopy studies. Liposome and Invasome were formulated containing anticancer and antiacne agents. Formulations designed in this study hold promise in intracutaneous delivery of active agents which could be useful in management of conditions like skin cancer, acne, psoriasis or keratinization. The systems investigated are scalable and there is good scope for its translation into medically feasible outcome.

Indo-INSERM Collaboration on Research in Oncology

Effect of Cellular and Immune Response in Mice and patients with Acute Promyelocytic leukemia treated with Arsenic Trioxide, CMC Vellore. The project was aimed to study the antibody response induced by all trans retinoic acid (ATRA) and arsenic trioxide (ATO) in mouse model of acute promyelocytic leukemia as well as their effect on immunosuppressive cells and describe the immune suppression status of APL patients in India and France using induction therapy. Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) which has an excellent prognosis. Single agent arsenic trioxide (ATO) based therapy is cost effective and efficacious as reported previously (JCO 2011). However, in high risk group of patient the risk of relapse is high and efforts to improve the outcome in this group are ongoing. In a mouse model of leukemia the synergistic effect between all-trans retinoic acid (ATRA) and a DNA plasmid vaccine was demonstrated. The absence of synergy was demonstrated with this vaccine when ATO was used as a single agent. This data has clinical implications on how one would plan protocols to make use of this DNA plasmid vaccine. A delayed recovery of NK cells was noted in patients treated with ATO along with *in-vitro* up-regulation of NK cell receptors and ligands on malignant promyelocytes in a direction that enhances NK cell mediated cytolytic activity against malignant promyelocytes. These observations also have significant clinical implications and potential for translation. Through

this project, successful transfer of the mouse model of leukemia and the use of DNA plasmid vaccine to laboratory and considerably increased ability to further pursue research in leukemia has been demonstrated.

Indo-European Union Collaboration on Cancer

Following a collaborative workshop on cancer and neurodegenerative diseases; the Indian Council of Medical Research (ICMR), Department of Health Research (DHR), Ministry of Health and Family Welfare, New Delhi and the European Commission's Directorate General for Research and Innovation (DG RTD), Brussels have mutually agreed to enhance opportunities for coordinated activities in health research between European and Indian scientists. The Letter of Intent was signed and a call for proposals was placed on ICMR website and proposal were shortlisted so as to twin with consortium identified by EU side. Two proposals are initiated: (i) Role of HPV infection and other co-factors in etiology of Head and Neck Cancers in India & Europe and (ii) Role of HPV in Head and Neck Cancer in Rural and Urban India.

Memorandum of Understanding (MoU) on Cooperation in Cancer Research, Prevention, Control and Management

The MoU was signed between ICMR/DBT/NCI-India and NCI-US in June 2015 with the aim to establish general framework of intended collaboration for promoting and conducting high quality research to strengthen evidence base necessary for cancer prevention. The activities of mutual interest under this MoU have been initiated viz: ICMR-NCI Workshop on Presentation Skills at ICMR Headquarters and ICPO, Noida; ICMR-NCI Workshop on Media Training at ICPO, Noida.

DIABETES

ICMR-Indian National Diabetes Study (ICMR-INDIAB)-North East. The study was aimed to estimate prevalence of pre diabetes and diabetes among rural and urban population in north eastern region of the country. A sample size of 32,000 individuals covering the North-East regions representing eight states in India namely Sikkim, Assam, Meghalaya, Tripura, Mizoram, Manipur, Nagaland and Arunachal Pradesh was proposed.

Six states have completed their survey. The overall weighted prevalence of **diabetes** was 5.4% in Assam, 5.7% in Mizoram, 5.2% in Arunachal Pradesh, 8.6% in Tripura, 4.4% Meghalaya and 5.1% in Manipur. The overall weighted prevalence of **pre-diabetes** was 11.8% in Assam, 5.8% in Mizoram, 12.8% in Arunachal Pradesh, 4.6% in Tripura, 10% in Meghalaya and 7.5% in Manipur. In all the six states, it was observed that the prevalence of diabetes was higher in urban compared to rural areas. The prevalence of **hypertension** in the urban and rural areas was (Assam: 38.3% vs. 30%, Mizoram: 31.1% vs. 23.5%, Arunachal Pradesh: 30.1% vs. 30.3%, Tripura: 36.1% vs. 26.7%, Meghalaya 27.3% vs 20% and Manipur 36.3% vs 28.5%). Similarly, the prevalence of **dyslipidemia** in the urban and rural areas of the four states was (Assam: 89.9% vs. 87.9%, Mizoram: 79.3% vs. 78.9%, Arunachal Pradesh: 80% vs. 79%, Tripura: 86.2% vs. 78.7%; Meghalaya 86.25% vs 85.7% and Manipur 79.1% vs 79.7%). The prevalence of hypertension was higher in urban compared to rural areas, whereas, dyslipidemia was similar in urban and rural areas of all the four states. The prevalence of **coronary artery disease** is higher among diabetic subjects compared to subjects without diabetes in Assam (Diabetes vs. No Diabetes - 8.6% vs. 1.2%), Mizoram (Diabetes vs. No Diabetes - 5.9% vs. 2.6%), Arunachal Pradesh (Diabetes vs. No Diabetes - 1.8% vs. 0.2%), Tripura (Diabetes vs. No Diabetes - 3.5% vs. 1.1%), Meghalaya (Diabetes vs No Diabetes - 2.3% vs. 0.6%) and Manipur (Diabetes vs. No Diabetes-2.9% vs 0.4%) respectively. In terms of **glycemic control** in the rural areas, 25.6% of self reported diabetic subjects in Assam and 23.0% in Mizoram had poor glycemic control compared to 17% and 18.9% of the subjects in urban areas of Assam and Mizoram respectively. Similarly, 36% of self reported diabetic subjects in rural Arunachal Pradesh had poor glycemic control compared to 33.3% in urban areas whereas 15.5% of self reported diabetic subjects in rural Tripura had poor glycemic control compared to 21.6% in urban areas.

ICMR-Indian National Diabetes Study (ICMR-INDIAB)-Phase II

After completing the survey in four states during phase I; the rest of states were proposed to undertake

survey in phase II with funding from Department of Health Research. The methodology adopted was the same as in phase I. The ICMR-INDIAB study (Phase II) reports on the results obtained from five states; Andhra Pradesh, Bihar, Gujarat, Karnataka and Punjab; the states which got funding from DHR. A stratified multi-stage sampling design was used to survey individuals aged ≥ 20 years with the primary objective to determine the prevalence of diabetes and pre-diabetes in India. Of the 20,072 individuals selected for the study; 19,301 [96.2%] individuals participated. The weighted prevalence of diabetes (both known and newly diagnosed) was Andhra Pradesh, 8%, Bihar, 4.3%, Gujarat, 7.1%, Karnataka, 7.5% and Punjab, 9.8%. The prevalence of pre-diabetes was 10%, 9.9%, 10.7%, 11.6% and 8.1%, respectively. The prevalence of hypertension was higher in urban compared to rural areas in all five states except Gujarat. The prevalence of dyslipidemia and metabolic syndrome were higher in urban compared to rural areas in all five states and the highest in Punjab compared to other states. In terms of glycemic control, urban Andhra Pradesh and rural Gujarat had the highest proportion of subjects with good glycemic control. The proportion of subjects who reported that they knew about a condition called diabetes ranged from 17.8% in rural Andhra Pradesh to 81.9% in urban Karnataka. This study throws light on the health burden due to diabetes in India and will help plan measures for both control and prevention of diabetes in the states where the study is completed. Some of findings were presented in World Diabetes Congress 2015 (organized by International Diabetes Federation) in Vancouver, Canada.

Dissemination Workshop and Media Event on ICMR-INDIAB Results

The dissemination of results of 15 states completed under ICMR-INDIAB was held on 15th March 2016 at ICMR Headquarters, New Delhi. There was participation of endocrinologists, investigators, members of expert group, representatives from WHO, the Union, Ministry of Health and Family Welfare, Directorate General Health Services and other scientific agencies and media persons. The guests of honor were Dr. Jagdish Prasad, Director General Health Services, Dr NK Ganguly, former Director General Indian Council of Medical

Research and Dr. LM Nath, chairperson of Expert Group. The Secretary, Department of Health Research and Director General, ICMR handed over state specific data to respective state health secretaries/ investigator. After the 1971-72 ICMR study of diabetes, this is the first time that ICMR has undertaken such an extensive study on diabetes. The ICMR-INDIAB study is a landmark study as this is the first representative study providing authentic epidemiological data on diabetes, prediabetes, hypertension, dyslipidemia and obesity from the various states of India. Reliable population based data on the burden of NCDs such as diabetes is necessary to plan preventive and curative health services in the country. In this context, the ICMR-INDIAB study gains significance as it is collecting representative data from the various States and Union Territories in India. 15 states have now been completed and the data has been compiled as a report and shared with national as well as state health departments. This data provides a snap shot of the existing burden of disease in the country. The data can also be used to plan preventive interventions to prevent/delay progression from pre-diabetes to diabetes. This mainly involves changes in lifestyle, regular exercise and reduced consumption of sugar and salt.

Release of ICMR-INDIAB Report

On occasion of World Health Day (6th April); Hon'ble Health Minister, Sh JP Nadda released the ICMR-INDIAB Survey Report (phase I) in presence of Secretary, DHR and DG, ICMR; Dr S Swaminathan; Director General Health Services, Dr Jagdish Prasad; Secretary, Ayush (Addl Charge) and Minister of State, Sh Shripad Yesso Naik; Minister of State for Science and Technology, Dr Jitender Singh; Secretary, Ministry of Health and Family Welfare, Sh BP Sharma; Secretary, Ministry of Health and Family Welfare; WHO Country Representative Dr. Henk Bekedam; Additional Secretary, MoHFW, Sh Arun Kumar Panda and Sh CK Mishra, Additional Secretary, MoHFW.

CARDIOVASCULAR DISEASES

Management for Acute Coronary Events (MACE) Registry: A Web Based National Network of Registry for Patients Hospitalized with Acute Cardiovascular Events

To improve the understanding of ACS patient characteristics and to incorporate evidence based medicine in their treatment and to gather information on long term outcomes in these patients, up to date registry data on ACS cases is required in India. A pilot study was initiated in 11 centres in the study. As the patient reaches different levels of care pathway during the acute episode and these affect the final outcomes, it will be important to understand the management of these patients at secondary level. In view of this, the study has now been scaled to 39 centres. Each of the 11 participating centre in Phase II study has recruited sub-centres with and without Cath lab/ PCI facility. The study will help to answer important clinical questions and reasons for delay in symptom onset to needle/balloon time and its effect on patient outcomes. Any gender/ socioeconomic bias in patient management will be looked for.

Development of a health promotion model for prevention of non- communicable diseases in school settings

The Chandigarh Centre carried out a 20-week lifestyle intervention study as a cluster randomized trial where four schools (two public and two private) were randomly selected and allocated to intervention and control arms. A school-based lifestyle intervention package favorably affected anthropometric (weight, waist circumference and triceps and biceps thickness) and behavioral parameters. With the available evidence from this study, incorporation of at least 20 weeks (~5 months) of health promoting intervention period in each academic year on a long-term basis is recommended so as to bring about a desirable change in behavioral and anthropometric or biochemical parameters of school children and thereby have significant impact on health and well-being of population in future. ICMR has sent a policy recommendation to Ministry of Human Resource Development to introduce School based lifestyle interventions in school curriculum so that lifestyle intervention becomes part and parcel of schools.

Designing a biobank for biological samples collected from a mega study on acute coronary events in Indians. A project under ICMR INSERM

(Indo French) collaboration was completed. A protocol for undertaking a pilot study for a MACE registry based Biobank was designed with French investigator's support. A 'Manual of Operations' was developed for the biobanking facility and validated in 3 clinical centres and a laboratory at ICPO. The study suggested that developing a biobanking facility for acute coronary event cases is feasible in India.

NEUROLOGY

Population Based Rural Stroke Registry at Bangalore

The rural population based stroke registry at Bangalore was conducted in population of 2,93,393 residing in Chinthamani Taluk, Chikkaballapur District, Karnataka. The incidence of first ever stroke was 61.01 per lakh population. Around 16.2% stroke patients were below 45 years of age. Case fatality rate was: 27.37%. The study suggested that service providers (Anganwadi workers, ASHA, Health workers) with adequate training are able to identify and report cases of stroke in the community and it is a feasible option to develop stroke registries in the rural communities.

Population Based Rural Stroke Registry at Ludhiana

The 'Rural population based stroke registry at Ludhiana' includes all villages in Pakhowal block and Sidhwan Bet block. The rural registry will utilize the presence of Accredited Social Health Activists of the NRHM in the villages to obtain information regarding cases of stroke in the villages (ASHAs). The Phase I of the project was funded by DHR for one year but was reviewed by ICMR Task Force group. Health education material including pamphlets, a flex board with FAST translating into Punjabi as Lakwa- bul kar and awaj was developed. The stroke incidence was 177/100,000 population for age above 18 years. The Task Force has approved the phase 2 of the project for 18 months which will help to find the feasibility of integration of stroke registry into NRHM.

Development and validation of a comprehensive clinical and neuropsychological test battery for use in the Indian context for patients with Vascular

Cognitive Impairment

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. Test selection, finalization, acquiring test permissions to adapt and/or use in Indian context was done with an aim to have a test battery that is free of cost and available in the public domain. Pilot data was collected across the 5 centers to see if the test battery and CRF needed any further changes or modifications before commencing with data collection. A validation study was then undertaken in 1200 subjects to test the above neuropsychological battery. Cognitive tests were validated for dementia and stroke. An online electronic system for data collection has now been developed along with CDAC.

Evaluation of development of neurosurgery skills by hands on skills training and interactive virtual modules (web based, tele-education and real time simulation)

Hands on training modules on neuro-anatomy, neuro-endoscopy, high speed drilling, microsuturing, spine and spine instrumentation were developed and have been incorporated in NSI (India) and CNS (USA) joint Simulation Workshops. A Learning Management System and Content Management System based e-learning platform has been set up at IIT Delhi and 15TB repository has already been setup at AIIMS and IITD for the archival of the 3D modules. A software based real-time evaluation and assessment system to grade the trainee/ trained neurosurgeons based on their surgical skills has been developed and evaluated. A neuro endoscopic trainer was developed in this project and 2 patents were submitted. Eight publications have come out from this study.

Centre for Advanced Research for Innovations in Mental Health & Neurosciences: Manpower Development and Translation Research at NIMHANS, Bangalore

This centre was initiated in July 2014 and has 3 ongoing translation based projects and two PDFs per year in the area of 'Neurocritical Care'.

Under the project "Development of Neuroscience Educational Material for popularizing Neuroscience

under Human Brain Bank” a ‘Histological Atlas of the Common Infection of CNS’, along with set of 48 histological slides depicting the pathological features and CD containing the text & photographs in the Atlas has been prepared.

Under the project “Translation research in ALS-Development of biomarkers for diagnosis, monitoring disease progression and evaluation of toxicity”, an increase in CHIT-1 levels and its high biological activity in ALS-CSF at < 6 months of disease suggests possible use of this protein as an early diagnostic marker.

Under the project, “Development of ELISA and evaluation of potential of 14-3-3 protein as a biomarker of neuronal injury/ neuro-degeneration with special reference to Creutzfeldt Jakob disease”, an ELISA test or diagnosis of CJD has been developed and validated. Four PDFs in Neurocritical Care have been trained under this Centre.

MENTAL HEALTH

Suicidal Behaviour

The task force project on “Suicide Behaviour” was carried out at Delhi and Vellore with the aim to find out the prevalence of suicidal ideation and suicidal attempt in the community. The study has been carried out in the purposively selected two communities at each centre. Delhi: Prevalence of attempters was 0.36% which is somewhat below the range (0.4 -4.2%) reported in internationally published studies. Incidence of suicidal ideation in two field areas on analytic component over two year follow up was 2.09%. Female gender, unemployment, Economic stress, poor educational level along with high levels of psychosocial stress, hopelessness, relationship difficulties with spouse were the risk factors for suicidal ideation. The protective factor of suicidal ideation was education above secondary school level. The risk factors for suicidal attempt were found to be problems in relationship with the spouse, domestic violence against women, high levels of psycho social stress, stressful life

events, suicidal ideation and poor integration with family.

Table 3: Prevalence of Suicidal Ideation

Prevalence of Suicidal Ideation	Vellore (%age)	Delhi (%age)
Lifetime prevalence	11.6	11.1
6-month prevalence	3.2	3.36
1-month prevalence	1.1	1.1
Prevalence of attempters	0.36	0.36

Table 4: Pattern of Psychiatric diagnosis among suicide ideators

Psychiatric diagnosis- (urban)	Prevalence (%)
Mixed Anxiety Depression	5.4
Depression	2.4
Obsessive Compulsive Disorder	1.8
Generalized Anxiety Disorder	0.9
Phobia	0.6
Panic disorder	0.5
Any Psychiatric diagnosis	9.8

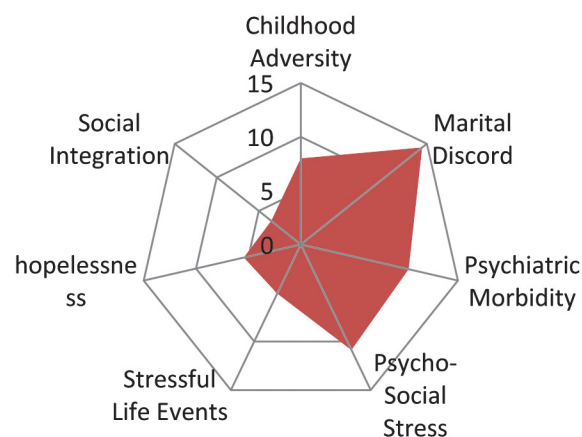


Fig. 14. The Risk factors for recent suicidal ideation which emerged after multivariate adjustment.

The study also revealed causes of death as per Verbal autopsies of 36 deaths of which death by suicide were: 3 due to failure in exam; poisoning associated with alcohol dependence; poisoning and depression due financial problems; drug overdose. The study also revealed correlates of new suicidal ideation (n=87) being marital discord, domestic violence in women, alcohol use in men, lower socioeconomic status. The study also provided insight into the correlates of suicidal attempts.

OTORHINOLARYNGOLOGY

ICMR's task force project on prevalence and etiology of hearing impairment

On the recommendation of the high powered committee meeting and after clearance from DHR, the National Task Force Project on Prevalence and Etiology of Hearing Impairment was initiated in January 2015 across six regions of the country viz. north, south, east, west, central and north-east with sample size of 14,000 at each center from urban and rural areas. The protocol, questionnaire and manual were developed. Technical committee meeting was organized for procurement of audiological equipment and sound proof van by Bangalore centre. Following this, coordinating centre developed digitalized questionnaire. After the initiation of project at 4 centres, coordinating centre at AIIMS and coordinating unit at ICMR were established. Two centres central and east were changed to AIIMS, Raipur and AIIMS Bhubaneswar. Project at these centres was initiated on 15th November and 1st December 2015. Further, a meeting of the investigators and consultants of this task force project was held in May 2015, another expert group meeting in July 2015 and a training workshop was organized for research staff along with investigators (PI and co-PI) in January 2016 at Bangalore. Sampling plan and area were identified based on census information 2011. Pilot testing was done by each centre. Translation of questionnaire was done in respective local language which was used in pilot testing in hospital setting and in the field. All the centres have been provided with the audiological equipment and sound proof van. Main study has been started at all centres.

ICMR's task force project on Congenital Deafness in Dadhkai village of Doda district of Jammu & Kashmir

This study is a research cum intervention project which aims at assessment of hearing impairment in Dadhkai village of Doda district of J & K which is inhabited by large number of deaf persons and genes responsible for such deafness. This project has emanated after lengthy deliberations among Medical experts, Public health experts and Geneticists. While the initial phase has helped the affected people in terms of diagnosis and getting

due attention for medical and rehabilitation support, subsequent analysis of genetic basis will help in developing and implementing counseling to prevent transmission of relevant genes so that the future generations could be saved. ICMR has sanctioned the above mentioned project at two centres at Dr. R.P. Government Medical College, Kangra, Himachal Pradesh which will be doing estimation of disease burden and its management and JNCASR Bangalore, Karnataka will do detailed genetic studies and genetic counseling and overall coordination centre is at the division of NCD, ICMR, Hqrs in Dec 2013. The study was conducted on all individuals residing in Dadhkai village of district Doda. The first stage of the study (screening phase) was entirely devoted to carry out a baseline survey on the prevalence of hearing impairment in village Dadhkai. For this, a house to house survey mapping of the entire Dadhkai village was carried out. The screening phase was conducted by two field investigators and an audiologist as per structured questionnaire. All individuals residing in the village under went audiometry for assessment of hearing impairment at the audiometry unit specifically established for the same. A clinical team comprising of ENT specialist and public health specialists confirmed that all patients included in the study were diagnosed as suffering from hearing impairment. They have been able to identify 80 hearing impaired out of a total population of 2522. All hearing impaired have been provided with hearing aids. The sustenance of rehabilitation was ensured by encouraging continuous usage of hearing aids and all patients are being continuously monitored for improvement. Further, a pedigree chart of entire village has been constructed and family groups with relatively higher number of hearing impairment have been identified. A Focussed Group Discussion among study participants on their knowledge about their problem was completed. 540 blood samples taken from the affected deaf cases and their relatives, have been sent to Molecular and Genetics unit of Jawaharlal Nehru centre for advanced scientific research (JNCASR), Bangalore for genetic testing.

National Burden of Non Communicable Diseases

A collaborative initiative on state-level disease burden estimation in India was launched between

Indian Council of Medical Research (ICMR), the Public Health Foundation of India (PHFI), and the Institute for Health Metrics and Evaluation (IHME) at the University of Washington under the aegis of Ministry of Health and Family Welfare, Government of India on October 12, 2015. In this effort, the robust methods of the Global Burden of Disease (GBD) study, developed by a global network of researchers coordinated by IHME, will be utilized to generate rigorous estimates of all the major drivers of health loss at the state level in India. In addition to the scientific papers, the initiative will produce multilevel disseminations through policy briefs, workshops, and seminars to raise the discourse and monitor changing disease trends. State-of-the-art GBD interactive visualization tools will be used to bring to life the initiative's findings. This will allow a variety of contrasts between states, which will be particularly useful for policymakers in understanding trends of disease and risk factors in order to plan further action. This initiative would work closely with a network of academic partners across India, and importantly with policymakers at the national and state levels, for guidance and utilization of the findings to improve health programs and planning.

ORAL HEALTH

Cleft Lip and Palate Anomaly in India: Clinical Profile, Risk Factors And Current lot & Pilot Phase

The study was essentially aimed

- i. To utilize the study tool *Indicleft* developed in the prepilot phase for evaluation of clinical profile of CLP anomaly.

- ii. To establish methodology for nationwide collection of data for clinical profile for Cleft lip and palate anomalies and its major treatment needs including the logistics, feasibility and difficulties expected in the execution of the multicentric project.

A total of 164 cases with cleft lip and palate anomaly were recorded from three hospitals involved in the project (55 from AIIMS, 54 from Safdarjung, 55 from Medanta-The Medicity). The Key observations of the study included:

- Wide variation in age at primary lip (range 2 to 180 months) and palatal surgery (3 to 228 months) were noted.
- A significant percentage of cases required lip and nose revision surgeries (36% and 35%, respectively)

UROLOGY-NEPHROLOGY

Multicentric Study To Find Out Prevalence Of Chronic Kidney Disease In Indian

ICMR sanctioned this study at seven centres Delhi, Mumbai, Jaipur, Hyderabad, Guwahati, Bhubaneswar, Bhopal. It has been initiated from September 2012. The Bhopal Centre during the course has been closed due to PI withdrawal from the study. The study has been able to collect data from 10,000 study participants. Preliminary results indicate prevalence ranging between 5-7%.

BASIC MEDICAL SCIENCES

Research in the field of basic medical sciences was carried out mainly in two permanent institutes of the ICMR namely National Institute of Pathology (NIOP), New Delhi and National Institute of Immunohaematology (NIIH), Mumbai, as also in various other centres. The areas covered included tumour biology, pathology of infectious diseases, environmental toxicology and haematological disorders under intramural research. Extramural research was done in the area of pharmacology, biochemistry, cellular and molecular biology, genomics, stem cell etc.

Intramural Research

NATIONAL INSTITUTE OF PATHOLOGY, NEW DELHI

At National Institute of Pathology, the thrust areas of research are tumor biology, infectious diseases including *leishmaniasis*, *tuberculosis*, *leprosy* and *chlamydiasis*, stem cell biology and environmental toxicology. The scientists conduct both basic as well as translational research leading to development of Vaccines for prevention and Biomarkers for screening, diagnosis, prognosis and prediction of drug response/resistance for various diseases with mission to bring lab to bed.

TUMOR BIOLOGY

Study on micro RNA Signatures associated with Breast Cancer Stem like Cells(CSCs) and their role in Drug Response

The project is aimed to identify miRNA and gene expression signatures associated with breast cancer stem cells to understand the molecular mechanisms involved and their contribution to response chemotherapeutic agents. In the year under report, in order to evaluate the contribution of SOX family genes to formation of breast cancer stem cells, we

have evaluated the expression of 8 SOX family genes in breast cancer stem cells derived T47D and MDA-MB-453 and compared them with their corresponding bulk cells. We found differential expression of *SOX1* and *SOX3* in cancer stem cells compared to bulk cells suggesting their possible involvement in formation of breast cancer stem cells. The expression of SOX family genes will be further extended to more number of cancer stem cells to confirm such involvement.

Targeted resequencing of breast cancer specific genes in early-onset breast carcinoma

This study is aimed to identify sequence variations and chromosomal rearrangements of deregulated genes in early onset breast cancers.

Whole exome sequencing has been done from 12 cases belonging to early and late onset tumors and analysis of data has been done to identify genetic variations associated with early onset and late onset tumors, using partek flow and partek genomics suite. We identified 2886 single nucleotide variations and 239 indels associated with early onset breast tumors, 5232 single nucleotide variations and 521 indels associated with late onset breast tumors. In addition, 1991 single nucleotide variations and 137 indels were found common in both early and late onset tumors. On analysis of chromosome wise distribution of the variants, highest number of variants were found in chromosome 1 followed by chromosome 2 and 6. We have analysed variants of early and late onset tumors to identify pathways that are disrupted in these tumors. Pathways involved in early onset tumors include cAMP, axon guidance, ECM receptor signaling TNF signaling etc., while in late onset tumors: regulation of endocytosis, regulation of keratins, Rap1 signaling pathways are uniquely disrupted. Further, we have analysed the whole exome data for gene fusions

in these samples. Three chromosomes, 1, 7 and 17 were having more than 30 gene fusions, rest of the chromosomes have shown 4-8 fusions. No gene fusions were found in chromosomes 13, 18, and 21. Further, whole exome sequencing in 22 breast tumors and 4 controls, including 9 early onset and 13 late onset tumors had also been carried out; analysis of genetic variants is under process.

Differential Protein Profile for Identification of Markers in Recurrent Urothelial Cancer

The present study was planned to identify differentially expressed tumor proteins in tumour for use as biomarkers of recurrence. The differential protein profile for identification of markers in recurrent urothelial cancer was evaluated by processing tumour and normal mucosal samples (n=16). The cases included low grade non-invasive, high grade non-invasive and high grade invasive tumours. Proteins were extracted from tumor and normal tissue and quantitated by BCA method. Proteins from tumour and normal tissue were labeled and subjected to liquid chromatography and mass spectrometry (MS/MS). Ratio of protein/peptide expression in tumor to paired normal was determined and fold change was calculated. A total of 3984 proteins were identified in tumour tissue, of which 1895 proteins (15381 peptides) were deregulated and included, 1137 downregulated and 758 upregulated proteins. Further data analysis showed 64 proteins common to all tumour samples with 100 proteins unique to low grade and 298 proteins unique to high grade invasive tumours. The gene ontology (GO) terms showed most of the proteins are involved in cell part (GO cellular component) catalytic activity (GO Biological process), and metabolic process (GO molecular function). Pathway analysis showed that the largest number of proteins were involved in integrin signalling pathway and the largest group of proteins belonged to the oxidoreductase class.

Genome-wide analysis of genetic alterations and gene expression profiles in hormone sensitive and hormone refractory prostate cancer

Study of genome wide analysis of genetic alterations in patients with prostate cancer of varying histological grades and aggressiveness was performed using array CGH based method. Array

CGH gives a much higher resolution of closely spaced aberrations and allows linking of ratio changes to genomic/genetic markers. CGH+SNP based study was carried out on biopsy samples of prostate cancer tissues using Agilent Sureprint CGH+SNP arrays. The data was analyzed using Agilent Cytogenomics software. The results showed marked DNA sequence copy number changes in 75% of primary prostate cancer tissue samples. 75% of the cases showed loss on chromosome 8 (p23.3) and chromosome 10 (q11.22). Loss was also observed on chromosome 6 on both p and q arms of chromosome 15 (q11.2). Large chromosomal loss was found on chromosome 13 (q11-q34). Gain was seen in chromosome 16 (p11.2) in 50% of the cases. Array CGH results indicated that losses of several chromosomal regions were common genetic changes in primary tumors, suggesting that deletional inactivation of putative tumor suppressor genes in these chromosomal sites is likely to underlie development of prostate cancer. These chromosome aberrations may have prognostic utility as markers of prostate cancer progression. Study is being continued for analyzing more number of cases.

Understanding the role of Chemokines in development of Glioblastoma Multiforme

Glioblastoma multiforme (GBM) is the most lethal neoplasm of Central Nervous System with 12-15 months survival. Chemokine signalling pathway is involved in myriad of biological processes and could directly influence the tumor growth by activating pathways related to cell survival and cell proliferation or indirectly by promoting angiogenesis. This study has been undertaken to identify important chemokine axis associated with GBM growth and development which could be used for therapeutic intervention. Chemokine genes involved in biological functions such as cell proliferation and cell cycle were selected. Those genes which were upregulated in GBM but not astrocytoma were sorted. These genes were *CXCR4*, *CCRL2*, *CCR5*, *PF4VI*, *CXCL6*, and *CXCL8 (IL8)*. For further validation at protein level, IL8 and its receptors were selected. A tissue microarray of 55 DA and 91 primary GBM tissues stained with Anti-Human CXCL8 was analysed. In DA only 30.90% (17/55) cases were positive for CXCL8

while 67.03% (61/91) positivity was observed in GBM. Difference in immune positivity for CXCL8 between GBM and DA was found statistically significant with $p < 0.001$. Expression of CXCL8 was primarily found in tumor astrocytes confirmed by co-expression study done with the help of immuno-fluorescence. However, no significant difference was observed between GBM and DA for its receptors CXCR1 and CXCR2. This project is ongoing and will continue further with validation on cell lines and targeting with antibodies and drugs *in-vitro*.

Expression of Gonadotropin releasing hormone receptor in Glioblastoma cell line-derived exosomes and its potential as circulatory biomarker

The present study aimed at investigating the expression of GnRH receptor, belonging to the rhodopsin-like Gprotein coupled receptor (GPCR) family in glioblastoma cell line, LN229 and cell line-derived exosomes to explore its potential as circulatory marker for post-treatment monitoring of GBM patients. GnRH receptor was observed to be expressed both at the gene and protein level in GBM cell line, LN229 and was also found to be expressed at protein level in LN229 cell line-derived exosomes. Interestingly, we observed significant enrichment of GnRH receptor protein in cell line-derived exosomes in comparison to cell lysate. Expression of GnRH receptor protein in cell line-derived exosomes opens up the opportunity to explore the potential of GnRH receptor as circulatory marker for post-treatment monitoring in GnRH receptor positive glioblastoma patients.

Molecular regulation of mTOR signaling in acute lymphoblastic leukemia (ALL)

Despite major improvements in understanding of the molecular genetics of ALL, the mechanisms that lead to the abnormal proliferation and survival of T and B lymphoblasts remain largely unknown. Therefore, treatment of leukemia still remains a challenge for clinicians. Major efforts have been made to develop new compounds targeting signalling pathways implicated in ALL cell proliferation and survival. One such pathway is represented by the mammalian target of rapamycin (mTOR). This study has been undertaken with the

objective of studying the expression of mTOR gene in acute lymphoblastic leukemia (ALL) samples using real time PCR to identify subset of patients having high expression of mTOR and its association with response to chemotherapy. Peripheral blood samples from 50 patients of acute lymphoblastic leukemia (ALL) admitted to the Departments of Haematology & Paediatrics, Safdarjung Hospital, New Delhi for induction chemotherapy, were collected during the current year. Expression of mTOR gene in response to induction chemotherapy was studied in 50 ALL samples and 20 Healthy control. Response to chemotherapy was determined at the end of completion of induction chemotherapy. Expression of mTOR was found to be significantly up regulated in non-responder patients of ALL as compared to responders. mTOR inhibitor in combination with conventional chemotherapeutic drugs used in ALL will be evaluated in leukaemic cell lines to study the effect on cell cycle, apoptosis and mTOR signaling. This study is expected to give insight into incorporation of mTOR inhibitors into the treatment regimen of ALL.

INFECTIOUS DISEASES

LEISHMANIASIS

Analysis of clinical efficacy of oral miltefosine in treatment of post kala azar dermal leishmaniasis (PKDL) in India

Recent studies have shown significant decline in the final cure rate after miltefosine treatment in visceral leishmaniasis. We evaluated the efficacy of miltefosine in the treatment of post kala-azar dermal leishmaniasis (PKDL) patients, recruited over a period of 5 years with 18 months of follow-up. In this study, 86 confirmed cases of PKDL were treated with two different dosage regimens of miltefosine (Regimen I- 50mg twice daily for 90 days and Regimen II- 50 mg thrice for 60 days) and the clinical outcome assessed monthly. Cure/relapse was ascertained by clinical and histopathological examination, and measuring parasite burden by quantitative real-time PCR. *In vitro* susceptibility of parasites towards miltefosine was estimated at both promastigote and amastigote stages. Seventy three of eighty six patients completed the treatment and achieved clinical cure. Approximately 4% (3/73) patients relapsed by the end of 12 months

follow-up while a total of 15% (11/73) relapsed by the end of 18 months. Relapse rate was significantly higher in regimen II (31%) compared to regimen I (10.5%) ($P < 0.005$). Parasite load at the pre-treatment stage was significantly higher ($P < 0.005$) in cases that relapsed compared to the cases that remained cured. *In vitro* susceptibility towards miltefosine of parasites isolated after relapse was significantly lower (>2 fold) in comparison with the pre-treatment isolates ($P < 0.005$). Relapse rate in PKDL following miltefosine treatment has increased substantially, indicating the need of introducing alternate drugs / combination therapy with miltefosine.

Studies on miltefosine resistance in visceral leishmaniasis

Increasing incidence of relapse in VL cases treated with miltefosine raised the concern for its immediate surveillance in the field to safeguard efficacy. We investigated the parasitic factors apparently involved in miltefosine unresponsiveness in natural population of *Leishmania donovani* using isolates from pretreatment group LdPreTX ($n=6$), relapse cases after miltefosine treatment (VL and PKDL) LdRelapse ($n=5$) and in experimental MIL resistant (LdM30, $n=2$) parasites. LdRelapse and LdM30 parasites exhibited significantly lower accumulation of miltefosine ($p < 0.05$) compared to LdPre-TX parasites. MIL induced ROS levels were significantly low ($p < 0.05$) in macrophages infected with LdM30 and LdRelapse parasites compared to LdPreTX parasites, also intracellular thiol content was significantly higher ($p < 0.05$) in LdRelapse and in LdM30 indicating better tolerance for oxidative

stress in unresponsive isolates. Transcriptome profiling revealed that several genes involved in antioxidant defense mechanism, metabolic process, transporters, cell component and cell motility are preferentially expressed in LdM30 and LdRelapse parasites than wild type *L. donovani* parasites. Several other genes mainly transporters like ABCF2, amino acid transporter, surface acylated putative protein, APH and mitochondrial precursor peptide, chaperon TCP20, clathrin coated assembly protein, C5 sterol desaturase, autophagy protein ATG10 were preferentially expressed in LdPreTX parasite compared to LdRelapse case and LdM30 parasites. The study provides the understanding of parasitic factors and pathways responsible for miltefosine unresponsiveness in VL and PKDL.

Mechanism of resistance towards paromomycin in *Leishmania donovani*

Paromomycin (PMM) is a new treatment option for VL control in India as a monotherapy and in combination therapy. Microarray was successfully exploited to analyze the genes showing modulated expression in PMM resistant parasites. We identified a total of 267 genes (approx. 2.9%) differentially modulated based on 2 fold cut off in PMM-R parasites. 174 genes were up-regulated and 93 genes were down-regulated in PMM-R isolates. The plot \log_2 transformed expression ratio of K133 PMMR (red line) compared to K133 WT (green line) as function of the chromosomal location of microarray probes is shown in Fig. 1. Up to 4 fold up- or down-regulation in drug resistant parasite was observed.

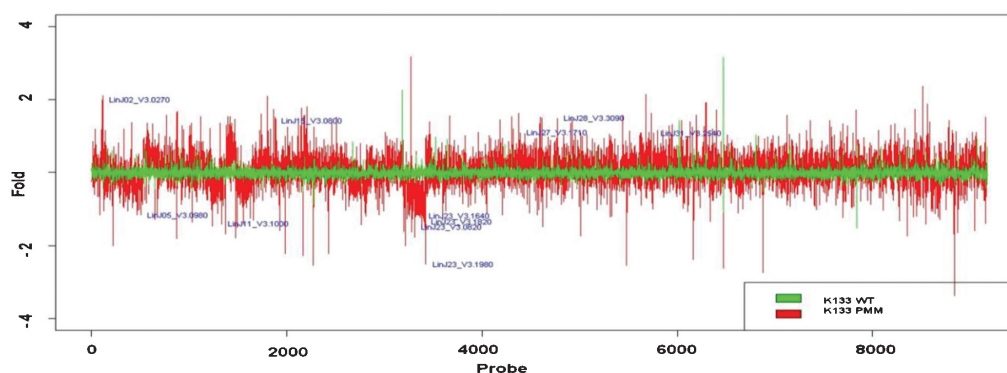


Fig. 1. Comparative transcriptional responses following PMM adaptation in *L. donovani*. Overlap of \log_2 transformed PMM-T and PMM-S expression ratio plotted as a function of chromosomal location of probes represented the full genome microarray. The plot represents the average values of three independent hybridizations for each isolates.

To analyse gene expression level on genomic scale, chromosome map was generated using Custom R program. Analysis of chromosome map identified that higher number of up regulated genes are located on chromosome 6, 12, 32, 35 and 36. There were no up regulated genes on chromosome 3, 13, 20 and 23. Maximum number of down regulated genes were located on chromosome 23 in PMM-R isolates.

TUBERCULOSIS

One of the important facets in deciphering pathogenesis of tuberculosis () is to understand the role of molecular three-dimensional structure of a protein and its function.

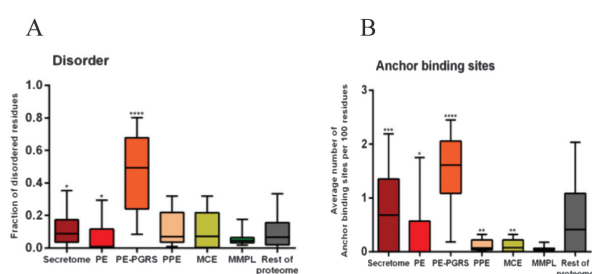


Fig. 2. (A). Boxplots shows Protein disorder content and average number of ANCHOR binding sites per 100 residues and **(B).** Secretome of PE, PE_PGRS3, PPE, Mce and MmpL families with rest of the proteome. Disorder content and ANCHOR Binding sites of each group is plotted on Y-axis and Families on X-axis.

It is now established that fragments of some proteins and sometimes the entire protein do not usually have a well-defined structure in solution, but such structures can be assumed only in a specific functional state. Such proteins are known as intrinsically disordered or unstructured. We performed the disordered protein analysis in the PE/PPE, Mce, MmPL and secretome of *M.tb* as well as prediction of protein binding sites and ELM search was also carried out. Experimental validation of *in-silico* analysis is important and it is being validated with one of the member of PE/PPE.

The virulence mechanism of mycobacteria is very complex. Broadly, the virulence factors can be classified as secretion factors, cell surface components, enzymes involved in cellular metabolism, and transcriptional regulators. The mycobacteria have evolved several mechanisms to secrete its proteins. RipA, possessing peptidoglycan

hydrolase activities is shown to be secreted by the TAT secretion pathway. Inhibition of this export system will prevent localization of peptidoglycan hydrolase and results in sensitivity to existing lactam antibiotics, opening up new candidates for drug repurposing. RipA is a secretory protein and has been shown to possess p60 domain that is capable of hydrolyzing dipeptide, D-glutamyl-meso-diaminopimelic acid. These properties of RipA protein such as virulence, invasion, secretion and cell-wall association make RipA an ideal candidate to evaluate the potential efficacy as a possible vaccine candidate. The physical interaction of RipA with MoxR1 protein, an AAA+ ATPase having chaperonic activity assists in proper folding of RipA in the cytoplasm prior to its secretion. Secreted RipA protein interacts with other proteins and gets cleaved to start its peptidoglycan hydrolase activity.

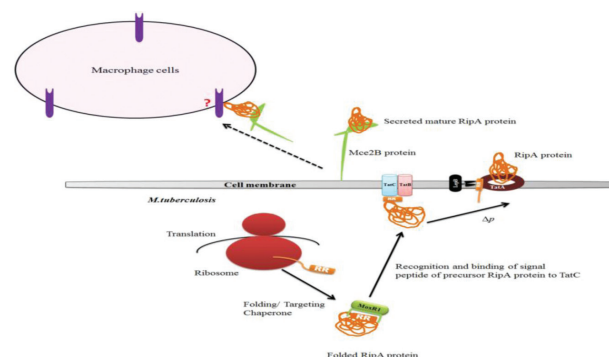


Fig. 3. Proposed Model for RipA secretion through TAT pathway.

M.tb has evolved mechanisms to survive in macrophages that represent one of the most stressful environments for bacteria. For successful colonization, *M.tb* forms a niche by establishing molecular interaction networks within the host system. *M.tb* has two cyclophilins PpiA and PpiB. It is a secretory protein and interacts with several host proteins such as those involved in iron regulation, immune defense mechanism and signal transduction

Peptidyl-prolyl cis-trans isomerases (Ppiases), are ubiquitously expressed enzymes that assist in protein folding by isomerization of peptide bonds preceding prolyl residues. We have described chaperone-like activity of mycobacterial Ppiases. We have also demonstrated its role in responding to host generated stresses like hypoxia and

oxidative stress by transiently expressing *M.tb* PpiA and PpiB in HEK293T cells. Presence of these proteins show increased survival as compared to control cells in response to oxidative stress and hypoxic conditions generated after treatment with H₂O₂ and CoCl₂. *M.tb* Ppiases play role in modulating host immune responses. Sera of TB patients showed high levels of antibody to *M.tb* Ppiases in the patient sera as compared to the sera of healthy humans. Treatment of THP-1 cells induced secretion of pro-inflammatory cytokines as a direct function of concentration of rPpiA. Alternatively, treatment with rPpiB inhibited secretion of TNF α and induced secretion of IL-10. Furthermore, heterologous expression of *M.tb* PpiA and PpiB in *Mycobacterium smegmatis* increased its survival in THP-1 cells as compared to vector control.

These results demonstrated that *M.tb* Ppiases are immunogenic proteins that can possibly modulate host immune response and enhance persistence of

the pathogen within the host by subverting host cell generated stresses.

CHLAMYDIASIS

Chlamydia trachomatis infects human host and tries to alter the host genomic environment in such a manner that favours its survival and successive proliferation. This is an important strategy employed by *C. trachomatis* to ensure its survival at genomic and proteomic level. It has been argued that Th1 and Th2 cytokines or blunting of initial cytokine response might also be important in the disease manifestation and its maintenance in *C. trachomatis*-induced Reactive Arthritis (ReA). Based on intra-articular and circulatory profile of key Th1/ Th2/ Th17 cytokines, viz.: IFN-gamma, IL-4, IL-6, IL-17, it was concluded that *C. trachomatis*-induced ReA patients have a Th-1 dominant profile. IFN-gamma levels were synergistically enhanced in both synovial fluid and serum in the infected group while IL-6 appeared to be the key player for this proinflammatory and

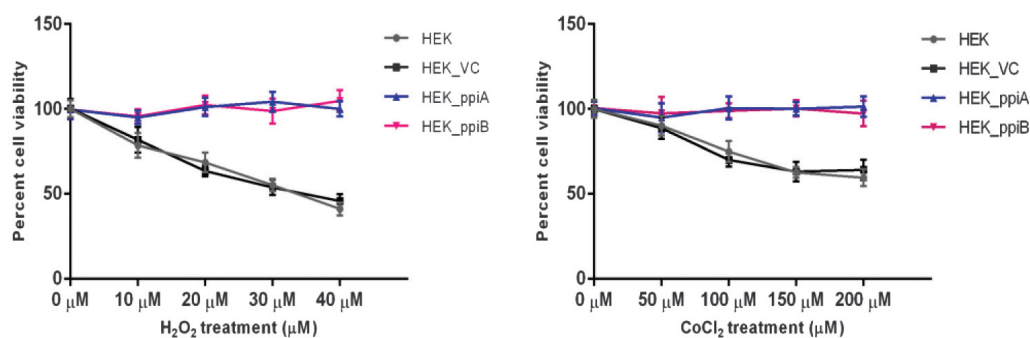


Fig. 4. Mycobacterial Ppiases impart resistance to the HEK293T cells against Oxidative and Hypoxic stress. HEK cells transiently expressing PpiA and PpiB could resist oxidative stress and hypoxia caused by H₂O₂ and CoCl₂ as compared to the wild type and vector control.

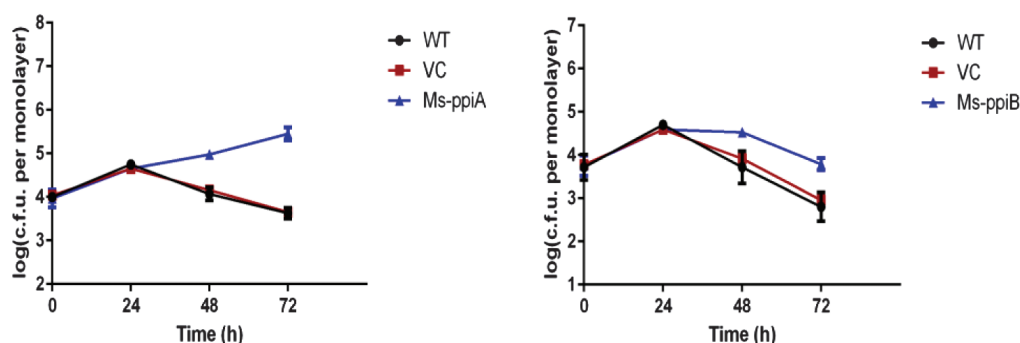


Fig. 5. *M.tb* Ppiases aid in intraphagosomal survival of the pathogen. *M. smegmatis* strain expressing PpiA and PpiB of *M.tb* exhibit increased survival in THP cell lines as compared to wild type *M. smegmatis* and vector control.

protective response. Upregulated level of IFN-gamma inhibit *C. trachomatis* multiplication and control the disease progression. Apparently, the role of IL-6 is important in regulation of Th1 /Th2 /Th17 cytokine pathway in *C. trachomatis*-induced ReA.

ADULT STEM CELL BIOLOGY

A new cell culture process for growing cultured epidermis for application in burns was earlier standardized at our laboratory. The technique involved culture of human epidermal keratinocytes in the presence of a specific sub-set of SWISS 3T3 feeders cells (Chugh et al 2015) which had 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 we found no detectable residues of mitomycin C in the final product. We have 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.

After obtaining the ethics committee approval, a small unused piece of autograft skin from a 26 years old male patient was obtained from the burns OT of Safdarjung Hospital, New Delhi, epidermal cells were isolated and plated over Swiss 3T3 feeder cells of 4-150 group prepared by our in-house technique. The cultures were subjected to serial passages and epidermal sheets were prepared by keratinocytes until six passages. The expansion logically proved that epidermal sheets to the tune of 60-100% coverage could be supplied in 22 to 28 days. The P5 cells were subjected to G-banding which showed no detectable Karyotype abnormalities in keratinocytes.

For preparing the injectable stocks of epidermal keratinocytes for 8 mice, the P4 cells in Matrigel were delivered to 8 nude mice in the range of 5 – 7 million per mouse by subcutaneous injections. A human embryonic kidney cell line, HEK293 at 90th passage, was similarly injected to 6 mice to serve as positive control. All six mice receiving HEK293 cells formed tumors at the site of injection at different post-injection time points. In the

keratinocyte group, two mice showed resolution of the nodule caused by primary injection in 49 days while in the other two mice it resolved after 59 and 91 days respectively. In the remaining four mice, the nodule showed initial rapid regression but a small and soft nodule remained unresolved. The dissection of the injection site showed only the residual Matrigel and histopathology of the nodule and the internal organs revealed no tumor cells.

FUTURE COURSE OF ACTION

Clinical trial with the cultured epidermis will be undertaken. Coordinated efforts are being made to simultaneously obtain grants and cGMP construction to facilitate such trial. Additionally, efforts will also be made to identify a suitable industry.

Human environmental biomonitoring of Polynuclear Aromatic Hydrocarbons (PAHs) in urban megalopolis of NCR Delhi and investigate the association between PAH exposure and Intrauterine Growth Restriction (IUGR)

Polyaromatic hydrocarbons are a ubiquitous group of environmental pollutants that have been shown to cause carcinogenic and mutagenic effects and are potent immuno-suppressants. Animal studies have shown that PAHs can cause harmful effects on the skin, body fluids, and ability to fight disease after both short- and long-term exposure. Mice that were fed high levels of one PAH during pregnancy had difficulty reproducing and so did their offspring. These offspring also had higher rates of birth defects and lower body weights. This study was designed to examine the association between IUGR and PAH exposure in expectant women. HPLC analysis of the extracts obtained from placental tissue and bloods for presence of the PAHs residues revealed presence of Acenaphthylene Phenanthrene and Pyrene in significant quantity in IUGR cases in comparison to control. The study revealed a positive correlation between presence of PAH in human placenta and/or blood and intrauterine growth restriction and low birth weight delivery.

Biomedical Informatics Centre of ICMR (Phase-II) at NIP, New Delhi

Biomedical informatics centre, NIP, has identified its major research focus as implementation/

development of biomedical informatics techniques for assisting disease diagnosis and therapies at the level of patient care, which is one of the major objectives sketched by ICMR for the taskforce project. Accordingly, it has initiated research and training facility for biomedical scientists, research scholars and students to promote and support informatics in medical research.

During this year, the activities of BIC included:

- Development of Psoriasis Associated Gene database: The database includes comparative genomic tool to assist biomedical scientist in detecting known psoriasis SNPs in an individuals' genome/gene sequence.
- Developed TiD: a standalone software for identification of putative drug targets from whole proteome of pathogenic bacteria.
- In silico design of IL6, IL23 and TNF alpha antibodies for biologic based therapy development against autoimmune and inflammatory diseases. It is TNF-alpha, JAK-2 and PDE4b inhibitor design.
- e-pharmacophore based inhibitor design for cytoplasmic tyrosine kinase.
- Organized National level workshops: 20 candidates (MDs, PhD and M.Sc.) selected from 11 states of India were trained in five-day workshop on National workshop on Next Generation Sequencing in disease diagnosis and therapeutic target discovery.

Patent filed: LK Yerneni and RM Chugh (2015). A method for processing of feeder cells suitable for adult stem cell proliferation. PCT WO2016067306, File Number PCT/IN 2015/000404, dated 29th October 2015

NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY, MUMBAI

Major activities of the Institute during the year are as follows

- Establishment of satellite centre for hemoglobinopathies at Chandrapur.
- Quality Assurance Programme for Molecular and Prenatal Diagnosis of Hemoglobinopathies.

- Interplay of miRNA expression and the epigenetic factors in sickle cell anemia patients following hydroxyurea treatment
- Centre of excellence for Research, Diagnosis and Management of Primary Immunodeficiency disorders (PID): a) Understanding Molecular pathology of Hemophagocytic Lymphohistiocytosis (HLH) in India. b) Understanding role of NK cells in immunopathogenesis of Dengue virus infection. c) Understanding the genomics of Primary Immune Disorders (PID) using Next Generation Sequencing (NGS)
- Reverse Dot Blot assay for detecting arginine hot spot mutations in von Willebrand Disease.
- Role of circulating procoagulant microparticles in women with pregnancy loss, both recurrent miscarriages and unexplained fetal loss
- Identification and molecular characterization of D variants among apparently RhD negative individuals
- 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
- Collaborative effort to understanding and characterization of novel molecular changes in fanconi anemia.
- To determine the prevalence of serological and molecular markers of Hepatitis B virus in voluntary blood donors from Maharashtra region
- Red cell membrane pathology in hereditary spherocytosis 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.

- Role of Erythroid Kruppel-like Factor (EKLF or KLF1) in Haemoglobinopathies.
- 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 -Thalassaemia and Sickle cell disorders.
- Understanding of molecular pathology of Chronic Granulomatous disease (CGD)
- Role of telomere length and telomerase activity in clinical outcome of patients with haemoglobinopathies
- Collaborative effort to study pathophysiology and molecular characterization of congenital anemia in India
- Prevalence and molecular characterization of NADH cytochrome b5 reductase deficiency in India
- Clinical, molecular and immunological characterization of common T-B+ and T-B-Severe Combined immunodeficiency (SCID) in Indian Population
- Does T cell Receptor Excision Circle Assay give an insight into various forms of Combined Immunodeficiency?
- Clinical, Immunological and Molecular Characterization of an Autoimmune Lymphoproliferative Syndrome (ALPS) and *B-cell expansion with NF κ B and T-cell anergy (BENTA)* in Indian Population.
- Fibrinolysis as a cause of thrombophilia in Indian patients with Deep Vein Thrombosis (DVT).
- A prediction model of preeclampsia using a combination of biomarkers
- Phenotypic and genotypic characterization of Antithrombin (AT) deficiency in Indian patients with thrombosis
- Modulation of the function of activated protein C (APC) to improve the clinical phenotype of haemophilia A.
- Microparticles versus thrombin generation assays as predictive tests for dosage adjustment of bypassing agents in hemophilia patients with inhibitors
- Mechanism of action of bypassing agents i.e. activated factor VII (rFVIIa; NovoSeven) and plasma-derived activated prothrombin complex concentrate (Factor Eight Inhibitor By-passing Agent [FEIBA]) in hemophilia patients with inhibitors
- Clinical spectrum and molecular basis of thrombotic microangiopathies
- Study on Human neutrophil antigens (HNAs) and associated antibodies
- Genetic Determinants for expression of Duffy Antigens on Red Blood Cells and their interaction with *Plasmodium vivax*
- Study of Fucosyltransferases (FUT1, FUT2 and FUT3) haplotypes and their association with some autoimmune disorders.
- 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
- Immunoprofiling of Scleroderma patients in India with varied disease severity
- Identification of early biomarkers in chronic kidney diseases (CKD) from Western India
- Role of matrix metalloproteinases (MMPs) and it's inhibitors (TIMPs) in pathogenesis of Systemic Lupus Erythematosus (SLE)
- 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 INTERVENTIONS

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 79 families with inherited bleeding disorders including hemophilia A, B, vWD and others rare bleeding disorders, 152 families with thalassemia, 6 families with primary immunodeficiency disorders and 3 families with Fanconi anemia.

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 20,000 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 the institute:

- a. Hemoglobinopathies
- b. >300 inherited bleeding and thrombotic disorders were diagnosed this year including 222 patients with hemophilia A, 62 with hemophilia B, 24 with rare factor deficiencies and 18 with platelet function defects.
- c. 122 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.
- d. 590 cases of genetic defects and 939 cases of various hematological malignancies availed the karyotyping services by NIIH.
- e. 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 150 sickle cell patients have been enrolled which will be followed up regularly. Antenatal and newborn screening has also been initiated.

Prevalence of G6PD deficiency in some of the tribal groups in different states was found to be 3-5%. New variant causing G6PD deficiency was identified in tribal group of Madhya Pradesh.

Newborn screening programme for sickle cell anemia in collaboration with Valsad Raktadan Kendra has been very successful in providing early diagnosis and comprehensive care to with sickle cell anemia in high prevalence tribal population in Valsad. It has also given us important information on the natural history of sickle cell anemia patients.

A centre for hemoglobinopathy and red cell enzymopathy diagnosis, molecular analysis and newborn screening was established at Agartala Medical College under the DBT twinning programme. Babies with Hb E disease, E thalassemia, sickle cell disease and G6PD deficiency are being followed up and monitored.

Under the principally approved project of Centre of Excellence for Primary Immunodeficiency Disorders, advanced diagnostic facilities for these disorders have been established. Prenatal diagnosis is also provided for some of these severe disorders.

Study on leukemic stem cells in acute myeloid leukemia has shown that number of stem cells as well as the constitutive activation of the ERK, AKT and STAT5 signaling pathways are associated with significantly poor prognosis and can be utilized as markers of disease prognosis while evaluating patients with AML.

Significantly elevated cMP levels i.e. CD62e, CD146 and CD142 with high AUC and with high specificity and sensitivity were observed suggesting that these can serve as promising biomarkers for early prediction of adverse pregnancy conditions

The in vitro investigations using HEPG2 cell lines to partially silence the *PROC* gene by a siRNA based gene silencing approach have shown that three sequences specific to propeptide, serine protease and activation domain of Protein C partially inhibit the function of this protein. These in vitro investigations are now being replicated in experimental mice and the reduced expression of protein C will be analysed by ELISA using appropriate controls

Study on molecular characterization and mitochondrial DNA variations in Fanconi anemia patients 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.

Study on understanding mechanisms of drug resistance in chronic myeloid leukemia patients have revealed that only 35% patients have tyrosine kinase domain mutations while in remaining other mechanisms like genes involved in drug metabolism (CYP3A5*1/3 polymorphism) may play important role.

Study on aplastic anemia has shown importance of reduced telomerase activity and telomere shortening as a biomarker for disease prognosis.

Studies on molecular pathology of MDS have shown that chromosome 7 abnormalities and 20q deletions are frequent chromosome aberration in primary myelodysplastic syndromes. The study identified Epo and SEC-61 genes as new candidate genes susceptible to genetic losses in primary MDS by Quantitative Multiplex PCR of Short fluorescent Fragments (QMPSF) assay.

Techniques for serological and molecular characterization of rare blood group systems (Rh, Duffy, Kell, and Kidd) are established which will be extremely useful for patients of thalassemia and other multitransfused patients and also in establishing rare blood group donor registries. Molecular characterization of Bombay phenotype and study of various coagulation factors in Bombay phenotype has been carried out.

Indo-French collaborative project on Rh blood group variants will provide insight into molecular mechanisms for weak Rh antigens in Indian

population. Fetal Rh genotyping by non-invasive technique which is being standardized to offer prenatal diagnosis will help many Rh-negative pregnant women.

A set up has also been established for detection of catalytic antibodies to understand the immunoregulatory role of various autoimmune and infectious diseases under currently ongoing ICMR-INSERM project.

Under the DBT-NE task force project a basic autoimmune diagnostic laboratory has been set up at Department of Medicine, Assam Medical College, Dibrugarh, Assam and the advanced immunological laboratory set up for autoimmune workup has been set up at Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, Assam.

Study on matrix metalloproteinases (MMPs) like MMP3, MMP2, MMP7 and MMP9 with their respective inhibitors in SLE patients has shown that MMP/TIMP ratio profile is an indicator for SLE disease progression and can be used as a possible immune parameter for SLE disease prognosis.



Fig. 6. Inauguration of satellite centre for Hemoglobinopathies at Chandrapur on 25th October, 2015.



Fig. 7. Annual Training Course for Blood Bank Officers and Technicians was conducted from 1st March 2016 to 31st March, 2016. There were 18 participants from all over India. The objective was to give some more exposure to newer methods in Immunohaematology to upgrade the standards of their blood banks.

Extramural Research

CENTRE FOR MOLECULAR MEDICINE

Centre Exploring genomic disorders at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow.

The work at centre has provided insights into developmental genetics, DNA based diagnostics has helped in understanding mutation spectrum and has direct application in genetic counseling and prenatal diagnosis. DNA bank and expertise in genomic tools will lead to more novel findings in the coming years.

Emerging Areas in Molecular Medicine at Jawaharlal Nehru University, New Delhi.

The centre supports the JNU faculty in studying various aspects in basic research in different model systems including microbial, mammalian cells and animal and the implications of the same in human

health. Besides research activities the centre is also involved in human resource development and capacity building activities/programmes including Integrated M. Sc-Ph. D. in Molecular Medicine.

ICMR Centre for Excellence in Molecular Medicine:

Advanced program in Basic and Applied Immunogenetics at AIIMS, New Delhi. The centre has done pioneering work in studying the role of immune system and HLA typing in kidney allografts, HSCT for development of GVHD in Indian population. It also offers teaching and training modules in Molecular Medicine, Immunogenetics and Transplant Immunology to graduate students, Ph D research scholars, scientists and doctors at the national level.

BIOCHEMISTRY

To investigate the role of nitrosative stress in the pathogenesis of cyclophosphamide induced kidney damage and possible ways to reduce the damage by the administration of selective inducible nitric oxide synthase inhibitor, aminoguanidine at Christian Medical College, Vellore. The study examined the role of nitrosative stress in CP-induced renal damage and whether aminoguanidine (AG), ameliorates it. The results show that AG pretreatment can prevent CP-induced renal damage by attenuation nitrosative stress and oxidative stress, and restoring the activities of the peroxynitrite sensitive antioxidant enzymes.

Identification of dicarboxylic acid generated by the oxidation of Peroxidized omega-3 fatty acids and analysis of their biological efficacy at Amala Cancer Research Centre, Kerala The present study revealed that both DHA and EPA undergo autoxidation or lipoxygenase mediated oxygenation and the oxidized products subsequently undergo decomposition at physiological temperature *in vitro* indicating long term consumption of omega 3 fats could have far reaching toxic effect. Healthy individuals with a well-defined antioxidant system may be free from these deleterious effects however individual with challenged antioxidant defense system like aged population may suffer.

Studies on developing a novel antifibrotic strategy in the therapy for macular fibrosis using bioconjugation

of oligodeoxynucleotides at JSS College of Arts, Commerce and Science (Autonomous), Mysore. The proposal aimed at critical experiment and computing treatment strategies for macular fibrosis, including prevention of macular injury, reduction of inflammation, inhibition of fibroblast-like cells activation and inhibition of aberrant collagen synthesis. The present study indeed indicates that administration of conjugated ODNs–Chol with Lutein might reduce the formation of new blood vessels by inhibiting RPE migration. The results demonstrate that bioconjugation of ODNs with Lutein would significantly suppress HGF/cMet, p38MAPK expression, collagen type I, fibronectin and quench the associated reactive oxygen species contributing for the progression of fibrosis in ARPE-19 cells.

Age related changes in the expression of antioxidant enzymes and apoptotic proteins in testis of neonatal hypothyroid rats at Utkal University, Orissa. The present study examined effects of neonatal exposure of 0.05% 6-n-propyl-2-thiouracil (PTU) on expression of antioxidant enzymes and apoptotic proteins in cells of seminiferous tubules of adult (90 days of age) rats. Treatment of PTU to lactating mothers and pups till 90 days of age (persistent exposure) differentially modulated expression of antioxidant enzymes, augmenting Cu/Zn-superoxide dismutase (SOD1) and catalase (CAT) at transcriptional, translational and activity levels while Mn-superoxide dismutase (SOD2), glutathione peroxidase1 (GPx1) and glutathione reductase (GR) were unaltered. PTU-induced transient or persistent neonatal hypothyroidism leads to permanent disturbance of antioxidant defences of seminiferous tubule cells in adulthood with mild generation of oxidative stress in terms of disruption of redox signaling and control. Disruption of glutathione redox status leads to disturbance in Bcl-2/Bax concentration in seminiferous tubule cells. Disturbed antioxidant defence status and oxidative stress in STCs during hypothyroidism may be one of the major factors of apoptosis and decrease in number of sperms which leads to impaired fertility at adult stage.

Role of Mitochondrial Glutathione in the Survival of Young and Aged Primary Rat Hepatocytes at Periyar University, Salem. The aim of this study

was to compare the variable effects of reduced glutathione (GSH) pre-treatment on I/R liver injury in young and aged rats. Wistar male rats were sorted into young and aged. All groups except sham were subjected to 90min ischemia and 2hr reperfusion. The treatment groups received 200mg/kg bwt of GSH, 30min prior to I/R. These findings suggest that, GSH supplementation prior to surgery would be an efficient therapeutic strategy and can be used synergistically with other treatments to yield better postoperative outcomes in liver surgery, thus circumventing the age factor.

Analysis of Structural and Mechanistic Basis of Novel Antithrombin Variants in Indian Families with Thrombosis at Jamia Millia Islamia, New Delhi. In this study several variants of Antithrombin III (AT) are described for the first time in the Indian population. 1950 deep vein thrombosis (DVT) patients for AT activity and antigen levels, DNA sequencing was done to identify known and unknown genetic variations in the AT gene. Two families, one with type I and the other with type II AT deficiency was identified. Three members of a family I with low AT activity and antigen levels showed increased coagulation rates, rs2227589 polymorphism was identified as the sole factor responsible for recurrent thrombosis. Four members of family II spanning two generations were with normal antigen level but decreased AT activity. In conclusion, we report a novel point mutation (T280A), a novel single nucleotide insertion (13363insA) in the background of known variants R47C, C4-stop, and polymorphism of rs2227598, *PstI* and *DdeI* in AT gene of Indian population.

Metabolomic Role of Dimethyl- Larsinic Acid (DME) and Benzo- (A)-Pyrene on the Regulatory Aspects of Cholesterol Homeostasis, Cell Proliferation and Immune Responses at AIIMS, Patna. This study aims to find correlation of carcinogen (benzo-(α)-pyrene(BaP) and DME) stimulated cell proliferation with cholesterol homeostasis and cell defense system and the immune response. Carcinogen defeats immune system by lowering LDLR expression, decreasing cellular uptake of LDL and lowering IgG level through immune cell death.

Amyloid Fibrillation of Caprine Brain Cysteine Proteinase Inhibitor, Influence of Neurotoxic

Metals (As, Cd) and Possible Protective Role of Bioflavonoids with Reference to Neurodegenerative Diseases at Aligarh Muslim University, Aligarh. Caprine brain cystatin (CBC) was isolated with an overall yield of 26.29% and 322-fold purification. The inhibitor gave a molecular mass of ~44 kDa and inhibited thiol proteases reversibly and competitively, with the highest inhibition towards papain ($K_i = 4.10$ nM) followed by ficin and bromelain. CBC possessed 34.7% α -helical content as observed by CD spectroscopy. results show that favorable condition for fibrillation of CBC is in the range of 25–30% TFE. Curcumin and quercetin inhibit fibril formation, curcumin being better inhibitor against amyloid fibril formation of CBC. CBC was further explored for its behavior in the presence of neurotoxic metals arsenic & cadmium. CBC showed a concentration and time dependent decrease in inhibitory activity against papain which was more in case of arsenic than in cadmium. Altered intrinsic fluorescence, increased UV absorption and ANS fluorescence are suggestive for the conformational alterations and opening up of hydrophobic patches in CBC. Finally increased ThT fluorescence is suggestive for possible amyloid like aggregate formation. Above all the effect was more prominent in case of arsenic than in cadmium.

Identification of Novel Biomarker in Cardiac Arrhythmia Induced Rats and its Significance on Human Serum Sample at Madurai Kamaraj University, Madurai. The present study suggested that the differential expression of protein play an important role in cardiac regeneration as well as in cardiac conduction leading to cardiac arrhythmia and Sudden Cardiac Death (SCD) in humans. The novel protein Hyaluronan-mediated motility receptor (RHAMM) has been associated with many facets of biology and it was proposed to play a critical role in the definition and maintenance of body shape and form. Increased expression of an hyaluronic acid mediated motility receptor (RHAMM) could play a crucial role in cardiac regeneration. Thus the result of the present work paves a way in the diagnosis of patients with CA and Sudden cardiac death.

Significance of Serum Metalloproteases 2,4,9 Levels in Metabolic Syndrome at King George's Medical University, Lucknow. The study of genetic

polymorphism was done by PCR-RFLP method. All metabolic risk factors were statistically significant ($p < 0.01$) in patients having metabolic syndrome as compared to control group while no such significant relation was found with serum MMP 4 level. The study revealed higher expression and serum level of MMP-2 and MMP-9 in the patients with metabolic syndrome. Further, this study suggested that MMP-2 (-1575 G>A, MMP-2 (-1306 C>T) and MMP-9 (-1562 C>T) polymorphisms were strong predisposing risk factors for metabolic syndrome. The MMP9 enzyme activity might be a significant indicator in the screening of MetS patients.

CELLULAR AND MOLECULAR BIOLOGY

Targeted Correction of the Point Mutation of Sickle Cell Gene and Targeted mutagenesis of Nucleotide Associated with HPFH by RNA/DNA Oligonucleotides (RDOs) at National Institute of Immunohaematology, Mumbai. The results of the study show that gene silencing of MMR gene with MSH-2 siRNA selectively increased targeting efficiency by blocking the mismatch repair machinery. The Taqman SNP genotyping assay showed a greater sensitivity for detection of mutation correction. The oligo-mediated targeting of human cells is currently limited by low targeting efficiencies and low survival of modified cells.

The study of mitochondrial dynamics during *S. aureus* infection in human epithelial cells at The MS University of Baroda, Vadodara. The present study describes the role of mitochondria in immunity against *S. aureus* infection in epithelial cells. *S. aureus* infection results in increase in transcript level of PGC-1 α as well as NRF1 and NRF2 in HEK 293 and A549. Increase in transcript level of this transcription factor signifies mitochondrial biogenesis in epithelial cells (HEK 293 and A549) during mitochondrial biogenesis. These results clearly signifies mitochondrial biogenesis in epithelial cells (HEK 293 and A549) during mitochondrial biogenesis. *S. aureus* infection also results in clearance of damaged mitochondria by mitophagy .

Fuch's Endothelial Corneal Dystrophy (FCD)-Fine Mapping and Identifying Candidate Gene for the Novel Loci 20p12.1- q13.2 in Early Onset Family & Study the Association of TCF4 Gene In Sporadic

Late Onset FCD Cases In Our Population under Dr. N. Soumitra, Vision Research Foundation, Chennai. We had previously studied the role of *SLC4A11* and association of rs613872 (2.1×10^{-5}), rs2286812 in intron of *TCF4* in our late-onset FCD cohort. We had also ruled out the role of *COL8A2* in the large early-onset FCD family and identified linkage to novel locus at 20p12.1- q13.2. Here we have studied the contribution of *TCF8* and association of rs17595731 and rs9954153 in intron of *TCF4* to late-onset FCD in our cohort. We attempted to fine map the locus 20p12.1-q13.2 in the early-onset FCD multigenerational family to identify the candidate gene. Mutations in *TCF8* and *SLC4A11* contribute to about 2-4% and rs613872 and CTG18.1 trinucleotide repeat expansion show significant association and increased risk to late-onset FCD in our cohort. In the early-onset family,

Possible Role of Key Sialic Acid Biosynthetic Enzyme in Apoptosis at Jawaharlal Nehru University, New Delhi. In order to elucidate the role of GNE in cell apoptosis, HEK cell based model system over expressing pathologically relevant GNE mutations was used. Annexin V, tunnel assay, activation of caspases and increased PARP cleavage, altered mitochondrial transmembrane potential and morpho-structural (TEM) changes in mitochondria in cells over expressing different GNE mutants indicated a role of GNE in mitochondria dependent cell apoptosis and provide insights into the pathomechanism of GNE myopathy.

HUMAN GENETICS

A study on Molecular characterization of familial hypercholesterolemia in Indian population was conducted at Sir Ganga Ram Hospital, New Delhi. Molecular study of LDLR, ApoB100 and PCSK9 genes in cases of PH are reported in this study. 100 unrelated cases, satisfying modified Dutch Lipid Network Criteria (DLNC) were enrolled. It was observed that mutation carriers had higher LDL cholesterol and early presentation of CAD than the non- mutation carriers. The family specific mutation was observed in 91 (42%) of 216 subjects analyzed. The current study has added to the mutation spectrum of LDLR gene in India. One founder mutation was identified. DLNC criteria proved useful for selecting cases for genetic

studies. Cascade screening was well-accepted by family members.

A study assessed the role of DNA SNP microarray in the evaluation of FISH negative (normal FISH result or non informative FISH in clinically suspected 22q11.2 microdeletion syndrome carried out at AIIMS, New Delhi. 101 cases of FISH negative clinically suspected microdeletion syndromes were included in the study. It was observed that FISH in this format should not be the method of choice for clinically suspected microdeletion syndromes as cost, labor & time versus benefit is unjust. More strict clinical criteria should be followed. If clinical diagnosis is uncertain or doubtful then microarray should be first screening test. This is important especially with newborn/neonate in intensive care unit. Furthermore, aCGH provides associated CNVs (often many) as well as UPD/LOH/AOH and their significance is unknown at present. Whole genome screening by aCGH should be carried out as first tier test and FISH should be the choice of test for detecting mosaicism, screening family members and prenatal diagnosis in proven family.

A study of the frequency and distribution of 10 Toll like receptor (TLR) genes and Killer cell Immunoglobulin like receptor (KIR) genes in Gurkha and Rajbanshi populations of Sub-Himalayan Regions of West Bengal was carried out at Dept. of Zoology, University of North Bengal, West Bengal. 315 samples were taken from which 125 were Gurkhas, 140 were Muslims and 50 were Rabha samples. It was concluded that both the Rajbanshi and the Gurkha populations based on their KIR gene profile are much more associated with the Mongoloid lineages whereas in case of TLR it was found that Gene –environment interaction play a major in the patterns of frequency distribution in the aforementioned populations.

A study on genetic determinants of cognitive impairment in cerebral small vessel disease was conducted at National Institute of Mental Health and Neurosciences, Bangalore. Cerebral Small Vessel Disease (SVD) is characterized by the triad of microbleeds, lacunar infarcts and leukoaraiosis. 120 cases and 104 controls were recruited that satisfied the radiological criteria for SVD and evaluated with complete neuropsychological assessment. Genotype analysis for 10 SNPs, Angiotensin Converting Enzyme (ACE) activity and plasma nitric oxide (NOx)

levels were determined. No significant difference was observed in increased ACE activity and plasma NOX concentration in subjects with DD genotype. The SNPs analysis in relation to hypertension also did not establish significant role of hypertension to cognitive impairment in subjects with SVD. Comparison of cognitive function tests score with genotypes indicated significant variation in NNPB - scores of control subjects with ACE gene polymorphisms with mutant genotypes of the selected ACE gene SNPs showing higher score than wild genotypes. A larger sample study is warranted to conclusively ascertain the role of the selected SNPs in cognitive impairment in cerebral Small Vessel Disease (SVD).

A study to establish a “non-invasive” methodology by isolating fetal DNA in large amounts in different trimesters of pregnancy and confirm with Y-chromosome specific (*SRY* and *DYS*) and housekeeping (*β -actin*, *β -globin* and *GAPDH*) genes, to validate fetal DNA by real-time quantitative PCR (qPCR) using SYBR green system, to use fetal DNA as marker for screening preeclampsia and various aneuploidies & to characterize fetal DNA by mRNA-qPCR using *PLAC4* gene was carried out at University of Lucknow, Lucknow. The study included 607 subjects divided into 4 groups: (1) healthy pregnant women (n=500), (2) non-pregnant taken as controls (n=50), (3) high risk pregnancy cases (n=37) and (4) after delivery cases (n=20). The “non-invasive” method will reduce the risks of invasive diagnostic procedures, will enable all women to undergo prenatal diagnosis and society will be benefited at large. NIPD techniques can be implemented in the early phase of first trimester and can possibly help to a great extent in decision making and mothers undertaking a painless procedure for PND. This approach will be useful for the estimation of cfDNA/RNA as a possible marker of pregnancy-associated disorders. Diagnoses are primarily limited to paternally inherited sequences, but the use of unique expression of fetal RNA has a great potential for detecting other chromosomal aneuploidies. It has opened the possibilities of diagnostic utility of pDNA/RNA and cfDNA/RNA concentration and add them to the current panel of biomarkers. Therefore, the non-invasive technique of obtaining cfDNA from maternal blood will

enable all pregnant women to undergo prenatal testing thus revolutionizing fetal medicine.

HAEMATOLOGY

A study on phenotypic and genotypic characterization of the Bernard Soulier syndrome (BSS) cases from India was conducted at National Institute of Immunohaematology (NIIH), Mumbai. BSS is caused by the defects in GP1b/IX/V complex which is due to the biallelic mutations in the GP1BA, GP1BB and GP9 gene, more than 50 mutations have been reported so far. This study confirms the heterogeneity in clinical presentation in patients with BSS as described earlier, the age of clinical diagnosis varied from 4 to 24 years, diagnosis was delayed in some patients may be due to the lack of awareness or unavailability of the facilities for the diagnosis of this disorder, but most of them were diagnosed early in their life. The severity of the disorder is inversely correlated with platelet count. The mutation data obtained in the present study can successfully be used in carrier detection and antenatal diagnosis of the affected families besides being complementary to data from other parts of the country in maintaining a National database of these rare platelet function disorders like BSS.

A study on exploring the clinically relevant notch1 mutations and to sequence all the domains of Notch1 to detect mutations and their functional significance in South Indian T-ALL patient samples with the prognostic correlation in South Indian T-acute lymphoblastic leukemia was carried at Cancer Institute, Chennai. The mutational study of all the exons of NOTCH1 in 25 T-ALL samples revealed 52% mutations; of which 44% spans mutations in the hot spot exons and 16% in the rest of the exons [2 (8%) cases had mutations in both hot spots and other exons]. Though the study did not reveal any prognostic significance in correlation with NOTCH1 mutation, the results need to be studied in the larger cohort to elucidate the frequency of whole NOTCH1 mutations and their role in the T-ALL leukemogenesis and prognosis.

A study on identification and characterization of hemoglobin variants using mass spectrometry was conducted at St. John’s Research Institute, Bangalore. In this study, the method has been

validated with sickle Hb variant which had been unambiguously characterized by D-10 HPLC. Presently described NanoLC-ESI/MS^E method coupled to data analysis using customized peptide database might offer absolute answer in the hemoglobin variant identification. The above method was found to be robust and it required minimal manual intervention.

Another study on analysis of Wnt signaling molecules and their association with common mutation in Acute Myeloid Leukemia (AML) was carried at AIIMS, New Delhi. The present study included de novo AML patients (n=117) and either their bone marrow or peripheral blood sample was collected. The study showed that Wnt signaling molecules might have a clinical utility in diagnosis and in disease monitoring of AML. Further, it may allow the development of non-invasive diagnostic and prognostic tools for the stratification and may help to develop new platform for new molecular targeted therapy against AML.

A study on dynamic regulation of lymphocyte signaling of acute leukemia was conducted at National Institute of Pathology, Safdarjung Hospital Campus, New Delhi. 90 samples of Acute Leukemia Patients attending Haematology Department of Safdarjung hospital for treatment have been collected after obtaining consent from the patient. Flow cytometry was done on all samples for confirmation. The study elucidated the role of ROS in dynamic regulation of T cell in the pathogenesis of Acute Leukemia. Investigated redox regulated molecules (CTLA 4, PD1, CD28, CD27) that played an important role in Acute Leukemia Pathogenesis. NK cell based therapy may be advised to AML patient for greater success with further understanding of molecular mechanism using Acute Leukaemia Mouse Model.

IMMUNOLOGY

In vitro expression studies of immune-regulatory genes: significance in vitiligo pathogenesis at University of Baroda, Vadodara. The study explored the role of various pro and anti-inflammatory cytokines role in vitiligo pathogenesis. Increased levels of pro-inflammatory cytokines such as *IFNG*, *TNFA*, *IL1B* and decreased levels of anti-inflammatory cytokine *IL10* in the lesional skin

of vitiligo patients are reported *in vivo* i.e. in the skin microenvironment of vitiligo patients (lesional and non-lesional) and skin of healthy age matched controls. TNF- α among all four is the most potent cytokine and down regulates the expression of *TYR*, *MITF* increasing cellular as well as mitochondrial ROS and inducing autophagy suggesting TNF- α in presence of H₂O₂ and other inflammatory cytokines (*IFNG*, *IL6*, *IL1 α* etc.) plays an important role in vitiligo pathogenesis.

Investigating the Molecular Basis of Characteristic Cytokine Profile in Systemic Lupus Erythematosus at Nizam's Institute of Medical Sciences, Hyderabad. In this study, a total of 678 subjects (219 SLE cases and 459 healthy controls) were included. There was a significant increase of cytokines i.e. TNF- α , interferon- α , IL-10, and IL-12 in serum of SLE patients as compared to control. Autoantibodies that are produced in SLE patients were tested and observed positive for ANA and anti-dsDNA antibodies whereas control subjects were negative for ANA, anti-ds-DNA antibodies, Anti-Ro, Anti-La, anti-cardiolipin (aCL), and anti-RNP antibodies.

NANO-MEDICINE

Targeted delivery of antitubercular drugs to the brain and their therapeutic efficacy against experimental tuberculous meningitis at Postgraduate Institute of Medical Education and Research, Chandigarh. The current study was designed to synthesize nanoparticles delivering antitubercular drugs to the brain by oral and intravenous route. Double coated nanoparticles administered via oral route were found to be most suitable strategy for brain targeting. Murine model for tuberculous meningitis was developed to evaluate the efficacy of the brain targeted PBCA nanoparticles containing antitubercular drugs. The work suggest possibility of using nanoparticle formulation through oral route which is more convenient and patient friendly mode of drug delivery

Nanoreservoir carrying Brugia malayi recombinant proteins as potential vaccine against experimental lymphatic filariasis at CSIR-Central Drug Research Institute, Lucknow. The results show that liposomized or nanoencapsulated Bm-TTP and BmAF-Myo triggered the production of anti

Bm-TPP antibody (Fig.2 a & b). As expected, the nanoencapsulated antigens triggered much higher IgG antibody secretion than the liposomized protein. In BALB/c, encapsulated recombinant proteins triggered a mixed Th1 and Th2 immune response as evidenced by the production of high levels of serum IgG1, IgG2a and IgG2b antibodies.

Nanotechnology based drug delivery and chemotherapeutic efficacy of Phenothiazines against active and latent tuberculosis at PGIMER, Chandigarh. The study was designed to evaluate the chemotherapeutic potential of nano-technology based drug delivery of phenothiazines against active and latent tuberculosis. The amylopectin-tagged PLGA-PEG nanocapsules were developed as a second generation lung-targeted drug delivery vehicle. The comparative pharmacokinetic and pharmacodynamic profiles of the free drugs and encapsulated drugs showed that the nanocapsules released the drugs in a slow and controlled manner, thereby resulting in the retention of the drugs in the plasma and tissues of the mice upto 14 days. The remarkable efficiency of amylopectin- tagged PLGA-PEG nanocapsules to target the drugs to the lung tissues and prominent dosage reduction underlines the importance of using a nanotechnology based targeted and sustained release drug delivery system for improving the therapy of tuberculosis.

Mesoporous silica nanoparticle for controlled delivery of insulin- design, fabrication, evaluation at Jadavpur University, Kolkata. The objective of the present study was to develop novel PMV coated mesoporous silica nanoparticles with improved hypoglycemic effect for oral insulin delivery. We have synthesized cylindrical MSN under acidic condition using non-ionic surfactant (Pluronic® P 123) and silica precursor (Tetra ethoxyorthosilane). After Post synthesis treatment (PST) surfactant was removed by calcinations. To obtain pH sensitive release calcined MSN was coated with pH sensitive polymer PMV (methacrylic acid-co-vinyl triethoxysilane) which was synthesized in a three neck flask under nitrogen atmosphere. Results showed significant release at pH 7.4. Cumulative drug release over a period of 6 hr was more than 48% at this systemic pH. Effect of this MSN-PMV-INS on blood glucose level was retained for 16 hr. This novel formulation shows 80% oral bioavailability of insulin.

Design and development of biodegradable nanoparticles for effective delivery of siRNA into heart: targeting the cardiac Natriuretic peptide Receptor- C at University of Madras, Chennai. The study aims to design and synthesis a biodegradable nanoparticle - chitosan nanoparticle (Chi-Np), for targeted delivery of Npr3 siRNA to the diseased heart, to silence cardiac natriuretic peptide receptor -C expression. The functionalized chitosan Nps could be a valuable tool to deliver desired siRNA and genes *in vivo*. The proposed studies of Chi-siRNA- Npr3 - nanoparticles mediated targeting will be a novel approach, and it has strong therapeutic application to treat cardiac hypertrophy and heart failure in human patients.

Development and Characterization of Functionalized Carbon Nanotubes for Tumour Drug Targeting and Therapy conducted at National Institute of Pharmaceutical Education and Research, Mohali. Surface oxidation of MWCNTs was optimized. It was demonstrated that oxidate MWCNT induced cytotoxicity and apoptosis increases proportionally with the density of surface carboxyl groups. The enhanced cellular uptake of negatively charged oxidized CNTs with increased degree of carboxylation induced higher intracellular stress response and cytotoxicity resulting in cell death. Optimal limit for MWCNTs' functionalization density that could be safely used for nanomedical applications while inducing minimal hepatotoxicity and bio-persistence in animal model. Hyaluronated tethered MWCNTs was developed and successfully explored for tumor-targeted delivery of Doxorubicin *in vivo*. Oxidized MWCNTs have been multifunctionalized with folic acid, methotrexate, Alexa-Fluor and Technitium-99m and successfully explored for tumor-specific drug targeting and therapy.

Magnetic Nanoparticles for Effective Target Drug Delivery of Carvacrol against Liver Carcinogenesis - A Molecular Approach at University of Madras, Guindy Campus Guindy, Chennai. For precise delivery of carvacrol, Poly (lactic-co-glycolic acid) (PLGA) based superparamagnetic nanoparticles loaded with carvacrol (Carv-MNPs) were synthesized and characterized. The magnetic nanocarrier enhanced the stability and activity of carvacrol. Release of carvacrol from Carv-MNPs

occurred in a controlled manner IC_{50} value was found to be $15\mu\text{mol/ml}$ for Carv-MNPs compared to $25\mu\text{mol/ml}$ for free carvacrol in HepG2 cell line. Clinical symptoms and signs associated with cancer such as increased liver weight, increased tumor nodule and tumor volume, ultrastructural alterations, abnormal lipid accumulation, elevated MMP-2 and MMP-9 expressions, increased expression of inflammatory markers like COX-2, TNF- α and NF- κ B and Akt signaling pathway proteins Akt, pAkt, GSK-3 β , Cyclin D1 and NF- κ B were attenuated upon treatment with Carv-MNPs in NDEA induced hepatocarcinogenesis. The results indicate Carv-MNPs exhibits anti-cancer, antimetastasis and anti-proliferative efficacy against NDEA induced HCC animals.

Evaluation of Antimicrobial Properties and Toxicological Effects of Nanoparticles in Mice at Aligarh Muslim University, Aligarh. These studies revealed that AgNPs could distribute from the exposure site and become systemically available. Once nanoparticles enter the body, they may become systemically available regardless of route of administration thus may cause toxic effects. For nanoparticles of medical applications, their efficacy largely depends on the control of their distribution within the body. The results obtained in the present study indicate that the short-term intraperitoneal exposure of AgNPs exhibited few toxic or adverse health effects in mice.

Development & Evaluation of Nanofibre Based Myocardial Patch for Functional Regeneration at Sastra University, Thanjavur. The study uses multidimensional tissue engineering approach for reconstruction of ischemic cardiac muscle tissue into healthy tissue after acute myocardial infarction by generating bioactive nanofibrous patch. A scaffold of heterogeneous fibres mimicking the cardiac tissue architecture and strength, was fabricated which influenced the cellular adhesion, proliferation and differentiation. The regenerative potential of the growth factor loaded nanofibrous patch was tested in vivo in an acute myocardial infarction rabbit model. The evidence for functional repair and possibility of regeneration was confirmed through histopathological and immunohistochemical analysis.

Bioadhesive Nasal Nanoparticulate Drug Delivery System for the Treatment of Osteoporosis at Jamia Hamdard, New Delhi. The objective of the present work was to develop and evaluate Polymeric Nanoparticles (NPs) of Risedronate Sodium (RIS) for the treatment of OP using Intranasal (IN) route in order to increase bioavailability and reduce peripheral toxic effects. The nanoparticles were prepared by ionic gelation and reverse micellar method. Placebo nanoparticles were formed by ionic gelation method and mean particle size of optimized nanoparticles was found to be 132.9 ± 9.6 nm with Poly Dispersibility Index (PDI) of 0.273 ± 0.022 which indicated narrow particles size distribution. Thus CG-NPs have great potential for delivering the RIS and may be a promising approach for IN delivery of RIS for the treatment and prevention of OP after clinical evaluation in near future.

Nanoparticle-encapsulated Bioactive Formulations for Mass Health Care at Indian Institute of Technology Delhi, New Delhi. The aim of this study was to develop *Calendula officinalis* extract based nanoformulations and to study the antibacterial activity of either calendula extract loaded chitosan nanoparticles or calendula extract coated silver nanoparticles for increased bioavailability and their long term effect. The demonstrated time dependent antibacterial activity against different microbes studied. Both calendula extract and calendula extract loaded chitosan nanoparticles have shown good antimicrobial activity against both Gram positive and Gram negative bacteria. Calendula extract loaded chitosan nanoparticles and calendula extract coated silver nanoparticles are potential antibacterial for their long term antibacterial effects.

Development of nanotechnology based drug delivery system for lipid lowering drugs at Manipal College of Pharmaceutical Sciences, Manipal

The project establishes that the model lipid lowering drug, rosuvastatin can be produced as nanoformulation using chitosan and hyaluronic acid to treat atherosclerosis more effectively. Two approaches were used to develop the nanoparticles of rosuvastatin: i) Rosuvastatin was encapsulated using chitosan and then dispersed in

a mixture of hyaluronic acid and tripolyphosphate. Nanoformulations were obtained by ultrahomogenization and ultrasonication techniques and ii) rosuvastatin was encapsulated in polymer matrix of PEGylated chitosan. *In vitro* drug release study showed that the optimized nanoformulations (Batches F3 and R9) release the drug in sustained manner. From the pharmacodynamic study, done in male Wistar rats, it was observed that rosuvastatin nanoparticles were able to reduce the serum cholesterol and triglyceride levels much more than the standard treatment bring them almost back to that of the normal animals.

PHARMACOGENOMICS

Impact of genetic polymorphisms of CYP2C19 and CYP3A4 on the pharmacokinetics and pharmacodynamics of proton pump inhibitors in Andhra Pradesh population (Telugu origin) at Asian Healthcare Foundation. In this study, to identify frequencies of different polymorphisms in CYP2C19 gene and study their impact on drug metabolism in healthy individuals. The results indicate that it is important to screen for CYP2C19*17 polymorphism and also optimize the drug dosage for individuals who carry the polymorphism as they failed to respond to the standard dose of the drug.

A comprehensive Pharmacogenetic Study of ABC Transporter Genes Implicated in Response to Antiepileptic Drugs at CSIR-IGIB, Delhi. Aim here is to identify ABC transporters variants which might potentially be used for optimization of treatment in individual patients, leading to a more targeted, efficacious and less harmful treatment. The study relives that the distribution of the associated variants supports the involvement of ABCC2 in controlling seizures in women possibly by lowering of its expression. The biological basis of this finding could be an altered interaction of ABCC2 with AEDs and estrogens as both AEDs and estrogen metabolites are substrates for ABCC2. These results necessitate replication in a larger pool of patients.

PHARMACOLOGY

A study was on Designed Synthesis and Biological Evaluation of Novel Agents for Management of

Benign Prostatic Hyperplasia was carried out at CDRI, Lucknow. Benign prostatic hyperplasia (BPH) is the major health concern in ageing males. Autopsy studies indicate that the evidence of BPH increases from 40% of men in their 50s to 70% in their 60s and 88% in their 80s. Variety of compounds belonging to different categories such as 5 α -Reductase Inhibitors, Selective Androgen Receptor Modulators (SARMs), Selective Estrogen Receptors Modulators (SERMs), etc. have been used for the management of BPH. Despite of having such a prevalence and significant advances in the diagnosis and treatment of BPH in recent years the available treatments for BPH are either highly invasive (surgical) or partially effective with unwanted side effects leading to a significant erosion in the quality of life; and additionally, put a significant burden on employees and their employers through direct medical costs and lost work time. Therefore, it is highly desirable to develop a potent molecule with fewer adverse effects. In continuation of our efforts to develop substituted aryl piperazines as potent agents for the management of BPH it was planned to synthesize N¹-aryl-N⁴-alkylpiperazines, bis(substituted phenylpiperazin-1-yl)alkane, piperazin-1-yl-benzothiazol-2-amines and substituted 2-phenyl-1-(substituted 4-phenylpiperazin-1-yl)alkanone as possible non-steroidal AR antagonists (SARMs).

Total 81 compounds have been synthesized in four prototypes and 39 of these exhibited EC₅₀ in the range of 7.6-79.8 μ M in androgen dependent LNCaP cell line. 8 compounds showed a significant decrease in the calcium level in alpha blocking analysis and significant reduction in prostatic weight in *in vivo* studies in BPH induced diseased adult male rats. Two compound exhibited higher levels in the prostate than that in serum and hypothalamus in pharmacokinetic studies and displayed similar to binding mode as that of hydroxyl flutamide. 1,2-bis(4-(4-nitrophenyl)piperazin-1-yl)ethanone and 1, 2-bis(4-(pyridin-2-yl)piperazin-1-yl)propane might be a promising lead candidate for the management of benign prostatic hyperplasia. A further lead optimization may lead to an agent acting both for reducing prostate size as well as relaxing smooth muscles i.e., both the disease burden and symptom relief by controlling Lower Urinary Tract Symptoms (LUTS).

The circadian pattern of blood pressure and cardiovascular autonomic regulation in patients with coronary heart disease and effect of melatonin supplementation on it was conducted at King George Medical University, Lucknow. The Melatonin supplementation was found to improve circadian pattern of blood pressure and heart rate in CAD subjects. It can be concluded that melatonin exerts protective effects on daily pattern of blood pressure and autonomic cardiovascular regulation in CAD subjects possibly by the action of high affinity G coupled receptors M1 and M2 via SCN. Furthermore, mounting evidence indicates that the melatonin rhythm has a crucial role in several cardiovascular functions. Melatonin has antioxidant, anti-inflammatory, and chronobiotic regulatory functions. It is recognized that melatonin is of special interest, being an endogenous molecule that can be used in humans, and is also safe.

A study on evaluation of disease modifying activity of plant lipids rich in omega-3 fatty acid in experimental models of chronic inflammation was completed at AIIMS, New Delhi.

The plants *Linum usitatissimum* fixed oil (LUFO) and *Ocimum sanctum* fixed oil (OSFO) were evaluated in experimental models of arthritis, viz. Cotton-pellet induced granuloma model and CFA induced arthritis. The fixed oil LUFO was found to be effective in both the above models. Additionally, LUFO also possess significant disease modifying activity by reducing the circulating levels of the inflammatory cytokine TNF-alpha in CFA models. At a dose of 3ml/kg, its antiarthritic activity was comparable to that exhibited by 3mg/kg indomethacin, thus demonstrating its potential in treatment of RA. Further the immunomodulatory activity of LUFO was confirmed by its ability to reduce granuloma formation in cotton pellet induced granuloma model.

In OSFO, a significant antiarthritic activity was only observed at the highest dose tested (3ml/kg). Its antiarthritic activity was inferior to the standard drug (as seen in CFA arthritis). Circulating levels of TNF-alpha were not significantly altered by the administration of OSFO.

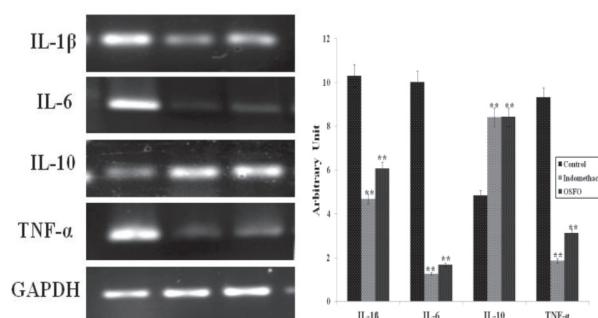


Fig. 8. Effect of OSFO treatment on proinflammatory and anti-inflammatory mediators gene expression in CFA-stimulated rat peritoneal macrophages.

To confirm the immunomodulatory activity of LUFO and OSFO, pro-inflammatory cytokine IL-1 & IL-6 and anti-inflammatory IL-10 cytokine were estimated in serum by Dot-blot. In this also both LUFO and OSFO showed a significant decrease in pro-inflammatory cytokine and increase in anti-inflammatory cytokine which further proves its immunomodulatory activity.

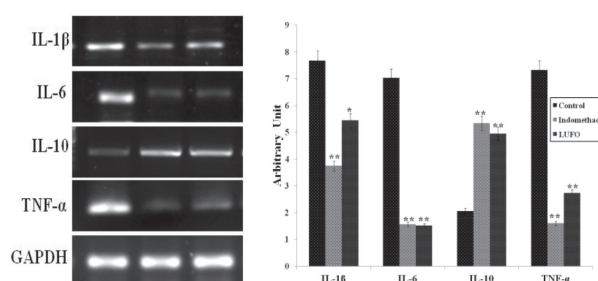


Fig. 9. Effect of LUFO treatment on proinflammatory and anti-inflammatory mediators gene expression in CFA-stimulated rat peritoneal macrophages.

To confirm the involvement of macrophage derived inflammatory cytokine in the activity of LUFO and OSFO, pro-inflammatory cytokine IL-1, IL-6, TNF-α and anti-inflammatory cytokine, IL-10 were estimated in serum by Dot-blot & ELISA, pro-inflammatory receptor, TNF-R1 and gene expression of IL-1β, IL-6, IL-10 and TNF-α in CFA induced stimulated macrophage model. In this model, LUFO and OSFO showed a significant decrease in macrophage derived mediators in serum and gene levels, thereby validating immunomodulatory activity of LUFO and OSFO by inhibiting stimulated and dysregulated macrophages. Based on these results, findings suggests that LUFO & OSFO have beneficial effect in chronic inflammatory disorders.

Effect of nalbuphine on opiate withdrawal in rats: behavioral biochemical and molecular study was studied at AIIMS New Delhi. The aim of the present study was to compare the acute and chronic effect of nalbuphine on naloxone precipitated opiate withdrawal in rats. The opiate dependent rats showed a significant increase in locomotors activity, Gellerts Holtzman rating scale, plasma corticosterone levels, tissue C-amp levels whereas protein expressions and relative mRNA expressions of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) were decreased significantly. Combination of acute doses of nalbuphine and morphine showed no effect at all doses on these parameters in opiate dependent rats. Moreover, chronic administration of morphine resulted in significant decrease in naloxone precipitated opiate withdrawal and up-regulation of protein expression and relative mRNA expressions. Nalbuphine might be used as an effective pharmacological adjunct in the treatment of opiate addiction.

Gene expression and protein profiling of group A streptococcus upon treatment with quorum quenching agents from marine bacteria was carried at Alagappa University, Chennai. In the present study, coral associated bacteria were screened for their ability to inhibit biofilm formation by *Streptococcus pyogenes*. Out of the 10 isolates screened, strain A1 was found to have greater potency in inhibiting the biofilm of *S. Pyogenes*. The strain A1 was identified as *Bacillus subtilis* based on 16S rRNA gene sequencing results (Genbank Accession number –KC433737). Even though *B. subtilis* is widely recognized as a soil bacterium, it is ubiquitous in the environment owing to its survival mechanisms such as motility, uptake of exogenous DNA, biofilm formation, sporulation and metabolic versatility. A number of studies have reported the presence of *B. subtilis* in marine environment as well as its immense potential to produce novel antimicrobial metabolites such as Gageotertrins A-C, Subtilomycin, ϵ -poly-L-lysine.

Ethyl acetate extract of A1 cell free culture supernatant was purified by TLC, gel filtration chromatography and HPLC and then the partially purified fraction was subjected to GC-MS analysis. GC-MS analysis of the active fraction revealed the presence of 5-hydroxy 2-furancarboxaldehyde (OHFCA) as one of its major constituents. OHFCA and its parent compound 2-furancarboxaldehyde

(2FCA) and its isomer 3-furancarboxaldehyde (3FCA) were assessed for their antibiofilm activity against *S. pyogenes*. Among the tested compounds, 3FCA showed strong antibiofilm activity (93% antibiofilm activity at 132 $\mu\text{g}/\text{ml}$ concentration) compared to OHFCA which showed only 80% antibiofilm activity even at 300 $\mu\text{g}/\text{ml}$ concentration. The compound 3FCA alone was selected for further analysis for following rationale: Novelty of the 3FCA for its antibiofilm activity; Ability to inhibit biofilm even at lower concentration and phytochemical and non-toxic nature of 3FCA. The proteomics results, most of the differentially regulated genes are involved in amino acid biosynthesis, peptidoglycan synthesis and membrane transportation activity, all of which plays crucial role in cell wall biogenesis. Hence, it is speculated that the 3FCA targets the proteins involved in cell wall biogenesis for its antibiofilm activity, which is also supported by the SEM results. Moreover, the proteins involved in amino acid biosynthesis specifically arginine could play crucial role biofilm inhibition, since arginine possesses an antibacterial activity against *S. mutans*. The result of proteomic analysis goes well in hand with that of *covR* mutant *S. mutans* wherein the proteins involve in amino acid production, ABC transporter and cell wall biogenesis were found to be differentially regulated.

STEM CELL RESEARCH COLLABORATION

To investigate the mitochondrial network dynamics during differentiation of human mesenchymal stem cells to cardiomyocytic and adipocytic lineage *in vitro* at PGIMER, Chandigarh. In this study, we studied the changes in mitochondrial biology & energy metabolism in human bone marrow derived mesenchymal stem cell (MSC) during differentiation to cardiomyocyte and adipocyte *in vitro*. We performed a temporal study to track the changes during cardiomyocyte /adipogenic differentiation and compared it with undifferentiated MSC. Cardiac differentiation hMSCs was induced with 5-azacytidine and bFGF mixture for 48 hr. So, our study showed that mitochondrial biogenesis was essential for both adipogenic and cardiomyocyte differentiation. The mitochondrial dynamics during differentiation can be utilized for selecting mitochondria with defined capacity that matches to the target cell type and effectively modulate their function. Metabolic interventions can also increase

the differentiation efficacy and may be a major step forward in terms of providing large quantities of purified regenerative cells that could be used for clinical applications.

Regulation of Mesenchymal Stem Cells towards Osteogenic Cell Lineage by microRNAs at SRM University, Kanchipuram. In this study, a selective number of miRNAs was investigated for their expression and intracellular regulatory networks involved in differentiation of human mesenchymal stem cells (hMSCs) towards osteoblasts. The results it is suggested that miR-15b promotes osteoblast differentiation by indirectly protecting Runx2 protein from Smurf1 mediated degradation. Thus, this study identified that miR-15b can act as a positive regulator for osteoblast differentiation and alteration in the expression of miRNAs would be a valuable approach for controlling osteoblast differentiation.

To Study the Association of Cardiac Specific Genes towards Generation of Functionally Active Cardiomyocytes Derived from Human Bone Marrow Mesenchymal Stem Cells at All India Institute of Medical Sciences, New Delhi. The study was designed to find out an efficient cardiomyogenic inducer for priming of these BM-MSCs. Flow Cytometry data and qPCR data showed that the expression of markers was significantly higher in oxytocin treated cells followed by TGF β 1 & 5-Azacytidine and least in CM group. The study was carried out for 3 groups and the results confirmed the same trend showing similar cardiac differentiation potential of Oxytocin and TGF β 1 followed by 5-Azacytidine. Oxytocin is a novel inducer and has earlier not been used for cardiomyogenic induction of human BM-MSCs. Calcium ion studies show that priming of stem cells with inducers makes them functionality active and Oxytocin & TGF β 1 are as good/efficient as 5-Azacytidine.

Role of Bone-Marrow Derived Angiogenic Cells in Chronic Liver Diseases at Institute of Liver and Biliary Sciences (ILBS), Delhi and at National Institute of Immunology, New Delhi. Our present study further investigates the role of the bone marrow derived EPCs in liver fibrosis in a mouse model system. Significant increase in the percentage of bone marrow derived endothelial cells in the liver together with an increase in the percentage

of collagen proportionate area (CPA) in the liver during the 4th week of liver injury suggested a substantial correlation between EPC numbers in migration and the degree of liver fibrosis. To conclude, this study illustrates an increase in the *in vivo* proliferation and migration of EPCs from the bone marrow to the circulation and then to the liver during CCl₄ mediated liver injury. The study also suggests that BM-EPCs do not participate in the *in vivo* endothelial repair in the liver via differentiating into resident endothelial cells; however they have an important secretory angiogenic function, which may serve as an important link between EPCs and liver fibrosis.

INDO-FOREIGN COLLABORATION

Proteolytic Antibodies in Immune-Mediated and Infectious Conditions: Role in Health, Disease and Therapeutic Implications at National Institute of Immunohaematology, Mumbai. In this International Associated Laboratories (IAL) program, Indian laboratory gained an insight into pathways related to mechanism of role IVIg in inflammatory and autoimmune diseases. The mechanisms of action of IVIg are complex, involving: Fc receptor blockade, interference with complement activation and the cytokine network, provision of anti-idiotypic antibodies, and modulation of T- and B-cell activation and effector functions. Such a broad range of activities reflects the numerous functions of circulating immunoglobulins in the maintenance of tolerance to self and immune homeostasis in healthy individuals.

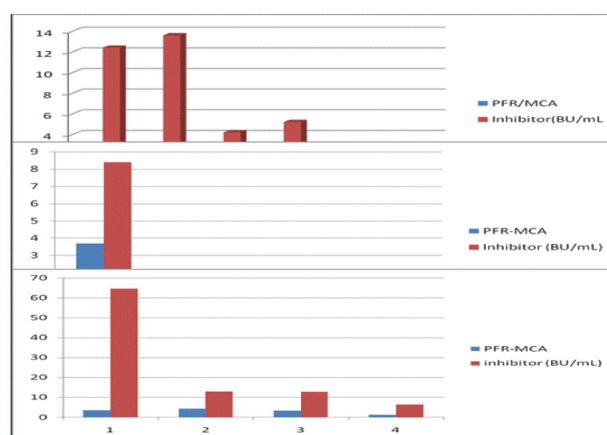


Fig. 10. Proteolytic antibodies in Immune mediated and infectious conditions.

A study on catalytic antibodies in autoimmune diseases such as Systemic Lupus Erythematosus (SLE) and Scleroderma as well as infectious diseases like Malaria and Tuberculosis to understand their role in immunopathogenesis and possible therapeutic immune modulation in these patients were done at National Institute of Immunohaematology, Mumbai. The majority of autoimmune diseases are characterized by the presence of high-affinity auto antibodies that form immune complexes with auto-antigens and stimulate innate immune cells via Fc receptors (FcR) to induce inflammatory mediators. Intravenous immunoglobulin (IVIg) exerts anti-inflammatory and immune-modulating effects through broad and possibly synergistic mechanisms. It can be understood that though various cellular, molecular mechanisms are involved are not mutually exclusive, they mainly contribute to the success of IVIg therapy in different inflammatory and autoimmune diseases. IVIg affects both cytokines and cytokine receptor levels. There is an increasing understanding of the immunological role of human regulatory T cells (Treg), but the direct effect of IVIg on this cell population is not known. While IVIg evidently inhibits T cell proliferation and T cell cytokine production, it is not completely clear to what extent these effects are dependent on the direct effects of IVIg on T cells or if they occur mostly through the inhibition of antigen-presenting cell activity including altered DC function. The

study of the IVIg mediated inhibitory signaling pathways on specific cells, including T and B cells and monocytes, is also an area where further explorations are needed. Thus to summarize, by this International Associated Laboratories (IAL) program, Indian laboratory gained an insight into pathways related to mechanism of role IVIg in inflammatory and autoimmune diseases. The mechanisms of action of IVIg are complex, involving: Fc receptor blockade, interference with complement activation and the cytokine network, provision of anti-idiotypic antibodies, and modulation of T- and B-cell activation and effector functions. Such a broad range of activities reflects the numerous functions of circulating immunoglobulins in the maintenance of tolerance to self and immune homeostasis in healthy individuals.

REGIONAL MEDICAL RESEARCH CENTRES

A total of 6 well established Regional Medical Research Centres at Jabalpur (MP), Port Blair (Andaman Nicobar), Bhubaneswar (Odisha), Jodhpur (Rajasthan), Dibrugarh (Assam) and Belagavi (Karnataka) are putting in their best efforts to improve 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 2015-16 is mentioned below.

REGIONAL MEDICAL RESEARCH CENTRE, BELAGAVI

Evaluating the effect of Shodhana procedure on Vatsanabha (*Aconitum chasmanthum* L.)

Vatsanabha (*Aconitum chasmanthum* Holmes, Ranunculaceae), is a widely used medicinal plant in Ayurveda and its roots are used for its medicinal value, but reported to be highly toxic. Traditionally the roots are purified, before its application, through the process called Shodhana. In classical text there are several purification methods but none are scientifically validated. In the present study, roots of Vatsanabha were treated with three classical preparations viz. Ajadugdha (Goat's milk), Godugdha (Cow's milk) and Gomootra (Cow's urine) along with plain water. The HPLC studies of Vatsanabha roots before and after Shodhana showed marked reduction in its toxin component Aconitin. The acute toxicity studies of treated and pre-treated groups in albino Wistar Rats of either sex using standard OECD guidelines, showed significant reduction in toxicity in all the post-purification groups.

Production of shikimic acid from in vitro cultures of selected plants of Western Ghats

Shikimic acid (SA) is a naturally occurring compound extracted from Chinese star anise (*Illicium verum*) fruits. The present study was conducted to identify viable sources to develop tools for production of SA using plant biotechnology approach. Out of 58 plants belonging to 25 angiosperm families only 14 yielded SA in excess of 1 mg/g dry weight, while the rest yielded low or no SA; and out of 18 families Clusiaceae, Ranunculaceae, Anacardaceae and Simaroubaceae had higher levels of SA. The protocol for the callus culture was developed for *Climatis gouriana* (Ranunculaceae).

Detection of adulteration by *Wedelia calendulacea* in *Eclipta alba* through ISSR and RAPD markers

Wedelia calendulacea (L.) Less is often used to adulterate the widely traded medicinal plant *Eclipta alba* (L.) Hassk, or 'Bhringaraja'. Inter Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD) assays were performed on 30 *E. alba* and four *W. calendulacea*. Samples of *W. calendulacea* were found to cluster in clades separated from *E. alba*, and one marker band was identified for each species with limited number of individual fingerprint. Despite variations in species phylogenetic analysis helped to differentiate both species as a cost-effective tool for detection of adulterants. The present analysis confirms that *E. alba* and *W. calendulacea* can be differentiated by the molecular phylogenetic inferences, and suggests that further evaluation of these techniques for authentication and quality control of crude drugs.

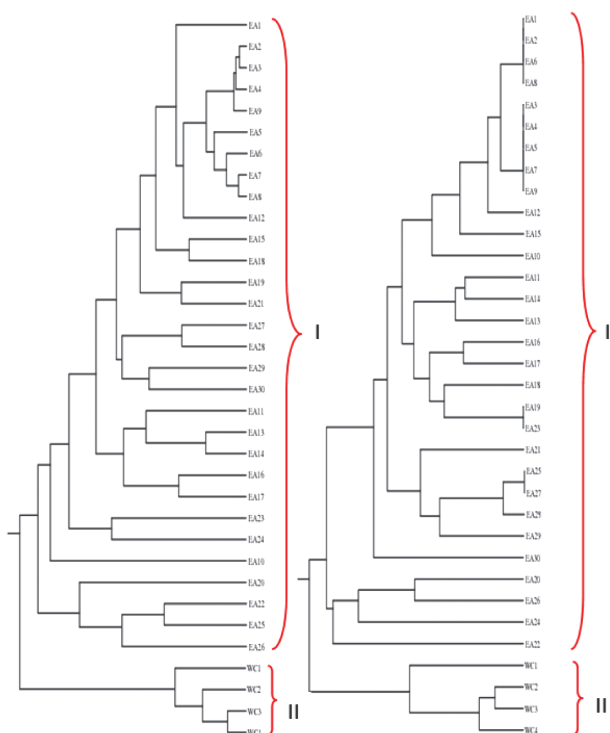


Fig. 1. Phylogenetic tree of *E. alba* (EA) and *Wedelia calandulaceae* (WC) samples.

Determining seasonal changes in phenolic compounds from *Saraca asoca* bark

Saraca asoca (Roxb.) Wilde (Fabaceae) or Ashoka is a rain forest tree found in Central Deccan plateau and Western Ghats. Its stem bark is used as a high valued medicine in gynaecological disorders, categorized as ‘vulnerable’ by IUCN. The present study aimed to investigate the seasonal variation of its polyphenolic compounds Gallic acid, Catechin, and Epicatechin with local market samples of *Saraca asoca* bark by reverse phase HPLC–diode array detector method. The results revealed that the sample collected during *Shishir Ritu* contained higher level of Epicatechin, while Gallic acid and Catechin were high in *Hemant Ritu*. Epicatechin was not detected in the market samples. However, Catechin content in market Sample was similar with the content detected from fresh bark sample of *S. asoca* in *Sharad* and *Vasanta Ritus*. The Gallic acid content was high in both market samples compared to fresh bark sample of *S. asoca*.

In-silico analysis of Gymnemagenin from *Gymnema sylvestre* (Retz.) R. Br. related to diabetes

Diabetes is a metabolic disorder characterized by higher glucose level in blood. Most oral hypoglycemic drugs produce adverse effects and thus companies are in continuous search for new agents with less or no side effects. Herbal drugs are considered as relatively safer alternatives and *Gymnema sylvestre* is one of the most well established remedy for diabetes world-wide. Here an attempt has been made to use *in-silico* techniques to predict the drug likeliness of Gymnemagenin, a major constituent of *G. sylvestre*, against 15 proteins having key role in carbohydrate metabolism. Gymnemagenin was found to dock well with crystallographic structures of 7 out of 15 selected targets and was found to be better than the known anti-diabetic compounds, Repaglinide and Sitagliptin. Thus, Gymnemagenin can be developed into a potent anti-diabetic drug.

In-silico network pharmacology study on bioactive-target interaction of anti-diabetic poly herbal formulation

Herbal remedies used for the management of diabetes, a complex multi-factorial metabolic disorder, but scientific evaluation of these remedies are hindered due to its polyherbal nature. In recent years, intricacies like drug-target interaction were understood with *in-silico* tools like system biology based bio-informatics or Network Pharmacology. Here we aimed to identify targets-bioactive interactions to understand the pharmacology of a poly-herbal formulation (*Curcuma longa*, *Embllica officinalis*, *Trigonella foenum graecum*). The data indicated that phyto-constituents/ bio-actives were interacting at multiple targets including Insulin and Glucagon receptors, Glucagon-like peptide-1, Peroxisome proliferator activated receptor alpha and gamma, SUR1-and SUR2-type K(ATP) channels, Sodium-dependent glucose co-transporters involved in glucose metabolism like intestinal glucose absorption, glucose dependent insulin release, cellular glucose transportation and excretion.

Establishment of Zebrafish Research facility

In the recent years, Zebrafish is increasingly used for preliminary evaluation of toxicity and bioactivity of test agents/drugs due to its simplicity, cost effective husbandry and maintenance, high

fecundity, translucent body which enable imaging and amenability to genetic manipulation. Moreover, Zebrafish has genetic and physiological similarity as well as response to drugs and chemicals with mammalian models. Additionally, Zebrafish is considered as a non-traditional model animal for evaluation of traditional medicines. A small scale preliminary facility for evaluation of herbal/traditional medicine has been established at RMRC Belagavi and several studies are expected to be conducted with this facility.

Molecular and immunological studies on lymphatic filariasis

Identification of a suitable marker for TAS is required to eliminate Lymphatic Filariasis (LF). Infective stage specific antibodies, mf and adult specific antigens hold promise. Thus, immunological and immunoepidemiological studies were undertaken in two LF endemic areas of Karnataka. Three genes of *Wuchereria bancrofti*: RMRC-BLG1, G2, and RMRC-BLP1 peptide, were identified as markers for the study. RMRC-BLG1 and G2 genes were cloned expressed and the recombinant proteins purified. Antigenic peptides of another protein antigen were identified through bio-informatics and synthesized. The RMRC-BLG1 recombinant protein and RMRC-BLP1 peptides were investigated for sero-reactivity in ELISA and both assays were able to differentiate the infected individuals from uninfected ones. An ELISA for detecting antifilarial antibodies was developed and is under evaluation for its efficacy in detecting microfilaraemic individuals. Community immunological surveys showed that new lymphatic filarial cases are emerging in endemic areas of Bijapur district, Karnataka. Analysis of mosquito vectors from these regions showed that LF is present in the mosquito vector. This raises concern that low level infection transmission is occurring in these villages which may explain the new cases we report. Similarly, evaluation of the immune status of the subjects living in these areas show that over 98% are non-immune, suggesting that there is a great risk of spread of LF infection, especially due to infective mosquitoes.

A prototype of miniaturized version of EC-biosensor using screen printed electrodes was developed that can detect filarial parasite DNA

in vector mosquitoes and in monitoring filarial elimination programme. This prototype needs to be assembled into a single unit for further application in filariasis elimination programme.

The analysis of genetic profiles based on the 29 bp tandem repeat sequences of Alt-2 gene indicated the existence of considerable genetic variability among *W. bancrofti* in two districts of Nepal. The study also showed that at least two genotypes, exhibiting high genetic differentiation existed in the areas. The two genetic 'variants' of *W. bancrofti*, may have important implications in filariasis epidemiology and control programme.

Micro mapping of G6PD deficiency among tribal's of India and its importance for anti-malarial therapy

About 200 samples were collected so far, from the Bedar tribe of Ningyanahatti, Belgavi District, and screened for G6PD deficiency, with one sample having indeterminate deficiency. Another 300 samples from Siddi tribe, Halyal division were collected and screened, of which 10% have G6PD deficiency. It is planned to collect more samples from different tribes of Karnataka state. Eventually, this multi-centric study will enable us to develop a micro map of G6PD deficiency among the tribes of India.

***Vibrio cholerae* O1 El Tor and emergence of Haitian *ctxB* variant strains from South India**

Cholera continues to be an important cause of human infection, especially in developing countries lacking access to safe drinking water and proper sanitation. We have reported the emergence of new variant form of *V. cholerae* O1 El Tor biotype with a novel mutation in *ctxB* from various outbreaks during 2010–2014 in Belgaum, Karnataka. A total of 14 occurrences of cholera were documented from Belgaum Division of North Karnataka during 2010 to 2014. All the *V. cholerae* O1 isolates were subjected to DAMA PCR to detect three different allelic subtypes of *ctxB* and PCR based detection of virulent genes, and subsequently 14 strains (one from each outbreak or sporadic case) were subjected to PFGE of the 14 *V. cholerae* O1 isolates from Belgaum Division *ctxB* gene sequence and pulsed-field gel electrophoresis (PFGE) analysis.

Out of 54 *V. cholerae* O1 strains obtained 21 strains isolated during 2010–2011 had classical *ctxB* and remaining 33 strains isolated during 2012–2014 belonged to Haitian variant. In the cluster analysis, PFGE profiles were divided into clades A with B. Clade A contained eight strains with 94 % similarity and Haitian type of *ctxB*. Clade B contained six strains and had Haitian type of *ctxB* except one with classical *ctxB*. To the best of our knowledge, this is the first report of the Haitian variant of *V. cholerae* O1 Ogawa causing outbreaks and sporadic cases of cholera in South India.

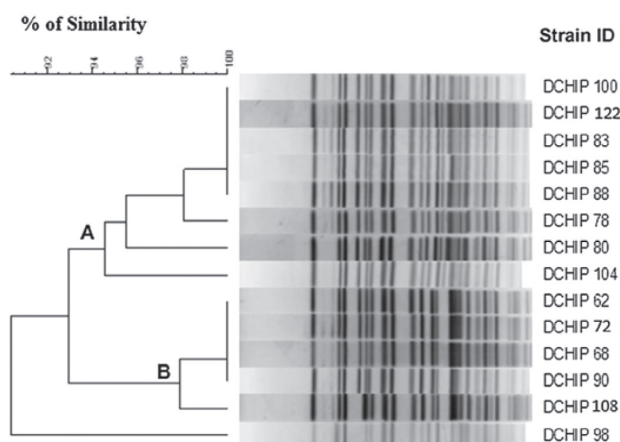


Fig. 2. Cluster analysis.

Developing a model of integrated approach for health in the selected tribal villages of Karnataka

A baseline survey was carried out in Ningyanahatti and Bommanahalli villages as part of developing a self-sustaining ‘Integrated Approach for Health’ model. It has a total population of 629 and 110 households. The major occupation in the village is agriculture (37%). The major drinking water sources for the villagers are open wells (76%), followed by bore wells (22%), and tanker supply (2%). Seventy four percent families drink water without any purification. Forty six percent of the families have the toilet facility in Ningyanahatti village; however 16% among them still practice open defecation, while 54% of the families practice open defecation because of lack of toilet facilities. Common cold and cough (30% each) were the most frequent problems in the village followed by fever (28%), body pain (5%), dysentery or vomiting (3%), weakness (2%) and blood pressure (2%). Thirty percent of the families face alcohol addiction among the family members. Forty seven

of the families in Ningyanahatti village visit private doctors for ill health conditions, where as 14% families prefer to go to Government Primary Health Centre at Handiganur, while 7% of the families use traditional medicines or home remedies. Thirty one percent of the families prefer both PHC (9 to 10 Kms) and private doctors (6 to 7 Kms @ Rajagoli, Daddi, Bombaraga) as their first choice of treatment for ill health conditions. The data for the last one year in the PHC indicated that the people in Ningyanahatti village have skin problems (Scabies) and loose stools as major ailments. Fifty one percent families in the village know home remedies for primary health care. However, only 5% use home remedies or follow traditional medicine and 12% know uses of few medicinal plants. Currently, 6% of the people grow medicinal plants for their use, as a result of activity initiated by RMRC, Belagavi. It is encouraging to note that 63% of families are ready to participate in training programs on traditional medicine/ home remedies, if conducted at the village level.



Fig. 3. Training Program in villages.

The Participatory Rural Appraisal activity was also conducted at Ningyanatti, which was resulted in prioritizing the primary health conditions, for

which the community preferred to have traditional medications.

II. MAJOR ACHIEVEMENTS HAVING PUBLIC HEALTH IMPORTANCE

- (i) Identification of pathogens and other services: RMRC has been providing identification & characterization services for various infectious diseases/pathogens to State/Govt. Hospitals, District Health Units and Medical Colleges throughout Karnataka.
- (ii) Outbreak investigation: Multidrug-Resistant *Vibrio cholerae* O1 was identified for a Cholera Outbreak in 2013 in Bagalkot, North Karnataka
- (iii) Demonstration of NDM-1-producing multidrug-resistant *Pseudomonas putida* in a pediatrics case of acute gastroenteritis, India

REGIONAL MEDICAL RESEARCH CENTRE, BHUBANESWAR

LYMPHATIC FILARIASIS

In order to support the strategy adopted in filariasis elimination programme, the centre has made an attempt to find out whether filarial infection in mothers during pregnancy has any role in increasing susceptibility to infection in the off springs. It is known that active filarial infection is associated with T cell hypo-responsiveness which is mediated by regulatory T cells (Tregs). Since the Tregs develop in the thymus from CD4⁺ CD25^{hi} thymocytes at an early stage of the human fetus, we have analyzed the role of maternal infection during pregnancy in development of Tregs in children at birth as well as early childhood. We have observed a significantly high as well as a positive correlation of Tregs and IL-10 levels in children born to infected mother than the children of uninfected mother at the time of birth as well as early childhood indicating that Tregs and IL-10 contribute to immune modulation during pregnancy. Since ongoing MDA excludes pregnant mothers and children below 2 years of age, hence implementation of supervised therapy at the time of adolescence through MDA may help the programme in achieving the target of global elimination of LF by 2020.

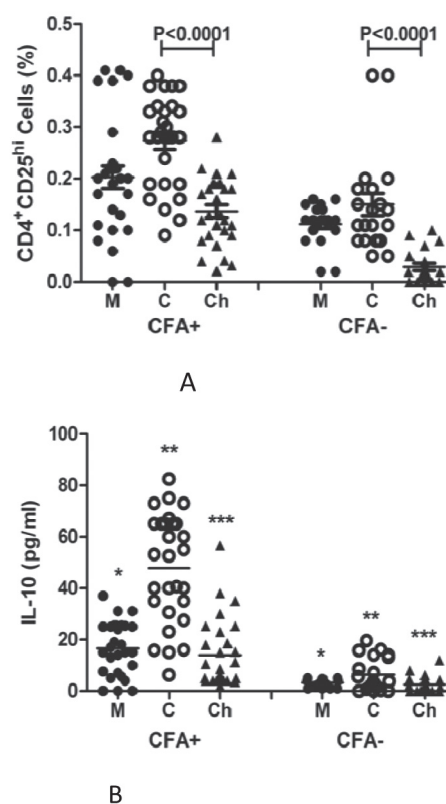


Fig. 4. Tregs (A) and IL-10 (B) in mother (M), cord (C) at baseline and in children (Ch) at the time of follow-up in enrolled infected (CFA+ve) and uninfected (CFA-ve) mothers.

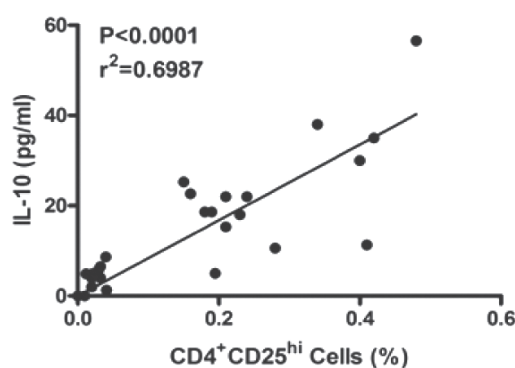


Fig. 5. A positive correlation between Treg and IL-10 in children born to filarial infected mother.

Further, it is a fact that immunization is key to control infectious diseases but efficacy of certain vaccines has been observed to be poor in tropical and developing countries. There are reports that helminthic infections induce impaired cellular and humoral responses to non parasite vaccine antigens. But no report is available on immunological response induced by filarial infection that biases

immunity and diminish the efficacy of vaccination. The study conducted by the centre during this period has shown decreased levels of IgG antibodies to TT in cord samples born from *W. bancrofti* infected mothers compared to uninfected mothers indicating that infection with *W. bancrofti* is associated with an impaired immune response to TT vaccine antigen, as reflected by relatively impaired antibody responses to TT. Further decreased levels of IgG antibodies to BCG in cord samples born from filarial infected mothers compared to uninfected mothers indicates down regulation of IgG response in cord blood of infected mothers. In addition to this Ig G isotypes antibodies response to BCG in filarial infected and uninfected mother reveals a decreased level of IgG3 antibodies to BCG in cord samples born from uninfected mother compared to infected mother while IgG2 and Ig G3 immune responses to BCG are down regulated in cord samples of infected mothers. Impaired antibody responses to TT indicate that infection with *W. bancrofti* is associated with an impaired immune response to a vaccine antigen TT and BCG. The study is on progress and the observations will provide a scope to re look in to immunization policy in filarial endemic area.

MALARIA

Though Odisha is endemic for malaria, yet coastal belt (Nayagarh, Ganjam, Balasore, Puri, Cuttack, Jajpur, and Jagatsinghpur districts) is known to be free from malaria except few sporadic cases reported till 2012. However, gradual increase in malaria cases has been reported from the above districts. An entomological survey conducted as per the request of NVBDCP, revealed a high density of *a. culicifacies* with presence of sporozoite indicating indigenous transmission. A separate study conducted in hilly and forest areas reveals two peaks of malaria transmission in these areas with *An.fluviatilis* as predominant vector. Further, the seasonal mosquito collection data of Dhansuli and Sastiguda Subcentre of Kalahndi district revealed that *Anopheles culicifacies* was prevalent throughout the year with peak density in summer season. In Ichapur Subcentre *An.culicifacies* density was found higher in summer and rainy seasons. *An. annularis* is found with maximum density during winter season in foothill, *An.*

culicifacies is found with maximum density during winter season in riverrine and *An. fluviatilis* is found with maximum density during winter in hilltop ecotype. Our study recommends two rounds of Insecticidal Residual Spray (IRS) with DDT spray for hilly /forest areas and two rounds of IRS with synthetic pyrethroid(SP) spray for plain areas where *An.culicifacies* is the predominant vector. The LLIN may be continued in high/priority areas as per the NVBDCP plan to check the transmission.

Recently there is a changing trend not only in the clinical manifestations but also the pattern of complications in malaria. Over a decade ago, cerebral malaria was the predominant manifestation of severe malaria, where as today the combination of jaundice and renal failure are more common world over. Since the incidence of acute kidney injury (AKI) is increasing and all the four species of malaria parasites are found in Odisha, the present study has been undertaken to know which parasites are involved in the AKI manifestation and the host and parasite genetic factors associated with it. The preliminary observation reveals that the most prevalent malaria parasite associated with AKI in this geographical area was *Plasmodium falciparum* followed by *P vivax* and mixed infections of *Pf+Pv+Pm*. With a limited sample (healthy control: 98, acute kidney injury: 45 and malaria attributed AKI: 46) we predict a strong association of all three regions of Pf EMP DBL α (FR, F1R2 and F2R2) of the parasite with malarial acute kidney injury, while individuals with eNOS ED (p value=0.032) polymorphism to be associated with AKI and individuals with ACE2 TC (p value=0.01) polymorphism more prone to develop MAKI.

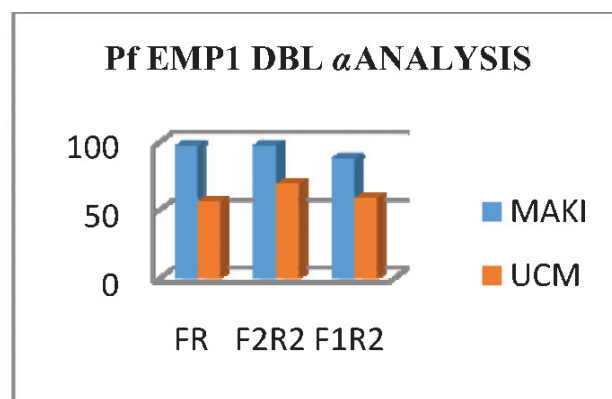


Fig. 6. Genotypes of Pf EMP DBL α in cases with MAKI and UCM.

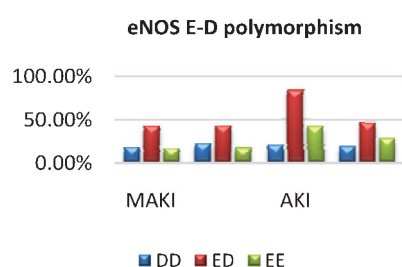
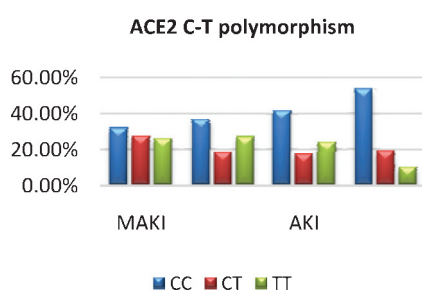

Fig 7 (A)

Fig 7 (B)

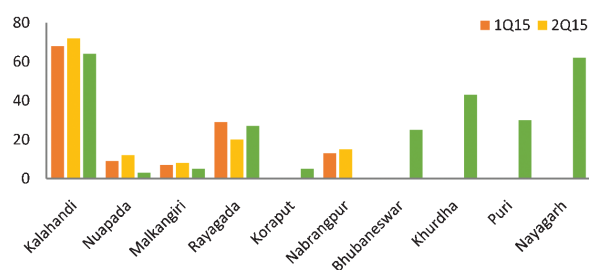
Fig. 7 A & B. Genotypes of eNOS ED and ACE 2 CT polymorphism in MAKI and AKI cases.

DIARRHEAL DISORDERS

Cholera, a devastating disease is caused by *V. cholerae* O1 and O139 serogroups. Currently *V. cholerae* O1 belonging to El Tor biotype is causing all the outbreaks while O139 serogroup has declined considerably over the past few years. The centre is continuously monitoring to identify the bacterial pathogens causing diarrhoea and their drug susceptibility pattern to help the local health authorities to reduce morbidity and mortality due to diarrhoea especially cholera.

TUBERCULOSIS

The RNTCP approved tuberculosis drug susceptibility testing laboratory has been providing support to the state of Odisha in MDR TB diagnosis for 10 districts and follow up culture for MDR TB patients on DOTS Plus treatment for 10 districts. Up to 3rd quarter of 2015, we have detected 23 MDR TB by Line Probe Assay and 2 MDR TB additionally by solid culture (Fig. 1).


Fig. 8. MDR TB diagnostic sample processed.

VIROLOGY NETWORK LABORATORY

This Centre is continuing surveillance and outbreak investigation of important viral diseases in the region through the established Grade-I viral lab as a part of network of viral research and diagnostic laboratories in India. During 2015-16, forty outbreaks were investigated that revealed Hepatitis A & E, Measles, Chickenpox, Dengue, Chik and JE causing morbidity and mortality. Hepatitis E outbreak was confirmed in Khurda, Kalahandi, Bolangir and Puri districts. JE outbreak has been confirmed from Jajpur, Keonjhar and Puri districts. Measles infection was investigated in Raygada and Ganjam district. The investigation report along with recommendation for control supported the State Public Health system for taking early prevention measures to control further spread.

From hospital surveillance activity, 4908 numbers of blood samples were collected for suspected AES, Dengue, Chik, JE, Chickenpox, Hepatitis, Diarrhoea and H1N1 from different hospitals of the state. Patients suspected of viral illness admitted to different secondary/ tertiary care hospitals of the state were provided diagnosis that supported case management. The observations on clinical presentation and viral agent identification improved the clinical diagnosis by the physicians of the region through information sharing and publications. One of the major findings showed HSV I and II as commonest viral agent for AES that promoted use of antivirals in management of suspected cases reducing morbidity and mortality.

Manpower training and capacity building in the field of viral diagnosis an important aspect of the centre. The lab provided skill development and training through Ph.D (4), M.Sc. dissertation (17) M.D. (3) and summer training (20). Training imparted

to 26 laboratory technicians from 12 district head quarter hospitals and NVBDCP on Dengue, Chik and JE virus diagnosis which created independent facilities at the respective districts. Also this lab is providing technical support to medical colleges, public health labs in different districts of the state and outside state (Andhra Pradesh). Quality control has also been initiated with these labs.

The centre has initiated the surveillance programme for rotavirus in children below 5 years as one of the selected site of a multicentric study in the virology grade –I laboratory facilities. The objectives of the investigation were to examine trend and pattern of diarrhea attributable to rotavirus, seasonal distribution, outcome assessment, prevalent strains and estimate economic burden of rotavirus gastroenteritis. The study started in 2014 and its continuing with regular project activity including filling of clinical report form (CRFs), sample collection and genotyping. During the period of 2015-2016, 438 cases were enrolled and 249 samples were collected. Overall prevalence of rotavirus infection for this period was 40%. The most common genotype observed was P [8] & G[1].

During the investigation period 2014 to 2016, 1244 cases were enrolled. Most of the samples that could be typed were P[8] and G[1]. 30-40% of the P and G types could not be typed. . The data generated from this study from this and other centres helped in deciding to introduce Rotavirus vaccine in national vaccination programme by the government.

TRIBAL HEALTH

Clinical, Anthropometric and Biochemical (CAB) survey has been carried out first time to produce district-wise data on prevalence of hypertension diabetes in adults and under-nutrition- and over-nutrition, micronutrient deficiencies in all age and physiological groups. The survey revealed that the state population is experiencing double burden of malnutrition and emergence of lifestyle disorders (hypertension, diabetes) that may result in structural changes in disease patterns. Under-nutrition is widely prevalent in southern KBK and tribal-dominant districts, while overweight/obesity and lifestyle diseases are emerging to be public health problems in coastal (Cuttack, Puri, Jagatsinghpur, Jajpur,

Baleswar) and northern (Jharsuguda, Sundargarh) districts. These biomarkers can serve as diagnostic tools to identify diseases in their early stages and can be used as surveillance tools to track changes in disease patterns or to evaluate intervention programs and can also be used at a macro level to measure the long-term effect of policies and programs. By using this data, programme implementation plans can be drawn up, funded and implemented at district level, enabling to bridge the gap between poor and good performing districts and by demonstrating good quality assessment of health and nutritional status in community setting. The baseline data of CAB survey is useful for district specific programme implementation plans serve to contribute immensely to rapid improvement in health and nutritional indices in Odisha by making available district level data, enabling to bridge the gap between poor and good performing districts and by demonstrating good quality assessment of health and nutritional status in community setting.

OPERATIONAL RESEARCH

To find out the effectiveness of diet and lifestyle intervention through Information Education Communication (IEC) tools with AnganWadi Centres (AWCs) as the centre of knowledge dissemination for hypertension (including hypercholesterolemia and diabetes) risk reduction- a cluster randomized controlled trial has been initiated in Kalahandi district. Baseline data collection was followed with an intervention over a period of 18 months and endline survey on the tablet software. The prevalence of hypertension was found to be as high as 20% in Kalahandi district. During intervention 1048 households were visited and meetings/activities were conducted among the village population, ward members, GKS members, Self Help Groups (SHG) and Mahila Mandal available in the respective villages. IEC materials in local language were provided with training to AWWs and training of staff on IEC delivery at household and village level. Health care workers were trained to create awareness about changes in diet like salt restriction, decreased oil consumption, decrease in consumption of tobacco and alcohol and increasing physical activities.

TRANSLATIONAL RESEARCH

Improving health of under five children in Raygada and Kalahandi district

A translational research program was undertaken for improving health of under five children in Raygada and Kalahandi districts of Odisha through health system strengthening. Morbidity survey, health care facilities and immunization coverage was assessed by household level interview. This indicated diarrhea, URTI and malaria to be the most prevalent morbidities, home delivery still being reported in around 16% registered mothers. Knowledge of health care providers in IMNCI illnesses and care of children were assessed that reflected deficiency in diagnosis, severity assessment as well as care of illnesses. Community behavior and perception towards modification in the program, if any, were assessed by PLA approach conducting group discussions with community and community leaders. It was planned to strengthen the existing IMNCI program by skill development of the present health workers, community participation for improving patient referral mechanism and improving logistic supplies and information/feedback system in the existing district health plan. Taking into consideration the education level of the ASHAs, training module and ready reckon was developed in local language and IEC materials prepared. The first round of training was given to ASHAs and AWWs on IMNCI illness management skills. To improve logistic supply, interactive meeting was held with local health system and to facilitate referral of cases to PHCs village level plan was made for utilizing local funds like GKS funds and local transport mechanisms. As preventive measure and hygiene practice by school children was initiated and it is planned to deliver the IEC messages on common illnesses prevention through school going children educating their own community. For effective delivery of family and community interventions we have selected school children as vehicle. We have introduced a “Thought of the Day” discussion session for 10 minutes after the prayer everyday on hand wash practice, use of mosquito net, use of toilet and seeking of treatment at hospital for any ailment by the family members. We have created awareness among the community through the students by arranging procession with

certain slogans and placards on prevention and control of IMNCI related diseases. Community awareness campaign on hand washing practice, use of safe drinking water, hygiene, use of mosquito net, immunization and treatment seeking in case of sickness due to diseases classified in IMNCI were organized through local health work force in each village. The intervention is ongoing which will be evaluated in mid-term for any medications in consultation with district health authorities.



Fig. 9. Focus Group Discussion



Fig. 10. Hand Wash practice.



Fig. 11. Social mobilization through school children.

Development of Quadruplex PCR Kit to identify of *V. cholerae* O1

Since untreated cholera leads to the onset of serious outbreaks and potentially great devastation, quick diagnosis is necessary from public health point of view for appropriate antimicrobial therapy and to chase the spread of the outbreak. Conventional methods of diagnosis are not

suitable for early detection because it needs a battery of biochemical tests, toxin assay, and slide agglutination with specific antisera. This takes long time about 72 hours. In this connection, to overcome these problems the centre has developed a Quadruplex PCR based molecular diagnostic Kit for simultaneous detection of serotype, biotype and toxigenic potential of *V. cholerae* O1. This is an easy, rapid, accurate and cost effective method. Third party validation of this Kit at NICED Kolkata and ILS, Bhubaneswar shows 100% sensitivity and specificity.

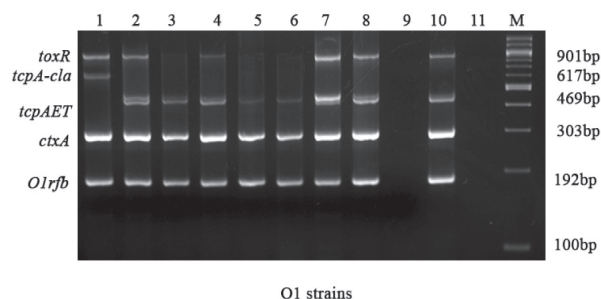


Fig. 12. Bands of various markers & virulent genes for detection of *V. cholerae* O1 employing PCR Kit.

FACILITIES

Designated Microscopy Centre (DMC) and DOTS site

The center has opened a Designated Microscopy Centre (DMC) and DOTS site in OPD for diagnosis of suspected TB patients with pulmonary and extra pulmonary symptoms. Till now 162 pulmonary TB patients and 52 Extra Pulmonary TB cases were tested by AFB microscopy and solid culture on Lowenstein Jensen media.



Fig. 13. Inauguration of DMC and DOTS site by DIC Dr N Mohapatra.

REGIONAL MEDICAL RESEARCH CENTRE, NE REGION, DIBRUGARH

EPIDEMIOLOGY AND COMMUNICABLE DISEASES

Prevalence of bacterial pathogens colonizing the genital tract of pregnant women and comparison with those causing early onset neonatal sepsis

A total number of 668 pregnant women were recruited during the study period and vaginal swabs were obtained from the recruited cases at different gestational period. Of the total 1137 swabs collected. 345 (51.6%) of them were obtained at 35-37 weeks of pregnancy. Colonization rate of Group B *Streptococci* (GBS), *Escherichia coli* and *Enterococcus* at 35-37 weeks was 15.1%, 7.8% and 33% respectively. The overall GBS colonization rate was 20.4% (136/668). The GBS colonized mother had also shown co-colonization with *E. coli* (22.1%), *Enterococcus* (53.7%) and yeast (45%). Among the neonates delivered to the colonized mothers, 125 of them were of normal birth-weight while 8 of them were of low birth weight. A total number of 8 neonates of these colonized mothers had developed sepsis, even though none of them were positive for GBS by culture, while one neonate delivered by a non-colonized mother was blood culture positive for GBS.

Study of interepidemic survival of *Vibrio cholerae* in outbreak prone areas of Assam

The study was conducted in three tea gardens one each from Golaghat, Dibrugarh and Jorhat districts of Upper Assam. A total of 203 rectal swab from diarrhoea patients were collected, of which 173 were collected from the study sites and remaining 30 during cholera outbreaks that had occurred in North Lakhimpur and Sibsagar districts. *V. cholerae* was isolated from the samples collected from both the districts during the outbreak. A total number of 113 environmental samples (water & plankton) were also collected from Sibsagar, Golaghat and Jorhat districts of which 30 were positive for *V. cholerae* specific ompW gene but were negative on culture. The frequency of positivity in the environmental samples was seen to gradually increase from January to June 2016 in the different sites which

coincided with the outbreak in June 2016 in a tea garden of Sibsagar district.

Epidemiological typing of *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* isolated from septicemic patients in North East India

This collaborative study aims to determine the prevalence, clinical spectrum and molecular characterisation of the lysine-positive NFGNBs (*B. cepacia* complex and *S. maltophilia*) amongst the septicemic patients. Standardization of phenotypic & molecular identification of *B. cepacia* complex as well as *S. maltophilia* at RMRC has been done. Ten isolates were confirmed as *S. maltophilia*. The isolates were obtained from Jorhat Medical College, NEIGRIHMS, Shillong and RMRC, Dibrugarh. One isolate of *Burkholderia* has also been obtained at RMRC, Dibrugarh. MLST of 4 isolates from different region of northeast were done and none of the isolates were completely similar to any of the STs available in the *S. maltophilia* MLST website. The closest similarity of the Dibrugarh isolate was to ST37, Jorhat isolates to ST119, ST23, ST 106, ST160, ST 168, and Shillong isolate to ST99. Further work is under progress.

Surveillance of Infection in neonates (0-28 days) (Multi-centric ICMR project)

A total of 162 bacterial isolates from neonatal septicemia cases were received from AMCH, Dibrugarh during the period for further testing. The predominant bacterial agent causing neonatal septicemia was found to be *Klebsiella pneumoniae* subsp. *pneumoniae* (38%) followed by *Staphylococcus aureus* (32%). High degree of drug resistance was observed in the bacterial isolates. Another 85 *Klebsiella* strains were received from AIIMS New-Delhi for NDM-1 testing at RMRC, Dibrugarh. Carbapenem resistance gene (NDM-1) was detected in 68.2%, of *Klebsiella* strains ESBL was detected in 37% of GNB. MRSA was detected in 43.1%.

Epidemiology of scrub typhus in selected areas of Assam and Arunachal Pradesh

289 human blood samples were collected which included 98 samples from hospitalized patients

presented with unidentified fever and Acute Encephalitis Syndrome (AES) suspected cases and 191 follow up clinical cases. 36.6% (106/289) of the samples demonstrated antibodies against *Orientia tsutsugamushi*, of which 75.4 % (80/106) and 24.5 % (26/106) were from Assam and Arunachal Pradesh respectively. Among the hospitalized cases, ST antibodies were detected in 38.7% (38/98), of which 33 cases were from the initially suspected AES patients. Fever was recorded as the primary symptom followed by headache and body ache. Phylogenetic analysis revealed three distinct clusters (I, II & III). One sequence formed a different clade, but was seen to be more related to Cluster III. The sequences closely resembled to the most prevalent strain 'Karp'. Entomological and rodent surveys were conducted in some scrub typhus affected sites. The collected rodents were identified as *Rattus rattus brunneusculus* (17 no.), *Mus musculus* (10 no.) and *Rattus nitidus nitidus* (5 no.). Ecto-parasites were collected from the trapped rodents as well as domestic pets. A total of 235 ecto-parasites were collected during the period. No *Orientia* DNA was detected in the mite (chigger) collection. However, rickettsial DNA was detected in 25% (4/16) *Ctenocephalides felis* for citrate synthetase (*gltA*) and outer membrane protein (*ompB*) genes respectively. Evolutionary analysis revealed divergence of 0.2% with *Candidatus Rickettsia senegalensis* strain of Senegal, Africa for *gltA* gene, whereas no divergence in *ompB* gene was observed.

Multi-site Epidemiological and virological monitoring of human influenza viruses in India, Phase-II

During the study period, 3171 samples were tested for influenza virus by real time RT-PCR. and 17.6% samples were found to be positive for influenza virus. Type-A/H3 (188), Type-A/H1 (165) and Type B (204) was detected. The maximum number of influenza virus detected was in the age group >5 to 30 years (335; 60.1%). Out of the 557 PCR positive samples, influenza virus could be isolated (in MDCK cell lines) from 231 (41.5%) samples; of which Type-A/H3 (58), Type-A/H1 85 and Type B (88) isolates.

National Hospital Based Rotavirus Surveillance Network at RMRC, Dibrugarh

The project commenced from Feb 2013 and till date 1316 subjects have been recruited from Dibrugarh and Dimapur. The overall prevalence of rotavirus was 37.4%. During the period a total of 383 subjects were recruited in the project and 333 provided samples for analysis. The rotavirus prevalence was 34.5% (95%CI:29.4% to 39.6%). Prevalence was slightly higher in Dibrugarh compared to Dimapur (37% vs 31%). The prevalence was also higher in infants compared to 4-5yrs old (40.2% vs 18.8%). The prevalence of rotavirus showed a distinct seasonal pattern with highest incidence in winter months. The positive cases were low in Jun to Nov. As per the project protocol, every 3rd rotavirus positive sample were subjected to genotyping for G and P types using type specific primers by multiplex RT-PCR for vp7 and vp4 genes respectively. During the period, G9P [4] was the predominant genotype, followed by G1P [8].

Establishment of a network of Laboratories for managing Epidemics and Natural Calamities (Regional Level Viral Diagnostic Laboratory)

This is an infrastructure building project cum development of a functional Viral Research and Diagnostic Laboratory funded by DHR. The construction for a two-storied Lab building has been handed over to the CPWD and work for construction of the building is undergoing. The project has the mandate to construct a 1206 SQM two-storied new laboratory building. During the period Jan – Dec 2015, viral diagnoses were provided to 1061 number of patients attending various hospitals and health centres. A total of 2115 tests were carried out over the total collected samples. The facility has developed capacity to provide diagnosis for upto 35 different viral infections. In 2015, five outbreak of suspected viral infections has been investigated including outbreak of Chickenpox, Measles in Goalpara, Rubella in Lakhimpur and Dengue in Pasighat. The last outbreak investigated was a massive outbreak of Dengue fever in Pasighat, East Siang, Arunachal Pradesh where > 6000 suspected cases have been recorded. A subset of suspected DF was analyzed by the VRDL at RMRC, among which 91 cases were positive for DENV NS1 Ag while another 24 were positive for DENV IgM ab. Among

the 91 NS1 Ag positive cases subjected to DENV typing by RT-PCR, 66 could be typed by RT-PCR. The predominant serotype of DENV detected in the 2015 outbreak in Pasighat was DENV-1 (60 /66).

Study of Host Innate Immunity and Hepatitis B persistence

A total of 619 subjects have been recruited for the study out of which 167 (27%) are from Assam, 309 (49.9%) from Arunachal Pradesh and 143 (23.1%) from Tripura. Among the subjects recruited 296 are cases with a median age of 32 years (range 6 - 80 years). HBV viral load was performed in 275 subjects. Low viral load (≤ 1000 IU/mL) was detected in 35.2%, intermediate viral load (1001-20,000 IU/mL) was detected in 18.1%, high viral load (> 20,000 IU/mL) was detected in 28.6% of cases and in 18.1% of cases no target was detected. Overall 30.7 % of control subjects were positive for HBeAg signifying past contact with the virus and have spontaneously cleared the infection. The prevalence of Hbc Ab was low among subjects aged upto 15 yrs. Genes downregulated in CHB compared to spontaneously cleared infection: IFNG, BTLA, CTLA4, CD274 (PD-1), CD40LG, TLR1, TLR2, TLR4, TLR6 (2 fold to 3.5-fold downregulation). Genes upregulated in CHB (HBeAg +ve) compared to HBeAg-ve: BTLA (3.2 times upregulated).

Multi-site Epidemiological and Virological survey of Nipah virus: Special emphasis on North East Region of India

A total of 253 (Male 139, Females 114) samples (serum / CSF) were collected from AES cases of different districts of Assam. Out of the collected samples, 24 were Nipah IgM Elisa positive and 3 PCR positive. Most of the Nipah IgM positive cases were from the districts near to Bangladesh border area. To identify the natural host, fruit bats were trapped by NIV, Pune team for detection of the virus and also around 29 pig samples were sent to NIV, Pune see the Nipah activity in Assam.

Synthesis of hybrid class Phenylthiazole 1,3,5 Triazine derivatives as novel Antifolate for Pf-DHFR-TS inhibition

During the reporting period a total of 15 compounds were screened for *in vitro* antimalarial activity

against chloroquine sensitive 3D7 strain at two fixed dosage i.e 5.0 and 50.0 µg/ml. Among these, 3 compounds have shown promising antimalarial activity.

A Study on eco-epidemiological perspectives of emerging West Nile Virus in Assam

A total of 21,422 mosquitoes collected during the reporting period were pooled species wise into 620 pools. *Culex vishnui*, *Ma uniformis* and *Cx tritaeniorhynchus* were the predominant species constituting 30.81%, 21.62% and 14.87% of the total catch respectively. All mosquito pools were subjected to reverse transcriptase-PCR and 2 pools (1 pool of *Culex vishnui* and 1 pool of *Cx. pseudovishnui*) showed amplification of 500bp of NS1 gene of WNV. Sequence similarity ranged from 97- 99% based on the WNV NS 1 gene. It was observed that the increase in abundance of three incriminated WNV vectors viz. *Ma. uniformis*, *Cx. vishnui* and *Cx. whitmorei* coincided with chicken seroconversion peak. Warmer temperature and rainfall were found to have a substantial impact on the abundance of WN vectors.

Surveillance of Chikungunya virus activity in Assam and Meghalaya

This project aims to find out morbidity pattern, social status, clinical spectrum, long term effect among the patients suffering from Chikungunya infection in North-East region particularly in Assam and Meghalaya, and to study the prevalence of mosquito vectors and their role in transmission of the disease in different ecological setups. Of the total 526 samples suspected but negative for JE, West Nile, Scrub typhus and Dengue, 32 (6.07%) were found positive for Chikungunya IgM antibody. Of these 24 samples were MAC-ELISA positive and 9 were RT-PCR positive.

Effectiveness of single dose of live attenuated SA14-14-2 vaccine against Japanese Encephalitis in adults over a period of three years in two districts of Assam, India

This study aims to evaluate the effectiveness of the single dose of SA-14-14-2 Japanese Encephalitis (JE) vaccine administered in adults in two districts (Sivasagar and Dibrugarh) of Assam over a period of 1 to 3 years post vaccination and in Sivasagar over a period of five years post vaccination. JE

positivity among them using IgM capture ELISA kit (NIV, Pune, India) was 26.92% (7/26) from Dibrugarh and 16.66% (2/12) from Sivasagar.

A study on the bionomics of the potential vector species of Japanese Encephalitis (JE) virus transmission in Assam

This study was newly initiated with the objectives to determine the seasonal abundance of potential JE vectors and other parameters like resting habits (endophily/exophily), breeding habits and habitat, incrimination of the vector by detection the virus in mosquitoes, identify the host blood meal preference and the extent of anthropophily of the vectors and to detect the insecticide susceptibility of the vector.

So far, 6 localities from each district were selected from where at least one JE case had been previously reported. A total of 13,057 female mosquitoes have been collected (dusk collection) comprising of 14 species. *Cx. vishnui* was the most predominant species comprising (39.67%) of the total mosquitoes collected. Among the known JE vectors, the most abundant species was *Cx. vishnui* (37.29%), followed by *Cx. tritaeniorhynchus* (13.20%), *Cx. quinquefasciatus* (10.56%), *Cx. pseudovishnui* (9.52%), *Cx. whitmorei* (2.61%), *Cx. fuscocephala* (1.98%), *Mn. annulifera* (0.65%) and *Cx bitaeniorhynchus* (0.02%)



Fig. 14. Collection of mosquitoes from field.



Fig. 15. Identification of mosquitoes species wise for blood meal analysis using dropnet and smearing blood on Whatman no-1 filter paper technique.

Adenoviral Vector based diagnostics for Japanese Encephalitis Virus

This is a collaborative study between RMRC, Dibrugarh and IITG, Guwahati which aims to carry out cloning of surface glycoprotein and non-structural protein genes of Japanese encephalitis Virus (JEV) in adenoviral vector, expression analysis of JEV proteins in different mammalian cell lines and optimization of enzyme linked immuno absorbent assay (ELISA) developed using the viral neutralizing glycoproteins with different clinical serum samples. During the period, the surface glycoprotein gene (E2) and non-structural protein gene (NS1) of JEV (SA-14-14-2 and Assam isolate of JEV) have been cloned in bacterial cells (DH10B) using pET- 28a (+) vector.

Lymphatic filariasis endemicity mapping in the state of North East India

During the reporting year field visit was carried out in West and North Tripura districts with the aim to collect information about disease awareness, knowledge about preventive measures and risk factors associated with lymphatic filariasis. The survey was carried out in West Tripura among 46 clusters (Gram Panchayat) selected by PPS sampling in West district. Among the surveyed population awareness about lymphatic filariasis was 11.4%, of whom only 9 (24.32%) knew that it is caused by mosquito bite. Entomological study was carried out at seven study sites by collection of morning indoor resting mosquitoes. A total of 567 mosquitoes observed for presence of larval stages (L1, L2, and L3) of *W. bancrofti* were found to be negative. In North Tripura, awareness about lymphatic filariasis is only 1.2%. Use of mosquito nets in the surveyed population (99.6 %) is significantly high. All of 267 mosquitoes collected were negative for presence of larval stages of *W. bancrofti*. Finding indicates that though clinical cases of elephantiasis are present, no active transmission of the lymphatic filariasis was found.

ICMR Virology network Lab- grade-1

The ICMR Virology network laboratory-grade-I at RMRC, Dibrugarh was sanctioned by ICMR on 25th March 2013. The project also under its infrastructure development part renovated approx. 3500 sqft

area of Laboratory building. After completing all the necessary ground work including recruitment of manpower in the August 2013, the project started with an aim to provide free diagnostics to some of the important viral pathogens prevalent in the region using current diagnostic technology including molecular techniques. The laboratory provided capacity to give diagnosis to a total of 30 viruses of different classes (comprising of classical, emerging and re-emerging) infecting human using latest techniques such as ELISA, conventional PCR and real time PCR. The representative viral isolates were further subjected to nucleotide sequencing for genetic characterization. A total of 1501 samples were given diagnosis for 2470 test, over a period of 2 years (2013-15). Additionally, outbreaks of measles, rubella and chickenpox were investigated and confirmed from Assam during 2015.

Prospective study of hepatitis B virus infection and its Genotypes in the North-Eastern India

This multi-centric ICMR study on HBV and its genotypes distribution on various types of liver disease, including chronic, acute, fulminant hepatitis, cirrhosis and HCC cases. The study also collected data on HBV prevalence among health workers and blood donors. The study recruited a total of 810 subjects from Assam Medical College, Dibrugarh, & Lower Dibang valley & Tirap district of Arunachal Pradesh. A total of 638 and 172 subjects were recruited from Upper Assam and the two eastern districts of Arunachal Pradesh respectively. The prevalence Hepatitis B surface antigen (HBsAg) among blood donors was 0.6% (2/355; 95% CI=± 0.8%). Only 25.3% (76/300; 95%CI=±4.92; 20.4 to 30.2%) prospective blood donors had protective level of HBsAb above 10mIU/ml. Among health workers, HBsAg was detected in one out of 128 (0.78%) screened for HBsAg (95% CI=±1.52). Overall, 67.1% of health workers doesn't have protective antibody for HBV infection. The most common genotype of Hepatitis B virus detected in Acute viral hepatitis (AVH) cases was genotype D (13/15) followed by genotype C (2/15). The most common HBV genotype detected in cases of CHB from Arunachal Pradesh was genotype C (42.1%; 16/38), followed by genotype D (36.8%; 14/38), genotype I (15.8; 6/38) and genotype A in 2 cases (5.3%), While

in Assam the most common genotype in CHB was genotype D in 72.8% (32/44) followed by genotype C (15.9%) and genotype A (11.4%). A total of 131 case of suspected cirrhosis cases from AMCH, Dibrugarh were recruited in the study. The prevalence of HBsAg was 48.1% (63/131) among the cirrhotic patient. HBV genotype was done in 33 cases of suspected cirrhosis and the most common genotype found was genotype D (84%) followed by genotype C (12%) and genotype A (3%). A total of 13 cases of HCC were recruited in the study out of which 10 cases were from Arunachal Pradesh and 3 cases from AMCH, Dibrugarh. HBV genotyping was done in two cases and was genotype I and the other was genotype D. A total of 135 HBV cases has been genotype using TSP-based PCR and HBV DNA Sequencing. S-gene partial sequencing (~400 bp) was performed on 56 samples for genotyping by sequencing method as well for screening for HBV surface gene mutation including the 'a'-determinant region of HBV S-gene. The most common genotype in Upper Assam was found to be genotype D (79%; 73/92) followed by genotype C (13%; 12/92) and genotype A (7.6%; 7/92). While in Arunachal Pradesh, the most common genotype was genotype C (42.9%; 18/42) followed by genotype D (35.7%; 15/42), genotype I (16.7%; 7/42) and genotype A (4.8%; 2/42). The difference in prevalence of genotypes in the two geographical regions is highly significant ($p=0.0001$).

With regard to mutations in surface protein gene of HBV, although escape mutant G145R was not detected, however probable escape mutant (A128V; N131T; G145A or G145P) were detected. A total of 33 precore sequenced at RMRC Dibrugarh in this study were screened for BCP for the above mutations. The mutations were screened against genotype A sequence (accession number AB014370) with mutations at position A1762T/G1764A. The prevalence of A1762T was found to be 36.4% (12/33), while the prevalence of G1764A was 42.4% (14/33). The prevalence of C1766T was 6% (2/33) while the prevalence of T1768A was also 6% (2/33) and prevalence of T1753C was 21.2% (7/33). Two samples had shown a mutation V173A in RT polymerase which may be responsible for partial resistance to lamivudine.

An exploratory study in visceral leishmaniasis endemic areas of Assam

In this study, prevalence of kala-azar was carried out by house to house survey in the endemic areas. A total of 845 households having a population of 4973 in four villages were surveyed and monitored. A total of 183 persons were found positive by rK39 including old and new cases (3.67% prevalence rate) of whom 46 (25.13%) were children. Microscopic examination of bone marrow biopsy samples from 5 persons showed *L. donovani* parasites. The disease progression of *Leishmania donovani* infection in the BALB/c mice was studied by establishment of the parasite load in the spleen by Real Time PCR. It was seen that there is a gradual increase in the LD load after day 14p.i that increases till day 45 and remain at the same level till day 60. Sequencing of the parasite collected from field sites revealed presence of *L. donovani* strain similar to those found in China and Sri Lanka. A questionnaire based survey on knowledge attitude and practices for Kala-azar was conducted in an endemic area of Assam which recruited about 240 participants. About 85.83% of them had heard of Kala-azar of whom 52.43% believed that it is a communicable disease. Only 11.66% of the respondents were found to be aware that it is transmitted by sand fly.

Genetic diversity and drug resistance pattern of Mycobacterium tuberculosis in the tribal state of Sikkim

A total of 112 samples were collected from Sikkim so far. Drug Sensitivity Testing (DST) was carried out using conventional proportion method for all first line anti-TB drugs like rifampicin (RIF), isoniazid (INH), streptomycin (STR), ethambutol (EMB), and pyrazinamide (PZA) for 54 MTBC isolates (24 completed, 30 ongoing) to determine the prevalence of drug resistance in MTBC isolates from Sikkim. DNA was extracted from 50 MTBC strains so far and for the rest of the MTBC strains DNA extraction are going on using cetyl-trimethyl ammonium bromide (CTAB) method. For all the extracted DNA from MTBC isolates the 24-loci MIRU-VNTR typing and spoligotyping are going on.

Molecular typing of *Taenia solium*/cysticerci and comparative analysis of human and animal isolates from North and North East India

Field trips were conducted in Assam (Districts covered: Dibrugarh, Tinsukia, North Lakhimpur, Sivsagar, Tezpur, Kamrup, Dhemaji and Jorhat) for collection of *Taenia* metacestodes from infected pigs and adult *Taenia* worms from human cases. 27 infected pigs were found and from each pig approximate 30 metacestodes of *Taenia* were collected. Adult *Taenia* proglottids were also recovered from 18 patients.

DNA extraction, sequencing and phylogenetic analysis

DNA has been extracted from each *Taenia* metacestode isolates and proglottids. *ITS2*, *cox1*, *Cob*, *ND4*, *ATP6* and *ND2* gene of each isolates was amplified using our designed PCR primer. DNA sequencing is carried out using 3130xl genetic analyzer for the above mentioned amplified regions for each isolates. The DNA sequence and phylogenetic analysis were also carried out for each isolates. The work is under progress.

NON COMMUNICABLE DISEASES

Risk factors of Hepatocellular Carcinoma in Sikkim and Arunachal Pradesh, India

A total of 49 HCC cancer samples and 49 controls have been collected till March 2016. Heterozygous mutant of CYP1A1 frequency was slightly higher among HCC cases than in age-sex matched controls. CYP2E1 homozygous mutant in HCC cases was similar to age-gender matched controls. GSTT1 genotype is more among cases and GSTM1 Null genotype was more among both cases and controls. Differential gene expression of T cell energy and immune tolerance genes were observed in HCC cases compared to age-sex matched healthy controls. Though, initial data on gene expression has shown various degree of fold change in HCC cases compared to controls, the data has to be interpreted with caution as the number of control cases analyzed was very low compared to controls. The study is in progress, further recruitment of age-gender matched healthy control is underway.

Pattern of Survival and Quality of life of the Oesophageal & Stomach Cancer patients in North Eastern Region of India

Kaplan Meier Survival rates shows the median time 15 months for Esophagus cancer patients and 12 months for Stomach cancer patients. Cox proportional hazard model shows significant hazard risk of both sites in the cases from rural areas. The Cox regression analysis result also shows the significant role in the survival of both cancer sites on continuation of treatment administrated to them. Cronbach's alpha (α) coefficients of internal consistency for the all multidimensional scales of EORTC QLQ-C30, EORTC QLQ-OES18 and EORTC QLQ STO22 questionnaire was tested for time to time evaluation of Esophagus and Stomach cancer patients and was found reliable. The non-parametric methods – Mann-Whitney U test with respect to sex of oesophagus cancer patients shows that male are experiencing more deteriorate condition than female counterparts. In stomach cancer patients it shows significantly higher problem in female's symptom scales than male.

Monitoring Unit of North East Regional Population Based Cancer Registry

Estimation of cancer disease burden through 12 population based cancer registries in north-east India is continued. Highest AAR among both males and females were seen in Aizawl and Mizoram. East Khasi Hills in Meghalaya had the highest relative proportion of tobacco associated cancers among both males and females. Meghalaya state as a whole showed the highest AAR in cancers of the oesophagus (in both males and females) and of the hypopharynx and larynx in males.

Assessment of Health Related Quality Of Life (HRQOL) and functional status of community dwelling elderly persons in rural settings of Assam: Ongoing

A total of 430 Participants (Male: 209; Female: 221) from 8 villages were enrolled. Mean age of the participants was 68.7 ± 7.4 years ranging from 60 to 102 years. The majority of the participants were from the age group 60-64 years followed by 65-69 years and 70-74 years. Majority of the participants reported their health as fair (52.3%) followed by good (33.7%). None reported their

health as excellent and 11.9% had reported their health as poor. Of the 8 domains highest Short Form (SF-36) scoring was found in case of social functioning followed by pain, role functional (emotional), emotional well-being and physical functioning. Participants from older age revealed lower SF-36 scoring. There were no significant differences of SF-36 scoring according to gender except physical functioning and social functioning. Of the 10 different types of activities of daily living most of the activities had highest (>85%) level of scoring except climbing stairs.

Effectiveness of diet and lifestyle intervention through Information Education(IEC) tools with Angan Wadi Centres (AWCs) as the centre of knowledge dissemination for hypertension (including hypercholesterolemia and diabetes risk reduction-a cluster randomized controlled trial: Ongoing/Extramural

The targeted sample size of the study was 3600 i.e. 300 per cluster (total number of cluster -12). Blood samples were collected from the consenting individuals and sent to central laboratory at ICMR, maintaining cold chain. Project staffs were recruited and trained at Centre for Chronic Disease Control, New Delhi. Twelve Anganwadi workers were engaged to assist project field works after one-day training organized at RMRC, Dibrugarh during September 2014. House hold listing and mapping were completed for the 12 identified clusters of Dibrugarh district. Baseline survey started since 26th September 2014; and completed.

OUTBREAK INVESTIGATIONS

Outbreak of diarrhoeal disease in Kanaigaon, Dibrugarh (October 2015)

A diarrhoeal outbreak was investigated at Kanaigaon, under Dibrugarh District in October 2015. Out of the total 10 samples collected four showed growth of *Shigella flexneri*. The strains were sensitive to norfloxacin, gentamicin, amikacin, cefotaxime, intermediately sensitive to doxycycline, tetracycline and resistant to ampicillin and cotrimoxazole. The report was intimated to local health authority for taking appropriate measures.

Diphtheria outbreak investigation: Dibrugarh District (October 2015)

There was an outbreak of diphtheria in three tea gardens of Dibrugarh district. Epidemiological data including blood samples, throat & nasal swabs were collected and processed for laboratory investigations. Culture for diphtheria bacilli were positive in 10 subjects of older age groups (>10 years). Antibiotic sensitivity was done for all positive cases and reports have been submitted to state health authority for taking necessary action.

Outbreak of vesicular rash in Dibrugarh (Chandmari ghat) in Jan-Feb 2015

The outbreak involved about 15 cases affecting mainly children below 10 yrs but also adult cases were also detected. The outbreak was confirmed to be due to Chicken-pox (VZV) which was detected via IgM ELISA.

Dengue fever outbreak in Pasighat (August-October 2015)

Dengue outbreak was reported from Pasighat District of Arunachal Pradesh during August-October 2015 and a total of 6195 suspected dengue cases were tested for DENV NS1 Ag & IgM ELISA and 1866 were found to be positive for either NS1 Ag or DENV specific IgM. A subset of 613 cases of suspected DF were analyzed at RMRC, among which 91 cases were positive for DENV NS1 Ag while another 24 were positive for DENV IgM ab. Among the 91 NS1 Ag positive cases subjected to DENV typing by RT-PCR, only 66 out of 91 could be typed by RT-PCR. The predominant serotype of DENV detected in the 2015 outbreak in Pasighat was DENV-1 (60 /66). DENV-2 was detected in 4 cases, while mixed infection with DENV-1 & 2 was detected in one case and mix infection with DENV-1 & 4 was detected in another. There was one death of an adult female with DENV NS1 Ag positive who expired within hours of admission to the general hospital, Pasighat in the month of Sept 2015. The outbreak peaked in the month of August with a total of 2201 suspected cases of DF reporting to the General Hospital, Pasighat.

NATIONAL INSTITUTE FOR RESEARCH IN TRIBAL HEALTH (NIRTH), JABALPUR

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 *pfhrp2* and *pfhrp3* gene variations / gene deletions in *P. falciparum* samples from malaria-endemic states of India. Study sites two sites one high transmission and one low transmission from each state were selected from eight states (North East, Orissa, Madhya Pradesh, Chhattisgarh, Jharkhand, Maharashtra, Gujarat and Rajasthan). Screening of malaria parasite was done by microscopy and mono infection of *P. falciparum* positive samples were collected after taking their written informed consent. *P. falciparum* infection was confirmed by species specific nested PCR amplification of the 18S ribosomal RNA gene. The merozoite surface protein 1 (*msp1*), merozoite surface protein 2 (*msp2*) gene and *glurp* were also amplified to ensure good quality DNA. The laboratory isolate Dd2 was used as a negative control for all *pfhrp2*/flanking genes experiments because this isolate lacks all three genes. Similarly, parasite isolate HB3 was used as the negative control for all *pfhrp3*/neighboring genes experiments because the isolate has deleted all three genes.

Among 1521 microscopically positive *P. falciparum* samples screened, 50 were negative by HRP2 based RDT test. PCR amplification showed 2.4% samples were lacking the *pfhrp2* gene. Only 1.7% samples showed absence of the *pfhrp3* gene and both *pfhrp2* and *pfhrp3* genes were not found in 1.6% samples. Though overall prevalence of HRP2 and HRP3 deletion is low, it varies between states ranging from 0-25% for HRP2 deletion and 0-8% for HRP3 deletion. Further analysis revealed that 14% of isolates lacked all the genes including their flanking genes. A total of 14 different amino acid repeats were identified from *pfhrp2* gene and 8 different amino acid repeat from *pfhrp3* gene. This indicates the need of regular molecular surveillance to ensure reliable performance of RDTs. The sequences were submitted to the Gene

Bank database (Gene Bank accession numbers KT 238913-KT 238939).

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

The study was carried out in 2 malaria prone districts *i. e.* Bastar and Korea of Chhattisgarh. Two CHCs in the district and 4 villages in each CHC were selected for this study. The overall objective was to study the bionomics of prevalent malaria vectors and their role in malaria transmission for development of evidence based sustainable malaria control strategy with special reference to vector control.

The average per man hour density of anopheles mosquito was 18.17 (ranging from 8.09 to 46.6) of which 44.3% were *An. culicifacies* and 4.6% were *An. fluviatilis* in indoor resting collection. The average per man hour density of *An. culicifacies* in Bastar and Korea district was 6.6 (95% CI 5.02-8.2) and 9.6 (95% CI 7.5-11.8) respectively. The average PMH density of *An. fluviatilis* in Bastar and Korea district was 0.39 (95% CI 0.02-0.8) and 4.34 (95% CI 1.82-8.84) respectively. The average PMH density of *An. fluviatilis* was significantly higher in Korea as compared to Bastar ($p < 0.001$), while *An. culicifacies* has shown no significant difference in Bastar and Korea in indoor resting collection. A total 2606 *An. culicifacies* (1489 from Korea and 1117 from Bastar) and 242 *An. fluviatilis* (185 from Korea and 57 from Bastar) were assayed by PCR. Of these 2 *An. culicifacies* one from each study site Korea and Bastar were found positive for the *falciparum* (Sporozoite rate = 0.07%). A total of 358 *An. culicifacies* samples and 153 *An. fluviatilis* samples were analysed by DNA sequencing. *An. culicifacies* C (38 %) was dominant followed by species D (23%) and B (22%). *An. fluviatilis* sp. T was dominant in the study area. Both *P. falciparum* positive *An. culicifacies* were identified as species C.

The corrected mortality of *An. culicifacies* was >98% against 0.1% Alpha cypermethrin in Korea district while 76.7-79.3% in Bastar district which comes under resistance (R) category. The corrected mortality of 0.05% of *An. culicifacies* against Deltamethrin (90.0-93.1%) comes under the category of verification required (VR) in Korea district while 73.3- 74.2% in Bastar district.

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). A training workshop was also organized at both CHCs for ASHA, ANM and anganwadi workers. Pregnant women (PW) who fulfill the enrollment criteria were enrolled in the study. Ranapur CHC is selected for all PW irrespective of their symptoms while in Meghnagar CHC only symptomatic PW were tested for malaria parasite. A total 678 pregnant women were enrolled from Meghnagar CHC and 26.6% women had malaria symptoms of which 24.3% were positive for malaria. In Ranapur CHC 398 PW were enrolled in the study and 5.2% women had malaria. During the subsequent follow-up 5 women were found positive from Meghnagar and 2 women from Ranapur for malaria. A total 575 placenta were processed and out of this one placenta was found positive for *P. falciparum* malaria.

Analysis of *in vivo* transcriptome of *falciparum* from Indian Patients suffering from cerebral malaria and its comparison with that from patients infected with severe malaria.

This study is carried out at Maharani medical college, Jagdalpur and District Hospital, Baikunthpur (Chattisgarh) to determine the *in vivo* *P. falciparum* gene expression profiling in cerebral and severe (non cerebral) malaria cases. All the patients admitted to the hospitals with fever history were examined for malaria using the microscopic examination of the blood smear.

During the year, a total of 14,083 patients were screened for malaria of which 904 cases were found malaria positive (778 Pf, 117 Pv and 8 mixed infection of Pf and Pv). Overall, 10.28% patients were recorded with Cerebral Malaria (CM), 11.50% with Severe Malaria (SM) and a total of 2.88% died. The, microarray data analysis was carried out by on 11 severe and 4 mild malaria samples. A total 358 and 293 genes are up-regulated in severe and mild malaria respectively. The ETRAMP 14.1 (early transcribed membrane protein 14.1) a

member of ETRAMP family of proteins is the most highly transcribed gene in severe malaria. The protein-interaction network predicts interactions of ETRAMP-14.1 with PfEMP1 (erythrocyte membrane protein) the major virulent protein. PfEMP1 is also a major vaccine candidate.

GENETICS

Establishment of prenatal diagnosis of beta-thalassemia syndromes and sickle cell disorders in Madhya Pradesh, Assam and the Andaman and Nicobar Islands.

Prenatal diagnosis of sickle cell disorders was established in NSCB Medical College, Jabalpur. During the period under report period, molecular studies involving ARMS- PCR and CRDB procedures have been standardized and initiated in the institute. A total of 24 high risk couples have been identified and followed up. Among these three high risk couples were referred to NIIH, Mumbai for chorionic villus sampling. The fetuses suspected for carrying homozygous sickle cell disease/Beta thalassemia were tested and found to be sickle cell or beta thalassemia carriers only.

Micro mapping of G6PD deficiency among the tribals of India and its importance for antimalarial therapy

Five districts namely Jabalpur, Mandla, Dindori, Chhindwara and Damoh have been selected for the study. Field trips were undertaken to Dindori district and 14 tribal school children were screened for various hemoglobinopathies and G6PD deficiency. A total of 912 children between the ages of 7-16 were screened. Among the 912 children 34 children were found to be deficient for G6PD enzyme. Two new G6PD variant (551 C to T) in exon 6 and 544 C-->G nt were observed among the tribal groups of Madhya Pradesh.

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

A total of 911 pregnant women were tested and among them 78 sickle cell trait, 2 sickle cell disease and 15 Beta thalassemia trait individuals were identified. The spouses (56) of these women

were also tested for sickle and β -thalassaemia carrier status. Five husbands were found to be heterozygous for sickle cell disease. These high risk couples (5) are being monitored and at the time of birth of their child, efforts will be made to test the new born for sickle cell disease. Among these 5 high risk couple 2 sickle trait babies have been born during the monitoring period. In addition, 397 cord blood samples have been screened. Till date 3 SS and 32 AS babies have been detected in these samples. Efforts are being made to register these babies for routine follow up. Overall 5 sickle homozygous babies were born of which 2 died within 30 days.

BACTERIAL DISEASES

IEC intervention to improve KAP related to tuberculosis and its impact on risk factors and TB disease burden amongst Saharia - a primitive tribe of Madhya Pradesh

The study was planned to execute a need based IEC intervention in saharia tribal population having high prevalence of tuberculosis and to assess the impact of IEC activities on KAP, risk factors and prevalence of TB. It was carried out in Shivpuri district of Madhya Pradesh in three phases; Phase I (baseline survey), Phase II (IEC intervention in study area) and Phase III (endline survey). The results of the study indicate that the TB disease remains a major public health problem amongst Saharia primitive tribal community with alarmingly high TB disease prevalence of 3003 per 100,000 in the baseline though it declined to 1995 per 100,000 in the endline. The knowledge on TB was low in the baseline. Though the overall awareness increased in both the areas but improvement in the intervention area was significant. The prevalence of tobacco smoking and alcohol consumption was high particularly among males (76.4% and 49.7% respectively) in the baseline and remained high in the endline though there was some decline in smoking prevalence in the intervention area. Malnutrition in adults showed improvement in intervention area during the endline. However, poor living conditions such as poor housing, overcrowding, cooking inside the living room using wood/crop residuals as the fuel remained the same by & large during the study period.

Estimate the burden of TB among the tribal population and develop an innovative health system model to strengthen TB control in the tribal areas A Multicentric ICMR Task Force Study

This cross sectional, multi-centric ICMR task force study is being carried out in randomly selected 16 clusters in the state in various phases: Phase I (Situational analysis), Phase II (Qualitative assessment), Phase III (Quantitative Assessment) and Phase IV (Intervention). Situational analysis, have been completed in 19 villages. Focus group discussions (FGDs) FGDs and interviews of key informants conducted so far, highlight their knowledge and perceptions about the TB disease, use of TB diagnostic & treatment facilities and the suggestions for improvement. TB disease survey has been completed in five clusters. Of the 8250 individuals eligible for screening, 7245 (87.9%) were screened and 394 (5.43%) individuals were found symptomatic. Sputum was collected from 386 (97.96%) symptomatic individuals and a total of 56 sputum positive cases (smear and/or culture) have been detected so who have been referred for treatment under RNTCP.

VIROLOGY

Virus research and diagnostic laboratory

During the period under report, the Virology Laboratory provided diagnosis for more than 4400 samples using 35 different serological and molecular testes for 15 different viruses of public health importance. Hepatitis, dengue and influenza contributed maximum number of samples. In the year, a total of 2023 samples were screened for different viral hepatitis. Maximum i.e. 646 samples were tested for hepatitis B of which 99 were positive followed by hepatitis E, (127 out of 569 samples were found positive), hepatitis A (62 positive out of 434) and hepatitis C (only 12 positive out of 374)

A total of 730 samples were tested; for which H1N1, of 186 were found positive.

National Hospital based Rotavirus Surveillance Network

This is a multicentric ICMR task force study on Rotaviruses. Being a regional laboratory, NIRTH

has 2 peripheral sites; NSCB Medical College Jabalpur and GMC, Bhopal. This year 200 and 254 children respectively were enrolled in the study. From Jabalpur 44/144 samples tested were positive for Rotavirus while in Bhopal 30/149 were positive. Out of 74 positive samples, 35 samples were processed for G and P typing for VP7 and VP4. G1 was the predominant strain found (18) followed by G9 (3) and G2 (2). Few mixed G types were also found; G1G4 (3), G1G4G9 and G4G12. In P typing P8 (19) was the most common type followed by P4 (5). Nine samples were not characterized in P-typing

SOCIAL AND IEC BASED STUDY ON MATERNAL & CHILD HEALTH

Impact assessment of an intervention package to improve maternal and child health services among primitive Baiga tribe of Dindori district in Madhya Pradesh

The study is being carried out in two phases to assess the impact of the intervention to improve maternal and child health services. Phase-I was implementation of IEC among women in reproductive age group in intervention villages and Phase-II was evaluation. It was found that antenatal care (88%) was significantly ($X^2=8.783, p<0.05$) higher in intervention group compared to control group (76.1%). So was Tetanus Toxoid (T.T) vaccination and consumption of iron folic acid (IFA) tablet (93.7%). Educated women had the highest percentage of adequate ANC use compared to those who were illiterate. The institutional deliveries were higher in intervention group (49.7%) compared to control group (40.0%). Use of postnatal services was higher in intervention group. Child immunization and Vitamin-A supplements were higher in intervention group (53.6%). About 85.0% of women knew of maternal and child health care services in intervention group compared to control group (42.7%). Utilization of MCH services and awareness was increased in 12 intervention villages due to effect of IEC interventions.

REGIONAL MEDICAL RESEARCH CENTRE, PORT BLAIR

RISK REDUCTION/ ELIMINATION 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 subperiodic *Wuchereria bancrofti* in Andaman & Nicobar Islands

Persistence of microfilaraemia was evident post six rounds of MDA in the Nancowry islands, endemic for diurnally sub periodic filariasis. Therefore, in order to hasten the process of elimination, one year mass distribution of DEC fortified salt is envisaged and to assess the technical and operational feasibility in the elimination of the lone foci of this infection in India.

The Chief Secretary, Shri Anand Prakash, IAS launched the distribution of double fortified salt (DEC+Iodine) for the elimination of diurnally sub periodic filariasis in the Nancowry group of islands.



Fig. 16. Chief Secretary, Shri Anand Prakash, IAS launching the distribution of double fortified salt to the tribal representatives.

Distribution of double fortified salt commenced from December 2015. All the 707 enumerated households with a combined population of 3351 have been covered. During the period Dec 2015 to March-2016, a total of 6,698 Kg of fortified salt have been distributed. The process monitoring is in progress.

OPERATIONAL FEASIBILITY OF ELIMINATION OF MALARIA IN CAR NICOBAR ISLAND

The annual parasitaemia incidence (API) decreased from 20.0 in 2008 to less than 0.3 in 2015. Two *anopheline* species viz, *Anopheles sundaicus* and *An. barbirostris* were identified as malaria vectors.

Investigation of the five malaria cases occurred in Car Nicobar in 2015 revealed that all these patients had travelled to the southern group of islands (endemic for malaria) prior to their infection. It is proposed to intensify the malaria control programme through innovative steps such as strengthening case detection by involving unconventional surveillance workers, transferring the technology of breeding larvivorous fishes and introducing to water bodies to the affected communities, introducing entomological surveillance and tracking down all cases of malaria in the island with the ultimate objective to interrupt local transmission of malaria. It has been observed that a large proportion of the patients approach the Traditional Knowledge Practitioners (TKPs). Therefore, it is envisaged to impart training to the TKPs of herbal medicine and health care practices towards active case detection and referring the suspected cases to the health seeking facilities.

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

The estimated prevalence per 100,000 population increased from 409.9 in 1986 to 735.3 in 2001-02 and then showed an equally sharp decline to 241.6 in 2013-14 and to 158.7 in 2015-16. Since the devastation of the Car Nicobar island during the Asian Tsunami of 2004, large scale reconstruction has been carried out and as a result there has been a great deal of improvement in the socio-economic and health status of the people. This has reflected in the TB situation also, which has been showing a constant downward trend. There is an opportunity to bring the TB burden in the island to very low levels so that a TB elimination initiative may be launched subsequently.

A study to generate community-based primary data on the prevalence of pulmonary tuberculosis, the challenges in accessing the health care services and the factors that influence their health care seeking behaviour was initiated as part of a multicentric project. A preliminary analysis of the data showed that the tribal villages of Car Nicobar and Little Andaman have good healthcare infrastructure and manpower with one hospital/PHC within 8-15 Kms, which are well connected with the villages. These villages also have a local subcentre with

1-2 DOTS providers, who are regularly detecting and referring TB suspects and providing treatment and monitoring. These providers are well aware of various aspects of TB and the control programme and face very little challenges in the implementation of various tasks under the programme. However, occasional instances of negligence and neglect due to alcoholism are causing some barriers in proper implementation of various measures of the control programme. Contact screening seems to be the most affected component of the programme.

A project has been developed to achieve a substantial reduction in TB prevalence in Car Nicobar through

1. Active case detection of latent TB
2. Develop strategies for treatment of such cases.
3. Impart training to TKPs, as a small proportion of the patients approach them
4. To screen and follow up Hepatitis B carriers among the TB patients and implement appropriate treatment strategies among TB patients with drug induced hepato-toxicity.

SURVEILLANCE OF DISEASES-HOSPITAL/ COMMUNITY BASED STUDIES

Assessment of disease burden among the rural population of Rangat, Middle Andaman

An analysis of secondary data on hospital attendance and deaths in Rangat during the years 2013-2015 was done. The most common causes of death were non-communicable diseases. Other common cause of death as per the death register was senility and suicide. Among the non-communicable disease, chronic renal disease was most common cause of death.

Among the communicable disease, the most common causes of hospital attendance were acute respiratory infection, acute diarrhoeal disease and skin and subcutaneous tissue infections. A large number of cases of viral hepatitis in 2013 indicated a possible outbreak that year. Although the peak occurrence of ARI was in the month of July during all the three years, the peak in July 2015 was much taller indicating an upsurge in ARI cases that year. Hypertensive heart disease was the most common non communicable diseases and this was followed by diabetes mellitus.

Assessment of nutritional status and prevalence of determinants of chronic non-communicable

diseases among the urban population of Andaman and Nicobar Islands-A survey under National Nutrition Monitoring Bureau

In view of the increasing prevalence of NCDs, particularly among the urban population and the influence of diet and lifestyle in the causation of these diseases, there was a need to assess the health and nutritional status and the magnitude of NCDs among the urban population of the country. The NNMB initiated such a survey nation-wide and the present survey is a part of this. The results of preliminary analysis of data on 2,139 study subjects aged above 18 years is presented here. About half of the analysed population (50%) were observed to be either overweight (36.9%) or obese (13%). About 14% of the population were diabetic and prevalence of hypercholesterolemia was about 22%. The study was the first nutritional survey among urban population in Andaman and Nicobar Islands and threw light into important aspects of chronic non-communicable disease risk factors among the urban population.

ICMR VIRAL RESEARCH AND DIAGNOSTIC LABORATORY-ICMR VIROLOGY NETWORK

Fever cases attending health facilities were screened for fourteen common etiological agents. Among the cases with identified etiological agents, DENV was the most frequent pathogen. The clinical syndromes observed were influenza like illness, lower respiratory infection and haemorrhagic fevers. DENV was the most frequent etiological agent detected in all clinical syndromes except that presenting with ILI and LRI together, in which case adenovirus and RSV were the commonest etiological agents.

National Hospital Based Rotavirus Surveillance Network at RMRC Port Blair, Andaman and Nicobar Islands

In 2015, a total of 337 cases of diarrhoea admitted to the wards of the selected hospitals were screened for rotaviral infection. Rotaviral etiology of the diarrhoea was confirmed by rotavirus EIA in 118 (35.0%). Among 297 inpatients from Andaman Islands, 109 (36.7%) were attributable to rotaviral infection, among the 40 inpatients recruited from Nicobar district, 9 (22.5%) were attributable to

rotavirus. In this study the most frequent G/P genotype combinations detected were G1P[8] (81.4%), G9P[4] (14.4%), G2P[4] (1.7%), G2P[6] (1.7%) and G1P[11] (0.8%) respectively. G9 genotype was reported in the previous years, however during the current year, the number of cases with G9P[4] increased and became the second most common circulating genotype in these Islands.

HEALTH AND NUTRITION OF MARGINALIZED COMMUNITIES

Micro mapping of G6PD deficiency among the tribals of India and its importance for antimalarial therapy

Studies of G6PD deficiency to determine the frequencies are important to develop a micro map of G6PD deficiency among the tribals of India. The clinical outcome of this study will also help to determine whether the routine G6PD screening is necessary in some of the tribal areas before giving the anti-malarial therapy. The molecular characterization of this gene is also necessary to determine the genotype – phenotype correlation. Based on the screening test, the observed prevalence of G6PD deficiency among the Nicobarese of Car Nicobar was 4.3% and that of the carrier state of the disease was 2.4%. The results were based on the results of screening test. The samples have been sent for confirmation to NIIH, Mumbai.

Elucidating the Richness and diversity of bacterial groups in the gut flora of Nicobarese Tribal Community – Perspective on their Lifestyle Transition

The gut microbiome of the Nicobarese people was studied using a metagenomic approach. Variations were observed in the composition of gut bacteria of Nicobarese population, with respect to changes in their lifestyle. In remote cohort, *Bacteroidetes* group of bacteria was dominant and *Prevotella* was found to be the largest contributor in this phylum. Urban cohort and rural cohort have decreased numbers of *Bacteroidetes*, while *Bifidobacterium* was dominant in the urban cohort. The remote Nicobarese gut microbiota has ancestral features as seen in rural communities of Africa, Venezuela and Hadza, but has transformed in the rural and urban cohorts indicating the transition in their lifestyle.

The gut microbiomes in the rural and remote populations are slowly being transformed due to the accessibility to modern diet and practices.

The Nicobarese present a unique set of features regarding the composition of their gut microbiota adapted specifically to their lifestyle and environment. However, they share some similar functions with the other rural communities pertaining their diet. They exhibit variations in the functions related to xenobiotic degradation, cellular motility and secondary metabolite synthesis concerning their remoteness and exposure to xenobiotics or infections.

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

The study was conducted to assess the preparedness of the health system in Nicobar district in addressing the increasing burden of chronic non-communicable diseases. As part of this a cause of death analysis using verbal autopsy was conducted. An interim analysis of 204 deaths was done. Among the deceased, 62% of male and 58% of females were above 60 years of age. Non-communicable diseases accounted for 71% of the deaths followed by other causes (16%) and infectious diseases (14%). Tuberculosis (10%) was the major infectious disease causing deaths. Neoplasm (8%), alcoholic liver disease (6%) and diabetes (5%) were the other major NCDs. The proportion of deaths due to NCD was higher among females (73%) as compare to males (69%). Further analysis of the data is in progress.

OUTBREAK INVESTIGATIONS

Emergence of Coxsackievirus A6 as a sole pathogen responsible for Hand Foot and Mouth Disease Outbreak in 2015, Andaman Islands

Clinically suspected Hand Foot and Mouth Disease (HFMD) cases were reported in various hospitals of the Andaman Islands, in the month of October-2015. Clinical samples were screened for the presence of enterovirus targeting 5'NCR followed by molecular typing based on partial VP1 gene region specific to enterovirus species. The

aetiological agent was confirmed by sequencing the partial VP1/2A junction region of enterovirus. Sequence and phylogenetic analysis were done using MEGA 6 software to know the genetic relationships of the aetiological agent with intra-serotype reference sequences. A total of 46 clinically diagnosed HFMD cases were reported from the month of October to December 2015. Children <5 years of age were maximum reported and all from South Andaman district. Enterovirus was detected in (n=17) 16 (94%) cases followed by molecular typing showed that the causal pathogen was Coxsackievirus A6 (CV A6). Phylogenetic analysis results implied that the CV A6 serotype was closely grouped with F genotype.

Shifting trend in serogroup composition in human leptospiral infection, Mumbai

During the monsoon season of 2015, there was an outbreak of leptospirosis in Mumbai. An investigation on leptospiral etiology of the disease and the causative serogroups was conducted. The case of death in the 12 fatal cases of suspected leptospirosis occurred in Mumbai in July 2015 was leptospirosis. A total of 12 fatal cases were reported to the Centre and among then 11 were males. Three-quarters of the cases were in the age group of 10-39 years. There seems to be a clustering of the cases in Malad, where 4 (33.3%) of the 12 fatal cases occurred. Acute respiratory distress syndrome that occurred in 11 (91.7%) of the cases, was the commonest severe complications in the fatal cases. Thrombocytopenic purpura and acute kidney failure occurred in 50.0% and 41.7% respectively. The predominant infecting serogroup was Tarassovi followed by Djasmin. A shift in the predominant infecting serogroup appears to be occurring in Mumbai now.

ECOLOGY AND ENVIRONMENTAL MICROBIOLOGY

Gene expression of GroEl and LipL32 in the different phases of Leptospiral biofilm

Recent studies on *Leptospira* have demonstrated biofilm formation on abiotic surfaces. A study was conducted to identify any proteins involved in the biofilm process. SDS-PAGE, immunoblotting in combination with MALDI-TOF mass spectrometry were employed, to analyse the protein expressed

during *Leptospira* biofilm phase in comparison to planktonic phase. Identification of proteins in these two phases reveals that, in biofilm phase *Leptospira* overexpresses certain proteins at 60 kDa. MALDI-TOF analysis of the protein identified stress and heat shock chaperone GroEL. Our findings demonstrated that GroEL is associated with the *Leptospira* biofilm formation. GroEL is conserved, known to be highly immunogenic and a prominent stress response protein among *Leptospira* spp., which may either directly or indirectly influence the colonisation.

BASIC AND APPLIED RESEARCH

Creation of maintenance of databases on medicinal plants, traditional knowledge practitioners and health profile of tribes

Under the ICMR Task Force project of 'Biomedical Informatics Centres of ICMR' a bioinformatics centre was established in the Centre. Developing National Repository of clinical information/data is one of the specific objectives of the project. A database on traditional healing practices and medicinal plants used by the indigenous tribes of the island was created. The database contains information about 250 species of plants belonging to 196 genera and 92 plant families. Information about the use of these plants to treat a total of 66 ailments is also included in the database. Centre has conducted 3 surveys in the tribal areas and clinical profile as well as ancient knowledge of Onges tribe has been recorded and digitalised in the database.

SUPPORT TO NATIONAL HEALTH PROGRAMMES AND WHO

Establishment of leptospirosis reference laboratories in Southeast Asian countries as a joint venture of the Centre (WHO CC) – follow up training to technologists from Bhutan

As per one of the terms of reference of the WHO Collaborating Centre during the last re-designation, the Centre was identified as the nodal centre for developing National Leptospirosis Reference Centres in WHO Southeast Asia Region. This activity is being undertaken as a collaborative project of the Centre and WHO. Last year National Reference Centre was established in Bhutan.

As a follow up action, training was provided to laboratory technologists from Bhutan this year.

Assessment of the ongoing MDA programme in Andaman and Nicobar Islands- consumption and compliance during MDA 2015

Eight rounds of MDA have been carried out to eliminate lymphatic filariasis. A cross sectional survey to assess the pattern of DEC distribution and compliance was undertaken. In all 2748 people were sampled. DEC was successfully distributed to 91.3% and 92.2% of these complied with the treatment. The overall effective drug coverage was 84.2%.

DESERT MEDICINE RESEARCH CENTRE, JODHPUR

DMRC has been working in the area of communicable diseases i.e., Malaria, Dengue, Tuberculosis; the non-communicable diseases i.e. Hypertension and Diabetes as well as Nutrition. DMRC has also focused its scientific studies as per the mandate of the centre i.e. to promote collaborative research with state Government and state medical colleges and universities and also to address region specific health issues. DMRC got adequate support from ICMR Headquarters for intramural activities, but also could generate funds by competing for extramural research grants. The centre received financial support from research activities with DST, DBT, MOEF, MOHFW, State Government as well as from ICMR Headquarters.

Eighteen studies have been carried out during the above period in the field of communicable and vector borne disease, non-communicable diseases and nutrition. Some of these studies, such as insecticide resistance among mosquito vectors in Rajasthan; use of insecticide treated nets in alternative forms for the protection against malaria transmission in desert region have provided important leads which can be pursued further.

A mega project jointly with Government of Rajasthan on screening of sickle cell disease in tribal students is under way and out of target 50000 students, 20000 have been screened. The project

covers tribal Students residing in Tribal hostels & Schools of five Districts (Sirohi, Udaipur, Banswara, Pratapgarh and Dungarpur) of Rajasthan and the tribal Students attending the Maa-Baadi Day Care Centre & Maa-Baadi Centres of these Districts. Appropriate counseling regarding Sickle Cell Disease (SCD) & Sickle Cell Trait (SCT), is being provided to normal students and concerned parents.. The project is likely to be extended at community level with appropriate intervention package.

A number of Interventional studies in the field of nutrition and hypertension are being carried out by centre in different parts of Rajasthan which have generated useful data and also helped in identifying gaps which is being addressed simultaneously.

A pilot study was undertaken in Jaipur district to assess the effectiveness of different modalities in making Breast Self Examination (BSE) a routine practice by the women. There was an increase in knowledge about breast cancer by 80% and 68% women started practicing self breast examination. The experience was shared with state health authorities and they have proposed to work jointly and extend the programme in all 33 districts of Rajasthan.

DMRC is also having two centres supported by ICMR. Biomedical Informatics Centre, which has been working for capacity building in the region and undertaken many tasks on malaria, diabetes, H1N1 etc, using applications of bioinformatics science. The Tribal Health Research Unit was established in January, 2015 and has been working for improving nutrition status of Sahariya tribal population.

DMRC has also established Model Rural Health Research Unit of Department of Health Research at Bhanpur Kallan, Jaipur (Rajasthan). The Unit has been carrying out three studies at present.

The centre is thus moving ahead in all aspects, addressing health issues of the region in collaboration with state Government, medical colleges and universities. Our major achievements have been: a) TB Lab of DMRC which has been accredited by Revised National Tuberculosis Control Programme, for culture and Drug Sensitivity Testing of *M. tuberculosis* using solid media; b) 32 MoUs signed on collaborative bio-medical research involving state medical colleges, state health department and universities, and; c) Model Rural Health Research Unit (MRHRU) has been established at Bhanpur Kalan, Jaipur with clinical biochemistry laboratory having sophisticated equipments.

SUPPORTING FACILITIES

During the period under report, the National Institute of Epidemiology (NIE) at Chennai and the National Institute of Medical Statistics (NIMS) at New Delhi provided statistical assistance to various ICMR Institutes. Health systems research (HSR) and social and behavioural research (SBR) were intensified by starting new projects and with the completion of previous projects. Various new agreements and letters of intent were signed with different national and international organizations.

Intramural Research

NATIONAL INSTITUTE OF EPIDEMIOLOGY, CHENNAI

ICMR School of Public Health

Master of Public Health (Epidemiology and Health Systems)

- 6th (2013) cohort: All 11 medical officers graduated in June 2015
- 8th (2015) cohort: 19 medical officers admitted in July 2015 [11 Tamil Nadu, 3 Maharashtra, 2 West Bengal, 1 each – Uttar Pradesh, Tripura, Arunachal Pradesh]
- Scholars' work resulted in 13 publications in 2015
- Scholars awarded 2 mini-grants by TEPHINET, CDC, USA:
- ◆ Innovations in Surveillance National Baseline Mini-grant: *Assessment of timeliness in detection and reporting of infectious disease outbreaks in 10 Indian States, 2003-2014*
- ◆ NCD mini grant: Evaluation of Referral mechanism in cervical and breast cancer

screening program for women, Tiruchirappalli district, Tamil Nadu State, India, 2012-15

Massive Open Online Courses (MOOCs)

- ◆ **NIE-ICMR e-Certificate (NieCer) – Courses** - The first in this series, *NieCer 101: Health Research Fundamentals*, is a basic level course in health research methods. 8 week course in collaboration with National Program for Technology Enhanced Learning (NPTEL), HRD Ministry funded initiative of IITs and IISc, Jan-Mar. 2016. 3000+ enrollments from across the country

◆ **Workshops**

- *Designing Public Health Program Evaluation* – 8th TEPHINET Global Scientific Conference, Mexico, Sep. 2015
- *Research Methodology* - North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Sep. 2015
- *Protocol Development*– Krishna Institute of Medical Sciences (Deemed University), Karad, Feb. 2016
- *Health Research Methods* - Multidisciplinary Research Unit, Madras Medical College, Chennai, Mar. 2016

Demographic and Health Surveillance-Ayapakkam Cohort Study

- GPS mapping and listing of 12,537 households
- Tablet based version of the questionnaire developed and survey initiated
- Demographic data collection for households: 2399
- Morbidity survey for the Individuals completed for 7843 individuals

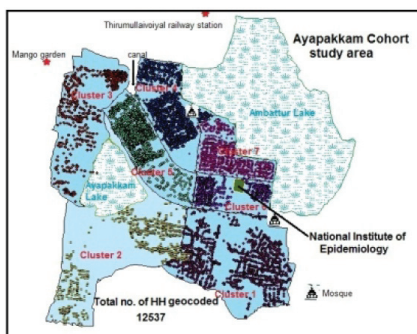


Fig. 1. Ayapakkam Cohort study area.

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

The study aims to (a) understand the level of health systems preparedness in terms of health facility infrastructure, human resources and training to manage hypertension, diabetes, chronic respiratory disease, cardiovascular diseases and cancers and (b) describe cause of death and estimate the proportion of deaths due to non-communicable diseases among tribal populations in selected states

During 2015-16, the study was conducted in Lunglei district of Mizoram and Nicobar district of Andaman and Nicobar Islands

Cause of death in Mizoram: Verbal autopsy done for 443 deaths. Non communicable diseases accounted for most of the deaths (68%) followed by other causes (16%) and infectious diseases (15%). Neoplasm (26%) followed by cardiovascular diseases (18%) and respiratory diseases (7%) were the leading cause of death due to NCDs.

Cause of death in Nicobar district, A and N: Verbal autopsy done for 262 deaths. Non communicable diseases were the leading cause of death (65%) followed by other causes (20%) and infectious diseases (15%). Circulatory system diseases were the cause of death for nearly one fourth (22%) of the deceased individuals while respiratory system diseases accounted for 18% of deaths. Among the infectious diseases, 10% of the deaths were due to tuberculosis. Neoplasm and alcohol liver diseases were responsible for 7% of the deaths

respectively.

National Rotavirus Surveillance Network

The objectives of the multi-site surveillance platform established by Indian Council of Medical Research and coordinated by National Institute of Epidemiology are 1) to estimate and monitor the rotavirus disease burden in children under 5 years of age hospitalized for diarrhea and 2) to generate data on molecular epidemiology of rotavirus in India.

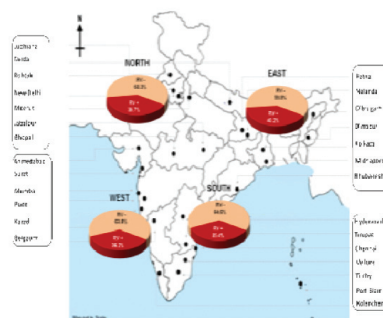


Fig. 2. Rotavirus disease burden [SEP 2012 - MAR 2016].

The Network includes four Referral and seven Regional laboratories in Southern, Western, Northern and Eastern/ North-Eastern regions of the country, and 28 clinical recruitment sites in 17 states and two Union Territories with inpatient pediatric facilities that contribute clinical data and samples.

A total of 22404 hospitalized children with acute gastroenteritis were enrolled and 18618 stool samples were collected in the study during September 2012 –March 2016.

Out of 18618 stool samples screened, 37.7% were positive for rotavirus by ELISA.

Rotavirus positivity rates across different regions are presented in figure.

Preponderance of G1P[8] was observed across sites, regions and age groups.

The other major genotypes were G2P[4], G9P[4], G12P[6], G9P[8],G1P[6], G12P[8]

Hospital based surveillance for bacterial meningitis

HBSSBM established 11 sentinel sites across the India

Generated data on disease burden of meningitis among children below 6 years of age attributable to *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type B etiology and also their trends for 4th year upto March 2016.

Data about circulating serotypes of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type B has been generated from the selected sentinel sites.

Literacy Immunization and health indicators of Chennai City

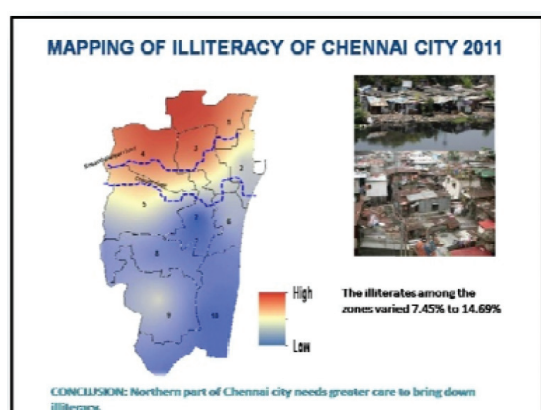


Fig. 3. Mapping of illiteracy of Chennai city 2011.

Objective of the study was to develop an index and a smoothed map of Kriging, simultaneously considering several covariates of illiteracy for Chennai city.

Data on illiteracy and its covariates for ten Zones of Chennai city were used for the analysis. The covariates were based on demography (population, average family size, proportion of slum population, proportion of illiterates, proportion of female illiteracy, proportion of births, proportion of person unemployed, proportion of tribal population).

Four zones had an index of above 50 (higher than average), which shows greater care should be emphasized in these zones to bring down illiteracy level. The smoothed map obtained using Kriging portrays the northern part of Chennai as higher risk of illiteracy.

The map with the index could help the health managers, social scientists and policy makers to

identify regions that require greater care to bring down the illiteracy level to a greater extent.

Health impact of quarry works in suburban areas of Chennai

The main objective of the study is to measure the health impact of quarry works among the residents within 5 KM radius from the quarries and to measure the environmental parameters in the study area.

1905 communities residing nearby quarries and 331 quarry workers were enrolled in this study.

The proportion of lung abnormalities observed in quarry workers is significantly (17.8% vs 4.5%; $P < 0.05$) higher compared to the general community.

The proportion of clinical symptoms for shortness of breath is relatively higher in quarry workers (14.5%) as against in the general community (9.2%).

The proportion of clinical symptoms for cough is relatively higher in quarry workers (16.9%) as against in the general community (7.4%).

The proportion of clinical symptoms for respiratory infection is relatively higher in quarry workers (20.8%) as against in the general community (4.1%).

The prevalence of TB observed among quarry workers is 1208 per lakh where as in the community it is 104 per lakh.

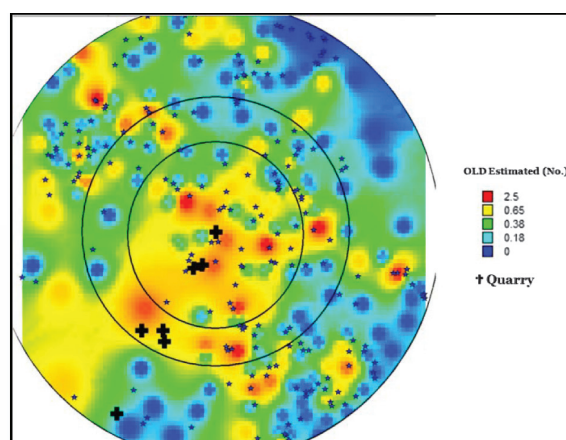


Fig. 4. Obstructive Lung Disease (OLD) is high among the participants living around quarries.

Coverage of childhood vaccination among children aged 12-23 months, Tamil Nadu, India:

The coverage of childhood vaccination in Tamil Nadu has been consistently high, however recently released findings of District Level Household Survey (DLHS-4) indicated vaccination coverage of 56% during 2012-13. NIE conducted surveys to estimate vaccination coverage in the state as well as for the rural, urban, slum, and hilly areas.

The coverage of fully vaccinated children (defined as defined as receipt of 8 vaccines by 12 months of age) was high (79.9%, 95% CI: 78.2-81.5).

The coverage for fully vaccinated children was not different across rural, urban, hilly, slum and non-slum areas.

About 12% fully vaccinated children were inappropriately vaccinated – either before the recommended age of vaccination or the interval between the 2 doses was less than the recommended interval.

Efforts to increase vaccination coverage in the State need to focus on educating vaccinators about the need to adhere to the national vaccination schedule and strengthening supervision to ensure that children are vaccinated appropriately.

Coverage and Missed Opportunity for JE vaccine, Gorakhpur division, 2015

NIE conducted surveys to estimate the coverage of JE vaccine and magnitude of missed opportunity for JE vaccination (MOV) in Gorakhpur division.

The coverage of one and two doses of JE vaccine in Gorakhpur division was 75% (95% CI: 71.0-78.9) and 42.3% (95% CI: 37.8-46.8), respectively.

Facility based exit survey indicated that 32.7% of the eligible children missed JE vaccine. Failure to administer vaccination simultaneously was the commonest reason for MOV for JE vaccine.

Training vaccinators about correct vaccination schedule and removing their misconception about administering vaccines simultaneously would substantially improve JE vaccine coverage in Gorakhpur.

Virus Research and Diagnostic Laboratory Network

DHR/ICMR have established the Virus Research and Diagnostic Laboratory Network (VRDLN) to strengthen the laboratory capacity in the country for providing timely diagnosis of disease outbreaks

As of January 2016, 34 VRDLs have been established in 23 Indian States

VRDLs have a facility to test up to 27 viral etiologies

Soil transmitted helminthes infection among primary school children in selected Indian states

NIE conducted surveys in Telangana, Tripura and Chhattisgarh to estimate the prevalence and intensity of STH.

The findings of these surveys indicated that the prevalence of STH infections in these states was high (Chhattisgarh: 74.6 (95% CI: 70.1-79.2); Telangana: 60.1 (95% CI: 53.1-67.1) and Tripura:59.8 (49.1-69.6)

High prevalence of STH in these states indicates a need to initiate a school based deworming program with bi-annual frequency in these states.

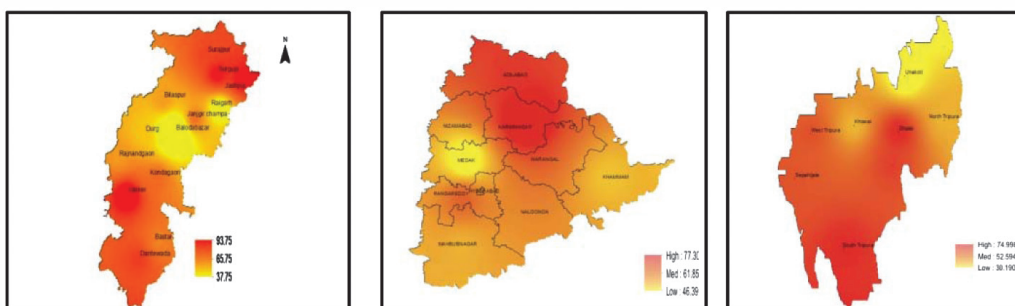


Fig. 5. Predicted prevalence map of STH infection in Chhattisgarh, Telangana and Tripura

Health-needs assessment of selected hill tribes (Palliyar and Muthuvan) in Western Ghats of Tamil Nadu - Tribal Health Research Unit

Conducted survey in 17 villages in Dindigul District among Palliyar tribes to estimate health status among the tribal population.

Qualitative data collected through 19 IDI has been transcribed and translated, 15 FGD has been transcribed and 5 FGD is in the process of translation. Data analysis has been initiated among IDI's of Traditional Birth Attendants (TBA).

NATIONAL INSTITUTE OF MEDICAL STATISTICS, NEW DELHI

The Institute continues to work in its thrust areas including human resource development in statistical research methodology by conducting a number of training programmes, carrying out research and providing technical support to programmes related to health research.

The Institute conducted following training programmes:

1. Summer Training Workshop for PG Students of Banaras Hindu University, Varanasi and Kurukshetra University, Kurukshetra, June-July, 2015 (20 students).
2. Winter Training Workshop for PG Students of Kurukshetra University, Kurukshetra, January 4-16, 2016 (20 students)
3. Internship to PG Students of AMITY University NOIDA, January-March 2016 (4 students)
4. Workshop on HIV Estimation of Working Group (25 persons), April 6-9, 2015; April 26-29, 2015; May 5-7, 2015; June 1-2, 2015; June 10-12, 2015; June 24, 2015; August 17-18, 2015; September 8-9, 2015; October 8-10, 2015; November 6, 2015 and November 17, 2015 (Meeting of the Technical Resource Group).

The Institute carried out following research activities:

Modelling and Estimation of HIV Burden

The Institute has been participating in NACO's HIV Sentinel Surveillance (HSS) and has been providing leadership for official estimates of HIV burden in India and its states. In the current year, it carried out HIV estimation for the year 2015 and submitted the Technical Report: India HIV Estimation, 2015 to NACO which was released by the Union Minister of Health and Family Welfare on World AIDS day, i.e., 1st December 2015.

National Integrated Biological and Behavioural Surveillance

This was the first nation-wide community based bio-behavioural surveillance among HRG and bridge population. It collected information on many key parameters of programmatic importance and included knowledge indicators related to HIV prevention, STI, HIV/AIDS services, risk profile and practices. The survey was not only the largest bio-behavioural survey of its kind but also a pioneer in aspects of use of technology, managing large scale surveys and strengthening the health survey system.

NIMS, besides providing technical support for the survey design (protocol development, pre-survey assessment (PSA) and sampling frame development) and implementation, acted as the Regional Institute (RI) for the states of Chhattisgarh, Madhya Pradesh and Odisha.

The Clinical Trials Registry – India (CTRI) With the help of the WHO, fourteen e-modules of CTRI were developed as the online training to end users covering complete process of trial registration with bottle necks for registration. They were successfully launched on 24th September 2015 by the Secretary-DHR & DG-ICMR and was uploaded on the CTRI web system. Now any researcher can have the training at their desk at anytime for successful registration of trials. The e-modules would help dissemination of CTRI process. With the increasing awareness regarding the setting up of the CTRI, about 6800 trials were registered by the end of March 2016.

Baseline Household Malaria Survey

This study was carried out in collaboration with the NIMR, New Delhi.

Ph.D. Guidance

The Institute is a recognized Centre of Guru Govind Singh Indraprastha University for the Ph.D. programme in Medical Statistics.

Extramural Research

HEALTH SYSTEMS RESEARCH

During the year, the Health Systems Research (HSR) under ad-hoc projects on understanding and strengthening of health systems in the country were funded.

Studies on health and health care of adolescents were undertaken.

- Adolescent health care services of government health system were studied in Karnataka. These studies covered domains of adolescent mental health, reproductive health, adolescent health seeking behaviour, knowledge about available services and suggestion for improvement. A qualitative study brought out several issues pertained to reproduction health, emotional changes and premarital sex from the perspectives of the adolescent boys. The study concluded that male adolescent reproductive health is a neglected area and needs to be more focused in government health programmes.
- Another study documented the access and utilization of various adolescent health programmes in Karnataka.
- Prevalence of smokeless tobacco use and its risk factors among adolescents were studied from Karnataka. A very small number (6%) of adolescents had the habit of chewing tobacco. Majority of adolescents (54%) got information from their parents/teachers, and about 17% got information from newspapers/magazines. And majority (94%) has a positive attitude on tobacco chewing.

Studies pertaining to various issues of health care delivery and access were undertaken.

- A study from Kerala, studied maternal and child health care issues among scheduled tribe population with a focus on health infrastructure and manpower deployment. The overall assessment of the health infrastructure facilities and health manpower availabilities

in the tribal dominated regions of Kerala revealed the shortage of all categories of health personnel in the public health system and it has been well recognized. The study warranted massive efforts from administrative, political, and people level. Also a policy direction from government was essential to provide attention to the destitute, who actually suffers from severe health ailments of chronic and communicable diseases on one side and acute anaemia and malnutrition on the other.

- Reasons for low utilization of health care services for treatment of sexually transmitted infections (STI) in Bhopal city were explored in a study. This study employed mixed-method methodology including the use of exit interview. Female predominance was observed among those who visited the government clinics and male predominance was observed in those who visited private clinics. Genital discharge was the predominant symptom among patients visited, both in government as well as private clinic. About 70% of the high-risk groups visited government hospitals for any health related problems. The knowledge about the various reasons for transmission of STI was good. Sex without condom was the most common reason for STI followed by the sex with multiple partners. The study showed that the NGOs played a major role in promoting a better health seeking behaviour amongst high-risk groups. Distance of health facility and high cost of treatment were found to be the major hindering factors in seeking treatment for STI.
- During this year, a study on health system's responsiveness during mass gathering was being studied by taking Sabarimala temple in Kerala, as a case.
- In Karnataka, another study has been initiated during this year to assess the capacity of panchayat raj institutions to administer the grass root health programmes.
- Another study that completed during this year is on impact of supervisory support training program for health assistants-female/lady health visitors (LHV) in enhancing performance in PHCs. This study was undertaken in Dakshina Kannada district of Karnataka. Study identified infrastructural

problems - lack of computer and internet access (60%), departmental transport facility (100%), training aids (80%), inadequate accommodation facilities (60%). Other problems were - LHVs expressed to be affected by time related problems (94%), motivation related problems (93%), personal problems (88%), problems related to guidance from superiors (100%), community related problems (100%) and subordinates related problems (77%). All auxiliary nurse midwives (ANM) expressed supervision is essential for improving the performance with majority (80%) expressing need for ongoing supervision. Majority (76%) received supervisory support from LHV and 24% from medical officer. Majority (100%) expressed job satisfaction improves with effective supervision. Majority (90%) of the ANMs felt their supervisors were supportive and 10% as very unsupportive. Satisfaction in the domains of approach/communication (67%), education and guidance (64%), problem solving/decision making (65%), management skills (67%) and overall satisfaction (66%). Nearly (35%) of female health workers were extremely satisfied, 30% were just satisfied, 25% were unsatisfied and 10% of them extremely unsatisfied with supervision by the LHVs.

A study from Chandigarh is ongoing to assess the approaches and factors in increasing attraction and improving retention of physicians in rural and remote areas. This study is analysing 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 analysing the perceived attraction factors and deterrents of final year MBBS students and interns regarding rural area posting. This study adopted the mixed-method approach covering samples of general physicians, specialists, final year MBBS students and interns, and health care administrators.

Geo-spatial analysis of accessibility of health care services by migrant pockets spread across the city of Mumbai, based on Geographic Information System (GIS) technology is progressing. This model focussed 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 affect utilization rates and population health is known. Among these less understood barriers is 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.

A study from Tamil Nadu developing an IT enabled tool for selection of desired health care services is currently under the phase of testing. This software is patient friendly and enables effective selection of health care services based on patients' requirements and needs. Broadly, this software has three technical modules – (i) Service Registration, ii) Service Selection, and iii) Service Delivery. For service registration, each hospital will provide different healthcare services based on their specialists (healthcare services) (example Ortho, Cardiology, Dermatology, Gynaecology, etc.) and there can be multiple specialists for each health care service. Each hospital will register with the Health Care Authority, their different health care services and their team of specialists. The Health Care Authority verifies and approves the registration and stores this information in the Health Care Database. With regard to the service selection, since many health service organizations provide almost identical healthcare services, selecting a desired specialist and the health service organization based on the patients' requirement/ criteria is an important process. The criteria that can influence the

specialist and health service organization selection can be experienced, success ratio, availability, cost, reliability, privacy, awareness, etc. This framework provides the patients to select the criteria of their own interest based on their requirement and also to give preferences to those criteria in terms of weights. Selecting a desired health care service, based on user preferences can be modelled as multi criteria decision problem. PROMETHEE Method (Preference Ranking Organization METHod for Enrichment Evaluation) is a multi-criteria analysis method that is used to solve multi-criteria problems, which are considered to be NP- complete. PROMETHEE method is one of the methodologies based on Outranking technique which works on pair-wise comparisons to rank the candidate specialists. The PROMETHEE methodology selects the best solution on the user's requirement. This methodology provides the users to select the criteria of their own interest and their requirement and also to give preferences to those criteria in terms of weights. The working principles of PROMETHEE includes – (a) information from the user, (b) information from the database, (c) calculation of preference function; (d) calculation of aggregated preference indices; (d) calculation of positive and negative outranking flows (e) calculation of net outranking flows and (f) selection of the best solution. The possible solution which has the highest net outranking flow is the best solution which has been selected as per users' preferences and requirements. With regard to service delivery, once the specialist is selected, the registration authority checks the schedule and the availability of the doctor and fixes an appointment with the doctor. This module then sends the service set to the patient. The service set consists of hospital id, doctor id, appointment no. and requests the patient to register for the same for confirmation.

Another ongoing study is developing a model for vital registration and communication among health care providers through mobile technology. The objective is to validate the innovative mobile based MIS application for timely and complete registration of vital statistics (i.e. birth and death). Mobile based application is operational in whole Doiwala block (Uttarakhand state) population with the help of 195 ASHAs, 9 village development officers and one block development officer. Data

was collected as per Government approved birth and Death certificate format. All the required data for the certificates are collected. As ASHA worker was already involved in collection of birth and death related data in their registers and this is reported in monthly meeting at their respective PHCs. All ASHAs were trained for sending birth and death related information via SMS in definite pre-decided format. Each ASHA 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. Each month, field assistant visits ASHA in respective PHCs monthly meeting and provide feedback on collected data, alleviates any difficulty. Beneficiaries can collect birth/death certificate from respective village development officer of an area on deposition of required proof document. Timely registration of data by ASHA avoids delay and thus no extra fee needs to be paid and timely data is available at all health care level i.e. CHC/PHC/SC. Preliminary data shows within 5½ months' time, model is successful in registering 969 births i.e. 176.2 births/month/block and 81 deaths that include 10 still births.

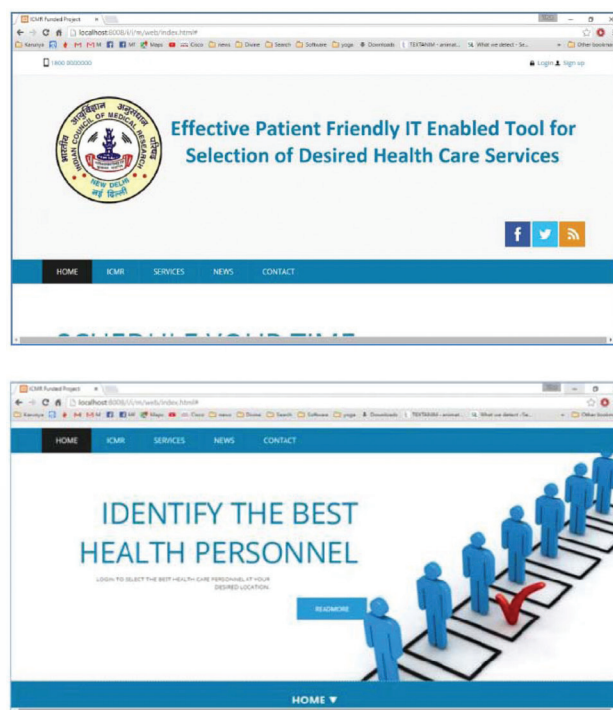
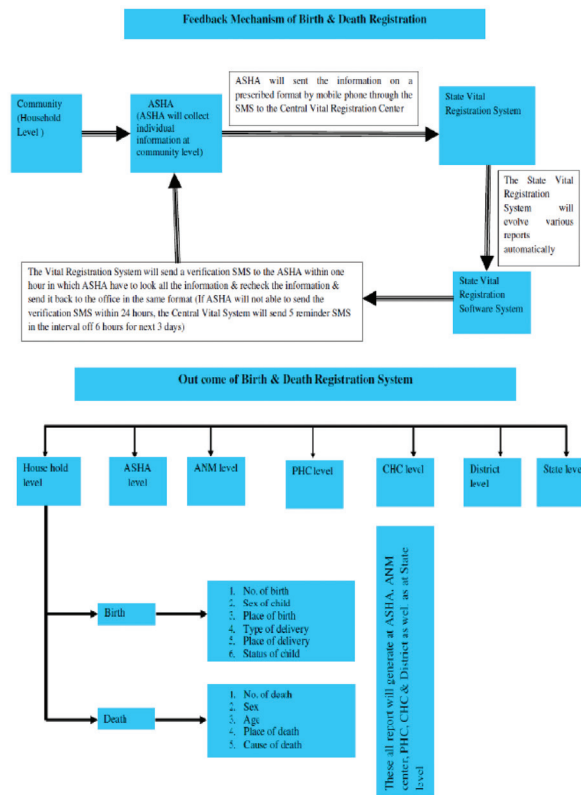


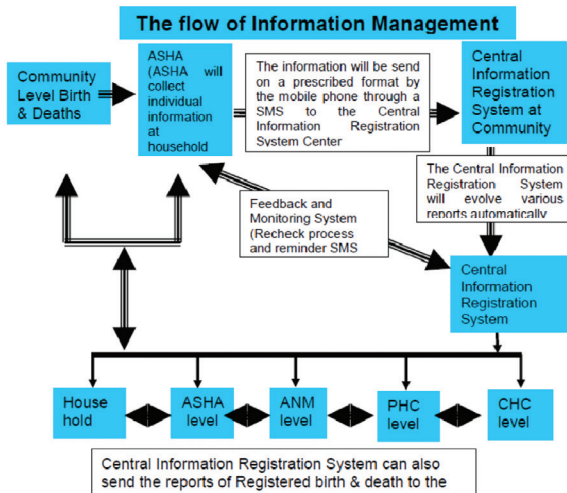
Fig. 6. Screen shots of the programme developed under project - Patient friendly IT enabled tools for selection of desired health care services.



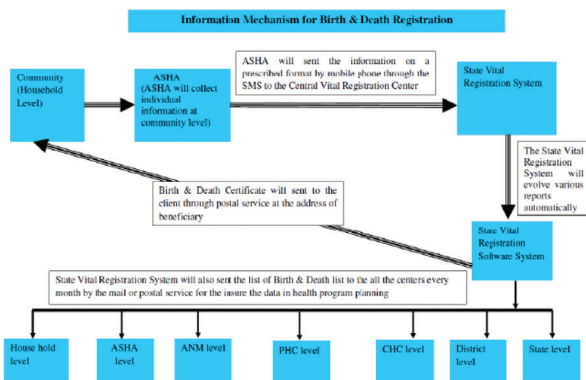
Fig. 7. Diagrams showing flow of information under project - A model Initiative for Vital registration & Communication by the use of mobile application among health care service Providers.



Vital Registration Model



Vital Registration Model



SOCIAL & BEHAVIOURAL RESEARCH

Unwanted Sexual Experiences and Victimization among Adolescents (Online and Offline): An Observational Study

An observational direct interaction cum questionnaire based study carried out to examine rates of online and/or offline unwanted sexual experiences (USE) amongst adolescents aged 14 to 18 years studying in both government and private schools across Chandigarh has shown alarming results. 60% of the adolescents have reported having faced either online or offline unwanted sexual experience or both. 36% adolescents reported having faced online unwanted sexual experience(s) while another 52% reported having faced offline unwanted sexual experience(s). 51% of adolescents have reported multiple such experiences. Majority of offenders were reported to be friends (50%) in case of online USE while it was strangers (38%) followed by friends (31%) in case of offline USE. One-fifth of the adolescents who have reported to have faced online and/or offline USE are still having such ongoing experiences.

Data Collected on internet access and misuse by adolescents is equally glaring, 99.5% adolescents

in present study had internet access. Majority (80%) were using mobile phones for accessing internet. 40% adolescents were using internet without seeking permission from parents. 80% adolescents have admitted to having account on social networking sites and 94% of these engage in chatting and a majority (53%) chat with strangers of both sexes (75%). One third of adolescents have also visited pornographic sites.

These figures point to urgent need of parental supervision of internet access amongst adolescents. Parents need to keep a check on their child's activities on social networking sites and openly discuss about unwanted sexual experiences, if any, being faced by their wards.

Understanding Reproductive Health Needs of Tribal Adolescents in Meghalaya

Reproductive health of adolescents is one of the important dimensions. The present study was undertaken with the objective of assessing the knowledge and practices of adolescents of Meghalaya regarding reproductive health. A total sample of 1260 school going adolescents in the age group of 13 to 19 years (630 boys and 630 girls) were randomly selected from 18 government schools of three districts of the state covering the Garo, Khasi and Jaintiya Hills. A pretested and modified self-structured interview schedule was used to elicit information on various aspects of reproductive health. The socio-economic background revealed that the respondents were mainly from 15 to 17 years age group, were Christians, had nuclear families with five to eight members. Although, most of the girls had good knowledge about menstrual aspects like duration of periods, interval between periods and various difficulties faced during periods, they did not have any knowledge about its meaning and importance. The mean age at menarche was found to be 12.6 years. All the girls faced problems like weakness, lower abdominal pain, backache, depression and moodiness during menstrual periods. They did not have any knowledge of menstruation before menarche and were afraid of the process. Majority of the girls shared their first reaction of menstruation with their mothers. Awareness regarding the concept of reproductive system, reproductive organs, external and internal changes during puberty and pregnancy related

aspects were found to be minimal in girls and boys. Results revealed that most of them knew only about condoms as family planning method and got this information from television and friends. They had fair knowledge about HIV/AIDS but were not aware of various sexually transmitted diseases (STDs) and their symptoms. Results indicated that adolescent girls suffered from reproductive health problems related to menstrual disorders like dysmenorrhoea, heavy bleeding and backache. Gender differences were found in the awareness levels of boys and girls regarding aspects of menstruation, STDs, reproductive system changes and puberty as revealed by significant associations through chi-square tests. Thus, it can be concluded that adolescents did not have information on various aspects of reproductive health and must be appropriately equipped with relevant knowledge and skills which will help them to develop healthy attitudes for future.

Effects of the Impact of Counseling Intervention on Life Situations of the Stigmatized Vitiligo And Psoriasis Patients

The high stigma scores in patients with vitiligo and psoriasis suggest reduction in quality of life and psychological distress. The significant reduction in stigma as measured after counseling mandates routine counseling for patients with such conditions. The counseling protocol developed for the study is transferable to health care professional and would require training of these professionals. It is recommended to train the health care workers in dermatology mostly doctors and nurses in administering the stigma-intervention individual counseling techniques; to replicate these techniques in at least two of the ICMR collaborative institutes, before recommending its usage to the stigmatized psoriasis and vitiligo population in India.

Mental Health of People Living with HIV/AIDS in India: Implications for Policy and Programmes

The study was aimed to assess mental health of the people living with HIV/AIDS (PLHA) in the context of psychosocial and economic realities in India. Three high prevalence states namely Maharashtra, Tamil Nadu and Andhra Pradesh were selected to assess the psychosocial burden

and their impact on mental health of PLHA. A total sample of 750 PLHA from all three study states was considered and the total sample was further subdivided into 250 per state. The research indicated that the majority of PLHA suffered from mental health problems such as anxiety, depression and other associated co-morbidities. The mental health disturbances must be treated concomitantly.

The people living with HIV/AIDS (PLHA) across different culture reported stigma and discrimination at the community level, work place as well as during the treatment and counseling in the health care institutions. The study revealed that the youth respondents were more in numbers as compared to middle aged or elderly. The data suggested that the female respondents had higher level of stigma and burden as compared to the male counterpart. The socio-economic status of female PLHA was lower as compared to the male PLHA. Reportedly, there is shortage of ART centres, the PLHA mentioned that there is need for more ART centres across various districts and subdivisions in all the states so that the waiting period and overcrowding may be reduced in those limited centres. The data also illustrates that there is difference in mental morbidities among the states. Regional and cultural differences exist in India at state level thus, policies should target this existing gap for wider implementation and higher health outcome.

Reproductive and Child Health Services: Examining its Utilization in Northeast India

The study was conducted for understanding the utilization pattern, health seeking behavior and other determinants that influence the accessibility of RCH care services for planning and implementing effective health policies and programmes in the Northeast. A total of 800 households were surveyed to collect the data. It was found that educated husbands were more supportive in seeking crucial services such as ANC and PNC. Although pregnancy registration was found to be quite high, 100 % compliance can still be achieved by improving the facilities.

The high number of home deliveries in rural areas must be reduced through special incentives aimed at rural area and awareness about RCH facilities must be widely disseminated. It was observed

that SCs and STs and BPL families opt more for government care services. Implementation of Janani Suraksha Yojna (JSY) and Janani Shishu Suraksha Karyakram (JSSK) can be improvised in order to ensure that the needy actually get these benefits. The lower utilization of PNC services among lower and lower middle classes was also observed during the study. The RCH programme under the aegis of NRHM has resulted in several positive experiences for the state on several parameters, ranging from institutional deliveries, full ANC, PNC, Immunization, birth control and use of spacing methods, awareness about RTIs/STIs and HIV/AIDS TFR, MMR and IMR have been gradually declining.

It is difficult for the Government alone to reach out to all the corners. NGOs can be identified to take up RCH projects which can be monitored and evaluated by the government. In order to reduce the burden on the tertiary care services, improving the primary and secondary care services are a must. The Sikkim Government's initiative of a token salary for ASHA is truly encouraging and can be adopted elsewhere too.

Equity Impacts of a Targeted Health Insurance Scheme: New Evidence from India's Rashtriya Swasthya Bima Yojana

The Rastriya Swasthya Bima Yojana (RSBY) has been successful in enrolling a fair number of targeted beneficiaries since its launch in 2008. This study probably is the first in this sequence that has attempted to evaluate the scheme in terms of not only coverage alone, its utilization, satisfaction and effect of reducing catastrophic health expenditure among urban poor. The study has adopted exhaustive methodology to identify the poor localities and household based on over 10,000 household screening across 52 JJ clusters. Finding of this study is based on the survey conducted in 3,350 households selected from the different socioeconomic background and spatial locations across Delhi. Results revealed that the prevalence of morbidities and illness is far higher in these low income communities than the general population. Study estimated that the overall, per capita health expenditure for all types of illness combined was widely dispersed around an average of Rs. 2148.9 and a median of Rs. 666.66. Results

further observed that for all type of morbidities and illness, self saving was the major source, followed by borrowing from friends and other family members. As far as the awareness of RSBY scheme is concerned, about 38% of the total households indicated that they heard about the schemes in last one year, with significant variations by background variables. Multivariate results after adjusting other confounders suggest that those households which used RSBY scheme in last one year during hospitalization, the catastrophic health expenditure rescued to half of level as compared to those who did not avail the scheme. There is an immense need at the policy level at Delhi government to provide sufficient support to the scheme so that it may help the urban poor to reduce the 'health shocks' that ultimately reduce the probability of households getting in severe poverty trap due to excess healthcare expenses.

INNOVATION AND TRANSLATIONAL RESEARCH

During this year, a total of 33 patent applications were filed, two in India, one each from intramural and extramural institute. Two patents granted during this period belonged to NIRT, Chennai and ERC, Mumbai.

With the help of PCT guidelines 10 PCT applications has been filed, four from extramural research done at AIIMS, New Delhi; KGMU, Lucknow and five from intramural research done at NIOP, Delhi; NIMR, Delhi; NICED, Kolkata; NIRRH, Mumbai.

21 international applications were filed in different countries viz. United States, Canada, South Africa, Europe, Australia, and United Kingdom etc in national phase while few applications were also filed in Non-PCT states viz. Nepal, Cambodia, Myanmar, Pakistan, Ethiopia, Bhutan.

Besides expanding patent portfolio, enormous efforts were being put towards commercialization of ICMR technologies initiating from website advertisement to showcasing at various exhibitions to finalizing the collaborators till transferring the technologies. As a result 12 different technologies were licensed out to two Indian companies for viral disease technologies (Chandipura, Crimean congo hemorrhagic fever virus (CCHFV), Japanese encephalitis, Kyasanur forest disease

virus, Hepatitis E) and other six technologies viz. Personal cooling garment, DBS kit, Ferritin kit, AV Magnivisualizer, Lung fluke test, Hepatitis B diagnostic, Detection of Blood Glucose.

Further handholding of a folklore practitioner is done for *Anti-JE herbal preparation* with the help of AYUSH.

For strengthening the commercialization activities of ICMR's technologies, the Council is in process of collaboration with Federation of Indian Chambers of Commerce and Industry (FICCI) for both national and international commercialization of health technologies.

The ICMR's technologies were exhibited at various conferences and summits to invite industrial partners for their commercialization : (i) 3rd edition of India–Africa Forum Summit (IAFS) summit which took place during 26th-29th October 2015 : showcased its technologies to companies, African delegates. Technologies were appreciated by all the visitors who showed keen interest in these technologies both from India and Africa. (ii) Participated in India International Science Festival (IISF 2015), a joint event of Ministry of Science and Technology and Ministry of Earth Sciences organized during, December 04-08, 2015 at Indian Institute of Technology, Delhi with Technology Information Forecasting and Assessment Council (TIFAC) as the nodal government institution, organized the event in collaboration with Vijnana Bharati (VIBHA).

(iii) Also participated in Global R&D Summit 2015, a two-day integrated conference and exhibition held at Vigyan Bhavan, New Delhi on 7th and 8th December, 2015, co-organized by FICCI and DST, Government of India with the objective of leveraging International cooperation to boost Indian innovation ecosystem and showcased our ready technologies for commercialization.

Organized workshop to sensitize biomedical scientists towards identification and protection of Intellectual property associated with their Research and Development at Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar on 15-16 March, 2016.

The IPR Unit of ITR continued its training of women scientists under the Woman Scientist scheme of TIFAC/DST. One woman scientist is being trained at IPR Unit for the year 2016.

RESEARCH METHODOLOGY CELL (RMC)

Research Methodology cell (RMC) has been established in ICMR in year 2010. The objective of the Research Methodology Cell in the ICMR Headquarters is to create awareness among new researchers about the different research methodologies being used by researchers in different fields of medical science, especially those belonging to state medical colleges and other academic institutions located in the periphery of different states. The major concept of Research methodology is to train the young faculty, professionals and students to write 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.

Activities undertaken during 2015-16

• Capacity Building workshops

Two Research Methodology Workshops were organised in year 2015 at All India Institute of Medical Sciences (AIIMS), Bhubaneswar and Directorate of Health Services, Itanagar, Arunachal Pradesh (North-East). In workshop at AIIMS, Bhubaneswar, 40 faculty members from various disciplines had participated which included the state level programme officers responsible for MRHRU research. Young faculty from SCB Medical College, Cuttack (MRU facility), VSS Medical College, Burla (MRU facility) had also participated. In the workshop held at Itanagar, Arunachal Pradesh, Army Medical Services deputed four professionals from Tejpur, Jorhat and Naharlagun to participate in the workshop which was well attended by state health services officials.

• Findings of the research work undertaken by young faculty:

The projects generated and discussed during the workshop are reviewed at ICMR and the suitable projects are supported as a seed grant to young

professionals of the medical colleges and research institutions.

In year 2015- 16, 6 projects were completed and the key findings are as below:

- Role of vitamin D and vitamin D pathway gene in breast cancer, at SMS Medical College, Jaipur. This study suggests that low levels of 25(OH)D may be modestly associated with an increased risk of breast cancer. The decreased expression of CYP27B1 in malignant tissues compared to adjacent normal may be important in their predisposition to the development of breast cancer. VDR gene receptor polymorphisms may lead to structural change of the receptor and further events of signaling pathway may be altered, therefore supplementation with vitamin D in polymorphic patients may not be beneficial.
- Genetics of childhood cataract: Functional candidate gene approach to study genetic polymorphisms in lens specific genes, at Iladevi Cataract and IOL Research Centre, Ahmedabad. Mutation screening in both genomic and cDNA revealed about a total of 18 nucleotide variations including reported (n=8) and novel (n=10). Out of the 10 novel variations, 3 novel variations c.92%>A of GJA3, c.34_359del326fs18* and c.313_42del114 of CRYBA1/A3 have not been reported previously or have not found in the public database. About 91 Nucleotide sequences have been submitted to GenBank (www.ncbi.nlm.nih.gov/bankIT).
- To Assess the role of micro RNA and epigenetic regulation of imatinib resistance in chronic myeloid leukemia, at The Gujarat Cancer & Research Institute, Ahmedabad. The drug resistance in CML and translation from CP to BC via AP has many molecular alterations including both genetic and epigenetic mechanisms. Some non-reported mutations were also identified which may add up to knowledge database specific for Indian population.
- Study of pouch colon affected probands in Rajasthan, at SMS Medical College, Jaipur.

Exome sequencing and the subsequent bioinformatic analyses revealed that the variants are distributed across six affected genes, viz. BAAT, DCC, DHCR7, FANCD2, COL14A1, TC1RG1, which are unique to CPC. However, validation of the Exome data with either transcriptomic or proteomic data is necessary before a prognostic or diagnostic test is devised using Exome data. It seems reasonable to conclude that Population having a mutation in BBS2 is predisposed to CPC as this is the only mutation known as Pathogenic in ClinVar database.

- Male involvement in maternal and child health care in Urban and rural areas of Uttar Pradesh, at Rural Institute of Medical Sciences and Research, Safai, Uttar Pradesh. In total, 160 married couples in rural area and 190 married couples in urban area were the eligible study subjects. Only one-fifths males of the urban & rural areas were aware that first trimester is the ideal time for first ANC visit to the health professional. Approximately 76% of the males & 64% of the females had no idea about the time of initiation of breast feeding after caesarean section while only 3% of the males & 5% of the females had correct idea about the time of initiation of breast feeding. Approximately 60% of the males & females were not aware about the high risk pregnancy signs.
- A follow up study of an established cohort in rural setting of HP, at Dr. R.P. Govt. Medical College, Kangra at Tanda, H.P. Increase of prevalence for obesity, systolic hypertension, and “at risk” level of total cholesterol was observed over the follow-up period. Majority was observed with use of extra salt while eating. At the reverse, the Framingham risk score based 10 year estimate for CVD showed overall decline for high risk i.e. 1-9% and ≥ 10%.

HUMAN RESOURCE DEVELOPMENT

Junior Research Fellowship (JRF)

During the period under report the Council conducted 15th National Level Examination for selecting JRFs

to augment Biomedical Research in the country. Every year 150 JRFs (i.e. 120 for Life sciences and 30 for Social sciences including biostatistics) are selected for doing Ph.D. in Biomedical Sciences in different institutions. Number of candidates appeared in the year 2015-16 was approx. 8,000. The examination was conducted at 12 centres (Bengaluru, Bhopal, Bhubaneswar, Chandigarh, Chennai, Delhi, Guwahati, Hyderabad, Kolkata, Mumbai, Srinagar (J&K) and Varanasi). A total of 720 JRFs is on-going (2011-2015) at various national level institutions. The value of existing fellowships is at present Rs. 25, 000/- p.m., The annual contingency grant is Rs. 20, 000/- p.a. + HRA.

Financial assistance to MD/MS/DM/MCh thesis in Biomedical Research

Financial assistance of Rs.25, 000/- is provided to MD/MS/DM/MCh students who are in the 2nd year of MD/MS course. The Selection Committee recommended financial assistance to a total of 614 MD/MS/DM/MCh thesis, out of 2329 proposals received so far. Out of 432 -thesis protocols, 82 protocols/candidates were awarded financial assistance during the reporting period.

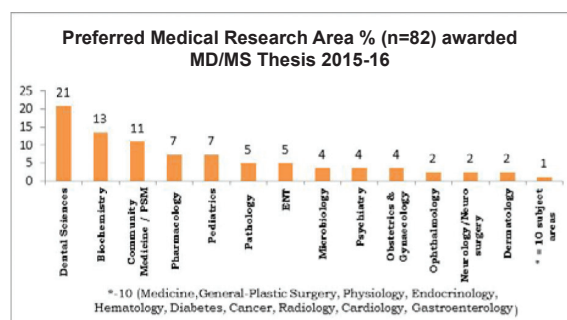


Fig. 8. MD/MS Thesis award distribution.

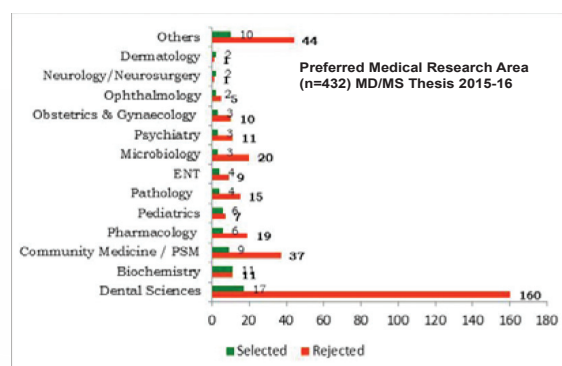


Fig. 9. Medical research area preferences.

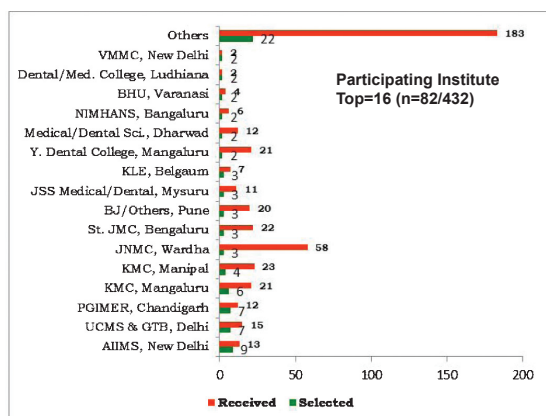


Fig. 10. Participating Institutes.

MD, Ph. D. Programme

Programme was revived to identify young medical graduates with brilliant academic record for pursuing post-graduation and later to absorb them in its research cadre. A candidate who passes all MBBS examinations in the first attempt with 60% or more aggregate marks is eligible for the examination. Under this programme selected medical graduates are provided financial assistance for 4 to 5 years. The eligible candidates were selected through national level examination.

Programme is on-going at three universities viz., King George’s University, Lucknow, NIMHANS, Bengaluru & Sri Ramachandra Medical College, Chennai. During 2015-16 out of 15 allotted slots 10 were selected. So far 99 candidates have joined the MD/PhD programme. 48 candidates have completed their studies in various areas.

International Conference/ Workshops to Non ICMR scientist’s Support Scheme

One of the major mandates of the Council is capacity building of biomedical scientist of the country by providing them financial assistance for participating in International Conference/ Training programmes/ Workshops, etc. Out of total 1284 applications, 327 (25.5 %) applicants were approved and 191 (15%) availed during reporting period.

An analysis of the availed 191 (out of 1284) applications shows that, 61.05 % i.e. 117 applications were male. Out of 117 male, majority of the applications i.e. 90 (77.00%) are young people i.e. ≤ 35 years of age. Similar trend is observed in the female availed applicants (74), i.e. 61 (52.13%)

are ≤ 35 years of age. At an overall level (males and females together), similar trend is seen i.e. 151 (79.05%) applications are from the < 35 years of age group & 40 (21.00%) > 35 year age group.

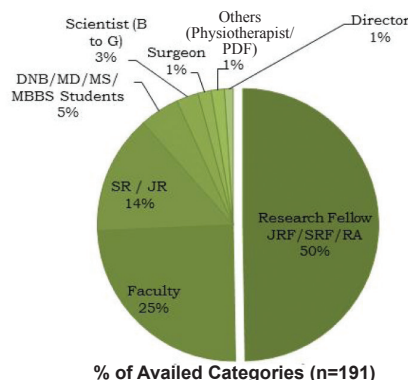


Fig. 11. Percentage of availed categories.

Designation wise analysis of the applications that were availed (191), reveals that maximum percentage of Research Fellows (JRF-SRF) i.e. 50.00% were benefitted from this scheme. Faculty (Lecture/Reader/Assistant/Associate Professor/Addl Professor/Professor Medical/Principal/Vice Chancellors/Dean) also were benefitted from the scheme and out of 191 apps, 25.00% were from this category followed by Senior/Junior Residents 14.00%, MD/MS/MCh students 5.00 %, Scientist (B to G) 3.00%.

AIIMS, New Delhi emerges as the top most institute, AIIMS, New Delhi - 50/23 from which people availed this scheme, followed by JNU, New Delhi-15/10, PGIMER, Chandigarh-16/9, KMC, Manipal-9/8, IITs-9/7, SGPGIMS, Lucknow-12/6, CDRI, Lucknow-11/6, BITS-Pilani-8/5, NIMHANS, Bengaluru-6/5, DRDO-DIPAS, Delhi-5/5, ICMR Instts.-6/4, ILBS, New Delhi-4/4, IISc, Bengaluru-5/4. etc.

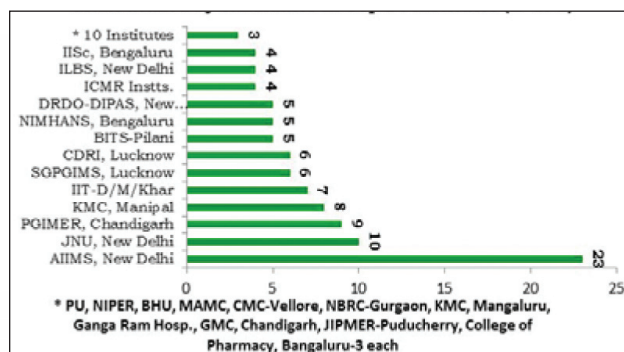


Fig. 12. Grant availed by candidates in top 14 institutes (n=191).

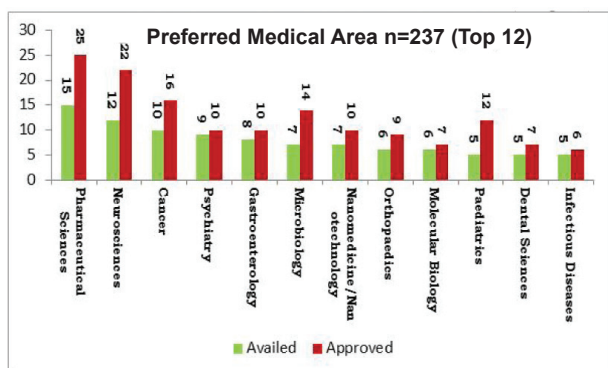


Fig. 13. Preferred medical areas.

An analysis of Research areas reveals that Pharmaceutical Sciences emerges as the top-most area for which this scheme was availed followed by Neurosciences, Oncology, Psychiatry, Gastroenterology, Microbiology, Nanomedicine/Nanotechnology, Orthopaedics, Molecular Biology, Paediatrics, Dental Sciences, Infectious Diseases, etc.

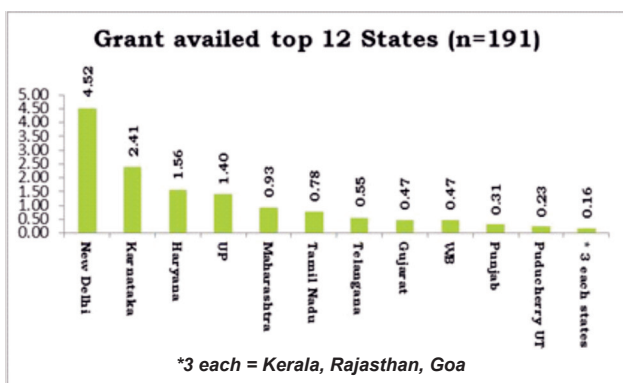


Fig. 14. Grant availed by states.

A State wise analysis indicates that New Delhi is the most active with maximum applications received, approved and availed followed by Karnataka, Haryana, UP, Maharashtra, Tamil Nadu, Telangana, Gujarat, WB, Punjab, Puducherry UT, etc. Detail of participating States is given in Table-1.

Sl. No	State	Received	Approved	Availed
1	New Delhi	348 (27.10%)	107 (08.33%)	58 (4.52%)
2	UP	148 (11.53%)	35 (02.73%)	18 (1.40%)
3	Karnataka	129 (10.05%)	38 (02.96%)	31 (2.41%)
4	Maharashtra	125 (09.74%)	22 (01.71%)	12 (0.93%)

5	Haryana	112 (08.72%)	38 (02.96%)	20 (1.56%)
6	Gujarat	69 (05.37%)	10 (00.78%)	06 (0.47%)
7	Tamil Nadu	63 (04.91%)	15 (01.17%)	10 (0.78%)
8	Telangana	62 (04.83%)	15 (01.17%)	07 (0.55%)
9	Punjab	51 (03.97%)	10 (00.78%)	04 (0.31%)
10	WB	46 (03.58%)	09 (00.70%)	06 (0.47%)
11	Kerala	22 (01.71%)	02 (00.16%)	02 (0.16%)
12	Rajasthan	16 (01.25%)	02 (00.16%)	02 (0.16%)
13	Assam	14 (01.09%)	04 (00.31%)	01 (0.08%)
14	Odisha	13 (01.01%)	02 (00.16%)	0
15	MP	11 (00.86%)	01 (00.08%)	01 (0.08%)
16	Puducherry	09 (00.70%)	06 (00.47%)	03 (0.23%)
17	HP	07 (00.55%)	01 (00.08%)	01 (0.08%)
18	UttaraKhand	07 (00.55%)	01 (00.08%)	01 (0.08%)
19	J&K	07 (00.55%)	01 (00.08%)	01 (0.08%)
20	Chhattisgarh	06 (00.47%)	01 (00.08%)	01 (0.08%)
21	Goa	04 (00.31%)	02 (00.16%)	02 (0.16%)
22	Bihar	04 (00.31%)	02 (00.16%)	01 (0.08%)
23	AP	03 (00.23%)	01 (00.08%)	01 (0.08%)
24	Manipur	03 (00.23%)	01 (00.08%)	01 (0.08%)
25	Jharkhand	02 (00.16%)	0	0
26	Nagaland	02 (00.16%)	01 (00.08%)	01 (0.08%)
27	Meghalaya	01 (00.08%)	0	0
	Grand Total	1284	327	191

Post Doctorate Fellowship Programme

To identify and support young Ph.Ds. for the conduct of research using ICMR Institutes working for priority areas of Health Research, so far 301 (2010- March 2016) applications were received by

the ICMR, 274 short listed were called for personal discussion and 124 were selected. 124 PDF's, 92 have joined different ICMR institutes, 61 PDF's completed their studies, 13 PDFs discontinued and 18 PDF's still continue their study for the year 2015-16. However, a total of 60 PDF's proposals were received by the Division during 2015-16. Out of 60 proposals, 20 were approved for funding and 15 PDF's were ongoing.

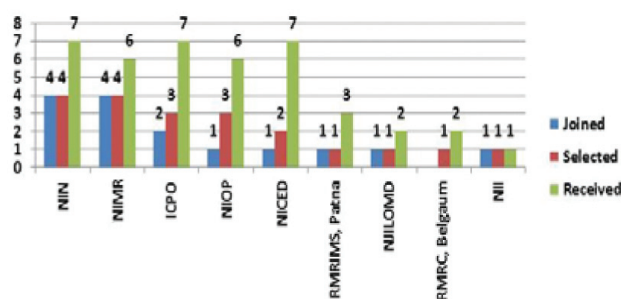


Fig. 15. Status of PDFs at ICMR's participating institutes 2015-2016 (n=60).

ICMR Awards & Prizes

A total of 223 applications were processed for evaluation for different awards for selection for the years 2013 & 2014. Award function was held on 19th Jan 2016 & 43 awards were given.

Grant-in-aid for Organising Seminars/Symposia/Workshops

To update knowledge, transfer from lab to land at national and international level in the concerned area is essential. To implement this policy, Conference/CME/programmes/ Workshops etc. scheme is ongoing. Out of total 921 applications, 418 applicants were approved & amount sanctioned was Rs. 2.05 crores during 2015-16.

INTERNATIONAL HEALTH DIVISION

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.

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.

Joint Working Group (JWG) and Joint Steering Committee (JSC) Meetings

1. Indo-US Joint Steering Committee (JSC) Meeting on Diabetes through videoconference at ICMR Hqrs, New Delhi held on 29th April, 2015.
2. 1st Joint Working Group Meeting between India & Japan at Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi held on 12th June, 2015
3. Indo US Joint Working Group Meeting on Environmental & Occupational Health at New Delhi held on 24th Feb, 2016.
4. 2nd Joint Steering Committee meeting between ICMR-National Institute for Health and Care Excellence (NICE), UK through videoconference at ICMR Hqrs. held on 1st March, 2016.

The International Workshops/ meetings held under Bilateral/multilateral programmes

1. Cancer Research in the Media: International workshop for Scientific Journalism -an interaction with the media at Institute of Cytology and Preventive Oncology, Noida held on 13th October, 2015.
2. Indo US Joint Working Group Workshop on Environmental & Occupational Health at Hyatt Regency New Delhi held on 24th Feb, 2016.

The following MoUs were signed:

1. A Memorandum of Intention (MoI) between ICMR and Swedish Research Council for Health Working Life and Welfare (FORTE), Sweden was signed on 2nd June, 2015 at Stockholm.
2. MoU was signed on 25th June, 2015 at New Delhi to promote and develop cooperation in the field of Environmental and Occupational Health, injury prevention and control research, education and training, infrastructure development and capacity building.

3. The Indo-US MoU among the NCI (AIIMS), MOH&FW, ICMR (DHR), DBT (M of S&T) and NCI (NIH), DHHS, Govt. of USA for Cooperation on Cancer Research Prevention Control and Management was signed on 25th June, 2015 at New Delhi for establishment of the general framework of collaboration for promoting and conducting high quality research to strengthen evidence base necessary for cancer prevention, treatment and management.
4. Letter of Intent (LoI) between ICMR, New Delhi and Department of Biotechnology, Ministry of Science and Technology of the Republic of India and the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, DHHS, USA on Anti- Microbial Resistance (AMR) research was signed on 25th June, 2015.
5. MoU between ICMR and Drugs for Neglected Diseases Initiative (DNDI), Switzerland was signed on 15th October, 2015 by the Secretary, DHR, MOH&FW, GoI & DG, ICMR and the Executive Director, DNDI at New Delhi for cooperation in neglected diseases.
6. MoU between ICMR –Medical Research Council, UK was signed by DG, ICMR on 10th Nov, 2015 at ICMR Hqrs, New Delhi.
7. MoU between ICMR and The National Health and Medical Research Council (NHMRC), Australia was signed on 18th Feb, 2016.
8. ICMR-London School of Hygiene and Tropical Medicine (LSHTM), UK was signed on 8th Dec, 2015 at London.

Dialogues initiated and MoUs forwarded to Govt. of India for approval of NIAID, USA & ICER, Chennai; and ICMR-Bill & Melinda Gates Foundation (BMGF), USA; Approval of GOI is awaited.

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 25 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 2015-16, eight meetings of Health Ministry's Screening Committee were organized, wherein 170 projects were considered and out of which 120 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 which, seven projects are co-funded by ICMR.

International Visitors / Dignitaries

ICMR also organized visits by various visitors to ICMR from foreign countries / agencies such as British High Commission, Sweden, Russia, UK, USA, RUCK, HGF-Germany, CDC-USA, Japan, French Embassy in India, Norwegian Team, Taiwan, BMBF, Germany, Korea, Australia and Sweden.

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 have been awarded to six Senior and twelve Young Indian scientists during the year 2015-16 of which 6 Senior and 10 Young scientists have undertaken the fellowship.

The reports of 6 Senior and 10 Young ICMR International Fellows who had undertaken the Fellowship during the year 2015-16 have been placed on ICMR website. Announcement for ICMR-International Fellowships for 2016-17 was

put up on ICMR website and published in National Newspapers and awarded the ICMR International Fellowship to 6 Senior and 12 Young scientists.



Fig. 16. A Memorandum of Intention (MoI) between ICMR and Swedish Research Council for Health Working Life and Welfare (FORTE), Sweden was signed on 2nd June, 2015 at Stockholm.



Fig. 17. Memorandum of Understanding (MoU) between India & USA on Environmental & Occupational Health signed on 25th June, 2015 at MOHFW in New Delhi.



Fig. 18. Memorandum of Understanding (MoU) between India & USA for collaboration of Cancer Research signed on 25th June, 2015 at MOHFW in New Delhi.



Fig. 19. Letter of Intent between India & USA ON Anti Mircobial Resistance (AMR) research signed on 25th June, 2015 at MOHFW in New Delhi.



Fig. 20. Signing of MoU between ICMR and Drugs for Neglected Diseases Initiative (DNDI) by Secretary DHR & DG, ICMR and Executive Director, DNDI on 15th October, 2015 at New Delhi.



Fig. 21. Signing of MoU between ICMR and Medical Research Council (MRC) by Secretary DHR & DG, ICMR and Director, RCUK India on 10th November, 2015 at New Delhi.

Joint Call for Proposals

Announcement made for submission of applications for transfer of human biological material for commercial purposes and/or research and development of commercial products with deadlines as 30th April, 2015, 31st July, 2015, 31st October, 2015 and 31st January, 2016 and 272 cases were considered by the Committee in its four meetings held on 26th May, 2015, 26th Aug., 2015, 26th Nov., 2015 and 22th Feb., 2016, of which 198 cases were approved.

PUBLICATION AND INFORMATION

During the year, Publication and Information division of ICMR continued to bring out both periodical and non-periodical publications and intensified its activities for dissemination of scientific contributions, achievements and activities of ICMR to various target groups. The Indian Journal of Medical Research with good impact factor among Indian biomedical journals continued with its uninterrupted publication. Dissemination of activities of ICMR and its 32 institutes through Social Media got intensified manifold.

Indian Journal of Medical Research

The Indian Journal of Medical Research (IJMR), completed 103 years of uninterrupted publication. Brought out in two volumes, 12 issues every year and covered by all global abstracting and indexing services, the IJMR is available full text free on the net (www.ijmr.org.in) with a searchable menu. The submission and processing of the manuscripts in the IJMR is online available at www.journalonweb.com/ijmr. IJMR archive is also available at www.ijmr.in with full text of articles available in PDF format since the inception (July 1913).

The IJMR continued to publish quality original research articles in the area of biomedical research as well as review articles (both solicited and unsolicited) on topics of contemporary biomedical interest. Other regular sections such as Editorials, Commentaries and Research Correspondence, View Points, Perspectives, Systematic reviews with meta analysis, and Students' IJMR were also published. Status Reports, Special Reports and Policy Documents were also published infrequently.

During the period under Report, a total of 282 articles were published. Category-wise distribution of articles is shown in Figure 1. The original articles contributed maximum to 48.6%, followed by correspondence (16%) and review articles (9.9%). Clinical Images accounted for 8.5%.

All articles processed in the IJMR are peer reviewed following a stringent peer review policy. The quality of peer evaluation process was maintained by involvement of experts from India as well as abroad. In all, 1138 reviewers were involved in the peer review process during the period under Report. Of these, 83% were from India and the remaining 17% were from countries other than India (Fig. 2). Majority of foreign reviewers were from USA and countries from Europe.

During 2015-2016, a total of 1976 articles were submitted to the IJMR for consideration for publication. Of these, 36% were contributed from countries other than India (Fig. 3). PR China, Turkey, Iran, Croatia, Malaysia, etc. were major contributors.

A special issue on Tribal health was brought out in May 2015. Dr Neeru Singh was the guest editor of this issue. Eighteen original articles and six review articles contributed by various experts were published in this special issue. A supplementary issue on Vector borne diseases was brought in December 2015 with 15 original articles and two correspondences.

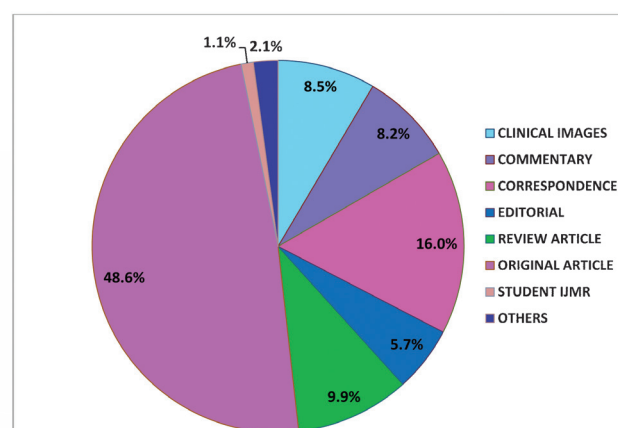


Fig. 1. Articles published in IJMR under different categories (2015-2016).

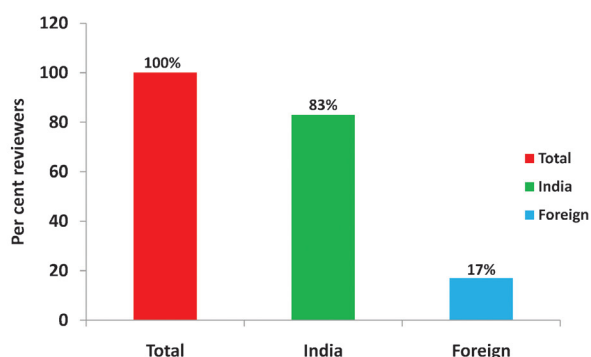


Fig. 2. Percentage of Indian and Foreign Reviewers of IJMR (2015-2016).

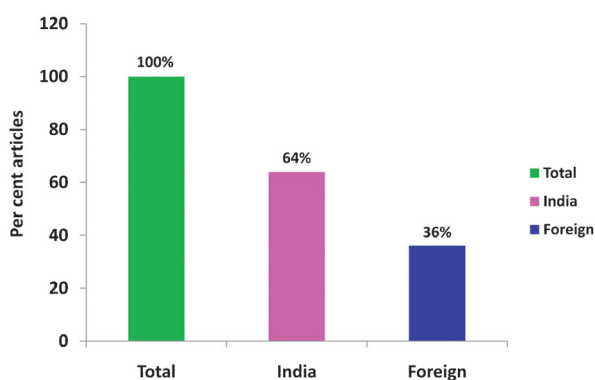


Fig. 3. Percentage of Indian and Foreign Submissions (2015-2016).



Fig. 4. IJMR Publications.

HINDI PUBLICATION

ICMR PATRIKA

During the period the publication of *ICMR Patrika* continued to be brought out. The article on Malaria, health hazards of electronic wastes, heart and environment, TB, etc were published. These included: *Swasthya Suraksha ke prati Bharat Sarkar ki Kary-Yojana* (April-May, 2015); *Bharat mein Malaria ke Nidan evam Ilaj hetu Disha Nirdesh 2014* (June, 2015); *Krishi aur Swasthya* (July, 2015); *Jaivayurvigyan Kachra*

: *Swasthya ke lie Hanikar* (Aug., 2015); *Adese aegipti ki Jaivparisthitiki evam Dengue se Bachav* (Sept., 2015); *Bal Yaun Shoshan : Ek Bahuayami Samasya* (Oct.-Nov., 2015); *Elektronik Apshishit : Swasthya ke lie Khatarnak* (Dec., 2015); *Ssasth Hriday ke lie Parivesh Mahatvapourn* (January-February, 2016), *Bharat mein Kshayarog : Sabhi ke lie Janch, Ilaj aur Upachar* (March, 2016).

Varshik Prativedan 2014-15

Annual Report of ICMR was also brought out in Hindi version as *Varshik Prativedan 2014-15*.

Two NIN Books in regional languages

The following two NIN books in two regional languages- *Odiya and Bangla* were brought out.

1. The *Odiya* version of *Dietary Guideline for Indians – A Manual* (Oct 2015); and
2. The *Bangla* version of *Diet & Diabetes* (Nov., 2015).

Hindi version of Citations and Certificates of ICMR Awardees for the years 2011 & 2012

The Hindi version of Citations and Certificates for 45 ICMR Awardees for the years 2011 & 2012 (20+ 25 respectively) (15th Jan., 2016) were brought out.

MONOGRAPHS ON INDIAN MEDICINAL PLANTS

Review Monographs on Indian Medicinal Plants

The programme aims at consolidation of Indian research contributions (published information) at the various National laboratories/institutions across the country in the area of medicinal plants and present the compiled information in series on Reviews on Indian Medicinal Plants which serve as comprehensive, informative & reliable source of information providing information on new leads, thus helping in systematic and planned evaluation of Medicinal plants, including drug design, basic and applied research.

During the current year, two volumes 14-15 (with botanical names L-Me) covering monographs on about 488 medicinal plants species carrying multidisciplinary information with 7500 citations

were published. Each Monograph includes regional names of the medicinal plant, its sanskrit synonyms as well as the Ayurvedic description (wherever available), ethnobotanical studies, apart from 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.

Work on other volumes is in progress

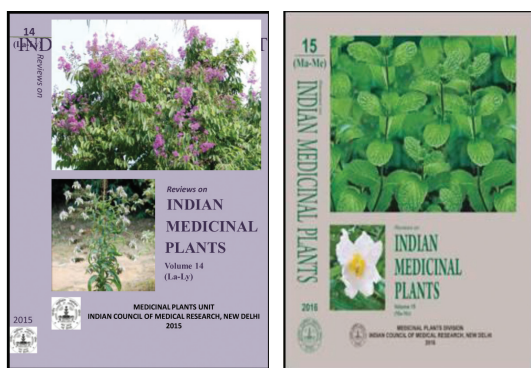


Fig. 5. Indian Medicinal Plant.

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. 13 as part of series on “Quality Standards on Indian Medicinal Plants”.

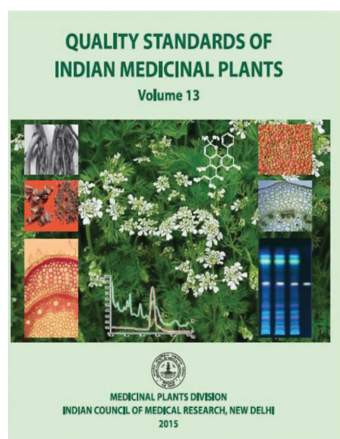


Fig. 6. Vol. 13 of Indian Medicinal Publication Plants.

The program is in progress at various national laboratories/ Institutions in the country. 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.

Training Program in Medicinal Plants

A National Workshop cum Training Programme on Plant Drug Discovery and Development was held at Arya Vaidya Sala, Kotakkal Kerala, from 7-9 April, 2016. The workshop focussed on the multidisciplinary integrated approach in the field of plant drug research leading to validation of therapeutic claims, establishing their safety and culminating in product. Eminent resource persons across the country shared their knowledge and came out with fruitful outcome.



Fig. 7. Training Programme on Plant Drug Discovery.

DISSEMINATION OF BIOMEDICAL INFORMATION

ICMR carried out large number of education and extension activities during the period between 1st April 2015 to 31st March 2016 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 given as under.

Indian Science Congress ICMR participated in Pride of India: Frontier Science & Technologies Mega Science Expo organized during 3-7th January, 2016 as part of 103rd session of Indian Science Congress held at Mysore and showcased its activities and achievements. Video films on the activities and achievements of ICMR were also shown to the visitors. ICMR Pavilion was given the Best Informative Pavilion Award.

2ND VISION JAMMU & KASHMIR 2016 – A MEGA SCIENCE EXHIBITION

ICMR participated in 2nd Vision Jammu & Kashmir 2016 – a Mega Science Exhibition held at Jammu from 22-24th January, 2016 and displayed its activities and achievements. Major focus was to create awareness among the masses particularly students regarding good food habits and having balanced nutritious diet.

Science, Technology & Industry Expo 2015: ICMR participated in Mega Science Technology & Industry Expo 2015 as part of India International Science Festival organized during 4-8 Dec., 2015 at IIT New Delhi. ICMR activities and achievements in respect to innovation and translational research and health technologies were displayed. Dr Harsh Vardhan, Hon'ble Union Minister of Science & Technology and Earth Sciences visited the ICMR Pavilion on 4th Dec., 2015.

Indo-Africa Forum Summit, New Delhi from 27-29th October, 2015: ICMR set up its stall at Indo-Africa Forum Summit-held in New Delhi from 27-29th October, 2015 and showcased ICMR Technologies by putting attractive posters.

13th Infra Educa 2015 : Educational Fair, at Solan, Himachal Pradesh: ICMR Participated in 13th Infra Educa- Educational Fair held at Solan, Himachal Pradesh from 26-29th June, 2015 organized by Friendz Exhibitions and Promotion Pvt Ltd and displayed ICMR Activities and Achievements through vibrant posters in the area of technological innovations and nutrition. Group discussions and video shows were also organized.

Other Exhibitions

ICMR is also participating in many other exhibitions to disseminate the research results for the benefit of the common man and create awareness as mentioned below:

- *Festival of Innovations at Rashtrapati Bhawan, New Delhi* from ICMR participated in festival of innovation held at Rastrapati Bhawan during March 12-18, 2016 and selected technological exhibits of ICMR were displayed in the same.

- *ICMR also organized an exhibition at ICMR Hqrs during on 14th Jan, 2016 displaying posters specifically on ICMR Innovations. This exhibition was originally planned for a day which was later extended for a week on the demand of visiting public.*
- *ICMR also participated in a mega science expo organized at Daman in 2015 for creating awareness among the rural population as well to disseminate useful information to students.*

World Book Fair

ICMR also participated in world book fair, held during 9-17 January, 2016 in coordination with the National Book Trust (NBT), New Delhi. All the books brought out by the ICMR and other health education material was displayed at the ICMR stall. Very good response was seen and many visitors visited the ICMR Stall and purchased the books. ICMR books worth of Rs. One lakh were sold with maximum sale of books of the title Nutritive value of Indian Foods followed by *Bharityon ke liye Ahar Sambandhi Margdarshika, Diet & Diabetes, Ahar aur Hridaya Rog, Dietary tips, etc.* There was a great demand for books in Hindi.



Fig. 8. Participation in Indian Science Congress.



Fig. 9. Engaging and creating awareness among Students/quiz competition.



Fig. 10. Participation in Festival of Innovations at Rashtrapati Bhawan, New Delhi.

BIOINFORMATICS CENTRE

Bioinformatics Centre (BIC) was established as Integrated Research Information System (IRIS) in 1983 under the Division of Publication & Information (P&I) at ICMR with a mandate to computerize extramural activities of ICMR. This was achieved in 1987, and launched on HP-3000 computer systems which was acquired with partial funding from WHO, other activities like computerization of information of intramural scientists, their publications and travel etc. were added, the IJMR was computerized. Starting in 2000 to 2004 a centralized network was designed and implemented across all institutes of ICMR and internet was provided to all scientists and administrative staff. ICMR website was also launched during this period. It was renamed as Bioinformatics Centre in 1999 with an extended mandate to promote Bioinformatics in medical research.

Various activities of Bioinformatics Centre (BIC) during 2015-16 can be described as under.

Revamping of ICMR Network

BIC has completed revamping of ICMR Local area network and the LAN can now support upto 10

Gbps speed, the issues of network saturation have been solved, link load balancer for amalgamating the various connectivity, installation of firewall and its configuration as per government policies has been carried out.

Task-Force Projects

ICMR initiated task-force 'Biomedical Informatics Centres of ICMR' in 2006 by setting up 8 Centres. The second phase of the Task Force was initiated in 2013 and the activity was expanded to 20, i.e. establishing 12 additional Centres in medical colleges and medical research institutes across India. The mandate of Centres is to support informatics in medical research by creating awareness through workshops and training programs, providing data management and analysis services and initiating large-scale collaborative projects with medical professionals from host institute and regional medical colleges.

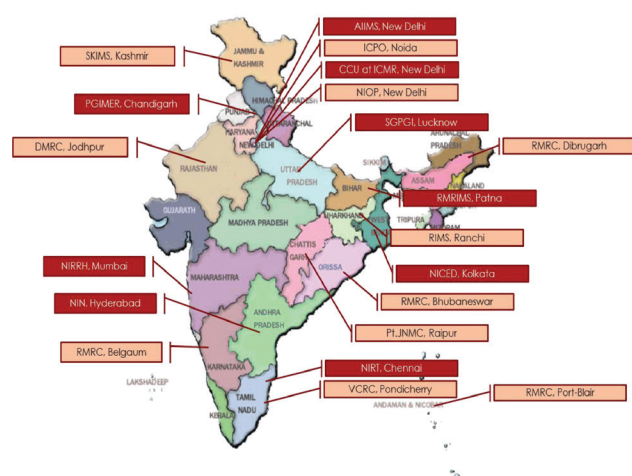


Fig. 11. Biomedical Informatics Centres of ICMR.

Activities and achievements of the task-force include:

Creating awareness

During the year Centres conducted 23 workshops and training programs on diverse themes; from basic bioinformatics and medical informatics to advance big data analytics for disease research. The workshops were attended by more than 400 medical professionals and researchers from regional medical colleges and medical research institutes.

Manpower development

The Centres assisted 27 young researchers in their short and long term training project.

Services to medical researchers

The Centres are created with mandate to provide consultancy to medical professionals from host institute and regional medical colleges in using modern biology tools and techniques for their research, providing data analysis and interpretation services and developing large-scale collaborative research projects. This year the Centres provided consultation in developing 3 large-scale proposals including proposal for developing a comprehensive knowledge-base on Smokeless Tobacco under the WHO Global Knowledge Hub on Smokeless Tobacco. The Centre at NICPR is managing the knowledge portal for KH established by Framework Convention on Tobacco Control (FCTC). The Centres also provided data analysis and interpretation services such NGS data analysis, Metagenomics data analysis, molecular modeling, Phylogenetic analysis, Docking and design and developing databases of clinical and biomedical data.

Collaborative projects

This year the Centres initiated 37 collaborative projects related to identifying disease targets through comparative genomics, protein modeling and analysis of identified target structures, docking and designing leads, in-silico ADMET modeling of identified leads.

Databases of clinical and biomedical informatics

Capturing clinical and biomedical information is extremely useful for developing prognostic and diagnostic models, estimating disease prevalence and evaluation of therapeutic regimens. Scientists of Biomedical Informatics Centre actively interacted with the medical professionals from host institute and regional medical colleges on developing databases of clinical and biomedical information.

The centres developed 34 databases of clinical and biomedical data. A few important databases developed include Sickle Cell Patient Data Management and Analysis Software for storage,

management and analysis of clinical and biochemical data of sickle cell disease patients, developed in collaboration with Sickle Cell Disease Institute, Raipur.

Data collection and analytics

During the year, BIC worked extensively on collection, management and analysis of research data for National programs of ICMR and GOI.

Indian Antimicrobial Resistance Surveillance System

To facilitate uniformity in collection, analysis and sharing of antimicrobial resistance data, BIC has developed an intelligent open-source antimicrobial resistance surveillance system. The system is robust, comprehensive, modular, and extendible with modules for Standard data collection (including administrative module for configuring users, hospitals, laboratories, antimicrobials, organisms, antimicrobial testing panels etc), Uniform XML-based format data sharing (both import and export) and dashboards for different stakeholders etc. The system has been implemented for collection, storage, maintenance and analysis of pathogen-specific antimicrobial resistance data being collected by National Coordinating Centres (NCCs) and Regional Collaborating Centre (RCCs) which are part of National Antimicrobial Resistance Surveillance Network of ICMR.

Leprosy Monitoring System

BIC in collaboration with CDAC has developed a Real Time Monitoring System of the Leprosy Eradication Programme System in pursuance of the joint endeavor of the Ministry of Health and Family Welfare (MoHFW) and the WHO to work for early detection and treatment of Leprosy in India as well as treat the known cases in a time frame of about two years. The solution has adopted the best practices to facilitate smooth roll out and data collection of the suspects using hand held devices, upload the data in central database directly from the hand held devices with the geographical location of suspects to facilitate validation of the data of suspects & early detection, treatment initiation, treatment completion and disability limitation as per the Scope in National Leprosy Eradication Programme. The system is intended to check and

minimize the cases that are lost from the point of referral to the Primary Health Centres (PHCs) as it will facilitate track back or follow up those cases which do not reach the facilities for diagnosis. In short, it will facilitate monitoring of detection, interventions, treatment initiation & completion and disability limitation as well as for having timely collection and use of data to improve the quality of services for responsive decision making.

The software will be interfaced with the National Leprosy Eradication Programme, authorized users shall be able to access the software on Desktops in PHCs as well as other similar institutions to facilitate them enter the data new cases & upload in Database on central server and update the data of patients at various stage of treatment and track each case.

Research Management

During the year BIC processed 14 projects in the area of Bioinformatics and medical informatics. BIC is taking initiatives to promote projects on medical informatics and projects involving use of primary patient data.

ICMR Website

The layout of the website is altered and the entire website is segregated into user friendly self-explanatory vertical bars. The Photo gallery section is supported with dynamic transition code. An audio/ video section is introduced which shows the clips and voice recording of various events. A new dynamic program is added to show the additional charge of the officers in case of the absence of another officer. Additionally, ICMR Website is updated on the regular basis with call for proposals, advertisements, announcements, IJMR, Intramural scientists detail, publications etc. Average page view of ICMR Website for the year 2015-16 is 17101. International visits are 43.88% and visits from India are 54.42% (As per Web Analytical Software designed by NIC).

Management of Servers and Network

The Internet and intranet are provided by BIC to ICMR Headquarters and its Institutes. Database Server for Short Term Studentship (STS), Management of Acute Coronary Event (MACE)

Registry, National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT), OPA (File Tracking System) Pay slip generation and GPF are also managed by BIC.

Video conferencing facility

Videoconferencing facility is managed and provided by BIC to ICMR Headquarters and its Institutes. It is used to conduct interviews and meetings in the country as well as abroad. This activity is planned to be expanded to cover all institutes.

LIBRARY & INFORMATION SERVICES

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 the most of the titles are available from 1998 onwards) has been renewed for one more year for six ICMR institutes including ICMR Hqrs.

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. J-Gate @ ICMR is a customized solution for ICMR Consortium to enable 30 members across India, to access consortia subscribed journals, individual library subscribed journals and full text journals available in J-Gate, through a single search discovery platform and a DDR (Document Delivery Request) functionality, for resource sharing among the consortia members.

Total Journals Indexed in J-Gate Biomedical Sciences is 11,237. This service is being provided by Informatics India (a professional) agency. It can be accessed by all ICMR libraries & Information Centres through desktop.

Subscription to DELNET institutional membership has been renewed. The following databases are available from DELNET which can be accessed online by the institutional members.

1. Union Catalogue of Books (2.28 crore bibliographic records)
2. Union List of Current Periodicals (37.8 thousand records)

3. Union Catalogue of Periodicals (20.3 thousand records)
4. Database of Periodical articles (9.22 lakh records)
5. CD-ROM Database (22.2 thousand records)

Training programmes on J-Gate Plus has been organized on 23rd July 2015 for Southern Region of ICMR institutes at RMRC, Port Blair. Training programme for ProQuest Health & Medical Complete and JGateplus was organized for Western Region of ICMR institutes at NIOH, Ahmedabad on 27th August 2015 and for Central/Northern region of ICMR institutes at NIREH, Bhopal on 23rd September 2015. The training programmes for J Gate Plus and ProQuest was organized at RMRC, Dibrugarh on 29th October 2015 and for JGateplus for Eastern Region of ICMR institutes was organized at RMRC, Bhubaneswar on 27th November 2015. The objectives of these training programmes were to promote the usage of digital resources available under ICMR e consortia.

WEB-BASED EXTRAMURAL PROJECT MANAGEMENT SYSTEM

ICMR provides financial assistance to promote biomedical and health research. In order to improve efficiency of processing of its Extramural Research Program and to save efforts of the Investigators, ICMR has shifted from manual receipt and processing of extramural projects to web-based interactive system. The system encompasses the complete life-cycle of a proposal funding beginning with the submission of project proposals including all required codal documents, project progress reports, utilization certificates, statement of expenditure etc. periodically, online, to its processing, sanction, funding till it's completion through web-based system. The main benefits of this system are : Paper less Process throughout the life of the project; Complete transparency; Results to Principle Investigator in real-time; In-built communication model; Reduction in time and efforts and increase in efficiency; Statistical analysis; Secured intellectual property rights; Easy tracking of proposal status by PIs; Ease in archival & retrieval; No postal liabilities on the Council etc.

In the year 2015-16, ICMR received 1392 pre-proposals using e-PPMS till 31st August, 2015, as

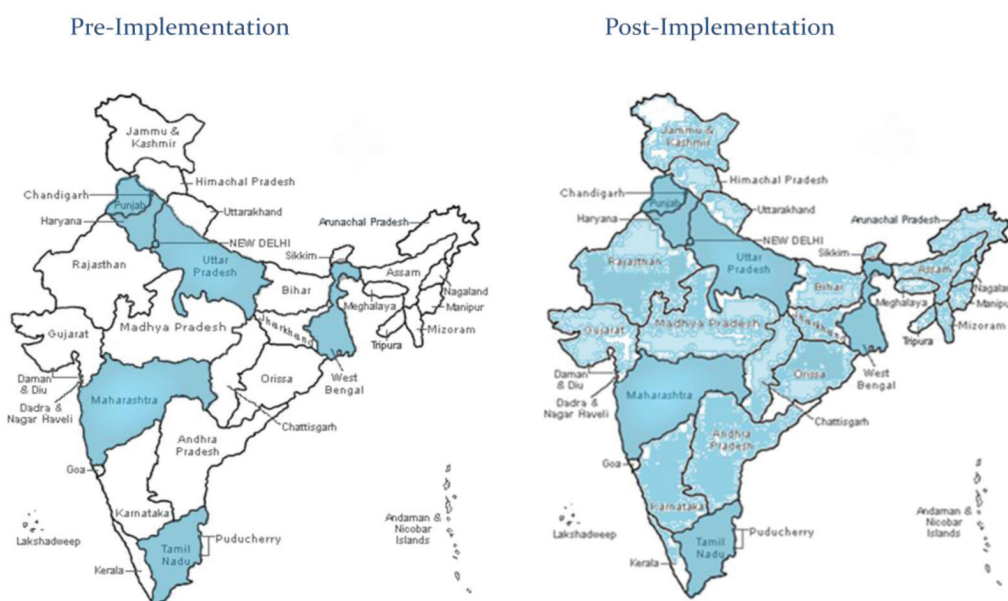


Fig. 12. Increase in outreach.

online submission of open ended extramural pre-proposals had been put on hold w.e.f. September, 1st 2015. These proposals were reviewed and of these, 299 pre-proposals were shortlisted for submission of detailed proposals. Many Call for proposals programmes were also launched during the year which resulted in receipt of several proposals. These included a) Call for proposal on Social determinants and management of Children in difficult circumstances (39 proposals), b). Call for proposal under North East Seed Grant Scheme (164 proposals), c). Call for Proposals on Interventions for Prevention of Prematurity (12 proposals), (d). Call for proposals on Quality of care in Pregnancy and Childbirth (86 proposals), e) Call for proposals for Focused Research on Management of Arsenic Related Diseases (73 proposals), f). Call for Proposals on Drug Utilization Research (51 proposals), g). Call for Proposals on Stillbirth (52 proposals).

Efforts were made to promote proper use of the system by Principal Investigators and reviewers, so that the system became much more user friendly. **Efforts were also made to make system more transparent and paperless in sync with policies of the government.** Online reviewing work was emphasized more during this period.

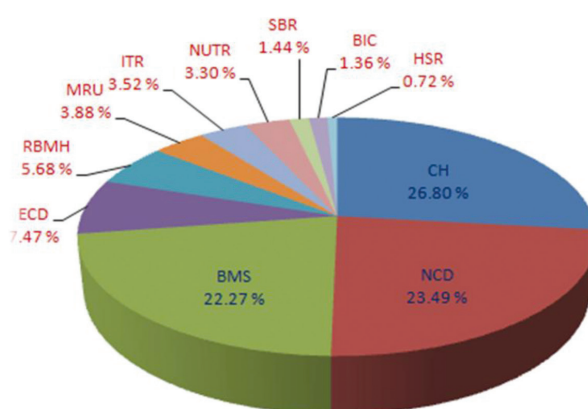


Fig. 13. Percentage share of pre-proposals 2015-16 (Division wise).

A total of 773 full proposals were received online and marked to respective Technical Divisions for processing. During the year, a new Collaborative Online Proposal Submission Scheme has been designed and developed for ICMR-ICSSR Joint Research Programme and a total of 271 Full Proposals were received on the web portal of this scheme.

During the year, recommendations of Experts/Project Review Committees pertaining to a total of 691 detailed proposals were communicated online.

Analysis of online data revealed that the top most subject discipline was Maternal Health which

was followed by North East Seed Grant Scheme, Pharmacology and Oncology. While, in the year 2014-15, Pharmacology (265 proposals) topped the list followed by Child Health and Oncology.

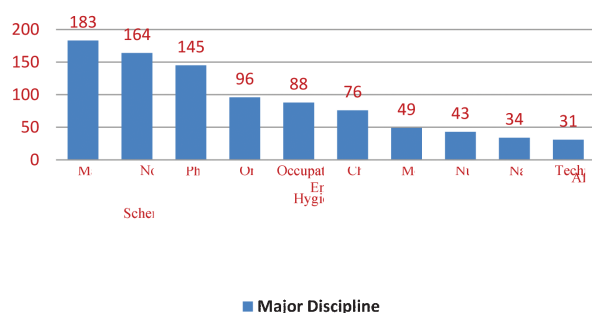


Fig. 14. No. of pre-proposals of top 10 subject discipline 2015-16.

The top Institute was AIIMS, New Delhi, by which a total of 37 proposals were submitted during the

year, followed by Sri Lakshmi Institute of Medical Sciences, Puducherry (30 proposals), King George Medical University, Lucknow (29 proposals), PGIMER, Chandigarh (29 proposals), PSG Institute of Medical Sciences & Research, Coimbatore (18 proposals) and Manipal College of Pharmaceutical Sciences, Manipal (15 proposals).

An initial analysis of the data culled out from the system for 'ad-hoc' proposals being submitted from the different parts of India, have clearly indicated change of productive institutions, subject areas being covered by investigators and the pattern of Cities and the 'Major Discipline' being chosen by investigators. Some of the remote cities, which have entered in the ICMR Extramural Project Scheme, are Amrawati, Anglong, Barpeta, Bhimavaram, Cachar, Gorimedu, Idukki, Una, Nanded, Palakkad, Palampur, Sangli and Rewa.

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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
Ahmedabad 380016
3. National Institute of Epidemiology
R-127, 3rd Avenue
Tamil Nadu Housing Board
Ayapakkam
Chennai 600077
4. National Institute for Research in Tuberculosis
No. 1 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
Beliaghata
Kolkata 700010
10. Centre for Research in Medical Entomology
4, Sarojini Street
Chinna Chokkikulam
Madurai 625002
11. Enterovirus Research Centre
Haffkine Institute Campus
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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 Multistoried 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
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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
Sus Road
Pashan
Pune 411021
21. National AIDS Research Institute
G-73
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
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25. National Centre for Disease Informatics and Research
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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



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