

Annual Report 2013-14



Indian Council of Medical Research
New Delhi

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Published by the Division of Publication and Information on behalf of the Secretary DHR &
DG, ICMR, New Delhi.

Designed & Printed at M/s Aravali Printers & Publishers (P) Ltd., W-30, Okhla Industrial Area, Phase-II,
New Delhi-110020 Phone: 47173300, 26388830-32

DIRECTOR-GENERAL'S MESSAGE

I am happy to present the significant achievements and activities of Indian Council of Medical Research during the year 2013-14. The ICMR has evolved over the years in line with changing health research needs of the country, effectively addressing the new challenges that have emerged especially those affecting the weaker section of the population. ICMR working through a countrywide network of 32 permanent institutes and over 110 field stations provides stewardship, conducts and supports health research, generates knowledge and ensures its utilization, and develops resources for health research in areas of national public health importance. ICMR, an important constituent of Department of Health Research (DHR), serves as the fulcrum for DHR by conducting and supporting research and development for the benefit of the Indian public. The seamless integration between the ICMR (generation of new knowledge) and the DHR (putting this new knowledge to public good) has progressed well. The vision of ICMR is to bring health care technologies to the people by encouraging innovations for development of affordable diagnostics, therapeutics-drugs and devices/treatment methods and vaccines into public health service.



The ICMR continues to march with rapid pace with focus on its flagship programmes on communicable (tuberculosis, HIV, malaria, Japanese encephalitis, leprosy, *etc*) and non communicable diseases like diabetes, cancer, cardiovascular diseases, environmental and occupational health, tribal health, vector borne diseases, mother and child health, malnutrition, *etc*. ICMR is also working for establishment of new centres in geographically uncovered and scientifically deficit areas. It works towards strengthening international cooperation.

The National Institute of Virology (NIV), Pune played a major role in providing assistance to SEARO countries in the region. These include supply of arboviral diagnostic kits, other diagnostic reagents related to respiratory infections in emerging diseases like MERS and H7N9. As a part of providing diagnostic kits for Japanese encephalitis, Chikungunya and dengue, in national/other programs, NIV provided a total of 5094 kits in the year 2013-14.

DNA vaccine development against leptospirosis by the RMRC, Port Blair has crossed another milestone when the project successfully showed that the DNA vaccine construct based on LipL45 sequence elicited both cell mediated and humoral immune response in experimental animals. The Centre is providing referral services in leptospirosis to various institutions in the country as well as to neighbouring countries. Diagnostic services (Microscopic Agglutination Test performance) was provided to 11 Centres, leptospiral reference strains were supplied to 22 centres and control sera to 3 centres.

EVRC, Mumbai is playing an important role in keeping a watch on polio in India and neighbouring countries like Bangladesh, Srilanka and Myanmar by testing stool samples from Acute Flaccid Paralysis (AFP) cases. Wild poliovirus has not been isolated in India in 2013-14 and the country has remained free from wild poliovirus since January 2011.

ICMR scientists published a total of 872 research papers in various national and international journals during this year. A total of 18 patents were filed and 3 patents granted during this period. A total of 190 new adhoc projects, 71 new TF projects and 238 new fellowship were sanctioned during the year under report. Currently a total of over 1200 adhoc projects, 390 Task Force projects and 950 fellowships are ongoing. In addition, the ICMR selected a total of 14 candidates for pursuing MD/Ph.D courses through national level examination. Besides, a total of 34 young Ph. Ds were selected for Post-doctoral fellowship programme of ICMR for the conduct of research in priority areas of health sciences in ICMR Institutes/Centres.

I am happy to report that ICMR has made steady progress in various fields of biomedical and health research. Scientists of ICMR have succeeded in developing indigenous and cost effective technologies for public use; eight of such technologies have recently been launched and work on several others is in final stages. I congratulate the scientists and other staff of ICMR for their dedication, hard work and commitment. I am confident that ICMR scientists will always be ready to face new challenges to solve health problems of mankind, especially faced by our country men.



Dr. V.M. Katoch
Secretary, Department of Health Research
and Director General, ICMR

OVERVIEW

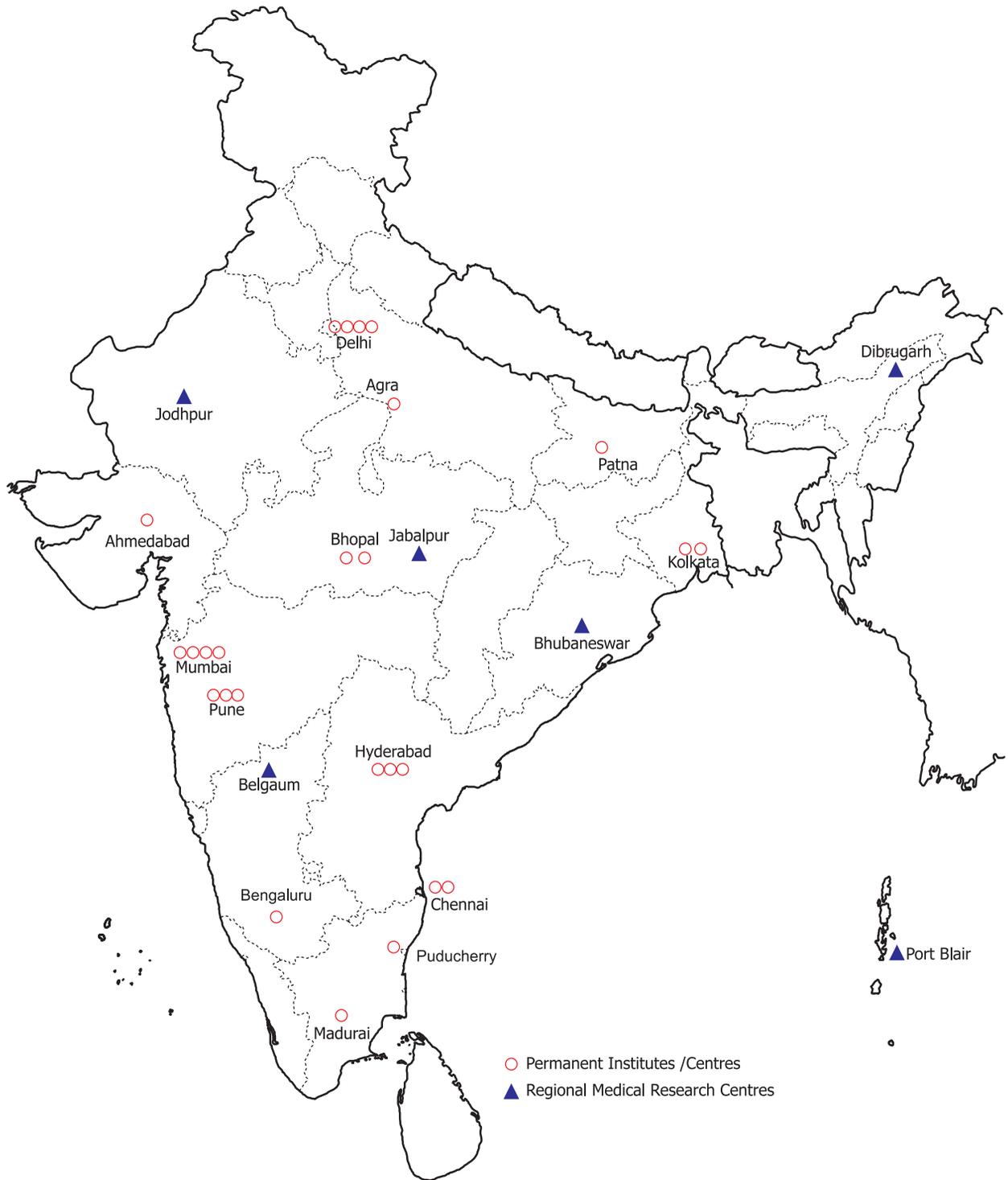
The Indian Council of Medical Research (ICMR), New Delhi, is over a century old autonomous organization, now working under the newly created Department of Health Research (DHR), Ministry of Health and Family Welfare, Government of India. The ICMR has evolved over the years in line with changing health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

ICMR promotes biomedical research in the country through intramural research (by its own Institutes/Centres) and extramural research (through grants-in-aid given to projects through a transparent rigorous review process mainly to non-ICMR Institutes). The extramural research is supported through (i) setting up Centres for Advanced Research in different research areas around existing expertise and infrastructure in selected departments of Medical Colleges, Universities and other non-ICMR Research Institutes; (ii) Task force studies which emphasise a time-bound, goal-oriented approach with clearly defined targets, specific time frames, standardized and uniform methodologies and often multicentric structure; (iii) long term projects targeting issues like vector control, nutrition, reproductive health, *etc*; (iv) Open-ended research on the basis of applications for grants-in-aid received from scientists from medical colleges and research institutes located in different parts of the country. The research activities carried out by the ICMR during 2013-2014 are highlight in the following pages.

Emergence of resistance to anti-TB drugs is one of the major challenges for the control of TB globally. Multi-drug resistant TB (MDR-TB) has now been reported in almost all parts of the world, and extensively drug resistant TB (XDR-TB) cases have been confirmed in 58 countries. An online resource called 'Database for Drug Resistant Tuberculosis (DDRTB)' is being developed at the ICMR Biomedical Informatics Centre, National Institute for Research in Tuberculosis, Chennai.

Wild poliovirus transmission in India and neighbouring countries like Bangladesh, Srilanka and Myanmar is detected by testing stool samples from Acute Flaccid Paralysis (AFP) cases at Enterovirus Research Centre, Mumbai. Polioviruses isolated in India, Bangladesh, Srilanka and Myanmar are also being received at the Centre for maintaining poliovirus bank, reconfirmation of the results and further characterization. A total of 4572 poliovirus isolates were received until November 2013. Wild poliovirus has not been isolated in India in 2013-14 and India has remained free from wild poliovirus since January 2011.

Analysis of 33 years climatic data at National Institute of Cholera and Enteric Diseases (NICED), Kolkata bestowed convincing information on relation between cholera infection and climatic factors by the influence of El Nino and La Nina-southern Oscillation (ENSO), which has shown to have positive role in controlling the prevalence of cholera in Kolkata. The year 2014 is strongest El Nino year ever observed since 1998, which may impact the climate in the adjacent land areas.



ICMR INSTITUTIONAL NETWORK

Effectiveness of heat killed multi serotype shigella (HKMS) immunogen against shigellosis in rabbit model has been established by the scientists of NICED, Kolkata. Challenge studies in animal showed broad spectrum protection against shigellosis. Results generated through this study suggested that the HKMS immunogens induced humoral and Th1 mediated adaptive immunity that caused complete protection against experimental *Shigella* infection in animal model. This could be a broad spectrum non-living vaccine candidate for human use in recent future which may stimulate long term adaptive immune responses against 50 serogroups of *Shigella* spp. Work with *Giardia lamblia* Kolkata isolates revealed mixed or recombinant strains. These strains also showed new metabolic cascade during its stress relief under oxidative environment. Identification and assessment of proper hygiene in relation to enteric parasitic infection among low socio economic classes have been identified as a major concern in preventing the disease.

Baseline results from “Gender Equity-Focused, Male-Centered Family Planning for Rural India” by National Institute of Research in Reproduction Health, Mumbai suggested the need to enhance spousal communication on family size and family planning to increase the use of modern contraceptive methods.

Severely ill MIC gas affected patients identified during epidemiological surveys, and in need of emergency care/specialized care are being examined at doorstep through community based health services being undertaken by the National Institute for Research in Environmental Health, Bhopal and if needed, being transported to Bhopal Memorial Hospital and Research Centre for investigation and treatment. Over 200 patients were benefitted under this programme.

The National Institute of Virology (NIV), Pune completed the restructuring of Virus Repository at par with international protocols of biosecurity and standards. The activity has been successfully launched as a part of the NIV Bio Banking programme and viruses given to researchers in the country.

The NIV played a major role in providing assistance to SEARO countries in the region. These included supply of arboviral diagnostic kits, other diagnostic reagents related to respiratory infections in emerging diseases like MERS and H7N9. As a part of providing diagnostic kits for Japanese encephalitis, Chikungunya and dengue, in national/other programme, NIV provided a total of 5094 kits in the year 2013-14.

Regional Medical Research Centre (RMRC), Port Blair investigated the (i) Rotavirus diarrhoea occurred in Radha Nagar of North Andaman; (ii) Hand foot mouth disease (HFMD) in Andaman – identified the causative agent as Enterovirus 71 and Coxsackievirus A-16; and (iii) Dengue among the employees of Wharf in Port Blair – identified dengue virus 3 (DENV-3) as the causative agent (this was the first report of DENV-3 infection in Andaman and Nicobar Islands) within 24 hours following the outbreaks and assisted in initiating the necessary measures for the containment of these outbreaks.

DNA vaccine development against leptospirosis by the Centre has crossed another milestone when the project successfully showed that the DNA vaccine construct based on LipL45 sequence elicited both cell mediated and humoral immune response in experimental animals. The study also identified the dominant T cell epitopes.

The RMRC, Port Blair is also providing referral services in leptospirosis to various institutions in the country as well as to neighbouring countries. Diagnostic services (Microscopic Agglutination Test performance) was provided to 11 centres, leptospiral reference strains were supplied to 22 centres and control sera to 3 centres. The Centre also established the facilities for development and production of diagnostic kits. The facilities comprise of four isolated laboratories for preparation of antigens, development of kits, evaluation chamber and packing and dispatch compartments.

The International Health Division of ICMR co-ordinates international collaboration in biomedical research between India and other countries as well as with national & international agencies. Division has facilitated

signing of two Memoranda of Understanding (MOU) with International Agencies during the period. This included DHR-National Institute for Health and Care Excellence (NICE)-MOU signed in UK on June 14, 2013 and Addendum two- Global Alliance for Chronic Diseases (GACD) Secretariat Funding and Collaboration Agreement as part of ICMR-GACD MOU signed in New Delhi on March 5, 2014.

A total of eight indigenous technologies were launched during 2013-2014. These included (i) The first indigenously developed Japanese Encephalitis vaccine (JENVAC) under Public Private Partnership; (ii) Thalassemia Detection Kit developed by the ICMR's National Institute of Immunohaematology, Mumbai; (iii) An "AV Magnivisualizer" for detection of cervical cancer developed by the ICMR's Institute of Cytology and Preventive Oncology (ICPO), Noida; (iv) Two simple and affordable glucose monitoring devices and testing strips developed under Extramural project; (v) Three indigenous affordable technologies namely, PCR based Food Pathogen Detection Kit, ELISA kit for Ferritin Estimation and Dried Blood Spot (DBS) collection kit for vitamin A analysis, all developed by the National Institute of Nutrition, Hyderabad. Work on several other technologies in final stage.

A total of 18 patents were filed during the year. Of these, 11 were from intramural institutes. During this period, three patents (2 from intramural and 1 from extramural institute) have been granted. ICMR has off shelved an old technology on *Vijaysar*, an antidiabetic drug generated in 1999 to a company after signing a tripartite agreement and assignment deed between the Indian Institute of Integrative Medicine (IIIM), Jammu and ICMR. This technology was an outcome of the joint effort of Indian Institute of Integrative Medicine (IIIM), Jammu (CSIR) and ICMR

ICMR scientist published a total of 872 research papers in various national and international journals during this year. A total of 190 new adhoc projects, 71 new TF projects and 238 new fellowships were sanctioned during the year under report. Currently a total of over 1200 adhoc projects, 390 Task Force projects and 950 fellowships are ongoing. In addition, the ICMR selected a total of 14 candidates for pursuing MD/Ph.D courses through national level examination. Besides, a total of 34 young Ph.Ds were selected for Post-doctoral fellowship programme of ICMR for the conduct of research in priority areas of health sciences in ICMR Institutes/Centres.

The ICMR has finalized three draft bills on (i) The Biomedical and Health Research Regulation Bill, (Ethical Issues pertaining to Biomedical Research); (ii) Assisted Reproductive Technology (Regulation) Bill ; and (iii) The Recognition of New Systems of Medicine Bill 2014 (Recognition of Alternate Systems of Medicine). Cabinet notes for all these three bills have been circulated.

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COMMUNICABLE DISEASES

Communicable and infectious diseases including vector borne diseases like malaria, filariasis, tuberculosis, leprosy, cholera, diarrhoea, dengue, chikungunya, Japanese encephalitis, leptospirosis and other viral infections still continue to become a major public health problem. ICMR's 16 institutes have focused their research to understand the complex epidemiology of these diseases and strive hard to find better solutions to control these diseases & contain outbreaks/epidemics for the benefit of the society.

The research activities carried out by ICMR's various institutes during the reported period in the area of communicable diseases is mentioned below:

Intramural Research

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS, CHENNAI

CLINICAL RESEARCH

A randomized controlled clinical trial was initiated for comparing daily vs. intermittent 6-month short course chemotherapy in reducing failures & emergence of acquired rifampicin resistance (ARR) in patients with HIV and pulmonary tuberculosis.

An open labelled, prospective, parallel arm, active comparator, randomized controlled clinical trial in HIV-infected patients with newly diagnosed pulmonary TB, is comparing daily and intermittent regimens of anti-TB treatment (ATT). Patients are randomized to receive one of the three regimens, (i) daily (2EHRZ₇/4HR₇) (ii) partly intermittent (2EHRZ₇/4HR₃) (iii) fully intermittent (2EHRZ₃/4HR₃), given for six months and followed up for a further period of one year. The primary outcome is

reduction of ARR among bacteriological failures. Interim findings from 212 patients indicate that the daily regimen has higher sputum smear and culture conversion at the end of intensive phase. The study is ongoing.

Predictors and immunologic characterization of tuberculosis-associated immune reconstitution inflammatory syndrome in HIV-TB patients started on antiretroviral therapy

Limited information is available on the evaluation of risk factors that could predict TB-IRIS among HIV-TB co-infected patients. This study was conducted in a cohort of culture confirmed rifampicin (RMP) sensitive pulmonary TB patients who are naïve to ART and ATT. The incidence of TB-IRIS was observed to be 56%; further, among a panel of inflammatory cytokines that were evaluated, a combination of IL-6 and C-reactive protein was the best possible predictor, since there was an increase at baseline and further increase during the subsequent IRIS event. The incidence of IRIS in patients treated with daily and intermittent regimens is also being studied. So far, 46 cases of IRIS have been evaluated. The study is ongoing.

Evaluation of newer diagnostic tools and feasibility of consensus case definition in the diagnosis of intra thoracic TB in children

This prospective study aims to determine the diagnostic accuracy of Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) in the diagnosis of intra-thoracic TB in children and to study the feasibility of utilizing the newly developed consensus case definition. It is proposed to include 2800 children aged < 15 yrs. Recruitment of children to this study is in progress.

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

A randomized clinical trial to study the efficacy and safety of 3- and 4-month moxifloxacin-containing regimens in the treatment of patients with sputum positive pulmonary TB is in progress in Chennai and Madurai. The aim of this study is to shorten the duration of TB treatment by supplementing the standard 4-drug TB regimen with moxifloxacin. Newly diagnosed sputum positive, HIV seronegative pulmonary TB patients are randomly allocated to 3-month or 4-month moxifloxacin regimens, or a control 6-month regimen. Treatment is given under direct observation and response to treatment is assessed with sputum culture examination every month during treatment and follow-up. The patients are also closely monitored for adverse drug reactions and followed up.

Interim analysis of the data from 931 patients has shown that those treated with the moxifloxacin-containing regimens become sputum culture negative earlier and to a greater extent (94%) than those treated with the control regimen (77%). Recruitment of patients to the trial is continuing, though intake to the 3-month arm was discontinued by the DSMB.

Evaluation of different strategies (pharmacologic intervention versus enhanced motivation vs. standard motivation) for smoking cessation in TB patients under treatment in the RNTCP

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

DMCs from two districts (Villupuram and Kanchipuram) were randomly selected to receive any of the three, such as (i) Bupropion SR along with standard counseling, (ii) enhanced counseling and (iii) Standard routine counseling (control arm). Smoking cessation is assessed by self reporting and confirmed by breath carbon monoxide testing, at 0, 2 and 6 months of ATT. TB outcome was recorded

at 6th month. The sample size was calculated to be 1200 patients, 400 in each arm. The study is ongoing.

Randomised clinical trial to study the efficacy and tolerability of a 4-month regimen containing ofloxacin compared to the standard 6-month regimen in the treatment of patients with superficial lymph node TB

A prospective, randomized (open-label) parallel arm, controlled clinical trial is ongoing that is investigating a four-drug (rifampicin, isoniazid, pyrazinamide and ofloxacin) daily during the intensive phase of two months, followed by three drugs (rifampicin, isoniazid and ofloxacin) thrice weekly during the continuation phase, in patients with TB lymph node. The control regimen is the standard 6-month thrice-weekly regimen of rifampicin, isoniazid, ethambutol and pyrazinamide for 2 months followed by rifampicin and isoniazid for 4 months. The study objectives are to (i) compare the efficacy of the regimens in terms of (a) Response at the end of treatment and (b) Relapse up to 24 months of follow-up after treatment and (ii) compare the incidence of (a) "Paradoxical reaction" during treatment and follow up and (b) Adverse reactions.

The study population comprises of patients attending the surgical, medical clinics of Madurai Rajaji Hospital and Govt. Hospitals and Corporation RNTCP Centres in Chennai. The estimated sample size is 320 patients; so far 33 patients have been enrolled in the trial.

HIV associated lipodystrophy syndrome in children: Role of nutrition, anti retroviral treatment and genes

A prospective observational multi-centric study is ongoing to determine the incidence and risk factors for dyslipidemia, abnormalities in glucose tolerance and body shape abnormalities, in HIV-infected children after initiating ART. Further, the role of genetic factors in the development of fat redistribution, insulin resistance and dyslipidemia is also being studied. So far, 277 HIV-infected children in Chennai, Madurai and Bengaluru between the age group of 2-12 years have been recruited against the targeted sample size of 440 children.

High density lipoprotein cholesterol and gene polymorphisms among HIV infected South Indians on First line antiretroviral therapy

This is a cross-sectional study to determine whether HDL-cholesterol gene polymorphisms (single nucleotide polymorphisms in ABCA1, CETP, LIPC, LPL and APOC3 genes) are associated with unfavorable blood HDL-cholesterol levels, in HIV infected adults in south India, after 12 – 15 months of a nevirapine-based ART regimen.

The study outcome targeted to examine the presence of single nucleotide polymorphism in ABCA1, CETP, LIPC, LPL and APOC3 genes, in individuals with low HDL-c after 12 – 15 months of ART. So far, 275 patients have been recruited against the required sample size of 300 patients. The study is in progress.

Study on the effectiveness and feasibility of TB preventive therapy for adults and children living with HIV in India

A prospective multicentric study with phased implementation is in progress to assess the effectiveness of IPT in PLHIV (adults & children). The study consists of two phases: Phase I: Enhanced TB surveillance (all sites, all ART centre attendees) for all HIV infected adults and children and Phase II: Provision of IPT: After 6 months (following the completion of Phase I), all eligible patients will be administered IPT.

The sample size required for the study is 6000 adults and 1800 HIV infected children. The study was initiated in five ART centers in Tamil Nadu, two in Karnataka, one each in Andhra Pradesh and New Delhi. So far, 4000 adults and 1000 children have been recruited.

Of the 1688 symptomatic PLHIVs, 111 were diagnosed with TB. Among the 111 TB patients, 31 were sputum smear positive, 30 were sputum smear negative (active TB) and 50 cases were extrapulmonary TB, mainly TB lymphadenitis.

Among children with HIV, overall prevalence of TB was 0.95% (4 cases) and TB incidence was 1.8 per 100 person-years.

EPIDEMIOLOGY

Effect on survival rate due to TB disease: study among TB patients treated under the RNTCP in Tiruvallur district, Tamil Nadu

A cross sectional study is being carried out in a TB unit in Tiruvallur district in Tamil Nadu, where, around 4000 TB patients were registered for treatment under Government health facilities in the age range of 15 to 64 years during 2000-2004. A control group, about 12,000 in number (not affected by TB disease) was selected in a ratio of 3 : 1.

A semi-structured and pre-coded interview schedule was used for data collection after pilot testing; quality of life was assessed using the WHO-BREF questionnaire. Person years were calculated for both the TB and control groups. An interim analysis based on data from 1166 control subjects and 640 TB patients showed that death among TB cases and controls was 26.8 and 17.0 persons per year in thousand population respectively. The study is in progress.

Screening of inmates and prison guards in the Puzhal prison, Chennai for TB

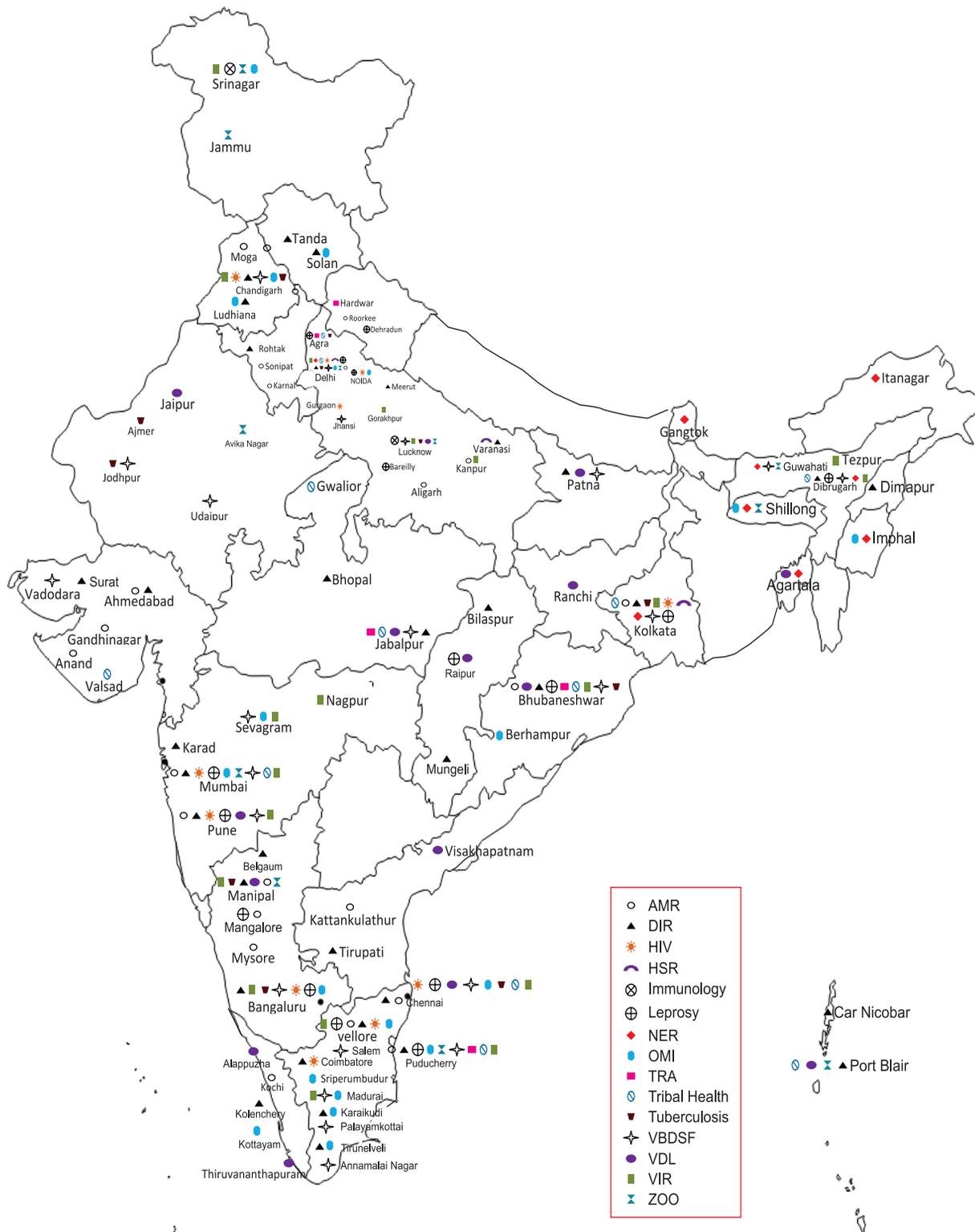
On the request of DG, Prisons, the inmates of the Puzhal prison, in-house staff and their family members were screened by X – ray and TB symptoms. Symptomatic persons and those with abnormal radiography and history of previous ATT provided sputum for AFB smear and mycobacterial culture.

A total of 2292 individuals were examined; 17 were diagnosed with TB. The rate was 10 per 1000 inmates in convicts, 8 per 1000 inmates in remand and overall rates for both groups combined was 8 per 1000. This rate is more than that observed in the general population (3.41 per 1000) in Chennai. This study highlights the need for TB screening at entry and for the presence of a medical officer in prisons.

BACTERIOLOGY

Performance of light emitting diode microscope in two different settings for TB diagnosis: a multi-centric study

The aim of this study was to evaluate the performance of Primo Star iLED microscope



MAJOR ICMR RESEARCH PROJECTS IN COMMUNICABLE DISEASES

in different settings for TB diagnosis. The study sites were the National Institute for Research in Tuberculosis (NIRT), Chennai and the Regional Medical Research Centre (RMRC), Bhubaneswar. The estimated sample size was 1350 samples collected from consecutive symptomatic patients at each study site.

So far, 499 sputum specimens have been processed at Chennai. Sputum smears were made from the samples and randomized by a statistician. Of these, 54 were smear positive and the remaining 445 were smear negative. There were 58 culture positive cases. The agreement between iLED and ZN for both direct and deposit smears is being evaluated.

International Centre of Excellence in Research Host immune responses in lymphatic filariasis

The Institute examined the frequency of Th17 and Th22 cells in individuals with filarial lymphedema, in clinically asymptomatic, infected and in uninfected individuals *ex vivo* and in response to parasite and non-parasite antigens.

Those with disease had a significantly expanded frequency of Th17 and Th22 cells compared to either infected or uninfected individuals at baseline (*ex vivo*) and in response to parasite antigen. This antigen driven expansion of Th17 and Th22 cells was dependent on IL-1, IL-23 and to a lesser extent on TGF β , since blockade of any of these cytokines resulted in significantly diminished frequencies of Th17 and Th22 cells. These findings suggest that filarial – parasite driven expansion of Th17 and Th22 cells is associated with the pathogenesis of filarial infection and disease.

Immunology of helminth-tuberculosis co-infections

The CD4⁺ and CD8⁺ T cell responses as well as the systemic (plasma) cytokine levels was examined in individuals with pulmonary TB with or without two distinct helminth infections - *Wuchereria bancrofti* and *Strongyloides stercoralis* infection.

There was a significant diminution in the mycobacterial – specific frequencies of mono - and multi – functional CD4⁺ Th1 and (to a lesser extent) Th17 cells when concomitant filarial or *Strongyloides* infection occurs. The impairment in CD4⁺ and CD8⁺ T cell cytokine responses was antigen – specific, as polyclonal activated T cell

frequencies were equivalent irrespective of helminth infection status. This diminished frequency of multifunctional CD4⁺ T cell responses was partially dependent on IL-10 as IL-10 blockade significantly increased the frequencies of CD4⁺ Th1 cells.

Co-existent helminth infection was associated with an IL-10 mediated profound inhibition of antigen - specific CD4⁺ T cell responses as well as protective systemic cytokine responses in active pulmonary TB.

Diminished systemic and antigen-specific Type 1, Type 17 and other pro-inflammatory cytokines in diabetic and pre-diabetic individuals with latent TB

The Institute examined the circulating plasma cytokine levels in individuals with latent TB infection (LTBI) with diabetes or pre-diabetes and compared them to those with LTBI with normal glycemic control. LTBI with DM or pre-DM is characterized by diminished circulating levels of Type 1 (IFN γ , IL-2 and TNF- α) and Type 17 (IL-17F) cytokines. This was associated with decreased systemic levels of other pro-inflammatory cytokines (IL-1 β and IL-18) and the anti-inflammatory cytokine (IL-10) but not Type 2 cytokines. There was no significant correlation between any of the cytokines measured (with the exception of IL-22) with HbA1c levels.

This study shows that LTBI in the presence of diabetes or pre-diabetes is characterized by diminished production of cytokines implicated in control of TB, allowing for a potential immunological mechanism that could account for the increased risk of active TB in diabetes mellitus.

BIOINFORMATICS

Homology modeling, docking, pharmacophore and site directed mutagenesis analysis to identify the critical amino acid residue of PknI from *Mycobacterium tuberculosis*

PknI is one of the 11 functional Serine/Threonine protein kinases predicted to regulate cell division in *M. tuberculosis*. Bioinformatics approach was used to identify the functionally active residues of PknI. A homology model for PknI was created and comparative structural analysis of PknI with other kinases was performed. Molecular docking studies

were done with a library of kinase inhibitors. T95 and B31 were found to be the most potent inhibitors for PknI. Based on structure-based pharmacophore analysis of kinase substrate complexes, Lys 41 along with Asp90, Val92 and Asp96 were identified as functionally important residues. Using site directed mutagenesis technique the institute mutated Lys 41 to Met and observed a defect in cell division in *M. smegmatis* mc²155, thus confirming *in silico* prediction that Lys41 is critical for the function of PknI.

Phylogenetics of HIV-1C isolates circulating in India

The Institute performed phylogenetic analysis for whole genomes as well as individual proteins of HIV-1 subtype C sequences from India, in order to clearly understand the phylogenetic dynamics of Indian HIV-1C strains.

Sixty five whole HIV-1 subtype C genome sequences sampled from 19 different countries during the years 2000-2010 and used as reference sequences in the HIV Sequence Compendium were downloaded from the HIV Sequence Database. Phylogenetic analysis was performed for the 65 full length sequences as well as individual proteins of HIV-1C using MEGA (Molecular Evolutionary Genetics Analysis, version 4.0 and version 5.0). It was found that, out of 20 Indian isolates, 19 (95%) were placed in a single cluster and one isolate (Accession ID: AF286223) was an outlier. Further, a lone HIV-1C isolate from China (Accession ID: AY967806) was part of the Indian cluster.

Phylogenetic analysis was also extended to individual proteins of HIV, and phylogenetic trees were constructed using MEGA. Of the 18 envelope sequences from India included for the construction of the phylogenetic tree, 17 formed a cluster into which a lone sequence from China was also included.

The present phylogenetic study confirms previous observations that HIV-1C strains from India are more closely related among themselves than with isolates circulating in other countries. This observation suggests for a possibility of designing a vaccine specifically targeting HIV-1C isolates circulating in India.

Identification of promiscuous epitopes in HIV-1C virus of Indian sequence using *in silico* tools

The HIV – 1C virus circulating in India is reported to be more closely related among themselves than with clade C viruses from other countries. The aim of this study was to identify promiscuous epitopes from consensus sequence of HIV-1C from India using multiple bioinformatics tools. Total forty nine potential promiscuous CTL epitopes were identified – about one third of these epitopes were from the envelope (9 from Gp120 and 6 from Gp41), 9 epitopes were from RT, 7 were from Gag and 5 from Nef. Out of the 49 epitopes, 43% (21/49) were found to be previously reported as immunogenic and listed in HIV Database.

Database for Drug Resistant Tuberculosis – DDRTB

Emergence of resistance to anti-TB drugs is one of the major challenges for the control of TB globally. Multi-drug resistant TB (MDR-TB) has now been reported in almost all parts of the world, and extensively drug resistant TB (XDR-TB) cases have been confirmed in 58 countries. An online resource called ‘Database for Drug Resistant Tuberculosis (DDRTB)’ is being developed at the ICMR Biomedical Informatics Centre, NIRT, Chennai.

The DDRTB contains data on 170 unique variables about drug resistant TB patients. The database has been developed using MySQL and HTML. In the initial phase, the database will be local and capture available data on all MDR-TB patients registered at NIRT since February 2008, and all XDR-TB patients registered at NIRT since February 2013. Currently, the database contains clinical and laboratory data on 21 XDR-TB and 26 MDR-TB patients.

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

With the initiation of the TB structural consortium, the protein structural space for *M. tuberculosis* has been steadily increasing. Currently, 969 experimental structures are available for 354 of the 4,018 proteins of bacteria. The institute has developed a web page to provide information such

as root means square deviation (RMSD), sequence identity, presence of mutations, torsional angles, *etc.* to understand the differences between multiple structures for each gene of *M. tuberculosis*. Torsion angles were used to perform Principal Component Analysis (PCA) to identify conformational differences between structures. The institute has identified three important *M. tuberculosis* drug targets *viz.* inhA, alanine dehydrogenase and FabH proteins having multiple crystal structures. This online resource would be useful for selecting appropriate protein structures for molecular docking and structure based drug designing studies.

Updating of *M. tuberculosis* Structural Database

The institute developed a database of all experimentally solved protein structures of *M. tuberculosis* which is available online at www.bmi.icmr.org.in/mtbsd/MtbSD.php (*Tuberculosis* 2011;91(6):556-62). The database contains systematically analyzed and categorized structural data of each protein and serves as a useful resource for structure based drug designing studies. The database has now been updated with 130 newly solved protein structures and their structural homologues, a functional annotation page has been provided for hypothetical proteins and a search option has been added for searching structures based on PDB id, country, authors, journals. The database is now integrated with BLAST, ClustalW tools. BLAST tool can be used for searching *M. tuberculosis* homologues in MtbSD database. Further, the user can select any number of Blast hits that are similar to the submitted sequence and submit for multiple sequence alignment using ClustalW. Option for saving both BLAST as well ClustalW results have been provided.

BIOCHEMISTRY & CLINICAL PHARMACOLOGY

Pharmacokinetics of anti-TB drugs in HIV-infected children with TB

The pharmacokinetics of RMP, INH and PZA was studied, with factors influencing drug pharmacokinetics and TB treatment outcome in HIV-infected children with TB, treated with fully intermittent regimens in the RNTCP.

HIV-infected (n = 77) children with TB aged 1 to 15 years from six hospitals in India were recruited. During the intensive phase of TB treatment, a complete pharmacokinetic study was performed, after directly observed administration of drugs. Treatment outcomes were taken from the RNTCP treatment card. Children with HIV & TB had significantly lower RMP peak concentration (C_{max}) and exposure (AUC_{0-8}) than those with only TB. Children below 5 years had lower C_{max} and AUC_{0-8} of INH and PZA than those above 5 years. C_{max} of RMP and PZA were significantly lower in children with unfavourable than favourable outcomes. Among all factors studied, PZA C_{max} influenced TB treatment outcome. The proportion of children with sub-therapeutic C_{max} of RMP, INH and PZA was 97, 26 and 34% respectively.

This study showed that younger age and HIV infection had an adverse impact on drug levels. PZA C_{max} significantly influenced treatment outcome in HIV-infected children with TB. The findings have important clinical implications and suggest the need to increase anti-TB drug doses in children with HIV & TB.

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

This study aims to examine the influence of several factors such as diabetes, HIV infection, malnutrition, age, smoking, alcohol intake, gene polymorphisms *etc.* on 2-hour concentrations of RMP, INH and PZA in adult TB patients treated in the RNTCP. The drug levels will also be correlated with TB treatment outcome. This prospective cohort study is recruiting adult TB patients with pulmonary or extrapulmonary TB started on category I or II ATT from the RNTCP treatment centres in Chennai Corporation. Interim analysis of data from 692 patients showed that 81, 11 and 17% respectively had sub-therapeutic RMP, INH and PZA. Further, patients with random blood glucose > 200 mg/dl had significantly lower INH and PZA levels than those with random blood glucose < 200 mg/dl. Recruitment of patients is in progress. All patients are being followed up and treatment outcomes will be obtained.

HIV/AIDS

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

The aim of this study was to examine differences in the codon usage patterns between HIV-1 and HIV-2, and to correlate these differences with the diminished virulence/pathogenesis of HIV-2 as compared to HIV-1. Analysis of genome composition of HIV-1 and HIV-2 showed that more than 50% of the HIV-1 & 2 genomes were composed of AT bases (AT-rich). Comparative analysis of the effective number of codons (Nc) for each of the nine genes of the two viruses revealed that the *tat* gene of HIV-2 had a comparatively higher Nc value than that of HIV-1 *tat* gene, implying lower levels of expression of the HIV-2 *tat* protein. Further, the GC composition of the regulatory genes of HIV-2 was >50%, unlike that of the structural genes which are AT-rich, indicating a firm effort by these viruses to adapt themselves to evolutionary survival. Thus differential codon usage might be one of the possible factors responsible for the diminished immunopathogenicity of HIV-2 in the host as compared to HIV-1.

Enhancing immunogenicity of HIV-1 epitopes by rational modification

The aim of this study was to identify a set of promiscuous, high affinity binding epitopes of HIV-1 subtype C isolates found in Indian population, and to evaluate a strategy to enhance the immunogenicity of the epitopes by rational modification of the amino acid composition.

Based on bioinformatics analysis and available literature, a set of 12 epitopes of HIV-1C as promising candidate epitopes were identified. Two of these epitopes that were widely recognized in the test subjects were subjected to *in silico* modification by substitution with amino acids other than the one present in the wild type form at position 2 (P2). The two wild type peptides as well as the eight analogs were tested *in vitro* for immunogenicity on PBMC obtained from HIV-infected individuals, by measurement of cytokines in culture supernatants using the Bio-Plex cytokine assay.

Of the 12 promiscuous high affinity binding epitopes tested using the IFN-gamma ELISpot assay, two

epitopes (E2 and E9) were widely recognized among the study subjects. It was found that all analogs of both E2 and E9 induced production of higher levels of Th1 cytokines, particularly IFN-gamma, TNF-alpha and IL-1 beta. Further, analogs with L and W stimulated >100-fold higher levels of IFN-gamma than the corresponding wild type epitope in at least one-third of the individuals. This study has demonstrated that immunogenicity could be improved by rational modifications of amino acid residues present at the anchor site in the epitopes.

IMMUNOLOGY

Construction of double knock out mutants of *M. tuberculosis*, characterization and their efficacy assessment as vaccine candidates

M. tuberculosis double knockout (DKO) mutant strain was constructed by specialized transduction protocol. The two genes which are knocked out in DKO strain are PknI, a Serine threonine protein kinase and DacB2, a Penicillin binding protein. The DKO strain showed smoother colony morphology and irregular cell wall structure and was defective in biofilm and cord formation. It was more sensitive to cell wall damaging agents and isoniazid. The virulence of the DKO strain was attenuated in guinea pig animal model. The DKO strain was injected via aerosol route. After 5th week and 10th weeks the animals were sacrificed and the organs were aseptically removed. A small portion of lungs and spleen was used for bacterial CFU counting and another portion for histopathology. Overall, these animal studies suggest that, both the DKO strain and Δ DacB2 strain were attenuated. These double and single knock out constructs have to be further tested for their efficacy as vaccine candidates.

Regulatory role of chemokine gene polymorphisms on chemokine expression in PTB

Chemokine gene polymorphisms have been shown to be associated with susceptibility or resistance to various infectious diseases. The aim of this study was to examine the association between chemokine gene polymorphisms and susceptibility or resistance to TB.

This study showed that (i) Healthy control subjects with -2518GG genotype of CCL2 gene showed a

decreased *M. tuberculosis* antigen induced CCL2/MCP-1 level when compared to subjects with AA genotype. (ii) *M. tuberculosis* antigen induced CCL5/RANTES was significantly lower in PTB patients with -403AA genotype of CCL5 gene as compared to PTB patients with GG genotype. These findings suggest that variant genotypes of CCL2 and CCL5 genes may be associated with decreased production of CCL2 and CCL5 at the site of *M. tuberculosis* infection and may affect recruitment of monocytes and T cells in granuloma formation.

Effect of vitamin D₃ on CD14 and CD206 (mannose Receptor) gene expression in macrophages infected with *M. tuberculosis*

To understand the effect of vitamin D₃ on the expression of CD14, CD206, CD209, Beclin and ATG-5 in monocyte/macrophages in pulmonary TB patients a study was undertaken. The study showed that Vitamin D₃ significantly up-regulated the CD14 and CD206 (Mannose Receptor) mRNA expression in *M. tuberculosis* infected macrophages in healthy controls and pulmonary TB patients. These findings suggest that vitamin D₃ may enhance the macrophage phagocytosis of *M. tuberculosis* in healthy control subjects and pulmonary TB patients.

Use of alternative biomarkers other than IFN- γ

The suboptimal sensitivity of IFN- γ Release Assays (IGRAs) emphasizes the need for alternative markers for diagnosing active TB. The aim of the study was to evaluate whether *in vitro* Quantiferon TB Gold assay (QFT-GIT) antigens specific IL-1 β , IL-6 IL-8, IL-2, IL-12 (p40) and TNF- α production is associated with active TB.

In a cohort of 77 pulmonary TB patients (PTB), 67 healthy household contacts (HHC) and 83 healthy control subjects (HCS), the antigen specific cytokines levels were determined in supernatants generated from QFT- GIT tubes. Results suggest that antigen specific IL-1 β levels were associated with active TB. Further studies are required to evaluate the role of IL-1 β in active TB diagnosis.

Dormancy associated antigens of *M. tuberculosis*

The aim of the study was to identify differentially regulated genes under hypoxia from laboratory

strain H37Rv and two clinical strains (S7 and S10) of *M. tuberculosis*.

Genes such as fad4 (Rv0214), Rv0719 (50S ribosomal protein L6) and Rv0347 were expressed almost 4 fold in all three strains. Highest level of upregulation (9.85 fold) was observed for Rv2293 in H37Rv, Rv1307 (atpH) 4.84 fold over-expressed in S7 and Rv2300c (hypothetical protein) was overexpressed in S10. These variations in gene expression highlight the need for studying the most prevalent strains.

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

The aim of this study was to analyze whether antigen specific IFN- γ response against Rv2204c, Rv0753c, Rv0009 can be used for the differentiation of latent TB infection (LTBI) and active TB (PTB).

Rv2204c, Rv0753c, Rv0009 and Standard antigens induced significantly higher IFN- γ production in HHC compared to active TB. Test antigens showed more sensitivity when compared to standard antigens. This preliminary study shows that these three antigens are differentiating LTBI from active TB disease in terms of IFN- γ levels.

SOCIAL & BEHAVIORAL RESEARCH

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

The aim of this study was to understand the psychosocial issues facing MDR-TB patients and to gain insight on the factors that influence treatment adherence and quality of life.

This was a qualitative study that was part of a larger experimental study to design an acceptable intervention for MDR TB patients. Four Focus Group Discussions (FGDs), 68 in-depth interviews among patients and 16 interviews with Health care providers (Doctors, Health visitors, Sector Health Nurse, Lab technicians and Treatment supervisors) have been completed. The findings from this phase will be used to develop an intervention programme that is feasible and acceptable for MDR Patients which will be tested through an experimental design. The study findings have brought out the psychosocial needs that challenge MDR patients

and health care providers. There is a need for intervention by trained counselors to deal with these issues.

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

This study is an outcome of a previous pilot study that explored the frequency of alcohol use among TB patients. 29% of the 490 TB patients (all male) consumed alcohol, the prevalence of AUD among them being 52%. The qualitative component of the study highlighted the need of an intervention among TB patients with AUD and a study to test the feasibility and the acceptability for such an intervention. This randomized experimental intervention study has been planned to enhance treatment adherence in TB patients who consume alcohol.

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

This study was done in different phases. Phase 1: comprised of Situational analysis and a Formative phase. Phase 2: A cohort of 1400 SHG representatives from 2 blocks, representing various areas in the district were randomized to an experimental and control group. 1560 participants have been recruited (764 in the experimental and 796 in the control group). The data analysis is in progress.

NATIONAL JALMA INSTITUTE FOR LEPROSY & OTHER MYCOBACTERIAL DISEASES, AGRA

LEPROSY

Clinical research

Large number of patients having leprosy and other skin diseases (10729) continued to attend the OPD services at the Institute. Of these, 7665 were leprosy patients. New leprosy patients who were put on treatment were 3192. Of them, PB patients were 1170 and MB patients were 2022. In addition, there were 4473 old leprosy patients receiving monthly treatment. Most of the patients who attended the

OPD wanted confirmation of the disease diagnosis and/or the status of the disease.

The studies showed the utility of the *in-situ* PCR procedure in the diagnosis of leprosy patients with AFB negative skin smear result. The *in situ* PCR contributed significantly to the diagnosis of leprosy. Studies on the predictors of therapeutic responses governing the regression and clearance of granulomas in response to chemotherapy alone and chemotherapy with immunotherapy in borderline leprosy are in progress. It is a randomized double blind therapeutic regimen study with *Mw* vaccine as immunotherapy.

Model Rural Health Research Unit, Ghatampur

The institute conducts extensive epidemiological activities primarily through its field unit *viz*, Model Rural Health Research Unit (MRHRU) at Ghatampur, district Kanpur Nagar in Uttar Pradesh. The field unit at Ghatampur continued to provide out-door services to the whole population of Ghatampur Tehsil in addition to the several field based studies. In the year 2013 the total OPD attendance was 9973 of which those with skin disease were 3529, with symptoms of filariasis 1540, and with chest symptoms 4286. Among these, 460 cases of TB, and 150 cases of leprosy were diagnosed and provided treatment from the MRHRU at Ghatampur. During the year, the unit has been serving the role of a Mentor Unit for establishing other Model Rural Research Units in the country under DHR. The long term follow up of the different leprosy and TB treatment cohorts is ongoing at the Ghatampur MRHRU.

The study entitled "Study of environmental *Mycobacterium leprae* and transmission link in leprosy" was completed on 31st March 2014. The key findings are as follows: From Ghatampur Tehsil, Uttar Pradesh, 169 patients were identified on the basis of physical examination, out of which 12 (7.10%) were found to be skin smear AFB positive and 157 (92.90%) cases were AFB negative. Out of the 97 PB cases, 30 (30.92%) patients were found to be RLEP-PCR positive while out of 72 MB cases 47 (65.27%) were RLEP-PCR positive indicating that RLEP-PCR is a better tool for case detection of leprosy for confirmation. From the surrounding

environmental area of these 77 smear RLEP-PCR positive patients showing skin smear RLEP-PCR positivity, 43 soil samples (25.44%) were found to be RLEP-PCR positive while 41 water samples (24.26%) were RLEP-PCR positive. Two soil and 3 water samples collected from non-patient area were also RLEP-PCR positive.

Using the RNA from the soil and water samples from the areas of all the RLEP-PCR positive patients, testing was carried out for 16s rRNA gene and in 32 soil and 29 water samples, positive results were obtained, showing the presence of viable *M. leprae* strains in the soil and water of patient dwelling area. The 16sRNA gene analysis indicates that *M. leprae* strain infecting the patients was detected in the soil and water samples of the patient dwelling area as well (Fig. 1).

positivity for RLEP-PCR showing the usefulness of the molecular technique of RLEP-PCR in studies on endemicity of leprosy.

The translational project on “Long term follow-up of cases on different drug regimens in leprosy and TB” is ongoing. *(i) Patients were put on various regimens for the treatment of leprosy and have been followed up for 60-84 months so far : UMDT; Standard PB regimen + daily CLF; Standard MB regimen + supervised Ofloxacin + Minocycline once a month; Standard PB and MB regimen. Eleven out of 360 patients (3.06%) on standard MB multidrug regimen plus supervised ofloxacin and minocycline had reaction during treatment compared to those on standard MB-MDT alone (36/895; 4.025%); Similarly, reaction after RFT was also less with ofloxacin and minocycline supplementation to

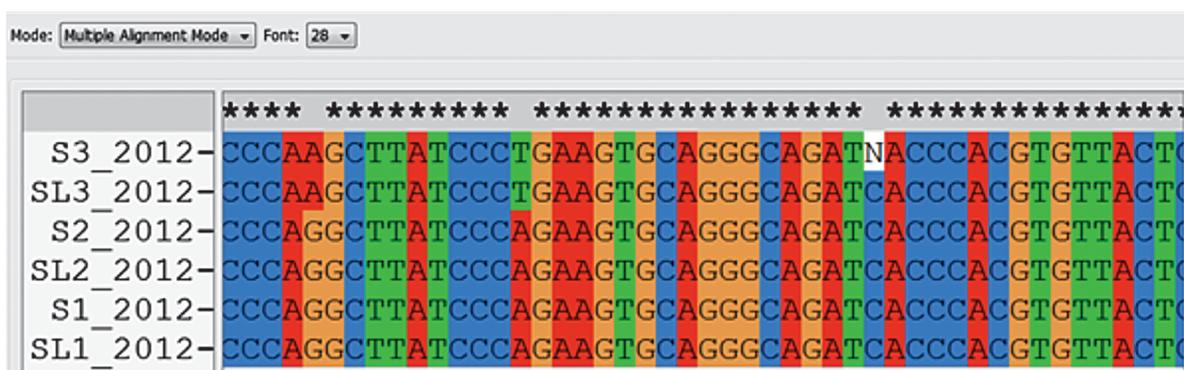


Fig. 1. Depiction of the alignment of the sequences from strains for 16S rRNA gene.

The STR typing of the strains collapsed 7 haplotypes indicating 7 type of strains are present in the area.

A study is underway on “endemicity of leprosy and utilization of health services in selected areas of Uttar Pradesh, Chhatisgarh and Tamil Nadu” with objectives to assess the disease burden in selected districts of Uttar Pradesh, Chhattisgarh and Tamil Nadu, study the profile of the disease, to identify sources of transmission by classical epidemiological and molecular epidemiological methods and to promote utilization of health services by leprosy patients and participation of the community. The skin smears from patients of Ghatampur and Banda (Uttar Pradesh) and Mahasamund and Sarguja (Chhattisgarh) showed his/her skin smear positivity for RLEP-PCR as compared against the AFB positivity. Soil and water samples in the patient dwelling localities of these areas also showed

standard MB-MDT and there was no case of relapse, compared to standard MB-MDT alone. Patients on standard PB-MDT with added clofazimine did not develop any reaction after RFT as also there was no case of relapse compared to those on standard PB-MDT alone. (ii) TB patients on various regimens of TB with immune modulator Mw and those put on treatment under DOTS detected outside the Mw trial through the TB prevalence survey are being followed-up. The initial results point out to the usefulness of Mw as an immunomodulator. The digitalization of the data for the Leprosy prevalence survey undertaken during 2002-05 and 2005-07 is underway. The data for the first prevalence survey has been completely entered while the demographic data for second survey has been entered for 155 villages against a total of 290 villages. Clinical data for all patients detected during both the surveys has been digitalized.

Establishment of Model Rural Health Research Unit (MRHRU) in Himachal Pradesh

A Model Rural Health Research Unit (MRHRU) is being established in Una, Himachal Pradesh. The State of Himachal Pradesh has signed an MoU with the Department of Health Research and has made provision of requisite land towards the same.

Ultrastructural studies in leprosy

A systematic ultra-structural form of peripheral nerves across the spectrum of leprosy was studied with an aim of understanding the pathogenesis of *M. leprae* and nerve involvement in leprosy by light and electron microscope. While in BT cases, hypertrophy of the endothelial cells occurs to such an extent that the lumen of the vessels become narrower and completely occluded causing ischaemia in patients, LL patients showed many intact bacilli in endothelial cell and lumen of the blood vessels is open facilitating vascular leakage for the entry of *M. leprae* into the nerves. Bacilli were found in Schwann cell even after receiving continuous MDT for more than two years.

Immunology and immunogenetics of leprosy

A study of the mechanism of regulation of T cell responses in leprosy has shown higher expression of DLL-1 by Th cells in HC, BT/TT and BL/LL patients and significantly lower DLL1 and Jagged2 in response to PHA in BL/LL patients; WCL and MLSA induced expression of Jagged1 in healthy individuals and TT/BT patients; increased lymphoproliferation by WCL in TT/BT in presence of anti notch1 antibody unlike MLSA which decreased the lymphoproliferation. However, in BL/LL cases, both WCL and MLSA decreased the lymphoproliferation; anti notch antibody along with *M. leprae* antigens leads to increased expression of CD25 and CD69 in BL/LL patients but not in healthy individuals, household contacts and BT/TT patients. This study has revealed an important role for Notch signalling during *M. leprae* infection and has opened avenues for further research on the role of Notch signalling for immunomodulation in leprosy patients.

Another study on the 'Role of FoxP3+ regulatory T cells in polarized immunity among leprosy patients (ICMR Task Force leprosy)' has shown significantly higher T regulatory cells in BL/LL

patients while PGL-1 increased the percentage of T regulatory cells in BL/LL.

A study on 'Association of *mcp1/ccl2* gene polymorphism with leprosy and analysis of underlying mechanism of susceptibility/resistance' was carried out to understand the role of CCL2 and associated genes in leprosy susceptibility and leprosy reaction. The study was aimed at finding out the frequencies of various genotypes due to *ccl2* and *nos2* gene polymorphisms in leprosy patients and in healthy controls. Three SNPs (-2518 A/G, -362 G/C and -2134 T/G) SNPs) at promoter region of *ccl2* and *NOS2* -954G/C and 1026G/T SNPs were analyzed in leprosy patients and healthy controls. The heterozygous genotypes (GC) at -362 position of *ccl2* gene was observed to be associated with leprosy patients. For *NOS2*, -(C1026T) (*rs2779249*) SNP, was associated with susceptibility to leprosy.

A study of the predictors of therapeutic responses governing the regression and clearance of granulomas in responses to chemotherapy and chemotherapy with immunotherapy with Mw in borderline leprosy has been completed and the results are being analysed. The objective of this study was to characterize the changes occurring in cellular phenotypes and mediators in granulomas in response to chemotherapy alone and chemotherapy with immunotherapy (Mw) by proteomic analysis to identify and characterize the proteins involved in granuloma clearance and to correlate these findings with histopathology and immunohistochemistry using markers of cellular phenotypes (*CD4,8,68*) and their mediators (IF-gamma, TNF-Alfa, IL-12 & TGF-Beta). A total 105 untreated clinically active borderline patients of leprosy (BT-36, BB-33, BL-36) were initially recruited in this project and 40 more untreated clinically active borderline patients of leprosy (BT, BB, and BL) were recruited in the 3rd year of this project. Ninety cases (BT-36, BB-23, BL-31) have completed their follow up. Skin biopsies were collected for proteomic, histopathological and immune-histochemical analysis. On follow up, all cases were showing improvement in clinical parameters. A total of 26 episodes of lepra reaction occurred during the period: 0-6 months- 12 cases (8 R/R, 4 ENL during 6-12months- 9 cases (7R/R, 2ENL); after 12months – 5 cases (3 R/R, 2ENL). Proteomic studies revealed that eighteen proteins were differentially expressed. Detailed analysis

is underway to associate this differential protein expression with granuloma clearance on Mw co-administration.

Tuberculosis

A study on 'Role of immune modulator along with chemotherapy in osteo-articular tuberculosis' is ongoing, in which, levamisole 1 mg/kg body wt. was administered every 15 days for three cycles along with ATT in Bone TB patients. Preliminary findings indicate that all patients with TB of the small joints heal in 6 months, while the spine and hip TB cases take 9-12 months in healing. Patients are under follow up for further 3 years. after the stopping of the anti-TB treatment.

Microbiology & Molecular biology of *M. tuberculosis*

The project entitled "DNA Fingerprinting of *M. tuberculosis* isolates from defined population by using IS-6110 probe" have shown that DNA fingerprinting based on insertion Sequence IS 6110 is well known marker for the molecular epidemiology of tuberculosis. IS 6110 based fingerprinting of 190 isolates of *M. tuberculosis* from the slums of Agra show that there is no correlation between drug sensitivity profile and distribution of IS6110 element. The studies on "Spoligotyping – A secondary molecular marker for typing of *Mycobacterium tuberculosis* isolates with low copy numbers of IS-6110" show that this molecular technique has the potential to differentiate the strains of *M. tuberculosis* complex isolates in the population. Using a well standardized system from a commercial source at NJIL&OMD genotypic differences have been identified among *M. tuberculosis* from defined geographical locations. The findings on 70 isolates of *M. tuberculosis* from slum areas of Agra region identified and spoligotyped, showed CAS-family was found highest and predominant There is no strains of the Beijing family in this region . The earlier studies from NJILOMD had showed eight spoligotypes from Jammu and Kashmir region. While a single isolate was found to be of the Beijing type in this region, interestingly a higher numbers of Beijing strains were observed from the Assam region.

A "Study on genetic polymorphism in *M. tuberculosis* isolates from tuberculosis patients from *Sahariya* Tribe of North Central India" was pursued

to identify different types of isolates including multidrug resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* infecting this tribe. No significant decrease in the magnitude of the disease was observed between *Sahariya* tribal people inhabiting close to Gwalior and those residing in the far flung areas of Sheopur district. Spoligotyping revealed the dominance of mainly two types of strains: East African Indian strain and Central Asian/ Delhi strain. The other shared types were also observed. MIRU-VNTR typing also helped in the identification of three additional strains which were not identified by spoligotyping and also strengthened the dominance of Central Asian strain/ Delhi lineage and East African strain.

The Study on 'Genetic polymorphism in *M. tuberculosis* isolates from tuberculosis patients among the victims of the Bhopal Gas Disaster' is ongoing and the findings indicate that the most predominant Spoligo-International Type (SIT) in this area was SIT11_EAI, and the next predominant Spoligo-International Type (SIT) was SIT26_CAS. This ICAR supported study (Outreach Programme on Zoonotic Diseases) aimed at providing original information about the distribution of *M. avium paratuberculosis* (MAP) strains present in specimens from animals, HIV patients and other human subjects has resulted in the development by NJIL&OMD of the first indigenous partial DNA chip/microarray against the selected genes (ORF) of MAP. This chip would greatly help in studying the strain variation in MAP isolates and the difference in expression profile of native MAP isolates. The novel findings of this ongoing study have potential application in the epidemiology of zoonotic infections (Fig. 2).

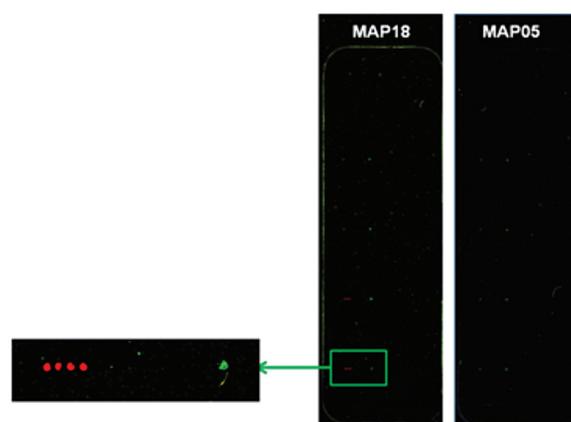


Fig. 2. Hybridization array of target gene; MAP18: Indian Bison type; MAP 05 Cattle Type isolate of MAP (Canada origin).

Mycobacterial Repository Centre

Mycobacterial Repository Centre established in 1995 with DBT support and maintained now with ICMR support has more than 3500 well characterized mycobacterial isolates from different parts of India and is providing reference and as well characterized Indian strains / DNAs / RNAs / Culture Filtrates/ Lysates to different investigators all over the country.

National Reference Laboratory for Tuberculosis Activities

The Revised National Tuberculosis Control Programme (RNTCP) of the Ministry of Health and Family Welfare is geared for the implementation of DOTS to all patients nationwide. The effective implementation is supervised by the Central TB Division through a network of four National Reference Laboratories (NRL's) and several Intermediary Reference Laboratories (IRL) spread throughout the nation. The main focus of the activities includes: Quality assessment of smear microscopy (EQA); Training of staff engaged in diagnosis and laboratory tests for TB in coordination with state IRL's; Mycobacterial culture and Drug sensitivity testing (DST); Quality assurance for culture and drug sensitivity of laboratories under it; Drug resistance surveillance (DRS); Providing DOTS plus centres with laboratory support; and Facilitating the establishment of more IRL's in the State and accreditation of Medical Colleges and other laboratories to undertake the RNTCP work.

BSL-3 lab for animal experiments

The facility is involved in working for different projects funded by DBT as well ICMR and during last one year, the work carried for different projects is given below:

(a) Evaluation of a recombinant BCG based vaccine for tuberculosis in the guinea pig model - ICMR funded. Ten rBCGs were tested for their protective efficacy. The rBCG elicited protective immunity which was similar to BCG in a H37Rv challenge study performed in guinea pigs. The rBCG constructs shown to be immunogenic in mouse models as well as eliciting protective immune responses in guinea pig model could be promising vaccine candidates for protection against

TB. (b). Therapeutic efficacy of Moxifloxacin and Econazole based drug regimen against MDR-tuberculosis: Evaluation of therapeutic efficacy of moxifloxacin / econazole alone or in combination against susceptible & resistant TB was done. Moxifloxacin and econazole alone as well as in combination are reducing the bacteria load from lungs and spleens of mice infected with MDR strain of *M. tuberculosis*. PLGA-Moxifloxacin nanoparticles and PLGA-Econazole nanoparticles were prepared and the same are being evaluated in mice models against MDR tuberculosis. (c) Development of novel nano-carriers for pulmonary immunization against tuberculosis: The potential of developed nanoparticulate vaccine formulation against tuberculosis was assessed. Moreover, the *in-vivo* data clearly indicated the efficacy of the developed formulations for safe and effective delivery via pulmonary route. The animals were challenged against tuberculosis after six weeks of primary immunization. The institutes observed better animal survival compared to the control group. The results were further supported by the elevated antibody titre and the histological studies. Significantly lower bacterial load was observed after two and four weeks of the challenge.

Biofilm formation in Mycobacteria, especially *M.tuberculosis*

The study entitled "Role of Biofilm in Mycobacteria: Correlation of Ultrastructural and Molecular profile" gave an insight into the process of biofilm formation in Mycobacterium species (*M. fortuitum*, *M. avium*, *M. tuberculosis* and *M. smegmatis*). In case of *M. tuberculosis*, the biofilm formation was significant at 37 °C but moderate at 30 °C whereas no biofilm formation was seen at 42 °C. The thickness of the biofilm was found to increase with the age of the biofilm and no consistent differences were observed on biofilm formation between the selected pH conditions. Antibiotic susceptibility of biofilms at ultrastructural level was also studied in fast growing clinical isolates. Isoniazid was found to strongly inhibit biofilm formation in fast growing and sensitive mycobacterial isolates. However, pyrazinamide and isoniazid inhibited biofilm formation in *M.tuberculosis* (H37Rv) and in MDR-*M.tb* isolates. Ethionamide and moxifloxacin inhibited biofilm formation in both slow growing and fast growing mycobacteria (Fig. 3).

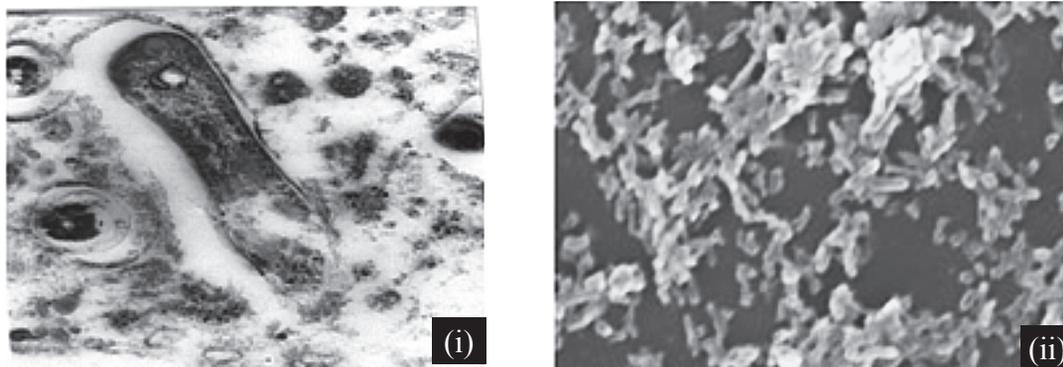


Fig. 3 (i) Transmission electron micrograph of complete mycobacterial cells surrounded by EPS. (ii) Scanning electron micrograph of biofilm of *M. tuberculosis* H37 Rv Biofilm.

Expression analysis using Microarray showed over expression of seven genes in MDR *M.tb* isolates (Rv 1032(*trcs*), 0903 (*prra*), 1308 (*atpA*), 1310 (*atpD*), 3411(*guaB2*), 0359 and 3526 (*kshA*)) and six genes in *M.avium* isolates (Mav 1175 (*trcs*), Mav 1022 *Prra*, Mav 1525, Mav 1527*atpD*, Mav 4356 and Mav 0633). The results were revalidated with Real Time PCR experiments in different conditions – presence of glucose and/ or OADC and drugs-isoniazid/ethionamide. It has been conclusively demonstrated that in *M. tuberculosis* MDR isolates seven genes were expressed and two genes Rv0359 and Rv3526 were homologous as earlier reported in *P. aeruginosa* and *M. avium* which might be responsible for biofilm formation.

Immunological and immunogenetic studies in tuberculosis

The study on ‘Analysis of SNPs in *TLRs*, *TIRAP*, *MCP 1*, *MiRNAs* in tuberculosis and understanding the possible role of these molecules in immunopathogenesis’ indicates that :

- While AG genotype at -2518 and GC at -362 of *ccl2* gene was observed to be associated with healthy controls, GG at -2518 and CC at -362 indicated association with susceptibility to TB.
- The levels of three cytokine variables (IFN γ , TNF α , TGF β) were significantly correlated with CCL2 levels in heterozygous TB cases and IL 12, IFN γ , TNF α level in homozygous mutant cases.
- Both the studied CCL2 SNPs are in linkage disequilibrium and AC haplotype is significantly associated with TB cases.

Proteomic approach to study drug in *M. tuberculosis*

A study on ‘Analysis of aminoglycosides resistance in *Mycobacterium tuberculosis* by proteomic approach’ revealed that twelve protein spots were found to be consistently upregulated in KM and AK resistant isolates. Among the twelve, five were chaperones, five were known proteins with their defined roles and two were with unknown functions. Two proteins were found to be involved in iron regulation/metabolism. It is assumed that iron regulation/metabolism might be playing some crucial role in contributing resistance to kanamycin and amikacin. As Rv3867 and Rv3224 were identified as proteins with unknown function in *M. tuberculosis*, the genes of these proteins were cloned and expressed in *Escherichia coli* BL21 host using pRSETb expression vector. E-test results showed significant change in MIC of recombinant cells of Rv3224 for AK and KM. In case of Rv3867 significant change in MIC was seen for KM while slight shift was observed for AK.

Another study entitled ‘Whole proteome analysis of aminoglycosides resistant isolates of *Mycobacterium tuberculosis*’ showed over expression of seventeen protein spots consistently in all resistant isolates as compared to sensitive isolates. However, five spots matched with already identified proteins and therefore, a total of twelve new proteins were found to be up regulated.

The ongoing study on ‘Pharmacoproteomic effect of fluoroquinolones on *M. tuberculosis* isolates’ showed that 11 proteins were over expressed with FQ induction of all drugs-sensitive *M. tuberculosis*

isolates and 10 proteins were over expressed in ofloxacin resistant isolates compared to sensitive isolates with/without FQs pressure. Three over expressed hypothetical proteins are being studied for functional significance.

HIV/AIDS surveillance studies

Diagnostic services for HIV infection (counselling and testing), Monitoring CD4 cells in HIV positive subjects, Blood group, Hepatitis B surface Antigen (HBsAg), Rheumatoid Arthritis (RA), widal tests and VDRL (Syphilis) testing facilities for cases referred by the OPD of the hospital are being provided at HIV/AIDS Laboratory of Institute. Out of 1627 tested in ICTC of HIV/AIDS Laboratory, 152 (9.3%) individuals were HIV sero-positive. Highest percentage (18.85%) of HIV seropositivity was observed among clients who were referred by various private and government hospitals. Six out of 232 screened for HIV (2.58%) were having co-infection with TB.

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES, KOLKATA

SURVEILLANCE, EPIDEMIOLOGY AND CHARACTERIZATION OF ENTEROPATHOGENS

Active surveillance of diarrhoea infection in infectious diseases and Beliaghata General Hospital and B.C Roy Post Graduate Institute of Paediatric Sciences, Kolkata

From April 2013 to March 2014, a total of 1226 fecal specimens were collected from every 5th patients admitted with acute watery diarrhoea at Infectious Diseases Hospital (IDH), Kolkata (during 24 hours a day from 2 randomly selected days per week) for etiological analysis (~5.77% of admitted patients). In case of B.C Roy Post Graduate Institute of Paediatric Sciences (BCRPGIPS), 1254 specimens were collected (every 5th systematic sample from OPD patients- Monday to Friday) (~20% of total OPD patients). Type of diarrhoea at presentation in IDH and BCRPGIPS were watery (77.2% vs. 15.6%), bloody (1.7% vs. 5.3%) and semi-solid (21.1% vs. 79.1%). In ID & BG Hospital 3.8% under five children presented with severe

dehydration and 95.9% with some dehydration. But in B. C. Roy Hospital these values are 0% and 2.5% respectively.

In children below 5 years of age, detection of rotavirus was found to be common in both the hospitals (~48%). *Vibrio cholerae* O1 (20%) and *Campylobacter* spp. (4.3%). *Vibrio fluvialis* (3.2%) were more in the IDH. In the BCRPGIPS, detection of adenovirus (15.3%), *C. jejuni* (13.8%), enteroaggregative *Escherichia coli* (5.6%) and *Shigella* spp. (5%) were high. *V.* remained susceptible for most of the fluoroquinolones. In both the hospitals, most of the *Shigella* strains were highly resistant to fluoroquinolones but were susceptible for ceftriaxone. NDM-type carbapenemase were detected in 27 strains of *V. fluvialis* strains isolated from 2011-2013. All these NDM-positive strains were susceptible to azithromycin. Weekly reports sent to Govt. and other agencies for control and improvement for better patients care and suggested treatment regime accordingly drug susceptibility patterns.

Surveillance and molecular characterization of group A rotavirus among children reporting with acute gastroenteritis

A total number of 353 and 390 stool samples (n=743) from hospitalized and OPD diarrhoeal patients (<5 Y) were screened for rotavirus during April 2013 to Feb. 2014. The stool samples were screened for rotavirus using VIKIA Rota-Adeno kit detecting the VP6 antigen. Among 743 total samples, 338 samples were detected as rotavirus positive (45.49%) suggesting it to be most important cause of diarrhoea related hospital visits in children. A large variety of genotypes were detected though G9 (40%) followed by G2 (37%) were the most common types among hospitalized children. In OPD cases, G2 was most common (41%) followed by G9 (23%) and G1 (22%) genotypes. High frequency of G9 strains suggests inclusion of G9 strain in rotavirus vaccines.

Antimicrobial resistance, virulence profiles and molecular subtypes of *Salmonella enterica* Serovars Typhi and Paratyphi A blood isolates from Kolkata

Enteric fever, caused by *Salmonella enterica*, remains an unresolved public health problem in

India and antimicrobial therapy is the main mode of treatment. This study was done to characterize the *S. enterica* isolates from Kolkata with respect to their antimicrobial resistance (AMR), virulence profiles and molecular subtypes. *S. enterica* blood isolates were collected from clinically suspected enteric fever patients attending various hospitals in Kolkata, and were tested for AMR profiles by standard protocols; for resistance gene transfer by conjugation; for resistance and virulence genes profiles by PCR; and for molecular subtypes by Pulsed Field Gel Electrophoresis (PFGE). A total of 77 *S. enterica* Serovar Typhi (*S. Typhi*) and 25 *S. enterica* Serovar Paratyphi A (*S. Paratyphi A*) from Kolkata were included in this study. Although multidrug resistance (resistance to chloramphenicol, ampicillin, cotrimoxazole) was decreasing in *S. Typhi* (18.2%) and absent in *S. Paratyphi A*, increased resistance to fluoroquinolones, the current drug of choice, caused growing concern for typhoid treatment (Fig. 1). In this study 28% of *S. Paratyphi A* isolates were azithromycin resistant. A single, non-conjugative non-IncHI1 plasmid of 180 kb was found in 71.4% multidrug resistant (MDR) *S. Typhi*; the remaining 28.6% MDR isolates were without plasmid. Various AMR markers

(blaTEM-1, catA, sul1, sul2, dfrA15, strA-strB) and class 1 integron with dfrA7 gene were detected in MDR *S. Typhi* by PCR and nucleotide sequencing. This was in contrast to the earlier reports which showed that one conjugative ca. 200 kb plasmid associated with multidrug resistance and belonging to IncHI1 type was regarded as the globally dominant plasmid in *S. Typhi*. Most of the study isolates were likely to be virulent due to the presence of virulence markers. Major diversity was not noticed among *S. Typhi* and *S. Paratyphi A* from Kolkata by PFGE. The observed association between AMR profiles and *S. Typhi* pulsotypes might be useful in controlling the spread of the organism by appropriate intervention (Fig. 2). The study reiterated the importance of continuous monitoring of AMR and molecular subtypes of *Salmonella* spp. from endemic regions for better understanding of the disease epidemiology.

Ecology and epidemiology of *V. cholerae* in riverine ecosystem

Dynamics of diarrhoeal pathogens (especially *Vibrios*) of riverine habitat in relation to environmental parameters, an enigma, was the prime subject of interest of research work. Accordingly, during 2013-14 the influence of hydrologic

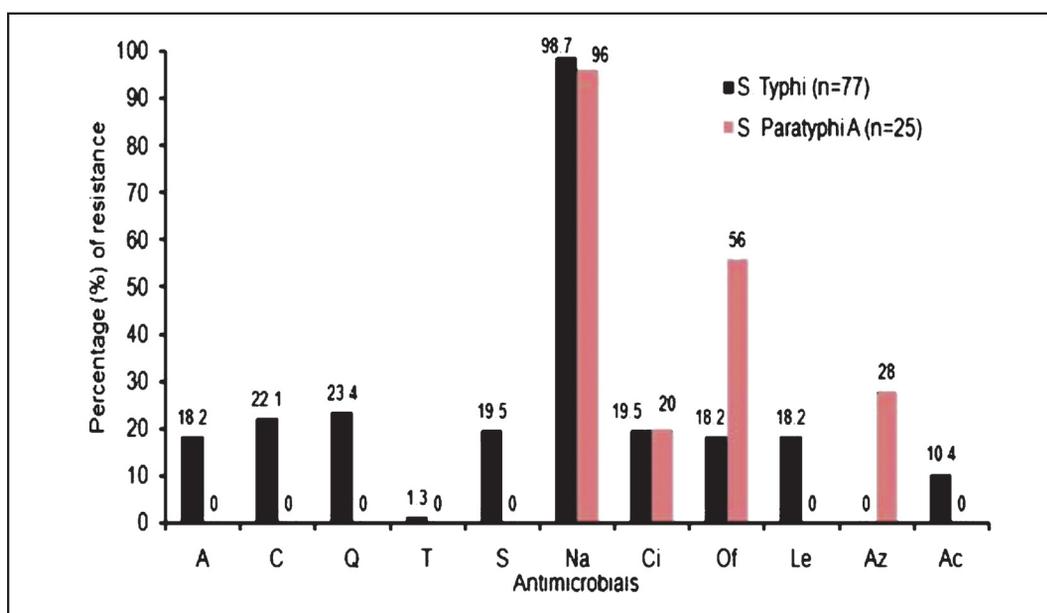


Fig. 1. Percentage distribution of antimicrobial resistance in *S. Typhi* and *S. Paratyphi A* Kolkata isolates. Interpretation was based on the MIC values of the antimicrobials. A, ampicillin; C, chloramphenicol; Q, co-trimoxazole; T, tetracycline; S, streptomycin; Na, nalidixic acid; Ci, ciprofloxacin; Of, ofloxacin; Le, levofloxacin; Az, azithromycin, Ac, amoxicillin-clavulanic acid.

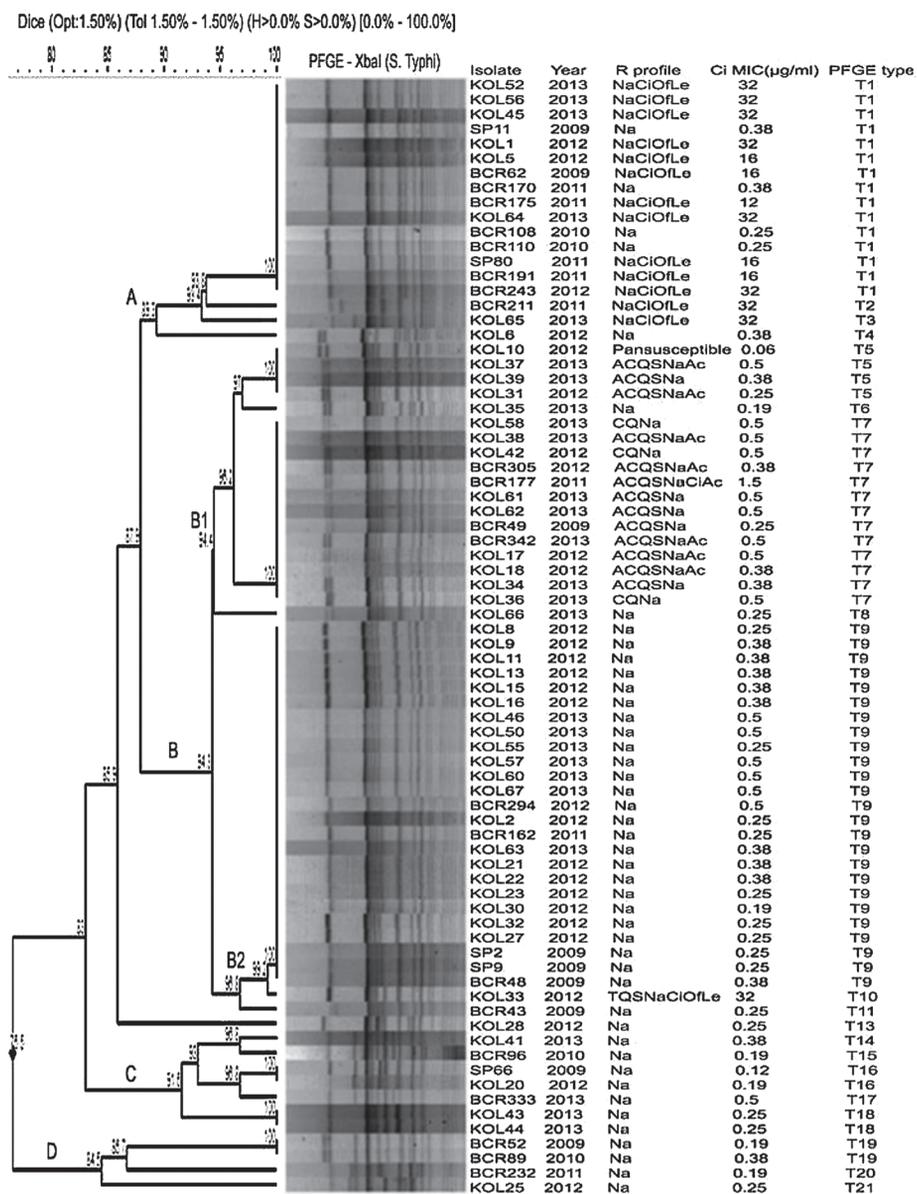


Fig. 2. Dendrogram showing the cluster analysis of 76 *S. Typhi* isolates from Kolkata, India, by *XbaI*-PFGE. Band comparison was performed by using the Dice coefficient with 1.5% optimization (Opt) and 1.5% position tolerance (Tol). Pansusceptible, susceptible to all 17 drugs tested; A, ampicillin; Ac, amoxicillin-clavulanic acid; C, chloramphenicol; Q, co-trimoxazole; T, tetracycline; S, streptomycin; Na, nalidixic acid; Ci, ciprofloxacin; Of, ofloxacin; Le, levofloxacin; Az, azithromycin.

and anthropogenic factors of riverine-estuarine ecosystem (especially the Ganges River) on the disposition of enteropathogens has been established for the cholera endemic foci of south Bengal. Benthic-pelagic coupling of *Vibrio* dynamics has been suggested in south Bengal riverine-estuarine ecosystem. Combination of tidal and seasonal variation of temperature, salinity and turbidity has been identified to be distinctly influencing the *V. cholerae* prevalence in riverine ecosystem. Further to this, influence of the Ganges riverine-estuarine

ecosystem on south Bengal cholera menace has been established. A positive correlation among inland cholera cases and toxigenic *V. cholerae* preponderance has been recognized in Ganges riverine-estuarine settings. Significant association of physico-chemical variants of riverine system and prevalence of toxigenic *V. cholerae* O1 and its phages in aquatic environment demonstrated to imply the role of riverine-estuarine ecosystem in cholera transmission. Conclusively, a “bio-monitoring tool” of physico-chemical stimulants,

tidal and climatic variants has been proposed collating *V. cholerae* and phage dynamics that can forewarn any impending cholera outbreak. Based on observed seasonal as well as hydrological influences on toxigenic *V. cholerae* dynamics and through unique longitudinal study, physico-chemical variation has been postulated as the inducing factor for *V. cholerae* abundance and toxin gene acquisition. Turbidity (<500NTU), temperature (30±2°C), salinity (2-5ppt) has been demarcated as conducive for toxin gene acquisition among environmental pool of *V. cholerae*. Chitinous fauna like crabs have been established to be the most suitable niche for genetic modification in *V. cholerae* and *V. parahaemolyticus*.

Haitian variant tcpA in *V. cholerae* O1 strains in Kolkata

The toxin-coregulated pilus (TCP) is a crucial determinant of the pathogenicity of *V. cholerae*. This bundle-forming pilus is an essential intestinal colonization factor and also serves as a receptor for CTXΦ, the filamentous phage that encodes cholera toxin (CT). Whole genome sequence analysis of *V. cholerae* strain isolated from the devastating Haitian cholera outbreak caused by *V. cholerae* O1 revealed a unique mutation at the 64th amino acid position of the matured TcpA subunit. Bioinformatics analysis showed that this mutation from Asparagine to Serine is positively selected. Moreover, this particular mutation is the result of a purine-purine transition which is evolutionarily preferred. This motivated to investigate the emergence and dissemination of these new variants in Kolkata, India. Our newly developed PCR assay revealed that the first appearance of Haitian type tcpA was noticed in Kolkata during October, 2003. Soon after its appearance; this new variant tcpA containing strain displaced the canonical El Tor tcpA containing strains completely in the succeeding years. Previous study indicated that the Haitian ctxB first appeared in Kolkata during April, 2006. So, this Haitian variant strain may be the result of the sequential genetic events in the evolution of *V. cholerae* strain in the Indian subcontinent. These results highlight a significant event in the evolution of recent variants of *V. cholerae*. Finally, this finding not only shows a cryptic change in the epidemiology of cholera but also raises questions about the origin of this variant of *V. cholerae* O1 El Tor.

Nationwide screening of phage types of *Vibrio cholerae* O1 and O139

A total of 645 strains of *V. cholerae* were received from different parts of the country during the current year for serotyping, biotyping and phage typing. All the 645 (100%) strains were confirmed as *V. cholerae* O1 biotype El Tor were included in phage typing study. During this period, highest number of strains was received from Maharashtra. Majority of the strains belonged to Ogawa 576 (89.03 %) followed by Inaba 69 (10.69 %). A total of 40 (6.2 %) strains were found to be untypeable with the conventional Basu and Mukherjee scheme. Using the new scheme, all of these strains were found to be typeable and could be clustered into a number of distinct types of which majority were grouped under type 27 grouped under type 27 (64.03%) followed by type 26 (5.42 %), type 20 (5.73 %), type 23 (4.49 %) and type 7 (2.94%), respectively. It has been shown that type 27 was the predominant phage type circulating in the country. During the current year, not a single strain of *V. cholerae* O139 was received for phage typing study from any parts of the country. Any noticeable change in this phenotypic marker may raise the suspicion of emergence of a new clone. The phages may play an important role as a potential predictor of outbreaks of the disease, which serves as an early useful signal for monitoring control measures of cholera.

Retrospective analysis of toxigenic traits of *V. cholerae* receiving for phage typing

Among two subunit proteins of cholera toxin (CT) encoded by *ctxA* and *ctxB*, much more attention has been attributed recently on *ctxB* due to genetic variability leading to defining several variants of *V. cholerae* O1. A total of 151 *V. cholerae*, biotype El Tor strains were taken for this study from 1990 – 2012. Most of the strains were found to be Ogawa (98%) and the Inaba (2%) serotype was dispersed among very few isolates. According to new phage typing scheme, type 27 was widely distributed throughout the study, followed by type 26, type 24 and type 20. Only 4.23 % strains were found to be sensitive to all of the antibiotics but 91.25 % strains were resistant to streptomycin and sulfamethoxazole/ trimethoprim. In this study, MAMA-PCR, and phage typing results concluded that the genotypic variation such as polymorphic

traits in *ctxB* genes did not influence the antibiotic susceptibility and phenotypic behavior of *V. cholerae* O1 biotype El Tor strains.

Detection and molecular characterization of complete nucleotide sequence of human picobirnaviruses causing acute watery diarrhoea among children in Kolkata

The genus, Picobirnavirus (PBV), Spanish 'pico' = 'small', 'birna' for 'bipartite RNA' genome, belongs to the family Picobirnaviridae. During this study, full genome of segment 2 of the picobirnavirus which encodes viral RNA-dependent RNA polymerase were sequenced for 5 strains. Sequencing data analysis have been made on strains isolated from porcine and equine fecal samples. Analysis revealed that the nucleotide sequence data of Kolkata (Indian) porcine PBV strains genetically related to European PBV strains and environmental PBV strains of USA. Further to this, detection of porcine-like picobirnaviruses in diarrhoeic children aged <5 years, suggestive of zoonotic transmission. These results insists that a zoonotic origin of PBV infections as well as a chance of water-borne route of transmission, which presents a wide spread of PBV across globe in various hosts ranging from humans, animals, birds, reptiles and environmental samples too.

Enterobacteriaceae from neonatal septicemic cases: a concern for transfer of blaNDM-1

An emergence of carbapenem resistance has necessitated the evaluation of resistance to other antibiotics such as aminoglycosides and fluoroquinolones. Studies have been carried out to understand whether there is an association of aminoglycosides and fluoroquinolone resistance determinants with carbapenem resistance among neonatal septicemic Enterobacteriaceae. New Delhi Metallo- β -lactamase -1 (NDM-1) was the only carbapenemase isolated in Enterobacteriaceae and co-resistance to other genes such as *rmtB* and *rmtC* for aminoglycoside resistance and *aac(6)-Ib-cr* for fluoroquinolone resistance were observed along with NDM-1. Most of these isolates were proficient in the transfer of NDM-1 along with other determinants. Molecular analysis showed that NDM-1 was not associated with any particular clone or plasmid type. This raised the concern on quicker spread of *blaNDM-1* to other organisms.

Virulent *E. coli* clone ST-131 in neonatal gut: role in spread of CTX-M-15 in *E. coli*

The gastrointestinal tract is a significant source of infecting organisms, with intestinal colonization being the prelude to bacterial infection. Studies on the neonatal gut have shown that carbapenem and cephalosporin resistance is high in neonatal gut isolates. The Institute also studied the prevalence of the virulent clone of *E. coli*, ST-131 in the neonatal gut isolates. This clone of *E. coli* combined extensive resistance in addition to multiple virulence genes. This clone of *E. coli* belonged to the highly virulent phylogenetic group B2. Distribution of ST131 isolates was low in comparison to the high percentage of CTX-M-15 producers which indirectly indicates that the clonal spread of ST-131 did not probably contribute to the high prevalence of CTX-M-15 in our setting.

Pathogenic implications of *Helicobacter pylori* strains in gastroduodenal diseases and gastric cancer

This study was aimed to investigate *H. pylori* morphology and subsequent histopathological and ultrastructural changes in this bacteria related gastroduodenal diseases in Indian population. Of hundred endoscopic gastric biopsy samples screened from a hospital in Kolkata, of which 70 specimens were studied. Based on clinical, endoscopic and histopathological examinations, 25 were diagnosed as non-ulcer dyspepsia (NUD), 25 duodenal ulcer (DU) and 20 gastric carcinoma (GC) cases. Only antral biopsy samples were further processed for culture, histology and ultrastructural study. *H. pylori* infection was evident in 80% cases of NUD, 92% DU and 40% GC cases. Vacuolating change was evident histologically in 72% of DU subjects. Activity of gastritis was more in DU (84%) cases compared to NUD and GC subjects but severity of gastritis and disease manifestation was not always correlated with the density of the organism in this study. The *CagA* was present in 89.1% (41/46) of the tested strains from this region and 69.5% (32/46) of the strains had *vacA* s1m1 allele. Invasion of *H. pylori* through tight junction and bacterial presence in intraepithelial intercellular spaces situated below the tight junction, vacuolating change in *H. pylori* infected areas of gastric mucosa and bacterial existence inside vacuole were few important ultrastructural observations. *H. pylori* invasion in

lamina propria confirms the intracellular pathogenic potential of the bacterium.

Helicobacter pylori dup A: A novel biomarker for duodenal ulcer in India

Recent studies have proposed the possibility of using genetic markers in the plasticity zone as indicators of pathogenicity for *H. pylori* infection. It seems that these determinants may play a key role in determining the virulence capacity of *H. pylori* strains either directly or by encoding factors that may lead to varying clinical outcomes. A novel virulence factor locating in the plasticity zone, duodenal ulcer promoting gene A (*dupA*) in *H. pylori* has been found to be associated with disease in certain population but not in others. So, the role of *dupA* as a virulence marker is still controversial. The debate of relevance of *dupA* for the prediction of clinical outcome has prompted to take this study in Indian population. A total of 140 *H. pylori* strains isolated from duodenal ulcer (DU) [n=83] and non-ulcer dyspepsia (NUD) subjects (n=57) were screened by PCR; Dot-Blot and sequencing to determine the presence of *jhp0917* and *jhp0918* and was also tested for *dupA* transcript. PCR and Dot-Blot results indicated presence of *jhp0917-0918* in 37.3% (31/83) and 12.2% (7/57) of *H. pylori* strains isolated from DU and NUD, respectively. Sequencing analysis showed an insertion of 'C' at position 1386 in 3' region of *jhp0917* forming *dupA* gene in 35 strains. RT-PCR analysis detected *dupA* transcript in 28 out of 35 strains. Expression level of *dupA* transcript varies from strain to strain as shown by Real Time PCR. Prevalence of *dupA* was significantly greater among strains isolated from patients with DU than NUD in this population. Our study in Indian population demonstrated that *dupA* gene was 6.5 times more prevalent in duodenal ulcer patients than non-ulcer patients. Based on our finding, *dupA* can be considered as one of the biomarkers for DU patients in India.

APPLIED AND TRANSLATIONAL RESEARCH

Assessment of perceived health needs and available health care facilities of Malda district, West Bengal

A community-based cross-sectional study involving 10,000 families (6000-rural & 4000-urban) was

conducted in Malda district. A total of 300 water samples were collected from 300 vulnerable block primary schools' tube wells and all samples were transported at Regional Occupation Health Center (ICMR), Kolkata, for testing the arsenic content of them. Additionally, data on morbidity/mortality from hospital admitted cases was collected as per protocol. Some of the observations based on interim analysis are given in Table I. Out of 300 water samples tested, 45.6% (n=137) showed presence of high arsenic level of greater than 10 microgram/litre as per WHO drinking water quality criteria (Fig. 3). A total of 16 studied schools reported arsenic poisoning cases (evidenced by skin manifestations) either in their schools or adjoining villages. Most of the schools were provided with arsenic filter few years back. But maintenance seems to be lacking in all of them. A need-based appropriate public health intervention programme must be targeted immediately to prevent this public health menace.

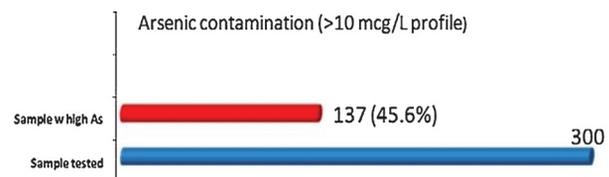


Fig. 3. Out of 300 water samples tested, 45.6% showed presence of high arsenic of >10 microgram/litre.

Assessment of nutritional status among primary & upper-primary school children in all districts of West Bengal

New initiative has been taken to look in to the existing nutritional scenario of school children along with its epidemiological variables in all districts of West Bengal, many of whom are highly backward areas, naxalite areas & hilly areas, bordering Nepal, Bhutan & Bangladesh. This project is aimed to understand the impact of the 'Mid Day Meal Scheme services' provided in West Bengal schools in future by establishing a nutrition surveillance system.

Live oral recombinant cholera vaccine VA1.4: a randomized placebo controlled study for assessment on safety and immunogenicity among healthy adults in Kolkata

Safety and immunogenicity assessment on two doses of live oral cholera vaccine VA 1.4 when given two weeks apart have been made through

a double-blind, randomized placebo controlled trial on adults (18-60 years of age) from Kolkata. The vaccine did not elicit any diarrhoea related adverse events. Other adverse events were rare and similar between vaccinee and placebo groups. Post 7 days of first dose, 66% subjects seroconverted (vibriocidal antibody response ≥ 4 folds rise in titre) in vaccinee group. No such seroconversion was observed in placebo group. No further increase in positive antibody response in vaccine group occurred after two doses. This study demonstrated that VA 1.4 at a single dose of 1.9×10^9 CFU is safe and immunogenic in adults from a cholera endemic region. No additional benefit after two doses was seen.

Development of modules to improve diarrhoea related knowledge and practices among non-qualified practitioners

A study has been undertaken for developing training modules (Fig. 4) for the non-qualified practitioners providing treatment and primary care to people living in the urban slums of Kolkata. This work was a sequel of previous study which showed that qualified and government physicians had better knowledge regarding diarrhoea and it was also seen that better knowledge was associated with a lower likelihood of prescribing antibiotics for diarrhoea. It was also established that diarrhoea-related knowledge and practice were poor with the exception of qualified physicians, in the slums of Kolkata; hence an improvement in the knowledge of pharmacists and unqualified practitioners is necessary for the overall improvement of diarrheal management in these slums.

Heat killed multi serotype *Shigella* immunogens induce both humoral and adaptive immune response in rabbit model

Protective efficacy of newly developed heat killed multi serotype *Shigella* (HKMS) immunogens prepared from six selected serotypes of *Shigella* was initially assessed in guinea pig colitis model. This newly developed HKMS immunogen also showed complete protection in orally immunized rabbits against challenge with homologous and heterologous strains. Serum antibodies titer were reciprocally increased during oral immunization which reached to the peak at 35th day post immunization of three doses at 0, 14, and 28 days and remained stable at an evident level up to 180 days (Fig. 5). Immunized rabbit's peripheral blood mononuclear cell (PBMC) culture supernatant showed increasing IgG titer during the immunization period indicating HKMS immunogens stimulated humoral immune responses. Up-regulation of IFN- γ and IL-10 mRNA was detected in the immunized rabbit PBMC upon re-stimulation with HKMS *in vitro*, whereas the level of IL-4 remained same as control PBMC. Memory B-cell proliferation was confirmed by rapid production of much higher level of HKMS specific IgG level in PBMC supernatant compare to control group. These results suggested that the HKMS immunogens induced humoral and Th1 mediated adaptive immunity and serves complete protection against *Shigella* strains in rabbit model. This could be a broad spectrum non-living vaccine candidate for human use in recent future which may stimulate long term adaptive immune responses against 50 serogroups of *Shigella* spp.

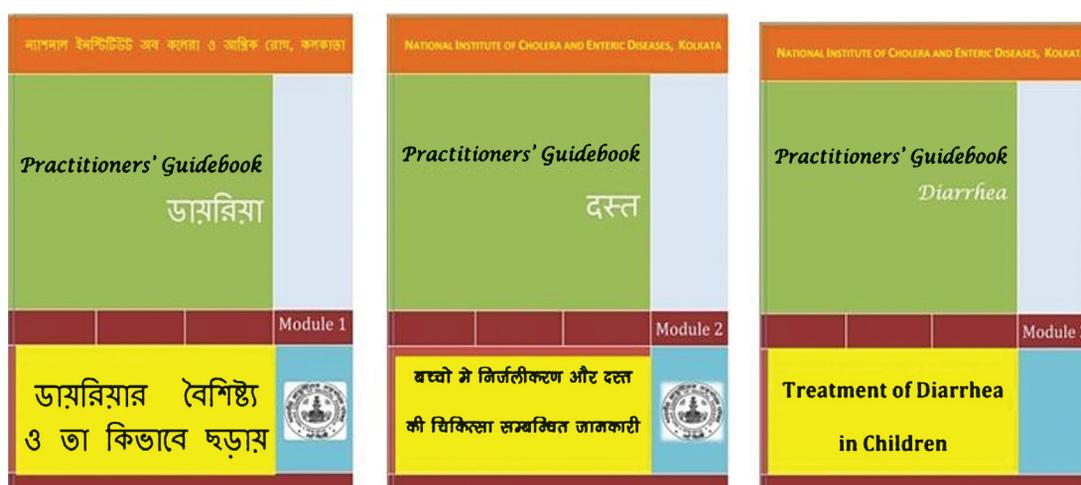


Fig. 4. Development of modules in three different languages and validated by NICED, Kolkata, Scientific Advisory Committee.

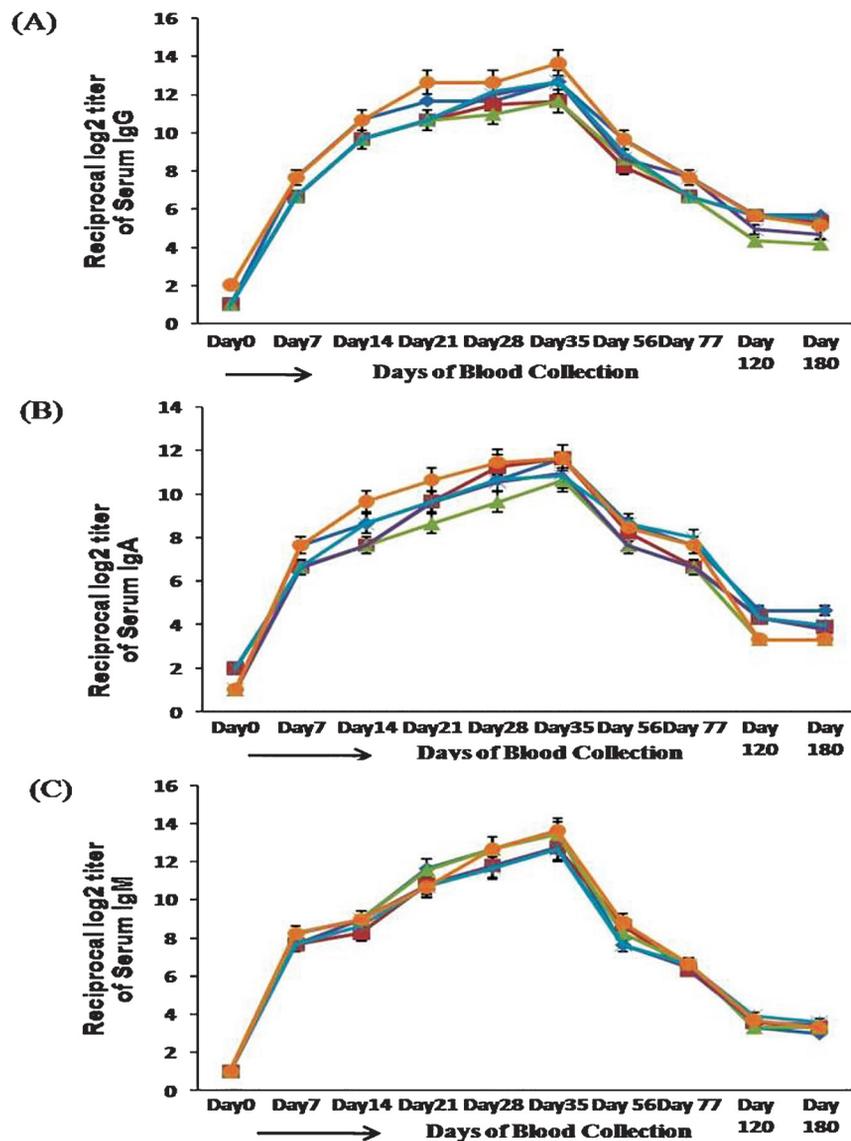


Fig. 5. Serum Immunoglobulins were measured (A) Serum IgG, (B) Serum IgA and (C) Serum IgM responses were measured against the WCL of *S. dysenteriae*1 (A1); *S. flexneri* 2a (2457T); *S. flexneri*3a (NK3758) ; *S. flexneri*6 (NK4025); *S. sonnei* (NK3918) and *S. boydii* type 2 (NK4023) at pre-immunization, immunization and post immunization periods. Blood was collected on the days indicated along the horizontal axis. Data represented here are the mean values \pm standard deviation (SD) of three independent experiments. The differences in post immunization day wise response of each of the studied antibodies against each of the six WCL were highly significant ($P < 0.005$).

Impact of Climate Change: EL Niño and La Niña thirty years persuade cholera in Kolkata

The analysis spotlight was on the EL Niño and La Niña impact in regional climate changes to relate cholera infection at Kolkata including 2013-14. At the regional level, seasonal outlooks necessitate to consider the relative impacts of both the El Niño/La Niña state and other locally relevant climate drivers. Active surveillance data collected during

1996-2013 and hospital cholera data recorded from 1980-1995 generated from the Infectious Diseases Hospital (IDH) were used in this study along with climate factors relative humidity (RH), temperature, rainfall and sea surface temperature (SST) of Bay of Bengal. Time series analysis of Auto-Regressive Integrated Moving Average (ARIMA) model has investigated relative impact of climatic on cholera. Relative humidity (RH)

seems to be the main influential factor to enhance *V. cholerae* infection when the sea surface temperature reached about 28°C and a positive correlation was detected between rainfall and *V. cholerae* infection. The yearly temperature has fluctuated considerably and an increase of 1°C has been observed during the 33 years. The highest observed rainfalls was 17.21mm, 20.78mm and 16.08 mm during the 1984 (82-83 El Niño), 1999 (La Niña) and 2007 (La Niña), respectively. However, the lowest mean rainfall (7.09) was recorded in 2011, which was one of the strongest La Niña year ever observed since last 30 years. The cholera infection was low during 2011. During 1996, 1997 and 1998, single cholera peak was recorded with more number of cases during the monsoon seasons due to highest rain fall while SST was below 28°C. Unusual rise of *V. cholerae* infection was noticed in December 2008. The mean days RH (morning) has drastically increased to 21% in December 2008, because warmer winter than normal owing to 2007-2008 La Niña event. Overall, 33 years data bestowed convincing information on relation between cholera infection and climatic factors by the influence of El Niño and La Niña -southern Oscillation (ENSO), which has positive role in controlling the prevalence of cholera in Kolkata. The current year 2014 is strongest EL Niña year ever observed since 1998, may impact the climate in the adjacent land areas.

Biomedical Informatics Center of ICMR supporting studies on host-pathogen interactions and identify small molecule inhibitors of potential drug targets

This activity has been initiated with support from the ICMR Hqrs and this center is currently engaged in providing valuable support to ongoing research activities of the Institutes. Some of the important support activities included (i) dynamic human protein-protein interaction network in *Salmonella* Typhi infection, (ii) prediction of interactions between viral and host proteins using supervised machine learning methods, (iii) identification of potential inhibitors of *H. pylori* DapE using *in silico* approach and (iv) identification of drug-like inhibitors of *V. cholerae* virulence gene activator AphB and their experimental validation.

BASIC RESEARCH

Studies on *Vibrio cholerae* adherence and survival in gut and environment

V. cholerae O1, a cause of epidemic diarrheal diseases, normally resides in aquatic environment utilizes chitin as the sole carbon and nitrogen source and remains associated with the chitinous exoskeletons of zooplankton. The principal objective of the study is to understand the mechanism how these bacteria adhere to the gut and survive in the environment using some common factors. Amongst these, the institute characterized one such chitinase ChiA2 and focused on its importance in *V. cholerae* pathogenesis. Purified ChiA2 from a *V. cholerae* pathogenic strain efficiently hydrolyzed mucin as a substrate, and released reducing sugars. Further, it has been demonstrated that the *V. cholerae* could utilize mucin as a nutrient source. In a *chiA2* deleted mutant strain, the growth was 60-fold less efficient compared to the wild type *V. cholerae* in a mucin-supplemented minimal media. The growth of the mutant strain was also 6-fold less in a human intestinal mucin-secreting cell line (HT29). Similar results were obtained in animal model experiments. Subsequently, pathogenesis was compared between these strains by analyzing the colonization ability and fluid accumulation in animal models. The ChiA2 mutant caused about 50-fold less fluid accumulation at 18 hours post infection. This was a result of poor proliferation of the mutant strain in the intestine. Results indicated that secreted ChiA2 helped *V. cholerae* to utilize intestinal mucin for their growth and survival in the host and eventually helping in pathogenesis.

Role of outer membrane vesicles (OMVs) in trafficking proteases in *V. cholerae*

Secretions of toxins and enzymes by *V. cholerae* are mediated by type I-VI secretion pathways (classical type). In addition, the organism utilizes non-classical pathway for secretion of toxins as well as extracellular enzymes through outer membrane vesicles (OMVs). Vesicle trafficking is involved in several cellular processes like extracellular delivery of toxic compounds, DNA, phage and periplasmic proteins. The most studied biological role of OMVs is their association with virulence

factors. Studies carried out with $\Delta hapA\Delta prtV$ *V. cholerae* O1 revealed presence of a novel 59-kDa serine protease. Functionally this protease caused hemorrhagic responses when assayed in rabbit ileal loop (RIL) model. In fact, this newly identified protease is also transported through outer membrane vesicles and induced changes in mice ileum. Therefore, OMVs of *V. cholerae* may be considered to play an important role in effecting a toxic response in the host towards the bacterial pathogens.

Molecular characterization of Enterotoxigenic *Escherichia coli* colonization factors

Colonization factor CS6 of enterotoxigenic *E. coli* (ETEC) is a prevalent one and is a vaccine target. However, CS6 exists as variants in different isolates. The unique combinations of CS6 subgroups were AIBI and AIIBII, where alterations in both subunits were observed. The AIBI was associated with clinical isolates whereas AIIBII was associated with asymptomatic infections. In order to understand the functional importance of this variation and association, the role of each amino acid variation has been analyzed by alanine scanning with help of site-directed mutagenesis. We used pCS6 (pSTV28-cssABCD) as template, expressed in *E. coli* BL21 DE3 and tested for the expression of CsaA and CsaB in heat saline extract by western blotting. Some of the mutations did not affect the expression of functional CS6, whereas some did. We further analyzed the periplasmic fractions of these defective mutants to locate the step in the assembly pathway that was hindered. As a result, it was noted that residues I22, V29 and I33 in the N-terminal of CsaA and residues G154, Y156, L160, V162, F164 and Y165 in the C-terminal of CsaB are involved in

the inter-subunit interaction during CS6 assembly. It was also observed that in absence of chaperone, CsaA is undetectable in the periplasm whereas CsaB is always present. This suggested that the stable existence of CsaA depends on the presence of chaperone. We further predict that T20, K25, F27, S36, Y143 and V147 amino acids in CsaA is involved in the interaction with the chaperone for its stable expression. It has been shown that AIIBII displayed different oligomerization pattern and had changes in polyacrylamide gel pattern. AIIBII bound to different epithelial cells in a CFU-dependent saturable manner 3.6-fold less adherence than AIBI in intestinal cells.

Role of commensal *E. coli* flagellin in the induction of intestinal regulatory responses and protection from experimental colitis

T regulatory (Treg) responses play critical roles in the maintenance of intestinal homeostasis and protect from the development of inflammatory diseases. It has been identified that intracolonic administration of commensal *E. coli* flagellin into BALB/c mice induces regulatory cytokines (TGF- β , IL-10) as well as CD11c⁺CD11b⁺CD103⁺ tolerogenic DCs and CD4⁺CD25⁺FoxP3⁺ Treg cells. To investigate the functional significance of the above regulatory responses, mice suffering from colitis induced by TNBS were treated with commensal flagellins. The results showed significant amelioration of colitis as evident from macroscopic, histopathological and flowcytometric analysis that correlates with increased numbers of Treg cells in the intestine (Fig. 6). The latter protect from colitis upon adoptive transfer to the littermates. In parallel experiments, induction of regulatory responses and protection from colitis

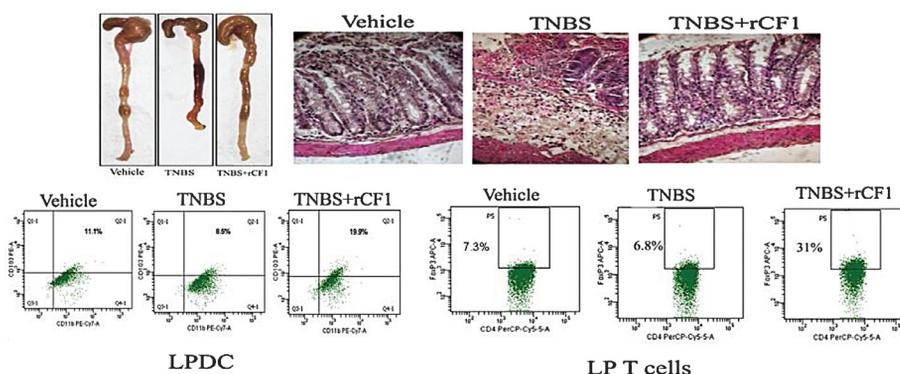


Fig. 6. Upper panel. (left). Colon of mice isolated 3 days after intracolonic TNBS administration. (Right). Histopathology of the colonic tissues isolated as above and stained with hematoxylin and eosin. Lower panel. Cells isolated from the colon of mouse treated as above.

by indigenous probiotic *Lactobacilli* were studied. In addition, role of microRNA in the generation of Treg responses is currently being studied.

Role of eukaryotic-like serine/threonine protein kinases (STPK) in the pathogenesis of *Salmonella enterica* serovar Typhi (*S. Typhi*)

Intracellular pathogens like *Salmonella* employ multiple mechanisms to subvert host-induced killing within macrophages. Eukaryotic-like Ser/Thr kinases (STPKs) have been shown to contribute to phagosomal survival of bacteria. It was noted that a putative STPK of *Salmonella Typhi Ty2* (T4519) was induced within macrophages and secreted into the cell cytoplasm. T4519 shows ser/thr kinase activities *in vitro* by autophosphorylation and phosphorylation of the universal substrate myelin basic protein (MBP) and promotes bacterial survival within macrophages. Complementary to this, *S. Typhi Ty2Δt4519* strain shows significantly reduced pathogenicity in mice that is reversed by gene or protein complementation of the strain. Further studies revealed that T4519 functions through the activation of NF-κB signaling pathways. This induces cathepsin B, leading to lysosomal membrane permeabilization (Fig. 7).

Regulation of cathelicidin antimicrobial peptide expression by Toll-like receptors

Toll-like receptor (TLR)-dependent regulation of cathelicidin antimicrobial peptide expression in the intestine and its role in the protection from infectious and inflammatory diseases has been studied. Results showed that microbial DNA, which contains CpG unmethylated motifs and signals through the host TLR9 receptor, induced cathelicidin expression

in the human intestinal epithelial cells (IECs), but not the haematopoietic cells, such as macrophages. Further, bacterial DNA transcriptionally regulates cathelicidin expression through the activation of MEK-ERK-c-Jun signaling pathways. Interestingly, MEK-ERK activation leading to cathelicidin expression in the intestine was Tpl2-independent and regulated by cAMP-PKA-cSrc-Rap1-B-Raf signaling pathways. Another study revealed that viral dsRNA transcriptionally regulated cathelicidin expression in the intestinal epithelial cells through the activation of PI-3Kkinase, PKC and SP-family of transcription factors.

High prevalence of serine protease autotransporters of Enterobacteriaceae (SPATEs) in *Escherichia coli* causing neonatal septicemia (NSEC)

Serine protease autotransporters of Enterobacteriaceae (SPATEs) are secreted proteins demonstrating diverse virulence functions. This study was undertaken to evaluate the prevalence and phylogenetic distribution of different subtypes of SPATEs among neonatal septicemia causing *E. coli* (NSEC). The presence of SPATEs was significantly higher among the septicemic isolates (89%) than the fecal (7.5%) and environmental isolates (2.5%) (Fig. 8). Vat (vacuolating autotransporter toxin) and Sat (secreted autotransporter toxin) was found as the most predominant SPATEs. The incidence of SPATEs was high in septicemic isolates of phylogroups A and B1 (87%) lacking other VFs. The high prevalence of SPATEs in the non-B2 phylogroups of septicemic isolates in comparison with fecal and environmental isolates indicates an association of SPATEs with NSEC. The septicemic

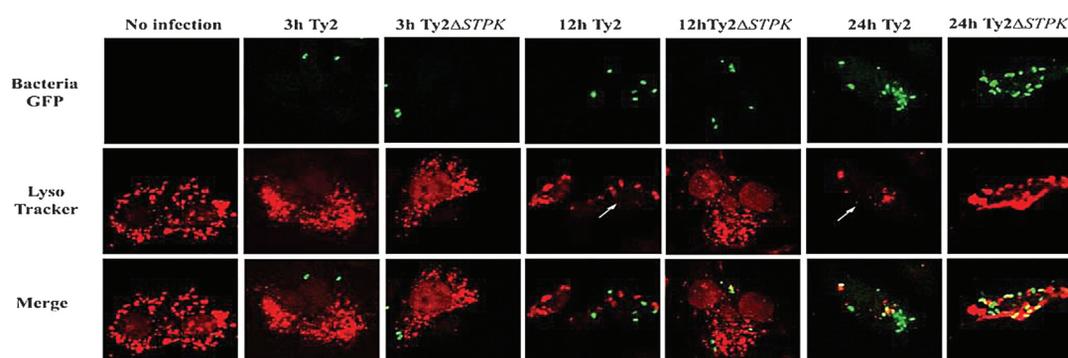


Fig. 7. Confocal microscopic images of THP-1 cells-derived macrophages infected with the wild type or mutant *S. Typhi* for 1h, washed and cultured in the presence of gentamicin (50 μg/ml) for the indicated durations. Cells and bacteria were stained with lysotracker (red) and polyclonal *Salmonella* antisera followed by FITC-conjugated secondary antibody, respectively.

isolates were found to be clonally distinct, suggesting that the high prevalence of SPATEs was not due to clonal relatedness of the isolates. This study is the first to show the association of SPATEs with NSEC. The presence of SPATEs in the septicemic isolates may be considered as the most discriminatory trait studied here.

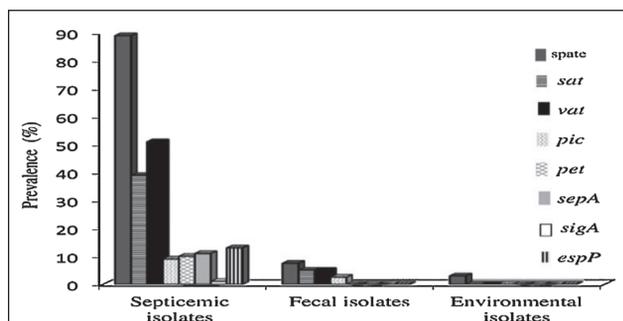


Fig. 8. Comparison of the prevalence of SPATEs among three groups of isolates. Prevalence of “spate sequence” (as determined using the generic primer pair recognizing the conserved sequences corresponding to the C-terminal domain of different SPATEs) and its 10 subtypes was significantly higher among the septicemic isolates compared to the fecal [$P < 0.0001$; OR 95% CI = 95.58 (21.03-510.09)] and environmental isolates [$P < 0.0001$; OR 95% CI = 263.50 (30.42-5926.55)]. No isolate was found to be positive for genes *hbp/tsh*, *eatA* and *espC*.

Development of novel screen to identify small molecules with either virulence suppressive or growth inhibitory properties against *V. cholerae*

This study was done in collaboration with Helmholtz Centre for Infection Research (HZI), Germany as a part of Council Indo-German Science Centre for Infectious Diseases (IGSCID). Data generated through this project helped in achieving development of i) a novel high throughput screening (HTS) assays to screen compounds libraries for identification of active compounds with either growth or virulence suppressive properties against *V. cholerae* using fluorescence based as well promoter reporter fusion based assays, ii) successful application of newly developed HTS resulted into detection of 6 compounds with growth inhibitory property and such property was specifically against *V. cholerae* but not against other bacterial enteropathogens like pathogenic *E. coli*, *Salmonella* spp., *Shigella* spp., iii) detailed analysis on mode of action of one active compound showed novel role of *V. cholerae* histidine kinase KdpD in growth and this could be a specific drug target, iv) identification of 6 other compounds

with potential to suppress virulence phenotype of *V. cholerae* have also been achieved, and v) working with virulence suppressive compound C₉ along with observation from other study, helped in identification of existence of functional Entner Doudoroff (ED) pathway in *V. cholerae* which was never been reported earlier by any group.

Analysis of rotaviruses and their interactions with the host: A viral proteomics approach

To identify the virus induced differential expression of proteins, 2D-DIGE based proteomics was used. For this, HT-29 cells were infected with rotavirus strain SA11 for 0h, 3h and 9h post infection (hpi) and differentially expressed spots were excised from the gel and identified using MALDI-TOF/TOF mass spectrometry. 2D-DIGE based proteomics study identified 32 differentially modulated proteins, of which 22 were unique. Calmodulin (CAM), a calcium regulating protein was induced by RV during early infection (3-4hpi). Co-immunoprecipitation experiment confirmed that Calmodulin directly interacted with RV-VP6 protein in absence of other viral proteins. CaM-VP6 interaction was also Ca²⁺ dependent as reduced interaction was observed in presence of Ca²⁺ chelators. W-7, a Ca²⁺/CaM antagonist had no effect on CaM-VP6 interaction but was able to inhibit rotavirus infection by down regulating expression of viral protein. This is the first report where cellular proteins which are necessary for RV infection have been identified. Inhibitors against these proteins are being analyzed for developing future antiviral therapies.

Characterization of parasitic pathogens isolated from diarrheagenic population

During the calendar year 2013-14 high extent of genetic diversity among the *Giardia* isolated from diarrhoeagenic population of Kolkata were identified along with identification of mixed or recombinant genotypes. The possibility of zoonotic transmission among *Giardia* and *Cryptosporidium* between human to other mammals as well as environmental transmission through contamination of drinking water source were evident. High resolution genotyping of *Entamoeba histolytica* isolates using tRNA linked short tandem repeat as the molecular

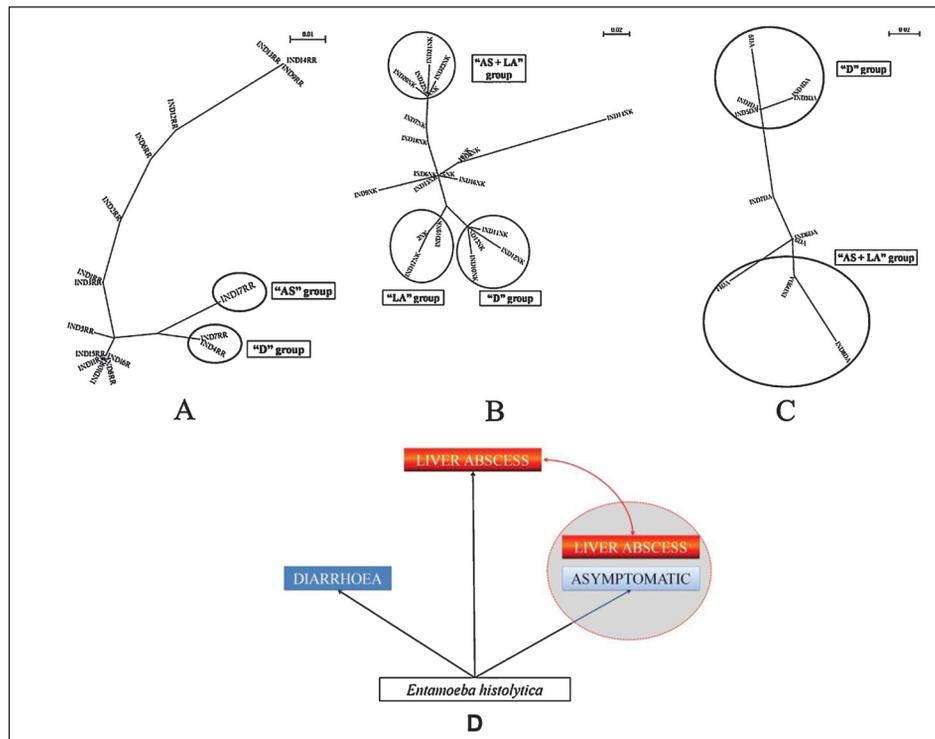


Fig. 9. (A) Phylogenetic relation among STR patterns of R-R loci obtained with ClustalW multiple alignment program. The alignment was used for constructing phylogenetic tree through “Generalized Time Reversal (GTR) + gamma” substitution model of SeaView Graphical Interface Ver.4 software. Two distinct (named as “D” group and “As” group) as well as a mixed cluster can be assigned. “D” group contain STR patterns found exclusively in diarrheal outcome, whereas “As” group contain STR patterns exclusive for asymptomatic outcome. (B) Phylogenetic relation among STR patterns of DA-H loci: One mixed “As + LA” group and a distinct “D” group can be assigned. Mixed “As + LA” group contains STR patterns found in both asymptomatic and Liver abscess outcomes. (C) Phylogenetic relation among STR patterns of N-K2-H loci: One mixed “As + LA” group and two distinct “D” group and “LA” group can be assigned. Mixed “As + LA” group contains STR patterns found in both asymptomatic and Liver abscess outcomes. “D” group contain STR patterns found exclusively in diarrheal outcome. “LA” group contain STR patterns found exclusively in liver abscess outcome. (D) Schematic representation showing lineage of *Entamoeba histolytica* based on disease outcome. Where Liver abscess and asymptomatic cases showing close association where as diarrheal cases showing distant or no association.

marker showed presence of new pattern of repeat orientation which is unique to Indian origin. Significant relation between genotypes and disease outcome in amoebiasis could be addressed and institute could also prove that genotypes found in asymptomatic isolates is evolutionary much closer or have significant association with the isolates found in Liver abscess cases as per our genotyping results (Fig. 9). It has been observed for the first time that *Giardia* trophozoites at high oxygen environment produces higher reactive oxygen species (ROS) in a time dependent manner. It is also evident that mitochondrial remnant proteins are not the key proteins from stress regulation, rather a cascade of other mitochemical pathways and proteins are involved in stress relief. Surveillance study revealed that *Giardia* still remained as the major parasite in the diarrheagenic population of Kolkata. A new method of KatoKatz screening was

successfully used in estimating the soil transmitted helminthic burden in the state of western India. The survey estimated the prevalence of *Ascaris* around 20% in the study region.

NATIONAL INSTITUTE OF MALARIA RESEARCH, NEW DELHI

EPIDEMIOLOGICAL STUDIES

Evidence based assessment of biophysical determinants of malaria in the north-eastern States of India and development of framework for adaptation measures for malaria control under climate change scenario

This study is being carried out in selected sites of Uttarakhand, Assam, Mizoram and Jharkhand for generating entomological, parasitological and

climatic aspects. Over a span of study period, changes in temperature were noticeable in study areas of Karbi Anglong and Almora districts up to ~2.5 °C in some months.

Based on temperature, rainfall and observed density of *Anopheles culicifacies*, the density was predicted for Bhorsa (Nainital) applying Genetic Programming mathematical model. Correlation coefficient of 0.77 between observed and simulated density was observed. Similarly, model for other study area is in progress. Ecological change detection using satellite data in 2011 as compared to 2001 in respect of study villages is in progress.

A total of 949 *An. fluviatilis* & 4470 *An. culicifacies* mosquitoes were assayed through ELISA for sporozoite detection from selected sites. Of 263 *An. culicifacies* and 278 *An. fluviatilis* from Uttarakhand, three samples (2 Pv & 1 Pf) were found +ve for sporozoites in the month of August 2012 and September 2012 in *An. culicifacies*. Of 4423 *An. culicifacies* and 671 *An. fluviatilis* from Jharkhand, eight samples (3 Pv & 5 Pf) were found +ve in January, April and August months.

Micro stratification of malaria in problematic districts of Rajasthan for development of strategic action plan

Field surveys were undertaken for generation of entomological and fever survey data and for ground truth. Mosquitogenic conditions were found as underground Tanks, cemented tank for *An. stephensi* and ponds for *An. culicifacies*. Using LISS IV satellite images with 5.8 metre resolution, distinction between mosquitogenic conditions in high and low malarious villages could be possible. In high malarious villages, in general, the proportion of water bodies, natural vegetation and agricultural land was more as compared to low malarious villages. In low malarious villages, the proportion of sandy/rocky area was more than high malarious villages. The statistics of landscape features differed between the studied districts. In Barmer district, the proportion of water bodies and vegetation was high in low malarious villages as compared to high malarious ones which needs further analysis. The analysis of Cartosat-II satellite data with 2.5 metre resolution revealed that breeding habitats

like Tanka and smaller habitats close to human settlements could be detected.

Survey for eliciting information on socioeconomics, Knowledge, Health seeking behaviour and behavioural practices in 144 households was undertaken in August 2013. Results of fever survey indicated the need for categorization of high/low endemicity based on present study using ecological and epidemiological approach.

Data are being analysed for development of action plan for control of vectors and malaria.

Monitoring the therapeutic efficacy of antimalarial medicines in India

Therapeutic efficacy studies of ACT (AS+ SP) in *Plasmodium falciparum* and CQ in *P. vivax* are being conducted at 15 sites in the country (13 for *P. falciparum* and 2 for *P. vivax*) in collaboration with NVBDCP and State Health Authorities using new protocols of World Health Organization. The studies have been continued in the fourth year (2012-13) in different malaria endemic regions of India where percentage of *P. falciparum* malaria is high. These studies (2012-13) have shown the efficacy of chloroquine for *P. vivax* as 100% at 2 sites. The PCR corrected cure rate of ACT (AS+SP) for *P. falciparum* ranged between 74.1 – 100% at 12 sites.

A random 20% samples were analysed for molecular markers of partner drug-resistance. Majority of samples (53.7%) showed *dhfr* double mutation followed by single (20.1%), triple (15.4%) and quadruple mutation (0.7%). Also, majority of the samples showed *dhps* wild pattern (40.5%) followed by triple (22.9%), double (20.9%), single (9.2%) and quadruple (6.5%) mutation. The K76T mutation in chloroquine transporter *Pfcr* was observed in 68.4% samples followed by 12.3% wild and 9.0% mixed type response.

These results were shared with ICMR and NVBDCP which led to change of drug policy for malaria in North-eastern (NE) region by NVBDCP. Current ACT (artesunate + sulfadoxine pyrimethamine) has been replaced with co-formulated tablet of ARTEMETHER (20 mg) - LUMEFANTRINE (120

mg) [ACT-AL] for treatment of uncomplicated *P. falciparum* malaria in NE region w.e.f. 30th April 2013. Although efficacy of combination therapy is fully effective in other regions of country, regular monitoring and vigilance is required to detect any development of artemisinin resistance as per WHO guidelines (Fig. 1).

Comprehensive case management pilot (CCM) programme in Odisha

The project was initiated in April 2013 with aim to assess the impact of a comprehensive case management system for uncomplicated malaria on the incidence of malaria in different transmission settings in the state of Odisha, India. The project is being carried out in four districts of Odisha: Dhenkanal (low endemic), Bolangir (mesoendemic), Angul (high endemic) and Kandhamal (hyperendemic). Of the two blocks selected for each district, one acts as a control while another is intervention block. The components of the comprehensive case management system have been implemented rigorously in the intervention areas, where as in the control areas, the current

routine case management system are continuing (Fig. 2).

Microscopy has been introduced at all the PHCs of the intervention blocks. CCM implementation activities like project staff training and sector level training of all health staff are over. Sufficient RDTs and age wise ACT dosage has been made available upto the ASHA level in the intervention Blocks. The buffer stock of RDTs and antimalarials has been made available at the CHC level and to avoid stock outs. The monthly data from the intervention block are received in the reporting system developed for the block level manager (BLM). Monthly review meetings of the BLM are taking place in the state headquarters.

According to the reports of July- October 2013 there has been an increase in the surveillance of malaria cases in the intervention Blocks of Bolangir, Dhenkanal as compared to the previous years of 2011 and 2012. The detection of *P.vivax* cases as compared to previous year has also increased in all intervention Blocks as Bivalent kits are available at the periphery level now.

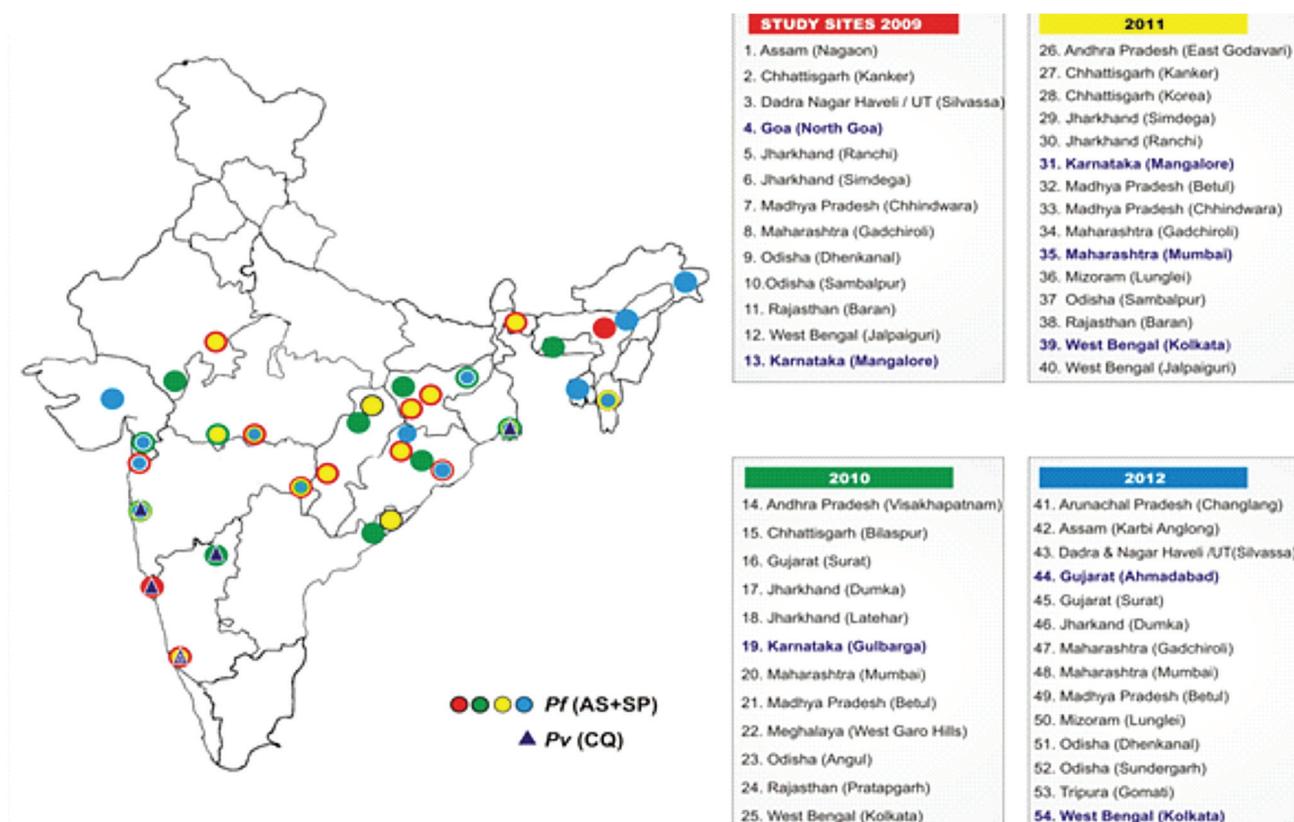


Fig. 1. Study sites during 2009-2012.

The project would enhance capacity building for malaria control in India through both the implementation experience of the State Programme Office in Odisha as well as for NIMR in large-scale pilot studies.

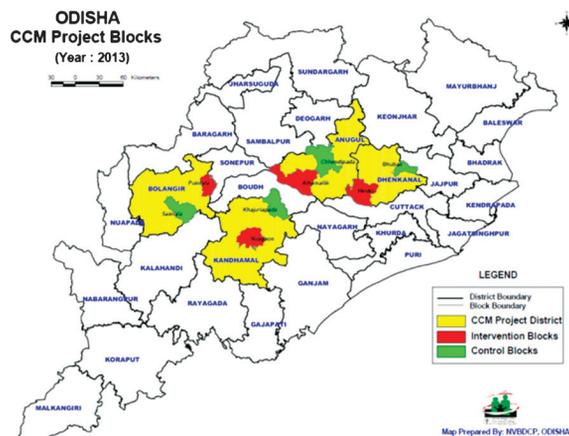


Fig. 2. Comprehensive case management pilot programme in Odisha.

Health impact assessment of Narmada Basin dams and resettlement & rehabilitation colonies in Madhya Pradesh

Health Impact Assessment was initially started in 2004 in 3 major dam's area in MP, which was extended further for 5 years in 2010 to cover entire Narmada Basin. Three study centres each working at Jabalpur, Bhopal and Narmada Nagar carry out entomological, parasitological and microbiological routine survey in the affected area of Narmada Basin.

Engineering problems were found as dam seepage, damaged canals and blockage with vegetation and stones in the command areas of all the projects. Other domestic problems were mosquito breeding in stagnant pools, cemented tanks, and absence of drainage system, swamps and water logging near hand pumps and in gutter surrounding the houses etc. It results in high breeding potential of vectors and vector borne disease. Control measures were suggested to NVDA and State Health Department *i.e.* dewatering, introduction of larvivorous fishes, channelization of pools in main River and larvicide 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.

The information of blood slides and water testing was given to concerned Health Centre for immediate action. In each survey, detailed

recommendations were submitted to NVDA and State Health Department for necessary action for control of vector borne diseases

Control of dengue and chikungunya by controlling *Aedes* breeding in key containers in pre-monsoon season endemic zone of Delhi

Based on data from 2007 – 2013, it was observed that the average container index (CI) of Delhi is about 30% whereas CI of West Zone of Delhi is 28% and there is no significant difference between CI of Delhi and West Zone. Four teams comprising of 2 NIMR field workers and 2 to 3 MCD field workers to identify *Aedes* breeding and for source reduction carried out surveys in 55 colonies of 25 localities in West Zone of Delhi. During non-transmission season (December – May) and during transmission season (June-November) fortnightly surveys were carried out for identification, detection and reduction of breeding in key and secondary containers respectively. Cross-checking was done by NVBDCP officials. Intervention methods included community awareness, vector surveillance & source reduction, introduction of Temephos granules and covering tanks broken lids with cloths.

Since December 2012 to October 2013, 116702 containers from 64177 houses were checked, out of which 1078 containers from 976 houses were found positive. During pre-monsoon season (Dec'12-May'13), maximum CI was found in tanks (6.08%) followed by coolers (5.09%) and solid waste (3.65%). Whereas, during post-monsoon season (June'13 – October'13) maximum CI was found in solid waste (36.92%) followed by tanks (11.84%) and mud pots (1.47%). As compared to 30% CI of Delhi, 28% CI was observed in West Zone and only 2% in selected localities. Percentage of Dengue Case in West Zone also reduced from 13% (2011) to 5% (September 2013).

PARASITE BIOLOGY

Molecular characterization of 4-diphosphocytidyl-2C-methyl-d-erythritol kinase (IspE) gene from *Plasmodium vivax* – ligand recognition in a template for antimalarial drug discovery

It is planned to amplify, clone, sequence, express and purify recombinant *P.vivax* IspE (PvIspE).

The genetic diversity of the IspE across seven different geographical regions of India will be characterized by biochemical properties and inhibition kinetic data, and structure determination of enzyme in complex with 4-diphosphocytidyl (CDP), 4-diphosphocytidyl-2C-methyl-d-erythritol (CDPME) and ADP. Novel compounds will be designed to mimic a fragment of the substrate and the complex structure will be determined with catalytic centre in culture *in vitro* and *in vivo* assays, by structural 3D modelling and docking studies *in silico*. In preliminary studies, the institute was isolated parasite DNA from filter paper blood spot from Bangalore region of India. IspE gene has been amplified and amplified PCR product is shown in Fig. 3 (EK-1 and EK-2). Phylogenetic analysis and optimization of enzyme activity assay is in process.

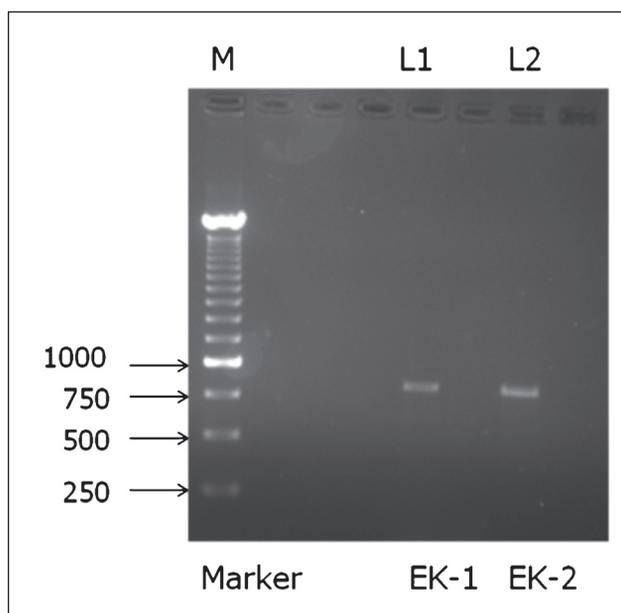


Fig. 3. PCR amplification of EK-1 gene (838 bp, L1) and EK-2 gene (829 bp, L2).

MALARIA PARASITE BANK

Parasite Bank is supporting a large number of organizations working on various aspects of malaria. Biological materials including non-human and human *Plasmodia* preserved/ maintained in Malaria Parasite Bank are supplied to various research organizations.

Expression of *vir* genes and drug resistance genes in severe *Plasmodium vivax*

Severe *P. vivax* and its pathogenesis has been linked to the *vir* genes and chloroquine resistance (CQR).

The main transporter that has been studied with regard to CQR in *P. vivax* is the *P. vivax* chloroquine resistance transporter (*Pvcrt-o*) which has been identified as possible genetic marker of CQR. Increasing *P. vivax* severity has been known to be associated to increased expression levels of *Pvcrt-o* CQR gene. In this study, the expression of five *vir* genes was compared in severe *P. vivax* infections and also the expression of *vir 12* gene along with *Pvcrt-o* gene was compared between severe and non-severe *P. vivax* cases. The severe *P. vivax* cases showed higher expression levels of *vir 12* gene and *Pvcrt-o* gene as compared to the non-severe *P. vivax* infections. Studying the virulence *vir* genes and transporter of CQR *i.e.* *Pvcrt-o* allows us to deduce that the increased expression levels of these genes in severe infections might be responsible for the changing trends of complicated *P. vivax* (Fig. 4 & 5).

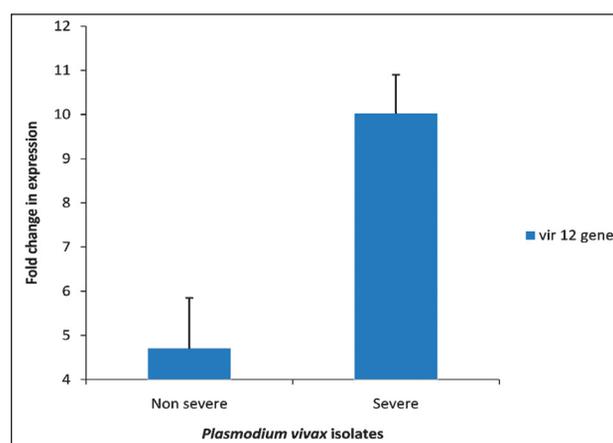


Fig. 4. Relative quantification of *vir12* gene between severe and non-severe *P. vivax* isolates normalized with endogenous gene (β -tubulin) $P < 0.05$.

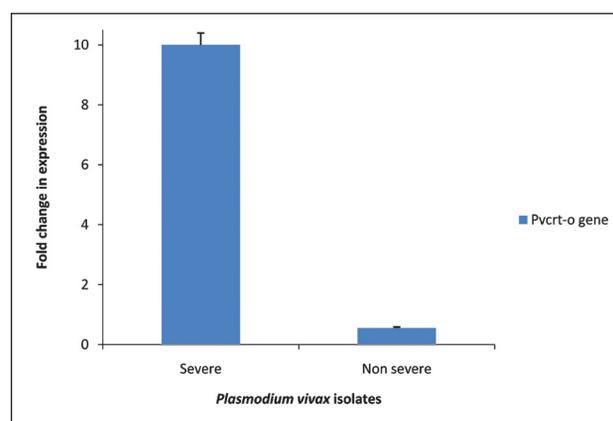


Fig. 5. Expression of *Pvcrt-o* gene in severe and non-severe *P. vivax* isolates normalized with endogenous gene (β -tubulin).

Multiplicity of *Plasmodium falciparum* infection in field isolates

P. falciparum attributes to about 50% of malaria infections in India but relatively little is known about the genetic structure of the parasite populations. This study reveals the genetic profile of the parasite population by molecular genotyping with merozoite surface protein- *msp1*, *msp2* and glutamate-rich protein (*glurp*) genes in selected regions across India with varying degree of endemicity among them. Genotyping the *P. falciparum* isolates using *msp1*, *msp2* and *glurp* gene loci have been known to distinguish treatment failures of *P. falciparum* infections and *msp1* and *msp2* have been used to assess the multiplicity of infection (MOI) for detecting the number of clones per isolate. Fifty-eight single *P. falciparum* infections after the species specific PCR were amplified for three allelic families of *msp1* gene (K1, MAD20 and RO33), two allelic families of *msp2* gene (FC27 and IC/3D7) and *glurp* gene. The study has demonstrated that more polymorphism was found in *msp2* gene than *msp1* and *glurp* genes. It was seen that 39.6% of the isolates studied were multiclonal in nature with two or more alleles present in *msp1*, *msp2* and *glurp* genes. Eleven multiple alleles were seen in *msp1* (18.9%), 16 were found in *msp2* (27.5%) and four multiple alleles were seen in *glurp* (6.8%) genes showing more genotypic variation in *msp2* than *msp1* and *glurp*. The *msp1* showed eight, *msp2* seven and *glurp* markers showed five distinct alleles present in the studied parasite population. These findings indicate that for detection of MOI, *msp1* served as a better marker as MOI with *msp1* was higher as compared to *msp2* (Fig. 6). The recent data of *msp1*, *msp2* and *glurp* markers for drug efficacy studies is highly important in malaria endemic areas for understanding the treatment criteria etc. These genes should be monitored regularly to know the present genetic structure of the parasite population.

Occurrence of multiple chloroquine-resistant *pfert* haplotypes and emergence of the S(agt) VMNT type in Cameroonian *Plasmodium falciparum*

The main objective of this study was to unravel (i) if other haplotypes are distributed in the central, littoral, eastern and southern regions of Cameroon

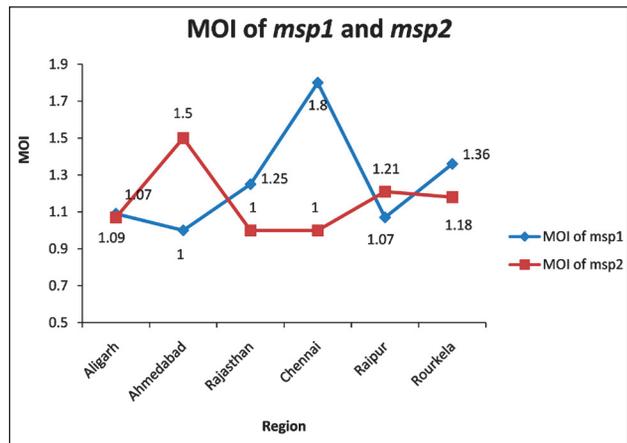


Fig. 6. Graph showing comparison of MOI for *msp1* and *msp2* genes in different regions of India.

and also in locations bordering Gabon and Equatorial Guinea. For this, we followed molecular approaches with DNA sequencing of the second exon of the *pfert* gene to identify single nucleotide polymorphisms (SNPs) in 180 *P. falciparum* field isolates sampled in five different locations in Cameroon (Fig. 7). The chloroquine-resistant *pfert* CVIET haplotype was most abundant, followed by the wild-type CVMNK haplotype. Five hitherto unreported chloroquine-resistant *pfert* haplotypes were detected for the first time in Cameroonian *P. falciparum*, including the surprise appearance of the S(agt)VMNT haplotype. The high observed haplotype diversity of the chloroquine-resistant *pfert* gene and the appearance of the S(agt)VMNT haplotype are daunting and can be attributed to drug pressure and/or the misuse of chloroquine and/or amodiaquine in Cameroon.

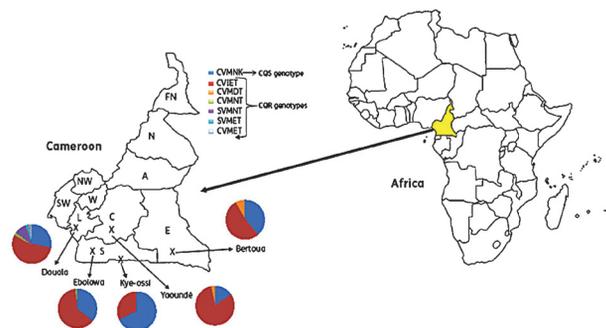


Fig. 7. A map of Africa showing the location of Cameroon, and a map of Cameroon showing the location of the different sampling sites in Cameroon. The pie chart against each sampling location indicates the frequencies of each of the seven *Pfert* haplotypes that were found in the respective locations. FN, far north; N, north; A, Adamaoua; C, centre; NW, north-west; W, west; SW, south-west; L, littoral; S, south; E, east; CQS, chloroquine-susceptible; CQR, chloroquine-resistant.

Mitochondrial genome sequence diversity of Indian *Plasmodium falciparum* isolates

Very recently designed novel primers to sequence the ~6kb of the whole mitochondrial genome of Indian *P. falciparum*. Here, we have used these primers to sequence the ~6 kilo nucleotide base pair whole mitochondrial (*mt*) genome sequences of four field isolates of the malaria parasite *P. falciparum* collected in from different locations in India (Fig. 8). Comparative genomic analyses of *mt* genome sequences revealed three novel India-specific Single Nucleotide Polymorphisms (SNPs) (Table 1). In general, high *mt* genome diversity was found in Indian *P. falciparum*, which comparable to African isolates. Population phylogenetic tree placed the presently sequenced Indian *P. falciparum* with the global isolates, while a previously –sequenced Indian isolate was an outlier (Fig. 9). Although this preliminary study is limited to a few numbers of isolates, the data have provided fundamental evidences on the *mt* genome diversity and evolutionary relationships of Indian *P. falciparum* with that to global isolates.

Table 1: Nucleotide sequence alignment of the whole mitochondria genome sequence of the four Indian *P. falciparum* isolates (excluding the previously reported PfPH10 isolate) with the reference sequence of the 3D7 isolate. Only sites showing nucleotide variations in the alignment were shown. To be noted that due to occurrence of four SNPs in four Indian isolates, three haplotypes are formed; GCTC (Blsp1), ATTT (Bet12) and ACCC (Goa2 and Mang2).

Isolates	Nucleotide positions				
	276	725	2175	2763	4952
3D7*	G	C	T	C	T
Blsp1 [#]	C
Bet12 ^ε	A	T	.	T	C
Goa2 ^ε	.	.	C	.	C
Mang2 ^ε	.	.	C	.	C

*Joy *et al.* (2003); [#]Tyagi *et al.* (2014); ^εSharma *et al.* (2001); ^εPresent study

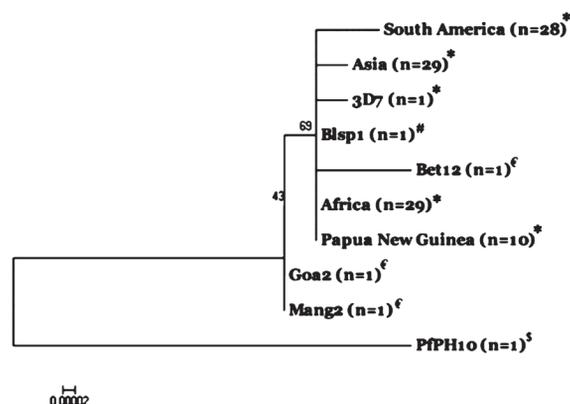


Fig. 9. Neighbor-Joining phylogenetic tree based on whole *mt* genome sequence alignment showing genetic interrelationships among *P. falciparum* isolates from different endemic localities of the globe. * represents sequences from Joy *et al.*, 2003; ^ε from Sharma *et al.*, 2001; [#] from Tyagi *et al.*, 2014 and ^ε from present study.

Immuno-modulatory role of mesenchymal stem cells (MSCs) in pathogenesis of malaria infection

Our previous reports suggest that recruitment of MSCs cells during infection and infusion of these cells into naïve mice was able to confer host resistance against malaria infection. MSCs augmented interleukin (IL)-6 productions whereas suppressed IL-10 production in recipient animals indicating that MSCs are host-protective, enhance pro-inflammatory cytokine production, and simultaneously inhibit anti-inflammatory cytokine production, hemozoin production, and Treg-cell accumulation in the spleen. In this context, we found that MSCs that accumulate in the spleen in response to malaria infection produce inflammatory cytokines such as IL-6 and MIP-1a as it is well known that IL-6 inhibits Treg-cell functions and differentiation; these findings provide a possible explanation for the reduced levels of Treg cells in the spleen of infected animals. While adoptive transfer of whole splenocytes was able to give 50% protection and to extend the survival. (Fig. 10)

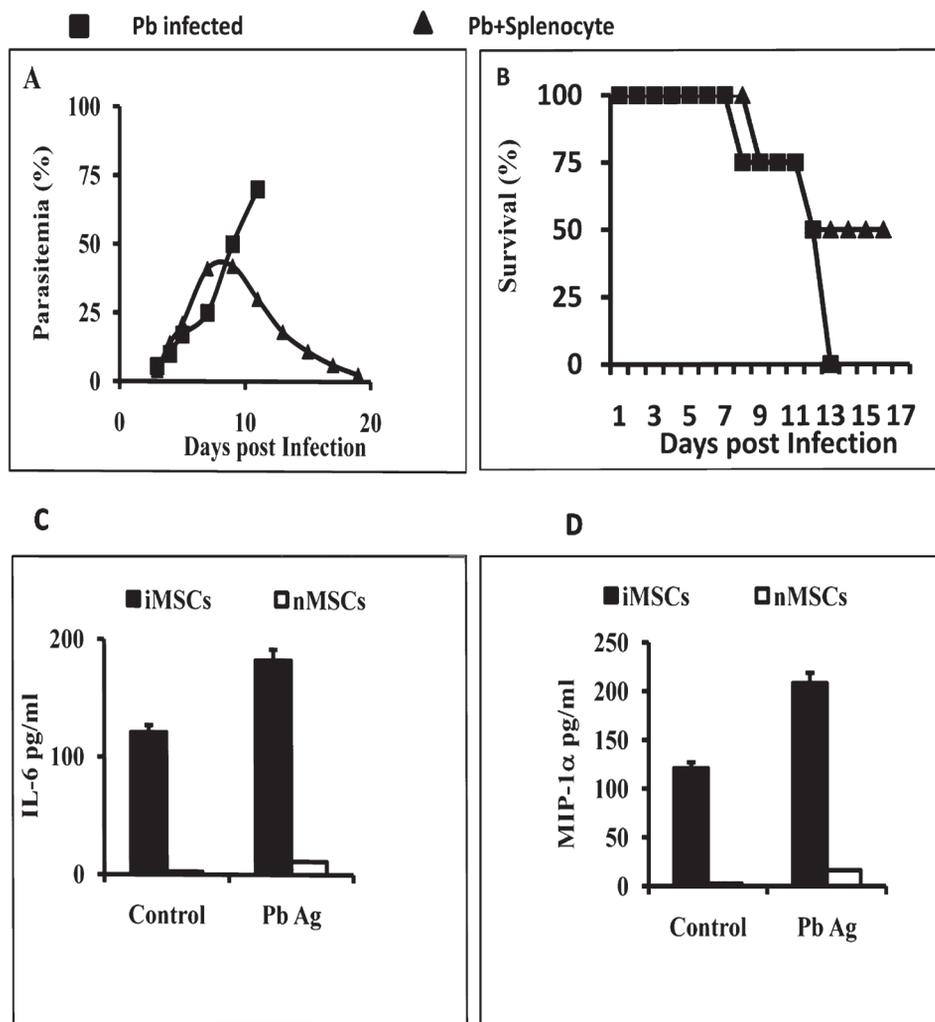


Fig. 10 (A-D). Immune response in *Plasmodium berghei* infected mice. Balb/c mice were infected with 5×10^5 *P. berghei* (Pb) parasitized erythrocytes via intra-peritoneal injection and isolated whole splenocytes (5×10^6 /mouse) from Pb infected syngeneic mice were injected into Pb infected mice intravenously. Balb/c mice infected with Pb parasites were divided into two groups. One group served as the Pb infected control group (square bar) whereas the second group of mice received whole splenocytes (Triangular bar). Blood collected from tail was used to prepare smears to determine the parasitic load. Survival of mice that received whole splenocytes vs control cells (A B). Data shown here is mean \pm SD of 12 mice from three independent experiments. The level of the pro-inflammatory cytokines IL-6 and MIP-1 α in the culture supernatant of MSCs from infected mice and normal MSCs from uninfected mice, stimulated with Pb antigen (50 ng/ml).

The repertoire diversity study of *var* gene family in complicated and uncomplicated falciparum malaria

Variable gene family (*var*) encoded protein called as *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) is highly diverse, multi-copy genes and have about 60 copies per haploid genome and situated on sub telomeric region of different chromosomes. Disease severity is strongly linked with the expression of *PfEMP-1* gene and different domains of this gene have very specific interaction

with the endothelial cell receptors. Degenerate PCR primers have been reported to amplify *dbl- α* domain of *var* gene family and we have used this reported PCR primers, however the PCR amplification protocol was not found suitable and hence we re-optimized amplification condition in the laboratory. We have tested better amplification using DNA obtained from *P. falciparum* clinical and field isolate. We have successfully optimized PCR amplification condition for *dbl- α* domain of *var* gene family, which is given in the Fig. 11a. The samples used for *stevor* study were also used for

var gene family repertoire diversity study. All 21 samples were successfully amplified for the *dbl-α* domain of *var* gene family. Amplified PCR products were cloned in TA cloning vector and 96 positive clones were sequenced per cloning experiment (Fig. 11 b).

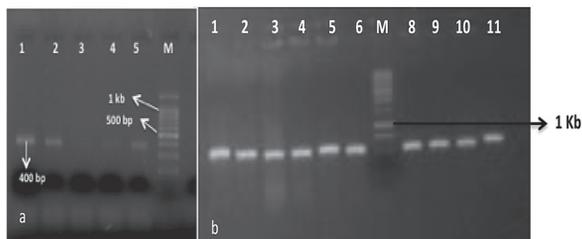


Fig. 11. PCR amplification of *dbl-α* domain of *PfEMP-1* gene (*var*) from *P. falciparum* isolates. **a:** 1-5: severe malaria samples, M: 100 bp DNA ladder, **b:** colony PCR, M: 1kb DNA ladder.

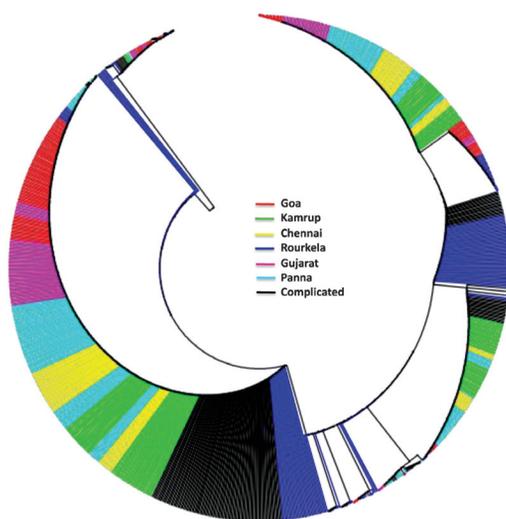


Fig. 12. Maximum likelihood phylogenetic tree showing genetic repertoire of *P. falciparum var* gene family.

A maximum likelihood phylogeny was constructed in order to understand the genetic repertoire of members of the *var* multigene family from complicated and uncomplicated malaria cases. A range of 40-60 unique sequences was obtained from of 96 clones sequenced for each isolates. ML phylogenetic tree clearly indicates for distinction between geographical isolates as well as complicated

samples (Fig. 12). DNA sequencing of *var* (*dbl-α* domain) gene from six complicated isolates are under analysis. Completion of sequencing of complicated samples work would lead to comprehensive analysis of complicated verses uncomplicated isolates to extend the distinction between geographical isolates to clinical isolates responsible for complication and uncomplicated malaria phenotypes.

Cysteine proteases as potential drug targets; A mechanism based approach for anti-malarial chemotherapy

Our recent study suggested the mechanism of activation of cysteine proteases. The sequence of events participated by these proteases are tightly regulated by a new class of endogenous cysteine protease inhibitor known as inhibitors of cysteine proteases (ICP). Structural studies of cysteine proteases inhibitors, chagasin and PbICP (cysteine protease inhibitor in *P. berghei*), clearly indicated that three loops (BC, DE, FG) are crucial for binding to target proteases (Fig 13a). Falstatin, an endogenous inhibitor of cysteine proteases of *P. falciparum* was previously reported to play a crucial role in erythrocyte and sporozoite invasion. However, the mechanism by which this macromolecular inhibitor inhibits and regulates cysteine proteases is unknown. Our study aimed to answer this question and we have identified a crucial loop as a hot-spot, (BC loop or L2 loop), which takes a center stage in the inhibitory function of falstatin (b,c, d). It is noteworthy to mention that falstatin is the first known endogenous inhibitor function as multimeric form. Using site-directed mutagenesis, hemoglobin hydrolysis assay and peptide mediated inhibition studies indicated that only BC loop inhibit cysteine proteases of *P. falciparum* and *P. vivax* via hydrogen bonds. This information is useful in exploring the mechanism of falstatin inhibition, and may be exploited to design small inhibitors based on protein-protein interactions.

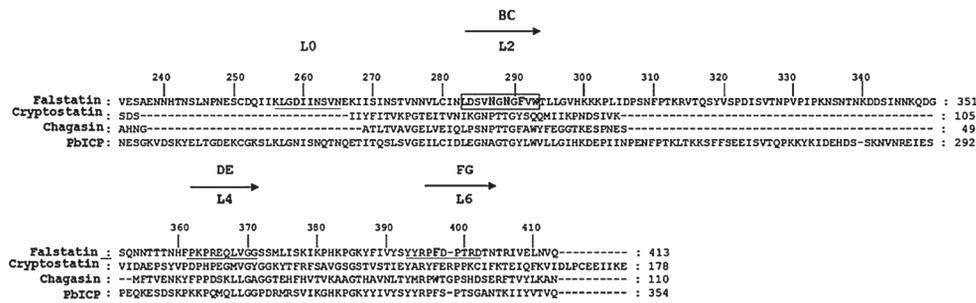
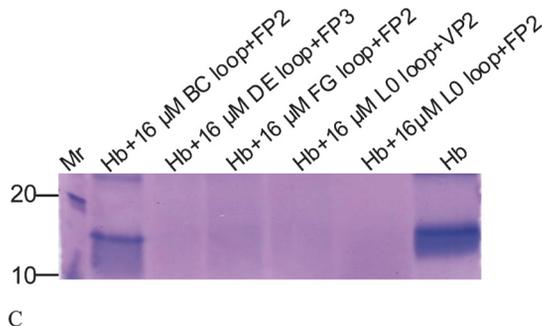


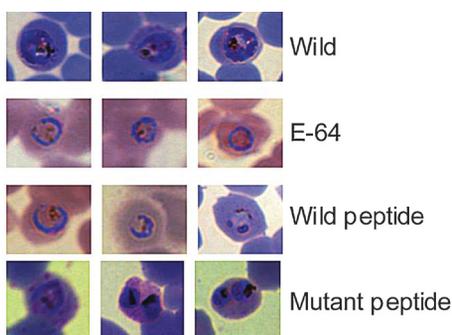
Fig. 13a. Alignment of falstatin with other ICPs. A multiple sequence alignment was performed with falstatin and ICPs from *Cryptosporidium parvum* (cryptostatin), *Trypanosoma cruzi* (chagasin), and *Plasmodium berghei* (PbICP). This alignment predicted three major loop regions; BC (L2), DE (L4) and FG (L6) in falstatin (labelled arrows). A peptide corresponding to the BC loop is indicated, and residues mutated in the described studies are in bold type. There are other loops present in the sequence alignment named L0, L3, and L5.

Models depicting the interactions of BC loop of falstatin and falcipain-3 (FP3) via hydrogen bonds. 3D model depict interactions of the BC loop peptide of falstatin (turquoise) with the active site (green) regions of FP3 via hydrogen bonds (yellow dashes).

Morphology of *P. falciparum* treated with wild type and mutant BC peptides. Synchronized ring-stage parasites were incubated with the wild type (LDSVNGNGFVW) and the mutated peptide (LDSVNGAGFVW) (25.0 μM) or E-64 (10 μM), and after incubation parasites were stained with Giemsa. Morphology of parasites was analyzed with two independent cultures of *P. falciparum*.



Only the BC loop appears to play a central role in protease inhibition. Peptides based on the BC, DE, FG and L0 loops of falstatin incubated with falcipain-2 (FP2), falcipain-3 (FP3), Vivapain-2 (VP2), and hemoglobin hydrolysis patterns were observed in 15% SDS-PAGE.



VECTOR BIOLOGY AND CONTROL

Bionomics and ecological studies of malaria vectors

Studies are being carried out in different ecological settings to understand the bionomics and prevalence of malaria vectors. Studies have assessed the changes in the ecology and impact on vector prevalence and behavior. The preliminary studies in tribal district Latehar of Jharkhand state (API- 19 to 40 and *pf%* 7.5 to 22) have shown prevalence of 3 vector species *An. culicifacies*, *An. fluviatilis* and for the first time *An. minimus* in human dwellings. The species have shown indoor resting behavior and studies are in progress on sibling species composition and their role in transmission of malaria.

Insecticide resistance and interventions

A WHO funded global project was launched in tribal district Kondagaon of Chhattisgarh State to assess the implications of insecticide resistance in malaria vectors on malaria transmission in the presence of combination of interventions. Baseline studies have shown presence of deltamethrin resistance in the main malaria vector *An. culicifacies* (~20%) and studies are in progress on other aspects namely

demography, topography, epidemiology etc. New interventions using insecticide molecules with novel mode of action, chlorfenapyr that was found effective for deltamethrin resistance management in malaria vectors is completed and was found effective but for short period. Similarly, trials with new long lasting insecticidal nets (LLINs), Duranet were completed and trial of another LLIN, Net Protect is being continued.

New studies are ongoing to establish immunological correlates of protection against malaria vaccine candidates using functional bioassays and proteomic deciphering of host-parasite interactions. Studies are also underway to understand molecular genetics of vectors and vector competence and on innate immune genes and immune modulators for cellular and humeral immunity in laboratory malaria vectors. This year the Institute was designated as “WHO collaborating centre on testing and evaluation of public health pesticides”.

Differential expression of salivary proteins between susceptible and insecticide-resistant mosquitoes of *Anopheles stephensi*

2D electrophoresis of salivary gland tissues of both *An. stephensi* sensitive and insecticide resistant strain were carried out. 17 protein annotations were found between resistant and susceptible strain when analysed by Protein PAGE software. Of the total of 17 spots, 9 spots were found to be up regulated and 8 spots were found to be down regulated in *An. stephensi* insecticide resistant strain. After analysis with MalDI-TOF with peptide mass fingerprinting and MASCOT search, total 42 proteins have been identified and among these 22 proteins are upregulated and 20 proteins are downregulated. Functional role of some of these proteins have been elucidated.

Phase III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal net DuraNet® with conventional insecticide treated nets in India

Over a period of three years, bioassay mortality after 24-hour ranged between 85-96 and 91% of DuraNet LNs resulted in producing >80% mortality in *An. culicifacies*. The remaining 9% of the failed nets were successful in causing >90% blood

feeding inhibition in tunnel test, thereby implying that 100% DuraNet LNs passed the WHO criteria of bioefficacy.

None of the distributed nets was found missing in all the surveys, thereby indicating full survivorship rate over a period of three years. The net usage rate also depended on mosquito nuisance during certain months but every night use of net remained > 53% (range 53.3-95.4%). Majority of the households (60%) washed their nets at six monthly intervals and a small proportion (6.7%) washed their nets at about monthly intervals. The proportion of washed LNs ranged from 83% to 95% after 18 to 36 months of net distribution. All the households used locally available detergent powder and cold water was used for washing the nets. The proportion of clean nets varied from 6.7% to 26.7% whereas that of very dirty nets was 3.3% to 26% during surveys undertaken after every six months up to three years of household use of nets.

The proportion of nets with holes after one year was 26.7% which gradually increased to 43.3% and 74% after two and three years of net distribution respectively. The mean hole index increased from 2.4 after six months to 92.9 after three years. Only few nets were found with repairs in the form of stitches (0.07 – 0.38 per net) and knots (0.03 – 0.34), open seams (0.02/net) and none with patches. Holes due to fire ranged between 0.07 – 0.4/net during intervals.

The trial results showed that DuraNet LNs maintained high efficacy against malaria vector for a period of three years under field conditions and can be used as a malaria control intervention tool in the community based malaria control programme.

Evaluation of NetProtect LN (impregnated with deltamethrin) against malaria vectors in the States of Haryana, Uttar Pradesh and Jharkhand

This is a multicentric phase III trial. Studies at Haryana site were initiated during September 2012 because of the closure of trial at Bangalore due to technical reasons. Baseline entomological and parasitological surveys were completed and villages were randomly assigned to NetProtect, untreated net or no net. Distribution of nets as per

demographic data were completed and intervention data collection was started from October 2012 onwards. During last one year, studies on wash-resistance showed that 100% mortality was observed in *An. stephensi* in cone bioassays on Netprotect LN up to 14 serial washings but in subsequent washing, there was a gradual decline in bioefficacy to 85.0% after 20 washings of Netprotect whereas 100 % mortality was recorded on unwashed Netprotect nets and mortality was ranging between 2.5-5.0% on serial washed untreated nets.

Changing ecology of anopheline mosquitoes in Dadri, U.P.

The study revealed that *An. culicifacies* continues to be the predominant malaria vector species in this area. The appearance of *An. fluviatilis* in high densities in the Dadri CHC area was observed only during Nov.-Dec. 2009 till July 2010 and again in Jan 2012 till April 2012. *An. fluviatilis* has not reappeared in this area again after April 2012 till date. It was also observed that the prevalence of *An. fluviatilis* in this area is not affected by annual seasonal changes, in contrast to other established anopheles species. An interesting observation was made that the appearance of *An. fluviatilis* was linked *An. fluviatilis* with the presence of thick vegetation of water hyacinth on the surface of slow moving water in the drain. These observations indicate that the appearance and disappearance of *An. fluviatilis* in this area is probably due to the presence of thick vegetation on the surface of slow moving water in the drain.

There is a declining trend in the overall density of *An. culicifacies* and other anopheline spp., except *An. subpictus*, in this area. This is probably due to land filling and increasing urbanization activities.

An inexpensive, simple and convenient approach to mosquito blood meal source identification

The benchmark method for mosquito blood meal source identification is based on counter-current immunoelectrophoresis (CCIE) using Barbitone buffer. The manufacture, distribution and sale of Barbitone are restricted by authoritarian laws in most of the countries and its procurement has become difficult and expensive. Therefore, the technique for mosquito blood meal source identification by CCIE using ordinarily available buffers in place of

Barbitone buffer has been devised. Seven different biological buffers (Tris-HCl, TBS, TBE, TAE, TE, Tris-Glycine, and Tris-Tricine) in the pH range (8.4-8.8) were tried along with Barbitone buffer in CCIE, wherein blood meal antigens move against respective antibodies to form precipitin arc and Tris Borate EDTA and Tris-HCl buffers were found to be suitable substitutes for Barbitone buffer.

VECTOR CONTROL RESEARCH CENTRE, PUDUCHERRY

LYMPHATIC FILARIASIS

Identification and characterization of potential immuno-diagnostic molecules from the L3 stage filarial parasite *Wuchereria bancrofti* for measuring the exposure to infection W

W. bancrofti L3 specific protein was identified which led to the selection of six peptides (P1 – P6) through epitope prediction. The peptides were synthesized and evaluated for their potential in detecting L3 specific antibodies. Initially they were tested against known filarial positive human sera samples and the peptides, P1, P2 and P3, showed 100% reactivity. They were also tested against BALB/c mice sera raised against *W. bancrofti* L3 and the two peptides, P1 and P3 showed 100% reactivity indicating their specificity to L3 antibodies. Subsequently, these peptides were tested against 10 endemic normal sera, P1 detected all 10 samples as positive and P2 & P3 detected 9 samples as positive. The utility of these peptides in detecting the filarial specific antibodies in human sera to be evaluated by testing further with more number of non-endemic normal and endemic normal sera samples. This will indicate the potential of these peptides in measuring the filarial exposure rate.

Immunological evaluation of the sensitivity and specificity of the L3 specific peptide, WbL3PP2 for monitoring filarial infection

Anti-WB3PP2 antibody was raised in BALB/c mice and the immunoreactivity of the peptide was tested at three concentrations (0.1, 0.05, 0.025 µg/well) against three dilutions of the mouse anti-peptide sera (1:100, 1:200, 1:500) with high titre (OD: 0.291-3.602) and all were found to show immunoreactivity. An optimum concentration of peptide (1.0 µg/well) and dilution of human sera

(1:200) were determined by titration against mf positive and negative sera and used for testing 34 coded human sera samples. The decoded results were compared with that of gold standard (Og4C3). Out of 12 antigen positives, six were detected positive for antibody, while among twenty two antigen negatives, 13 were tested antibody negative. The sensitivity and specificity of the peptide were found to be 50 and 59% respectively. Hence, it was concluded that the use of the peptide, WbL3PP2, in immunodiagnostic assay is limited.

Multi-centric evaluation of L3 stage specific RT-PCR assay for the detection of infective stage (L3) *W. bancrofti* in vector

A stage-specific RT-PCR assay was developed and validated at VCRC laboratory for detecting L3-stage of *W. bancrofti* in the vector *Cx. quinquefasciatus*. An independent multi-centric evaluation of this RT-PCR assay was carried out at different Centres of ICMR. Following hands-on-training of the scientists and technical personnel of the four participating Centres, assay conducted in the laboratory (Phase I) by the participants on coded pooled mosquitoes containing mixed stages of the parasites showed it to be stage specific detecting the L3 stage of the parasite. In Phase II, when the assay was tested independently by each Centre with coded samples of 25 infected pools and 25 uninfected pools of mosquitoes indicated 84-92% sensitivity and 92-96% specificity in two Centres, and 44-60% sensitivity and 40-60% specificity in other two Centres. In Phase III, evaluation was done for detecting infectivity in wild caught vectors. A total of 40-100 pools of 25 mosquitoes each (coded by a third party) collected from filariasis endemic areas were subjected to *W. bancrofti* L3 specific RT-PCR assay by each participating Centre. Simultaneously, 938-1550 female mosquitoes were dissected and detected L₃ stage larvae of *W. bancrofti*. The results from the Centres showed that the infectivity rate estimated by PCR assay (0.5%; 95% CI: 0.3-0.8) is comparable to that by dissection method (1.2%; 95% CI: 0.8-1.6) (95% CI overlaps). The III phase of the study indicated that the L₃ stage-specific assay has potential application in monitoring the transmission of LF. Operational feasibility required to be assessed for its recommendation in the national guideline for filariasis elimination programme monitoring and evaluation.

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

The VCRC devised a prototype electrochemical detector of *W. bancrofti* infection in vectors. This prototype is further refined in order to develop a miniaturized version to reduce the volume of the analyte and also to make the device user friendly. Synthesis of specific probe DNA of both sense and anti-sense strand of Ssp1 gene of *W. bancrofti* was followed by asymmetric PCR producing single stranded (ss) DNA. Further, the 188 base pair probe DNA sequence was cloned into TOPO blunt vector in order to have constant source of probe DNA, without relying on parasite material.

To prepare suitable chemically modified electrode (CME) for probing DNA hybridization process, cyclic voltameter (CV) differential pulse voltametry ((DPV) and electro chemical impedance spectroscopy (EIS) methods employing Ruthenium bipyridyl [Ru(bpy)₃²⁺] as redox mediator in PH 7.0. EIS method was optimized and found highly reproducible than the other two methods. EIS method will be extended on carbon screen printed electrode for miniaturization. Further detection and validation of real samples will be carried out.

National network for genotyping of human lymphatic filarial parasite, *W. bancrofti* from different endemic areas

In order to facilitate the application of molecular tools developed at VCRC for differentiation of genotyping of *W. bancrofti* parasite based on of the polymorphism of certain key genes (*b*-tubulin, Alt-2 gene and ITS2 region of rDNA) of the parasite, a national network has been established. This capacity building exercise is carried out for genotyping of *W. bancrofti* and *Brugia malayi* prevailing in endemic areas at different geographic locations and determining the frequency of alleles of different loci on different genes (*b*-tubulin, Alt 2 and ITS region of rDNA) among *W. bancrofti* parasite populations under a common platform. The collaborating centres of this approach, carried out night blood surveys for microfilaria and the morphometry analysis have been completed using 100 microfilaria from the slides with a minimum ten microfilariae per slide. Isolation and purification of microfilaria from archived blood smears, isolation

of genomic DNA from microfilariae, amplification, cloning and sequencing of Alt-2 and ITS region and detection of Albendazole resistance by real time PCR have also been carried out by the participating centres. The results show that the genotyping of the *W. bancrofti* population collected from Wardha region exhibited occurrence of 2-3 sub-population based on 29 bp tandem repeat sequence of marker-Alt2 gene and the genotyping based on beta tubulin gene of *W. bancrofti* indicated the occurrence of Albendazole insensitivity alleles among parasite population collected from Wardha (Maharashtra) region.

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

More sensitive and rapid tests are necessary to monitor filarial infection in human and vectors during post-MDA surveillance. PCR-based assays are more rapid, sensitive and specific for detecting filarial infection in mosquitoes. However, its application in programme (in place of human sampling) requires sampling methods for mosquito collection. In this study gravid traps were used to sample vector mosquitoes from randomly selected households in the 'hotspot' villages and in all the villages in a PHC (undergone 8 rounds of MDA) in Thanjavur district, Tamil Nadu. Two such surveys were independently carried out at two time points: immediately (stage 1 - 2010) and two-years after the cessation of 8 rounds of MDA (stage 2 - 2012). Vector infection and infectivity were assessed using qPCR and RT-PCR assays respectively. The changes in vector infection and infectivity rates based PCR-assays were compared between 2010 and 2012. The results of the study suggest that the proposed mosquito sampling strategy of collecting 7 pools of 25 gravid females from each of 33 clusters (aggregating to a total of 231 pools of at least 5000 gravid females) would suffice to assess the vector infection rates by PCR assays even when the infection rates in human and vectors are below the transmission threshold level of 0.5%.

Post-MDA surveillance of rural communities in south India

This study was carried out in four villages, located in Villupuram district of Tamil Nadu, in

which the Mf-prevalence was <1% and the vector infection was nil following six rounds of MDA (DEC+albendazole), to find whether adult age class can be monitored in place of children and monitoring infection in vector population can be an alternate to monitoring infection in human. Mass screening of targeted population in the two age classes (6-10 & 16-45 years) for filarial antigen using ICT and xenomonitoring carried out in 2011 and in 2013 showed that the Ag-prevalence declined from 1.0% in 2011 to 0.72% in 2013 among children (P= and from 2.5% in 2011 to 1.6% in 2013 among adults. While the reduction was not significant in the children age class, it was at its statistical limit in the adult age class. Comparison of Ag-prevalence between the two age classes did not show any significant relationship. A cohort of 202 children and 934 adults screened during 2011 were followed in 2013. About 1% of the individuals in both the age classes showed gain of infection and about 13.6% of the individuals showed persistence of filarial antigen. The loss of infection was about 86% in both the age classes. There was no positive in both the surveys in the age-class 6-7 years (targeted age-class for TAS) indicating continued absence of transmission even after two years of stopping MDA.

Out of 5282 mosquitoes dissected for filarial infection status, none was found with filarial infection indicating absence of transmission in the study community. Absence of recent transmission in two consecutive post-MDA surveys indicates that 1% Mf prevalence was safe to discontinue MDA. Post-MDA Ag-prevalence between children and adult age class is not related, and therefore adult age class cannot be targeted for evaluation.

MALARIA/LEISHMANIASIS/SCRUB TYPHUS

Tolerability, efficacy and operational feasibility of artesunate combination therapy (ACT) (Artesunate-Sulfadoxine + Pyrimethamine): as 1st line antimalaria drug for *falciparum* malaria control in a tribal area in Koraput district, Odisha State

This study is being carried out in a stable *falciparum* area, Laxmipur CHC in Koraput district of Odisha state to monitor the efficacy of artemisinin combination therapy (ACT). A total of 93 cases

were recruited for the study following inclusion and exclusion criteria. The mean age of the study subjects was 5.3 (+2.3) years. The patients were treated with ACT as per NVBDCP guidelines and supervised consumption was ensured. A total of 75 cases completed 28 day follow-up.

Three major clinical symptoms of malaria (fever, headache and vomiting) were monitored before and 1, 2, 3, 7 day after medication with ACT. About 3.2% of cases (n=75) continue to remain mild febrile on 3rd day but recovered on day 4 and hence no medication required. The overall incidence of adverse reaction was 75.3% (70/93). The major symptoms were abdominal pain and nasal irritations followed by fever, headache, myalgia and vomiting. Abdominal pain was observed up to 3 days post-treatment. The duration of other mild symptoms varied between 1-5 days. The drug regimen has good clinical response in uncomplicated falciparum malaria, although the fever resolution is by crisis. Reduction of parasite density by 96% by day 3 suggests adequate response to ACT. However, there is late parasitological failure of about 27% of cases studied as observed both by microscopy and PCR suggesting failure of partner drugs: sulfadoxine and pyrimethamine. The adverse drug reactions were mild and abdominal pain was the predominant observation. About 28% ASHAs either do not know or take others' help for performing RDT. 60% of ASHAs give ACT for 3-days' dose on the 1st day of patients' visit and the remaining ASHAs distribute ACT on daily basis.

Comparative assessment of the efficacy of two rounds of indoor residual spraying with DDT 75% @ one g/m² and DDT 50% @ one g/m² against, *Anopheles fluviatilis*, the malaria vector in Odisha State

DDT WDP 50% @ one gm/m² is currently in use for indoor residual spraying (IRS) in malaria control. Although, DDT WDP 75% is considered as the cost effective high performance formulation for IRS with long lasting residual properties, no information is available on its comparative efficacy DDT WDP 50% in Indian conditions. In response to the request from the Directorate of NVBDCP, this two arm study was carried out to compare the effectiveness of the two formulations in controlling *An. fluviatilis*, the primary vector of malaria in the selected endemic areas of Koraput district,

Odisha State at a subcentre level. The intervention sites comparable in terms of ecotypes, human population size and vector density, with vector density as the main parameter were randomised to receive either one of the two DDT formulations. The entomological parameters were monitored in six index villages randomly selected from each arm, in order to evaluate the impact of IRS.

First round of IRS was carried out in the two arms in July 2013 and the second round after three months in November 2013. The spray coverage was 83.4% 50% arm and 86.9% 75% arm during the first round. The corresponding values during the second round were 75.6 and 80.1% in the respective arms. At two months post-spraying, the mud plastering coverage was higher in both DDT 50% (27.7%) and DDT 75% (25.1%) arms.

Entomological and epidemiological investigations on leishmaniasis among the Kani forest tribes in the tribal settlements of Thiruvananthapuram district, Kerala

Following a cross sectional survey on the prevalence of cutaneous leishmaniasis (CL) infections and vector studies in 28 Kani tribal settlements, located in the difficult-to-reach areas of the Western Ghats, Thiruvananthapuram, Kerala, a longitudinal survey was carried out to monitor the seasonal distribution and behavior of sandflies and the extent of CL infection in the tribal settlements. A total of 768 individuals in 28 settlements were examined for CL / suspected infection of whom 12 had healed lesions with scars. Based on nodules or active lesions 15 cases were clinically suspected. Biopsy of specimens from the nodule/ lesion of 12 suspected cases were subjected for PCR assay as well as histopathological examinations. Six cases showed positive for *Leishmania donovani*. The lesions, mostly nodulo-ulcerative were present on the face, upper limb, lower limb, upper part of trunk and other parts. Both the genders and children were affected. Mucosal involvement in lesion which is considered to be a rare feature was observed. The cases are appeared to be indigenous as the individuals did not have history of movement to other areas.

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

Towards establishing laboratory facilities for diagnosis and developing vector surveillance

strategies for scrub typhus, a study has been initiated with experts from Medical colleges. Clinical profile of a total of 132 suspected Scrub typhus cases (96 adults and 36 paediatric age-groups from Pondicherry and nearby villages from surrounding Tamil Nadu areas) were collected and analysed. Out of 145 clinically suspected cases, 115 were anti-56Kda (Scrub typhus) IgM positive. Further, diagnostic assays on these samples with Weil-Felix test and Polymerase Chain reaction to detect 56 Kda antigen, 16s rDNA and GroEL genes of *Orientia* showed that among the two immunological tests the IgM ELISA tested highest number of samples positive, almost twice as much as the conventional Weil-Felix test. Of the three PCR assays tested, the one based on detection of GroEL gene yielded significantly higher number of positives. Nucleic acid sequencing indicated that all the three cases are that of *Orientia tsutsugamushi*.

Determination of genotypes was done on positive samples in 56kDA PCR, by nucleic acid sequencing. Twenty one out of the 34 positive samples have been sequenced and the genotypes that have been identified. A total of 6 genotypes were identified and most of the samples (11) belonged to genotype I SS-11. Phylogenetic analysis of the genotypes has shown that some sequences are more similar to the Madhya Pradesh genotype and a few sequences are of genotypes unique to Puducherry.

Surveillance of *Leptotrombidium* mites (Chiggers), the vector of scrub typhus was carried out in areas where confirmed human cases of scrub typhus reported. All the study locations were characterized by the presence of shrub and bush vegetation. A total of 391 mites were collected from 35 rodents/shrews trapped. *Leptotrombidium (L) deliense*, the vector of scrub typhus constituted 42% of the total mites identified (n=178). Scrub typhus continues to cause an acute febrile illness in this region as evidenced both by the antibody detection and the detection of the DNA of the bacterium. ISS-11 is the most common genotype identified so far along with other genotypes like Inha Kp1186344, CMC Scrub E6, UT219 and CBNU-19.

DENGUE/CHIKUNGUNYA/JAPANESE ENCEPHALITIS

Ecology and population dynamics of dengue/chikungunya vectors towards development and

demonstration of integrated vector management strategy in Kerala

The project was initiated at the request of Rubber Research Institute (RRI), Govt. of India, Kottayam and in collaboration with the Department of Health Services, Govt. of Kerala in the wake of the large scale outbreak of Chikungunya in Kerala State during 2007, particularly in the rubber plantation areas. The cost of the staff requirement was met by the RRI. The objectives were to study the factors involved in the outbreak of CHIKV and to demonstrate IVM for the control of Aedes vectors and prevention of Aedes transmitted diseases in rubber plantations. The sero-prevalence of CHIKV infection in the community was estimated to be about 68.0%. *Aedes albopictus* was found to be the predominant mosquito species and incriminated as the vector involved in the CHIKV outbreak. The peak abundance of the vector was recorded during pre-monsoon months. Intermittent rainfall and prevailing plantation practices (rubber and pineapple cultivations, removal of rain guards and intermittent tapping of rubber) during this season favored prolific breeding of the vector species in discarded/unattended latex collection cups. A community oriented IVM strategy was developed for the control of the vectors in the plantation sector and successfully demonstrated in the Aimcompu and Chethackal villages (Kottayam and Pathanamthitta Districts). The discarded and unattended latex cups and tree holes were targeted since these habitats contributed to more than 80% of the vector population in the area.

Long term morbidity due to Chikungunya and its impact on quality of life in Kerala

Under this project, to know the long-term impact of CHIKV infection, clinical, biochemical and auto-immune parameters were obtained from two sets of population where there was reported epidemic in 2006 (Kadakkarapaipally and Chetikadu PHC areas of Alleppy district) and in 2010 (Karinthalam and Kayur PHC areas of Kasaragod district). In total, 832 individuals were screened in a camp mode. This included 280 individuals in Kadakkarapaipally and Chetikadu PHC areas of Alleppy district and Karinthalam and Kayur PHC areas of Kasaragod district. However, clinical data were captured from 816 individuals. Data for the 16 individuals were not collected as these individuals wanted only

medications. A total of 280 samples from Alleppy district and 301 samples from Kasaragod district were screened with CHIKV IgG Abs. Sociological data was collected from 87 and 193 individuals in the above order of the population.

Clinical observation indicated that 89/280 (31.7%) and 66/301 (22.0%) report CHIKV epidemic related joint pain in Alleppy and Kasaragod district respectively. Among the 89 reported CHIKV related joint pain in Alleppy district, 20 (22.4%), 1 (1.1%) and 68 (76.5%) reported large, small and large and small joint involvement respectively. Among the 66 reported CHIKV related joint pain in Kasaragod district, 48 (72.7%), and 18 (27.3%) reported large, large and small joint involvement respectively. Immune-fluorescence testing for anti CHIKV IgG abs showed that 221/280 (78.9%) and 183/301 (60.8%) of the individuals were positive in Alleppy and Kasaragod district respectively.

Development of RS-GIS based model to forecast JE vector abundance and transmission risk

During the year 2013, field surveys were carried out to monitor the vector abundance and paddy growth in JE endemic villages of both Cuddalore (Tamil Nadu) and Bellary (Karnataka) districts. It was observed that the adult vector density (*Culex tritaeniorhynchus*) increased along with paddy growth up to flowering stage, and then it dipped when the crop reached the maturing stage during 'kharif' as well as 'rabi' seasons in the study villages in both the districts. The occasional vector, *Cx. gelidus* was also recorded in high numbers through both seasons in Cuddalore villages. The immature density of JE vector showed a similar trend as that of adult population in both the study villages and these variables significantly correlated with crop height.

The Indian Satellite [RISAT-1 dual polarization (HH, HV) MRS and FRS data] data available for the last one year were used for identifying the different stages of paddy growth in the study villages. Rice, being a semi aquatic crop, generates unique backscatter profile. Discrimination of different temporal stages of paddy growth is achieved by calculating backscatter coefficient (σ_0) derived from the RS imageries. The σ_0 values increase with

stages of paddy growth in 'kharif' season suggesting that the backscatter coefficient could be used as a proxy for monitoring paddy growth and hence can be used to explore its possible relationship with JE vector density. For 'rabi' season too, a similar exercise is being done to verify the consistency of satellite data corroborated for different stages of paddy growth.

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

Regular surveys for collection of entomological data were commenced in July 2013 in all the study villages. The overall per man hour resting density of the vector *Culex tritaeniorhynchus* during the period of July – December exhibited a similar trend in all the three blocks (two intervention blocks and one comparison block) with the highest per man hour density recorded in the months of September and October following which there was a decline in vector density in the months of November and December. In all the three blocks, the indoor resting density was higher in the cattle shed compared to that in the human dwelling. Analysis of 286 samples out of 770 blood meal samples obtained from wild caught engorged female vector mosquitoes from the study villages by agarose diffusion method showed 93% were positive for bovine blood, 1.75% for pig, 0.7% for mixed (human and bovine). Human blood index (HBI) was zero. Among the 75 pools (1535 specimens) processed for determining the vector infection rate, 7 pools showed positive reaction to RTPCR-JE showing a minimum infection rate (MIR) of 0.46%.

Studies on the transmission dynamics and control of dengue in forest fringe areas of Kerala

The study is aimed at identifying the factors responsible for the continued occurrence of dengue cases in Kerala, particularly during pre-monsoon season. Three villages in Kanjirappally taluk, namely, Kanjirappally (population: 42, 952), Koruthode (population: 18, 187) and Erumeli (population : 52, 997) located in the forest fringes of Western Ghats contributing about 80.0% of dengue cases in 2013 were the study sites. Entomological collections carried out (both adult and immature)

in these villages on fortnight intervals showed that *Ae. albopictus* is the predominant species (64.86%). Adults emerged from immatures collected from these areas were processed for arbo-viral infection and none was found infected.

MICROBIAL/CHEMICAL AGENTS FOR VECTOR/PARASITE CONTROL

Development of nanoparticle based formulation of *Bacillus thuringiensis* var. *israelensis* (VCRC B17) to improve efficacy and nanoparticles based detection system

Two types of nano-particle based aqueous suspension formulations of *B. thuringiensis* var. *israelensis* (*Bti*) were prepared and tested against *Culex quinquefasciatus*. The LC_{50} and LC_{90} values of the nano-formulations ranged between 30-35 μ l and 61-81 μ l when compared to 25 μ l and 54 μ l of the existing AS formulation. Hence, the nano-formulations did not enhance the activity over the already existing formulation. Antibodies (with titre 1:3200) were raised in BALB/c mice against *Bti* and conjugated with gold nanoparticles. The detection signal of *Bti* spores from environmental samples using this nano-conjugated antibody was found to be feeble. Hence, the nano-based *Bti* spore conjugate was not found to be efficient in detecting the antigen from environmental samples.

Development of nanotechnology based public health larvicides for effective mosquito control

The nano-particles based formulations prepared using temephos, pirimiphos-methyl (organophosphates) and an indigenous IGR, 2,4-Dichloro-2',6'-ditertiarybutyl diphenylether (DPE-28) were field evaluated. At a dosage of 1 mg/l, >90% reduction in immature density of *Cx. quinquefasciatus* in breeding habitats was observed but the effect lasted only for 1 – 2 days. Though, the nanoparticles are reported to play a definite role in taking the active molecules to the desired target in a closed system, especially in the drug delivery, the size of the nanoparticles does not have any positive influence on the biological activity of the larvicides especially in an open environment. Therefore, it is inferred that the use of nano-formulation for mosquito larvicides will have limited application in mosquito control.

Isolation and characterization of a lead molecule from the mosquito larvicidal *Euphorbia lactea* crude extract

This project envisages the isolation and characterization of the active principle (lead molecule) responsible for the mosquito larvicidal activity from *E. lactea*. The residue extracted from the latex of *E. lactea* plant was screened for mosquito larvicidal activity. After confirming the activity, it was purified by TLC and Column chromatography. Identical fractions when pooled and screened for mosquito larvicidal activity at 100 ppm, showed one fraction (A1) was effective against *Culex* larvae (100% mortality,) while fraction A2 was effective against *Culex* and *Aedes* larvae. The fraction B2 was effective against all the three species of mosquito larvae tested. Fraction B2 was subjected to GC/MS analysis and identified as 2,6-octadiene 2,4-dimethyl-an aliphatic hydrocarbon and 1H-Cycloprop[e] azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1a α ,4a α ,4a α ,7a α ,7a α ,7b α)]- a tricyclic. This is the first report of the mosquitocidal lead molecule from *E.lactea*. Further work is in progress.

Characterization of the bacterial toxins isolated from marine soil samples for the control of mosquito vectors

The density and toxicity of *Bacillus cereus*, VCRC-B520 (NCBI: KC-119192), a potent mosquitocidal agent isolated from marine soil, was compared between conventional culture broth (NYSM) and in chicken feather waste (CFW) media. Larval bioassays were performed against mosquito species (*Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*). The density of *B.cereus* in the CFW found to increase with increase in culture time. The multiplication process lasted upto 72 hours. The biomass production was also comparable with that of the growth pattern of the bacteria. The protein profile of *B.cereus* showed that the toxic protein "Cry4Aa" (85kDa) was distinct and conspicuous in the culture grown by both CFW and conventional medium (NYSM). Comparative toxicity analysis of *B.cereus* from CFW (LC_{50} : 0.47, 0.5 and 1.92) and NYSM (0.44, 0.54 and 1.95) media respectively for *Cx.quinquefasciatus*, *An. stephensi* and *Ae. aegypti* showed that the results are on par with one another.

Characterization of the specific polypeptide (s) in *Culex quinquefasciatus* (filariasis vector) causing resistance against biopesticides in mosquito control

Mosquito vector *Culex* species is resistant to *B.sphaericus* and this is expected to limit the use of this bio-larvicide in vector control programmes. Therefore it is important to understand the resistance mechanism and feasibility of reversing the same. The objective of the present project was to identify and characterize the specific polypeptide (s) in *Cx. quinquefasciatus* causing resistance against *B.sphaericus*. Laboratory selection experiments with *Bs* against the larvae were carried out up to 17 generations of *Cx. quinquefasciatus* and occurrence of resistance was reported (RR at LC₅₀ and LC₉₀ = 1987 and 2051 folds respectively). The protein profile of both resistant and susceptible larvae were qualitatively analysed by SDS-PAGE (12%) and the difference in the polypeptide pattern were analyzed. The resistant polypeptide was subsequently used for raising antibody in rabbits for acquiring anti rabbit polyclonal antibodies (IAEC/EM-0503/8/6/2010). Immunoblotting was carried out to visualize the factor responsible for resistance. The *Bs* resistant polypeptide was subsequently eluted from the gel and the mass spectroscopic analysis (M/S-MALDI-TOF) was carried out for protein sequencing.

The inheritance of resistance based on protein profiles from mosquito population was confirmed with expression of a conspicuous polypeptide (80kDa) in *Bs*-resistant population. This was again proved with western blot analysis for visualization of resistant protein. The protein was found to be reacting with anti-rabbit anti-sera. The result from MS analysis of peptides of resistant protein (80kDa) was submitted to NCBI and it revealed that the resistant protein was identical 100% with "hexamerin" (XP-001843494.1), a conserved insect protein that accumulate extraordinarily in the larval stages. Further supportive information on the matched peptides from hexamerin (bold red colour) was mentioned. The data indicated that hexamerin might play a vital role on the development of resistance in *Cx. quinquefasciatus*.

In vivo screening of six promising 1-N-methyl-4-(substituted) benzoyl/phenyl acetyl piperazides for macrofilaricidal activity against *Brugia malayi* in animal models

VCRC has identified six substituted phenyl acetyl / benzoyl piperazides, exhibiting moderate adulticidal activity against *Setaria digitata* under *in vitro* conditions. These compounds were further tested under *in vivo* conditions against adults of lymphatic filarial parasite *Brugia malayi* (sub-periodic strain) using suitable animal models. Out of the six promising compounds, five compounds, A₄, A₅, A₆, B₇ and B₈ were administered to gerbils transplanted with *B. malayi* adults at 100 mg/kg body weight. The gerbils treated with base and citrate salts of A₄, A₅ and A₆ died within 3 to 5 hours after administration on the first day, whereas the gerbils administered with base and citrate salts of B₇ and B₈ at 100 mg/kg b.w. for five consecutive days, survived. Animals administered with DEC citrate and normal saline were maintained as positive and negative controls. The animals were routinely monitored for weight loss, food intake and body temperature. Animals were sacrificed 45 days after administration of the drug. The citrate salts of B₇ and B₈ were found to exhibit 100% mortality of *B. malayi* adult worms but were not effective against microfilariae. The IP administration of free base of B₇ and B₈ was not effective against both microfilariae and adult worms. The *in vivo* screening of free base and citrate salt of the sixth compound B₁₄ is in progress. The effective compounds (citrate salts of B₇ and B₈ and B₁₄ if found effective) will be further tested in *B. malayi* infected *Mastomys coucha* through oral administration of citrate salts of the effective compounds in comparison with DEC citrate.

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

Secondary metabolite(s) produced by an indigenous strain of *B. amyloliquefaciens* (B483) were found to have mosquito larvicidal and pupicidal activity. The crude mosquitocidal metabolite(s) was also found to show anti-bacterial activity against multi drug resistant (MDR) human pathogens. To develop this mosquitocidal bacterium as a

biocontrol agent, the production processes *viz.* upstream and downstream need to be optimized for maximizing the yield of the metabolite. This property is expected to have additional value to this product. Upstream process for the production of the mosquitocidal metabolite(s) was optimized by designing culture medium using different carbon and nitrogen sources like lactose, maltose, sucrose, starch, groundnut oil, coconut oil, groundnut oil cake, coconut oil cake, peptone, tryptone and ammonium nitrate. Parameters studied to assess the suitable carbon source for maximum production of the metabolite was biomass, mosquitocidal activity and quantity of biosurfactant. Among the different carbon and nitrogen sources tested, sucrose and peptone were found to enhance the production of the mosquitocidal metabolite by the bacterium B483. Method for the separation of the crude metabolite was also standardised. Studies on purification of the metabolite and testing the metabolites against multidrug resistant bacterial pathogens of human are in progress.

MICROBIAL/CHEMICAL AGENTS FOR VECTOR/PARASITE CONTROL UNDER TRANSLATIONAL RESEARCH

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

This project was undertaken to assess *in vitro* and *in vivo* macrofilaricidal activity of the monoterpenes and the different combinations of monoterpenes present in the fruit extract. The seeds of *T. ammi* were extracted by two methods *viz.*, (i) by soxhlet extraction with methanol followed by solvent removal by rotary vacuum evaporation under reduced pressure (ME) and (ii) by hydro distillation using Clevenger apparatus (HD). The presence of four monoterpenes (TE-1-4) in both extracts were identified by TLC and HPLC analysis. The two extracts and four monoterpenes were screened individually for adulticidal activity against *S. digitata in vitro*. Eight combinations (MCT 1-8) containing monoterpenes were also prepared and screened against adult *S. digitata in vitro*. Two combinations *viz.*, MCT-6 & MCT-7 exhibited higher macrofilaricidal activity with ED₅₀ value of 0.006mg/ml. Mongolian gerbils

(*Meriones unguiculatus*) were inoculated with 100 - 200 infective larvae (L₃) of *Brugia malayi* harvested from mosquitoes. Animals with adult *B. malayi* worms were identified and treated with the compounds. There was no mortality of adult worm in contrast to the *in vitro* tests. This could be due to the low bio-availability of the active components on the drug due to poor aqueous solubility or fast metabolism. Hence, attempts are being made to develop suitable formulations to improve the aqueous solubility and the bioavailability of the two promising macrofilaricidal combinations.

Optimization of production and downstream processing for the improved yield of Thrombinase, a blood clot dissolving enzyme, from a *Bacillus sphaericus* (strain no. NRRL B 18949)

The production of Thrombinase was upscaled to pilot scale level and the activity was found to be superior to other commercially available thrombolytic agents. Further, molecular weight of the enzyme was determined, conducted peptide mapping and studied the stability of enzyme. The HPLC purified Thrombinase was subjected to peptide analysis after digestion. The peptide profile generated a total of 29 peptides of molecular weight ranging from 599 Da to 2716 Da and the molecular weight was determined to be 28.95 kDa by MALDI-TOF. The enzyme did not show any activity at pH 2-4 indicating that the enzyme may not work at the pH equivalent to that of human gastric juice. The optimum pH for the activity of the enzyme was found to be 7.0.

Development of formulation and evaluation of *Pseudomonas fluorescens* (VCRC B426) against mosquito vectors

This study was undertaken to scale up the production to large scale in 100 litre pilot fermentor to improve the yield, reduce the field dosage, study different downstream process to reduce the production cost develop a suitable liquid formulation and to test the product against different mosquito vector species, both in the lab and under field conditions. The culture medium was modified with respect to various physico-chemical factors and it resulted in 50 fold increase in the production of the mosquito pupicidal toxin of *P. fluorescens*, when compared to the defined medium. For the recovery

of maximum level of the toxin from the culture filtrate, different downstream process, viz., solvent extraction, ammonium sulphate precipitation and acid precipitation were attempted. Among the three methods, solvent extraction method yielded the maximum recovery of the pupicidal toxin from the culture broth. The results indicated that pH 7.0 and cultivation temperature 30° C are optimal for the maximum yield of the pupicidal toxin. The results of optimization of culture conditions in 100 l fermentor showed that the bacterium starts synthesizing the pupicidal toxin at 12 h (LC_{50} 555.80 μ l/100 ml) and reaches the maximum at 36 h. (LC_{50} 13.82 μ l/100 ml). Compared to this, in shake flask culture it took 66 h for producing the same level of toxin.

Laboratory bioassay results of the formulation showed that *An. stephensi* (LC_{50} 0.92 and LC_{90} 2.14 μ l/100 ml) was more susceptible followed by *Ae. aegypti* (LC_{50} 4.56 and LC_{90} 6.92 μ l/100 ml) and *Cx. quinquefasciatus* (LC_{50} 6.48 and LC_{90} 8.89 μ l/100 ml). In a preliminary field evaluation against *Cx. quinquefasciatus*, the formulation was effective at a reduced dosage of 90 ml/m² resulting >90% pupal mortality up to 9 days post-treatment. Field evaluations against other species of mosquitoes such as *An. stephensi* and *Ae. aegypti* are required for assessing its potential in controlling mosquitoes breeding in different types of habitats.

Pilot scale production and evaluation of a mosquitocidal product based on the lipopeptides of *Bacillus subtilis*

The secondary metabolites produced by a *Bacillus subtilis* subsp. *subtilis* (VCRC B471) were found to be effective on the various life stages of mosquitoes and more importantly, the pupal stages. Subsequent to the development of a cost effective production medium and up-scaling the production to pilot scale level, seven different types of aqueous formulations (I –VII) were prepared and evaluated against the pupal stages of *An. stephensi*. The LC_{50} values ranged from 2.12 to 12.7 μ l for different formulations. Formulation II was found to show the maximum pupicidal activity with an LC_{50} value of 2.12 μ l followed by formulation VII with an LC_{50} value of 2.6 μ l. As formulation II contained both spores and metabolite, while formulation I contained only metabolite, the latter was taken up for laboratory evaluation against important

mosquito vector species and the susceptibility pattern was found to be of the following order: *An. stephensi* < *Cx. quinquefasciatus* < *Ae. aegypti*. This formulation needs to be tested against different mosquito species under field conditions for its pupicidal efficacy.

Small and large-scale evaluation of Natular™ T30 and G30 formulations against immature of *Culex species* in polluted water habitats in India

Natular™ T30, a single layer tablet containing 8.33% spinosad and Natular™ G30, an extended release granule containing 2.5% spinosad were tested at four dosages of 25, 50, 100 and 150 mg (ai)/m² against *Cx. quinquefasciatus* in street drains and *Cx. tritaeniorhynchus* in abandoned wells through small-scale (phase II) and large-scale (phase III) field evaluations in Puducherry, India following WHO guidelines. In street drains, application of Natular G30 formulation produced $\geq 80\%$ control of *Culex* immature for one week period at 50 and 100 mg (ai)/m² and three weeks at 150 mg (ai)/m². The efficacy was three times greater at 150 mg (ai)/m² compared to 50 and 100 mg (ai)/m². In abandoned wells, the G30 formulation did not give effective control at the dosages of 25 and 50 mg (ai)/m². At 100 mg (ai)/m², though, the mean reduction of pupal density was >80% on days 3-14 post-treatment, the lower limits of 95% CI for the means were <80%. Only at 150 mg (ai)/m², the formulation yielded $\geq 80\%$ control of *Culex* immature for 3 weeks post-treatment period. Application of Natular T30 formulations at 25, 50, 100 and 150 mg (ai)/m² in street drains and abandoned wells, did not yield the desired level ($\geq 80\%$) of control of *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* immature.

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

Phase III testing and evaluation of polyester nets treated with a new LN treatment kit, ICON MAXX, (LN) in comparison with the conventionally treated nets using the same insecticide (lambda-cyhalothrin) (ITN) was continued in Koraput district, Odisha State, endemic for *Plasmodium*

falciparum malaria in India. Cone bioassays of *An. fluviatilis* on net (LNs) surfaces after 18 months of net distribution showed a 100% knockdown one hour post-exposure and a mortality of 100% after the holding period (24 hrs). At 24 months post-distribution of LNs, the knockdown and mortality of *An. stephensi* in the bioassays were 100 and 88.3%, respectively. One year after the net distribution, all ITNs were withdrawn from the households enrolled for the study and substituted with LNs. Therefore, the surveys conducted on 18th and 24th months of net distribution were related to LNs. After 18 months of distribution, 98.2% of the LNs (n=57) were available in the 30 households inspected, and there were holes in 17 (56.7%) out of the 30 nets randomly examined. After 24 months of distribution, 91.3% of the LNs (n=323) were physically present in the 180 households inspected, and out of the 180 LNs examined, 45 (25%) nets were found with holes of varying sizes.

The survey after 18 months of net distribution showed that in 93.3% of the holdings (n=30) people were using the nets regularly and at 24 months post-distribution the net regular use rate declined to 81.1% (n=180); occasional/ seasonal use of nets (1.6%-8.9%) were also observed. None of the household members who slept under the LNs reported any side effect. Free from mosquito bites and no malaria was the main perceived benefits reported by the net users. The study is in progress.

Transmission assessment survey in selected filariasis endemic districts

In response to the request from the Directorate of National Vector Borne Diseases, a joint exercise was carried out in two districts (north & south Goa) of Goa, Thiruvarur district in Tamil Nadu and Puducherry, Karaikal and Mahe regions in Puducherry to plan and implement Transmission Assessment Survey (TAS) for making decision on stopping further rounds of Mass Drug Administration. More than five rounds of MDA have been completed in all these districts. As the children enrolment in the schools was more than 75% in all the five selected evaluation units (EU,) school based survey was carried out to assess filarial antigenaemia in children (6-7 years) by ICT. The results of TAS showed that the number of antigen

positive children were below the critical cut-off required for stopping MDA In all the districts.

RAJENDRA MEMORIAL RESEARCH INSTITUTE OF MEDICAL SCIENCES, PATNA

EPIDEMIOLOGY

Serological screening of 1104 kala-azar endemic population (male 522, female 582) with rK39 strip test revealed 46 asymptomatic cases, of which 6 converted to symptomatic VL during follow-up. IL10, Hb% and neutrophil count were observed as possible markers for conversion of asymptomatic to symptomatic.

In continuation of the sentinel surveillance, altogether 2147 VL patients received treatment at the sentinel sites established in different districts (East Champaran 646, Muzaffarpur 612, Samastipur 366, Saran 523). Diagnosis with rk-39 (RDT) in about 99.81% confirms implementation of the national program guidelines. It was observed that about 57% VL patients were treated with miltefosine, followed by 27% with SAG, 10% with Combination therapy and about 6% were not treated at the site due to non availability of drugs and several other reasons thereof. Out of 948 women, 483 were in reproductive age group *i.e.* 16-45 years, and were treated with miltefosine as per the standard guidelines. During follow up of these females till six months for pregnancy and later on till child birth, no adverse event has been encountered as yet. About 98% of the total cases reported as new case is suggestive for either low relapse rate in these area or preference of private/higher level of treatment providers in case of relapse. Pharmacovigilance of miltefosine at peripheral level did not reveal any major side effects, other than known G.I. toxicity.

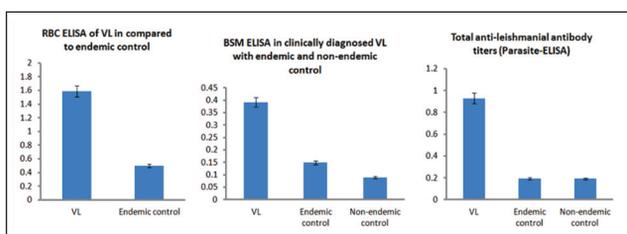
To assess the association of drug in progression of VL into PKDL development and time period of PKDL development after VL treatment, a study was initiated to follow-up all the VL cases (n=3647) treated at the Institute during the period 2007-2012. Till date, 54 patients of Muzaffarpur district were followed up, out of which 36 had been treated with Amphotericin B, 14 with Ambisome, 2 with Ampholyn, 1 with homeopathy medication and 1 with Amphotret. None of the followed up cases was

neither clinically suspected for PKDL nor had past history of PKDL after end of treatment for VL.

Diagnosics

Sensitivity and specificity evaluation of RDT kits being used in programme and other commercially available kits revealed that Dia Med's IT-LEISH has maximum sensitivity of 96.66%, followed by InBios KA-Detect kit (95.55%), Span Diagnostic's Signal KA and CTK Biotech's Onsite Leishmania Ab (93.33%) and Span Diagnostic's Crystal KA (90%). All the RDTs were found highly specific (100%) and no significant variability observed in thermo stability test at different temperature in any kits. Though, KA_Detect kit (InBios) and IT-LEISH (Dia Med) performed almost equally, the InBios Kit may be preferred due to simplicity and early test reading.

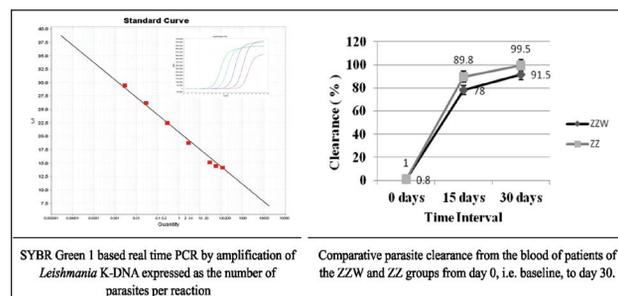
After confirming selective presence of 9-O-AcSA determinants on VL erythrocytes employing a 9-O-acetylated sialic acid binding lectin, Achatinin-H, the unique epitope has been identified on erythrocytes of VL patients, but absent in healthy individuals. A novel blood based antigen detection assay (RBC-ELISA), antibody based ELISA (BSM ELISA) and parasite ELISA for diagnosis and longitudinal follow up of VL patients have been standardized for screening a large population. Additionally, RBC-ELISA assay has prognostic potential that may be explored for early diagnosis of VL.



Therapeutics

Zinc supplemented Amphotericin B therapy (test arm) in zinc-deficient kala-azar patients was observed to be better than Amphotericin B alone (control arm) as initial cure in test arm was 100% as compared to 91% in control arm. In test arm, level of IFN- γ was considerably increased and IL-10 level was quite high at Day 1st which dropped steadily with treatment. Interestingly, TNF- α

level raised high at Day 14th but significantly dropped thereafter and almost reached to very low level at end of treatment. qPCR revealed earlier parasite clearance in Zn supplementation arm than Amphotericin B alone.



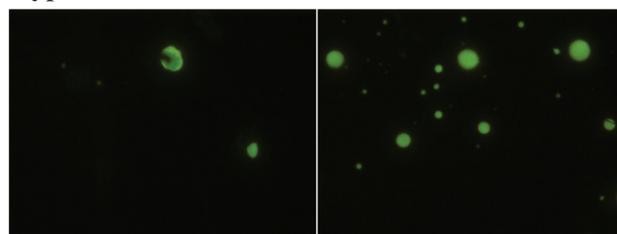
Being one of the centres of a multicentric DNDi sponsored combination therapies of ambisome, miltefosine and paromomycin, out of 22 enrolled patients (Male 15, Female 7), 4 were administered single-dose AmBisome (5mg/kg BW) IV infusion followed by oral Miltefosine 2.5 mg/kg/day for 7 days, 2 were given oral Miltefosine 2.5mg/kg/day + Paromomycin sulphate 11mg/kg/day IM for 10 days and 16 were given single dose Ambisome 10 mg/kg. One patient withdrew his consent during treatment. All patients achieved initial clinical cure. Further recruitment and follow-up of treated cases in progress.

Basic Research

After establishing direct correlation of anemia and parasite index with hypocholesterolemia, IFN- γ was found down regulated during hypocholesterolemic conditions in VL infection. The membrane fluidity study using DPH probe revealed disturbed red cell membrane integrity, might be due to down regulated G6PDH. Further, the observed role of HDL in plasma fluid mechanics causing anemia in VL infection is suggestive for good cholesterol diet in improving anemic condition and protection against VL infection.

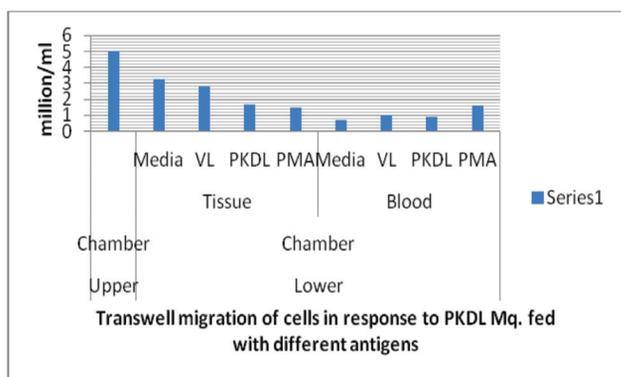
Hypocholesterolemic VL

Normal VL



Increased microalbuminuria in PKDL cases may be an early marker of subtle renal damage and glomerulonephritis. Pre-dominant MIP-1 α chemokine in all types of PKDL lesions with higher expression in macular lesions revealed its role in manifestation into different types of PKDL lesions.

The absolute number of circulating CD4+ T cell subpopulation and surface expression during active infection in PKDL cases was found markedly reduced in the dermal lesions as compared to healthy controls. The increased level of IL-10, IL-4 and TGF- β showed suppression of CMI. Activation and up-regulation of chemokine receptor particularly CCR3 and CCR4 was shown to be associated with PKDL. Further, it was observed that infiltration of CD4 cells more prominently decreases in macular lesions as compared to papular and nodular lesions. This pattern was also confirmed by *in vitro* transmembrane experiment. The migration of CD4+ cells was also lower in response to the PKDL parasites. However, no significant difference was observed in the response induced by PKDL and VL derived *L. donovani* parasite.



Bioinformatics

In-silico interaction between cytosolic TXN and TXNPx proteins was further confirmed *in vitro*. Further, mTXN interaction with mTXNPx was also observed through in-silico analysis. Up-regulated expression of cTXN in drug resistance *L. donovani* parasite in presence of 20 ng/ml of amphotericin B suggests its possible involvement in amphotericin B resistance.

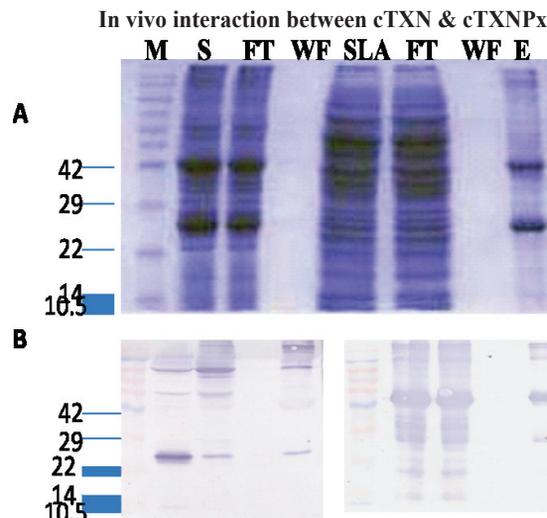
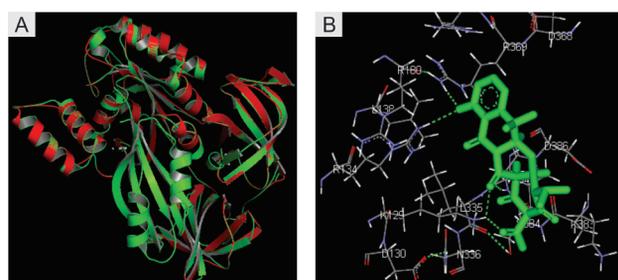


Fig A. - 12% SDSPAGE of cTXN and cTXNPx in vivo interaction
Fig B. - immunoblot analysis.

The constructed Elongation Factor-1 alpha (EF1- α) protein was superimposed with target protein (Fig. A). A hairpin of 12 amino acids, unique to the human EF1- α protein but missing in *Leishmania*, was modeled which may be explored to design novel, small molecule inhibitors that bind specifically to the region. Docking of EF1- α with various GTP analogues exhibited highest dock score (-10.8193) with Asp159. Protein-ligand interaction of EF1- α protein of *Ldv* with various tetracycline analogues showed the highest dock score 70.42 (Tetracycline n-hexylsulfamate) and involved amino acids are Arg 134, Arg180 and Arg369 (DS). Docking analysis with tetracycline analogues showed the highest dock score -27.9665 (Tetracycline n-hexylsulfamate) and involved residues are His15, Gly19, Ser21 and Asp91 (Fig. B). Thus, the active site architecture and certain key residues responsible for inhibitor binding of EF1- α were identified for designing novel inhibitors of leishmaniasis.



Physical encapsulation of amphotericin B, an antileishmanial drug, has been done with PAMAM dendrimer accomplished by thermo mixing of the polymer and drug solutions where the hydrophobic drug associates with the non polar core through hydrophobic interactions. Characterization of nano-particle by transmission electron microscope (TEM) revealed its size of 15-25nm and is uniformly distributed that may further be explored for its application in drug delivery system.

The downloaded genome sequences of all the leishmania species were screened to find out polymorphic repeats. Whole genome of a strain and SSR database was constructed to mine the SSR coordinates and conserved flanking sequences.

Operational Research

The host-preference analysis of *P. argentipes* revealed unequal preference for each host species during pre and post DDT spray ($P < 0.005$). During the post-DDT spray, bovine blood was found significantly preferred by vector in comparison to goat ($P < 0.003$) and mixed feeding ($P < 0.004$).

Out of 19 plants' extracts subjected to bio-assay test, 4 exhibited lethal effect to *P. argentipes*; 80% mortality in PK₄₈ leaves and roots (methanol extract), 87.5% in PK₈₁ leaves (methanol extract), 100% in PK₁ leaves (aqueous extract) and 80% in PK₉₀ root (hexane extract). Thin Layer Chromatography of Fraction 9 (methanol phase of PK₉₀ leaves, hexane extract) showed presence of a terpenoid, "Clerodin". HPLC analysis of 9th fraction of the extract of PK₉₀ showed presence of 9 compounds, of which 2 were identified as potentially active molecules for insecticidal effect.

In order to identify sustainable, cost effective and environmentally friendly alternative to DDT for vector control, three interventions *viz.* Indoor wall and floor plastered with lime (IWFPL), Durable wall lining (DWL) with deltamethrine (55 mg/m²) and KO TAB 123 Insecticide treated nets (ITN) were evaluated in altogether 1200 households of 4 highly endemic villages of kala-azar. DWL was found as the most efficient method for vector control in comparison to ITN and IWFPL. However, ITN, which was better than IWFPL, was the most acceptable method by study population.

An autoregressive integrated moving average (ARIMA) (1,1,1) analysis of the weather data in the study area revealed that mean temperature, sea level pressure, relative humidity and mean wind velocity play significant role in sand fly abundance at a lag of one month. However, the overall log likelihood ratio (X^2) of the predictive model showed significant result ($X^2 = -160.42$ $P < 0.0000$). The value of AIC of the predictive model is 328.84.

Kala-azar transmission risk map was generated based on index model considering the demographic characteristics such as population density, family size, non-working/unemployed population, illiteracy rate, agricultural density and nutritional density derived through census data and remote sensing data of the study sites. A pair-wise comparison matrix was built via Saaty's analytical hierarchy (AHP) process to differentiate sand fly abundance zone of the study district and the validated with the ground data to estimate its accuracy. The overall accuracy of the model in lean season was 63.33% while it was 68.63% in the peak season.

Others

Altogether, 59 cases of anophthalmia and or microphthalmia (including 35 reported cases) from different districts of Bihar *viz.* Bohpur, Buxar, Rohtas and Patna were identified; maximum (78%) from Bhojpur. All the cases and almost equal representation of controls ($n=61$) from each district were studied. 95% of cases had 38-42 week gestational age (On term birth). Fever and night-blindness was found as highly significant ($P < 0.001$) risk factor for anophthalmia/ microphthalmia. The percentage of deficiency in vitamin B-12, Retinol, α -tocopherol, Copper, and Zinc was higher in cases than controls. Further, positivity % of T IgG, T IgM, R IgG, R IgM, CMV IgG, CMV IgM, HSV1 IgG, HSV1 IgM, HSV2 IgG and HSV2 IgM was higher in control than cases except IgM R (Rubella). All the mothers of cases and controls were aged < 40 years at the time of child's birth.

In ICTC centre, out of 2337 [male 1391, female 942 and transgender (TG) 4] individuals tested for HIV, 547 (23.4%) were found positive for HIV.

Significant weather predictor variables for sand fly abundance				
Variables	Coefficient	Standard error	p> z	95% Confidence interval
Constant	-379.17	83.64	0.000	-543.35 – 214.99
Mean Temperature (°C)	0.85	0.09	0.000	0.68 – 1.02
Sea level pressure (mb)	0.35	0.08	0.000	0.19 – 0.51
Relative humidity (%)	0.16	0.02	0.000	0.12 – 0.20
Mean wind velocity (km/h)	-0.34	0.096	0.000	-0.53 - -0.15

181 cases of HIV/TB co-infection was found. The number of patients put on ART reached to 499.

In MDR/XDR TB Lab, out of 773 sputum samples of pulmonary tuberculosis (PTB) patients referred from TBD Centres of all the 38 districts of Bihar (300), RMRI OPD (444) and RMRI ART centre (29), altogether 34% were found AFB positive. About 37% of the samples referred from TBD centres were also found positive for *M. tuberculosis* by solid culture in Lowenstein-Jensen Medium.

The virology laboratory provides diagnostic support for several viral diseases viz. diarrhoea, acute encephalitis syndrome (AES), influenza, enterovirus, swine flu, HZV, herpes simplex I and II. A total of 1098 blood, sera, throat swab and stool samples were collected from different districts of Bihar and analyzed for different viral diseases. In the year 2013, this laboratory provided diagnostic services during various disease outbreaks in Bihar such as Diarrhoea outbreak in Patna, AES in Muzaffarpur and Dengue in Rohtas.

ENTEROVIRUS RESEARCH CENTRE, MUMBAI

Poliomyelitis Surveillance

Wild poliovirus transmission in India and neighbouring countries like Bangladesh, Srilanka and Myanmar is detected by testing stool samples from Acute Flaccid Paralysis (AFP) cases at Enterovirus Research Centre, Mumbai. In 2013, a total of 8102 stool specimens were collected from 4038 AFP cases reported in Maharashtra, MP, Goa and Chhattisgarh and tested until the end of November 2013, whereas in 2012 we received stool samples from 2897 AFP cases from

Maharashtra only. The samples were tested for the presence of wild and vaccine derived polioviruses. Polioviruses isolated in India, Bangladesh, Srilanka and Myanmar were also received at the Centre for maintaining poliovirus bank, reconfirmation of the results and further characterization. A total of 4572 poliovirus isolates were received until November 2013. Wild poliovirus has not been isolated in India in 2013 till date. India has remained free from wild poliovirus since January 2011.

Year	Total AFP cases tested	Polioviruses positive cases	NPEV cases
2012	2897	90 (3.1%)	783 (27.02%)
2013	4038	95 (2.47%)	1313 (32.50%)

Detection of vaccine derived polioviruses (VDPV)

The vaccine-derived poliovirus (VDPVs) are viruses from the oral polio vaccine (OPV) which regain wild-type transmissibility and pathogenicity resulting in large outbreaks. A total of 162 discordant isolates were molecularly characterized by complete VP1 sequencing. Type 2 VDPVs were isolated from 5 AFP cases during the period of January to November 2013, out of which 2 cases were from Maharashtra and 1 each from Bihar, Orissa and Delhi. Two of these cases (1 each from Maharashtra and Delhi) were identified as iVDPVs (immunodeficient VDPV).

Supplementary surveillance for detection of wild poliovirus

Supplementary surveillance was established at the centre in the year 2001 for detecting presence of wild poliovirus in Mumbai. The surveillance was

later expanded to Delhi, Kolkata and Patna in the year 2010 and 2011. In 2013, surveillance was further expanded in two additional states - Punjab and Ahmedabad. No wild poliovirus could be detected from 132 sewage samples collected from the three high risk blocks in Mumbai and 82 samples from Patna. Two poliovirus type 2 VDPVs were detected in sewage samples, one each in Mumbai and Patna. In addition, one poliovirus type 3 VDPV was detected in Patna. The results indicate absence of wild poliovirus circulation in India.

Real time reverse transcription PCR assay for detection of wild type 1 poliovirus

Real time RT-PCR assay for detection of wild poliovirus type 3 has already been developed at the centre. The Centre has developed a Real Time Reverse Transcription PCR assay specific for wild poliovirus type 1 (WPV1) using wild polioviruses isolated in India. The assay detected WPV1 in the presence of large excess of Sabin 1, 2 and 3 and non-polio Enteroviruses (Fig.1).

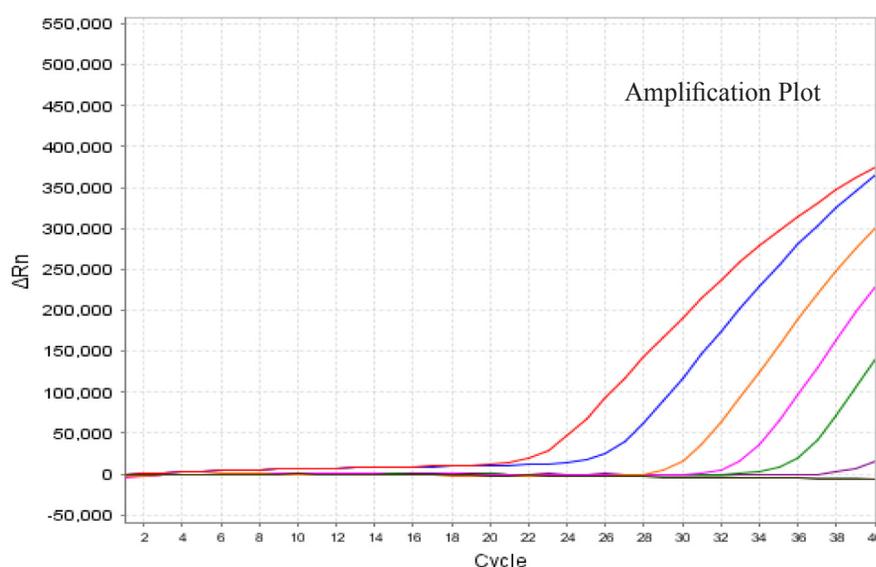
Enterovirus surveillance in healthy children in eastern UP, Gorakhpur

The prevalence of human enteroviruses (HEV) infections was studied in healthy children in JE/AES infested areas of Gorakhpur, UP during September and October, 2013. Stool samples collected from 994 children below 60 months were screened for

HEV using viral culture method. HEV serotypes were identified by complete VP1 gene sequence analysis. HEV-positivity was observed among 51% (508/994) of the children out of which 68% (675/994) were below 3 years of age suggesting high susceptibility of young children to HEV infection. An extensive circulation of high degree of Enterovirus diversity belonging to as many as 51 different serotypes was observed. HEV-B was present in 51% of the samples followed by HEV-C and HEV-A in 28 and 21% samples respectively. HEV-D viruses were not detected. The most commonly detected serotypes included CVA4, EV99, CVB1, E1, E24, E33, CVA13, CVA17 and CVA20. Newly identified HEV serotypes (EV76, EV89, EV90, EV91, EV99, EV101, EV102, EV107) were also detected.

Virological investigations of hand foot and mouth disease (HFMD) and herpangina (HA)

The Hand foot-mouth disease (HFMD) and herpangina (HA) are closely related but clinically distinct childhood infections caused by human enteroviruses (HEV). The HA is caused by multiple serotypes of coxsackieviruses including CVA2, CVA4-6, CVA8-10, CVA24, and EV71 where as HFMD is mainly caused by EV71 and CVA16. However in recent years CVA6 has also been reported to cause HFMD outbreaks worldwide.



Titre of undiluted WPV1 stock : 10^{5.4}/μl

Fig. 1. Sensitivity of WPV1 specific rRTPCR assay.

The HFMD and HA outbreaks occur simultaneously between May to November every year in Thane, Maharashtra. The outbreaks have been clinically, epidemiologically and virologically studied by virus culture and molecular diagnostic methods to identify the etiological agents. Multiple HEV serotypes were isolated from clinical samples of HA cases. CVA4 and CVA10 were the most predominant serotypes isolated during HA outbreaks in 2011 and 2013 where as CVA2 and CVA5 were the main serotypes causing HA in 2012. HFMD was mainly caused by CVA16 in 2010 and 2013 where as CVA6 caused HFMD outbreaks in 2009, 2011 and 2012 in Thane. The study suggested cyclic pattern of HA and HFMD outbreaks caused by different EV serotypes.

Outbreak of acute gastroenteritis due to norovirus in south Mumbai

During June-July, 2013 an outbreak of acute gastroenteritis was observed in children from high socio economic families (12 years of age or younger, median age = 18 months) in a paediatric clinic in south Mumbai. Stool samples from a total of 50 children were collected and tested for the presence of rotavirus (RV), norovirus (NoV), astrovirus (AsV) by RT-PCR. Human enteroviruses (HEV) were detected by isolation in RD cells and identification by RT-PCR and partial VP1 sequencing. Viral etiology was observed among 46% cases. A total of 42% cases tested positive for NoVs affecting mostly young children less than 1 year (43%). RV was identified from only one case (4.7%) and HEV from 4 cases (19%). AsV

was not identified from any case. NoV comprised genogroup GI 47%, GII 38% and GI +GII 14%. There were no hospitalizations and the outbreak subsided by August. The study highlighted that NoV genogroup GI and GII were responsible for causing outbreak in the young children from high socio economic families.

Host virus interaction and significance of apoptosis in infections by viruses causing hand-foot and mouth disease (HFMD)

Coxsackievirus A16 (CVA16) and Enterovirus 71 (EV71) are the major etiological agents of HFMD. CVA16 and CVA6 are the major pathogens of HFMD in India. Although genetically close to each other EV71 leads to severe fatal neurological complications but not CVA16 and CVA6. Mostly these viruses cause asymptomatic infection with blisters on hand, foot and mouth and do not cause AFP.

The Enterovirus Research Centre (ERC) studied the host pathogen interaction of CVA16 and CVA6 isolated at the Centre and compared that with EV71 prototype strain (EV71 BrCr). The mechanism of cell tropism, inflammation and apoptosis by the above viruses were studied by infecting human RD (Rhabdomyosarcoma) or SK-N-SH (Neuroblastoma) or Vero (African green monkey kidney cells) or HEp2 (Larynx carcinoma) cell lines. The extent of virus replication, presence of viral antigen and quantification of viral RNA were determined by single-step growth curve, confocal microscopy and real-time PCR.

Table 1: Enterovirus etiologies of HA cases in Thane (2011-2013)

EV serotypes	2011	2012	2013
Most Predominant	CVA10 (43.3%) CVA4 (28.3%)	CVA2 (51%) CVA5 (26.4%)	CVA10 (46.4%) CVA4 (35.7%)
Less Predominant	CVA2, CVA6, CVA8, E12, CVB4, CVA21 (28%)	CVA10, CVA6, CVA4, CVA8, CVB3, CVB4 (22.6%)	CVA8, CVA16, E6 (17.8%)

Table 2: Enterovirus etiologies of HFMD cases in Thane (2009-2013)

EV serotypes	2009	2010	2011	2012	2013
Most Predominant	CVA6 (61%)	CVA16 (64.3%)	CVA6 (41%)	CVA6 (40%)	CVA16 (96%)
Less Predominant	CVA16 (3%)	CVA6, CVA8 (2.8%)	EV71 (4.5%)	CVA2, EV71 (6%)	E6 (4%)

The EV71 replicates well in all the three cell lines, CVA16 replicates in RD and vero cell lines but shows delayed growth in neuronal cells which is detectable after first cycle of replication (Fig. 2). CVA6 replicates only in RD cell line and failed to grow in Vero and SK-N-SH cell lines. None of the viruses grew in HEp2 cells. Confocal microscopy study to detect the viral antigen corroborated the data where in EV71, CVA16 and CVA6 viral antigens could be seen in RD cells at 8 hpi (Fig. 3). EV71 and CVA16 antigens were observed in SK-N-SH and Vero cells also at the same time point

but not CVA6, corroborating the single step growth curve data. Although none of the viruses grew in HEp2 cells, slight patches of antigen could be seen for all three viruses at 8 hpi. Quantification of viral RNA by real time PCR showed presence of EV71 and CVA16 viral RNA in all the four cell lines at 8 hpi. CVA6 RNA could not be detected in SK-N-SH, Vero and HEp2 cell lines confirming that CVA6 viruses do not grow in SK-N-SH, HEp2 and Vero cells. The findings will provide insight into the nature of virulence and pathogenesis of CVA16 and CVA6 infection as compared to EV71.

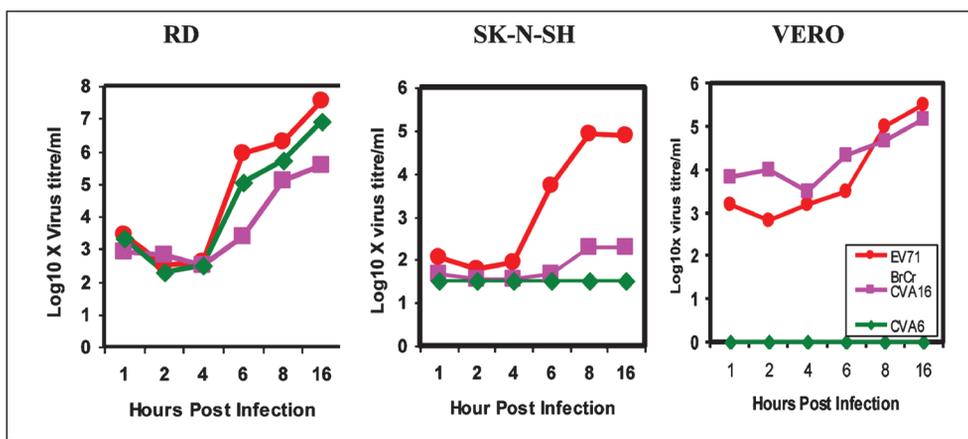


Fig. 2. Single step growth curve of EV71 BrCr (red lines), CVA16 (pink lines) and CVA6 (greens lines) in human muscle, neuronal and kidney epithelial cell lines.

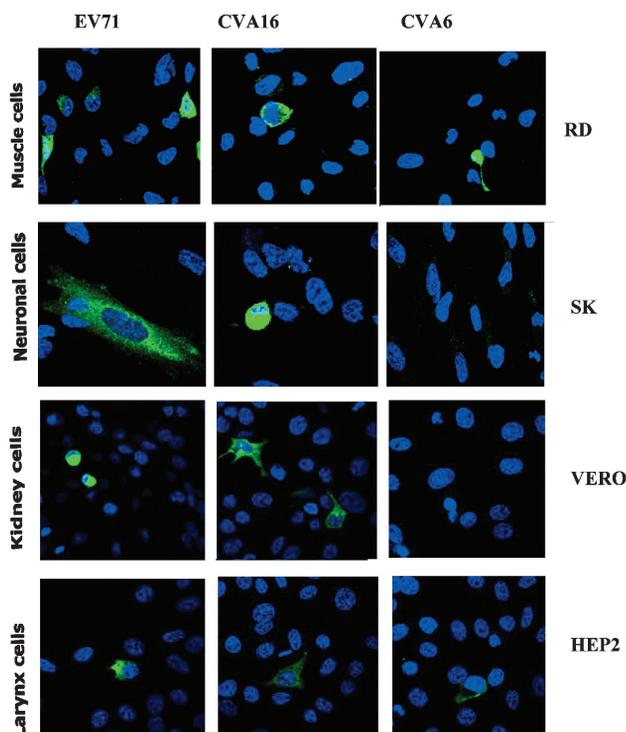


Fig. 3. Confocal microscopy of human cell lines infected with HFMD causing viruses at 8 hours post infection (8 hpi).

NATIONAL INSTITUTE OF VIROLOGY, PUNE

During the year the National Institute of Virology (NIV) focused its research on Crimean Congo hemorrhagic fever (CCHF) and Kysanur forest disease virus (KFD).

Crimean Congo hemorrhagic fever (CCHF)

Following the outbreak of CCHF in Gujarat, epidemiological studies and surveillance were conducted. These included : (a) Serosurvey of Crimean Congo hemorrhagic fever virus in domestic animals, Gujarat; (b) Outbreak investigation of Crimean-Congo hemorrhagic fever in the Amreli district of Gujarat State (3) Nationwide serosurvey of Crimean Congo hemorrhagic fever virus in domestic animals - which is ongoing.

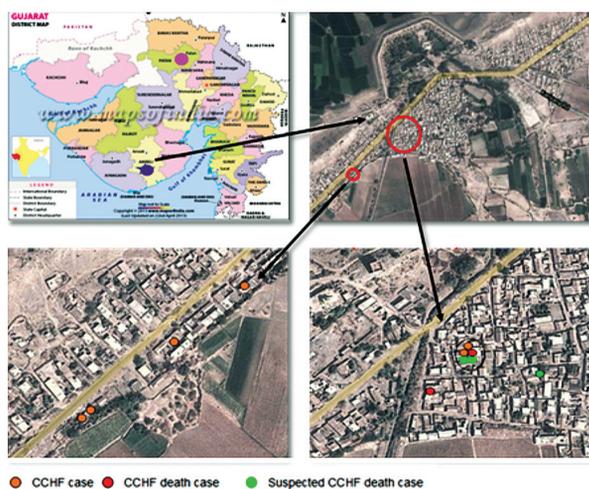


Fig. 1. Geographical location of CCHF cases in Karyana village, Amreli district, Gujarat.

Investigation of Kysanur forest disease (KFD) was carried out by testing referred samples of Thirthahalli, Shimoga district of Karnataka State.

- The following technologies are available with the ICMR for commercialization:
 - a) An ELISA based diagnostic kit for the detection of CCHF IgG antibodies in sheep, goat and bovine population,
 - b) An IgM assay for the detection of CCHF in humans
 - c) An ELISA based KFD antibody IgM detection assay and
 - d) A diagnostic assay for detection of Hepatitis E virus.

- e) An IgY based anti-rotavirus therapeutic technology was transferred to industry for commercialization.

The BSL-4 laboratory commissioned in 2013 and at par with international operational standards is currently fully operational. A total of 198 human samples were processed by real time PCR for CCHF from Gujarat and 19 were found to be positive. The 166 domestic animal samples tested by anti-CCHF IgG-ELISA, 55 were found to be positive for anti-CCHF IgG antibodies from Amreli (45/137) and Ahmedabad (10/29). All the 134 samples tested by real-time RT-PCR were found to be negative. Out of 192 tick samples processed by real-time RT-PCR, 5 tick pools belonging to Ahmedabad (1/5) and Amreli (4/5) were found to be positive. Out of all inoculated tick samples for isolation of the virus, two samples showed distinct cytopathic effect. They were passaged in Vero cells for three passages and then the tissue culture fluid was inoculated in suckling mice. The mice did not show any sickness or death. Both the virus isolates were tested using genus specific RT-PCR for genus *Flavivirus*, *Orthobunyavirus*, *Nairovirus*, *Phlebovirus* and showed no positive amplification. Both the virus isolates were inoculated in adult Swiss albino mice for raising immune serum. Further characterization is in progress.

From all the fifteen districts of the Gujarat State, anti-CCHFV IgG antibodies were detected in bovine, sheep and goat with positivity of 12.09, 41.21 and 33.62% respectively which confirms the widespread seroprevalence of CCHF in domestic animals in the State. Out of 1226 animal samples tested total 278 (22.67%) had IgG antibodies against CCHFV. Highest IgG antibodies positivity specific to CCHFV was observed in sheep (41.21%) followed by goat (33.62%) and bovine (12.09%). District-wise highest seropositivity was recorded in Porabandar (52.5), Junagadh (50%), Surendranagar (50%) followed by Amreli (33.82), Jamnagar (30%), Kutch (25.7 %), Ahmadabad (24.21 %), Mehasana (22.5%), Rajkot (20%), Patan (11.25%). While, low seroprevalence levels were detected in Gandhinagar (8.75%), Banaskantha (7.5%), Kheda (7.5 %), Anand (6.25%) and Sabarkantha (6.25%) respectively. This necessitates the surveillance and continuous monitoring of CCHF IgG antibodies in animals and hemorrhagic fever cases in human.

The overall prevalence of antibodies in all the 15 districts surveyed suggests that the domestic animals play a central role in the maintenance of the disease cycle and serve as host for both the virus and the tick vector. The data also emphasizes that domestic animal trading could have a crucial role in the spread of this disease. There is need of laboratory screening of all suspected severe human hemorrhagic fever cases for CCHV. The spread of this disease in Gujarat State should be taken seriously from public health point of view since it indicates possible existence of this disease elsewhere in India.

KYASANUR FOREST DISEASE (KFD)

During the year KFD virus positivity was recorded in Chamarajnagar district of Karnataka and Nilgiri district of Tamil Nadu which borders Wayanad district of Kerala. The serum sample of an 18 year old Male from Wayanad presented with fever and thrombocytopenia was referred to NIV, Pune for diagnosis. He was not responding to antibiotics. History of monkey death in the neighborhood had been noted. The referred sample was negative for Dengue IgM / NS1Ag, Leptospira IgM and Scrub typhus IgM. Serum sample was tested for KFD viral RNA using real-time RT-PCR and RT-PCR assay and were found to be positive for KFD virus. Presence of KFDV was never detected previously in monkeys, ticks and humans in this area *viz.* Alegowdanakatte forest area Anti-poaching camp, beside Kozhikode-Mysore-Kollegal highway, Chamarajanagar district. The Gundulpettaluk is connected with Mysore, Chamarajanagar, Kerala and Tamil Nadu State. It was important to note the positivity of monkey specimen from Nilgiri area, which is bordering Tamil Nadu State and adjacent to Karnataka State. This confirmed the spread of KFD virus in newer areas of adjoining states of Karnataka

Study on *Phlebovirus* (Malsoor virus) from wild *Rossetus* bats

A novel *Phlebovirus* (Malsoor virus) was isolated and characterized from wild *Rossetus* bats. This virus is found to be closely related to highly pathogenic human STFS and Heartland viruses and its possible role in human infections is being studied.

Diagnostic Virus Laboratory Resource Centre

The Diagnostic Virus Laboratory Resource Centre established and became operational in 2013 has completed first phase of training program for network diagnostic laboratories.

Virus Repository

Restructuring of the Virus repository has been completed to make it at par with international protocols for biosafety and biosecurity standards. This is a part of the NIV Biobanking program. Viruses have been given to various researchers in the country.

Outbreak investigations

NIV scientists participated in outbreak investigations at different parts of the country both independently as well as part of central teams constituted by the Health Ministry, Government of India. Major investigations include: (i) Dengue in Thiruvananthapuram and Kollam districts, Kerala; (ii) Acute encephalitis syndrome in Muzaffarpur, Bihar; (iii) Crimean Congo hemorrhagic fever in Amreli district, Gujarat in 2013; (iv) Scrub Typhus without Hantavirus evidence in Thiruvananthapuram, Kerala in 2013; (v) Hepatitis E in Pombardi village Bhor, Pune, Maharashtra in 2013; (vi) Hepatitis A in an Orphanage in Pune city, Maharashtra and (vii) Chicken pox among college students in Pune city, Maharashtra.

Surveillance for Influenza viruses

Surveillance for Influenza viruses continued through the multicentric project (Multisite epidemiological and virological monitoring of human influenza, Surveillance Network in India, Phase II). Also, community-based surveillance of viral diseases was undertaken in Janata Vasahat in Pune city, Maharashtra and in rural settings in Vadu, Maharashtra. Detailed operational preparedness for tackling potential threats from H7N9 and MERS were developed and being readied. Training was also imparted to personnel from various national and state level laboratories.

Significant contribution from the Influenza group included the new case definitions for severe acute respiratory illness (SARI) by WHO. The seasonality studies in Indian and SE Asia warranted the use

Enterovirus Research Center, Mumbai through development of documentation and field visit audits of key high risk laboratories for Laboratory Containment of Wild Poliovirus program.

Recognition as National Measles Referral Laboratory

The NIV has received WHO recognition as a national measles referral laboratory. The Measles Group has also developed a rapid and reliable assay for detection of measles, mumps and rubella neutralizing antibody.

Diagnostic Reagent Facility

The Diagnostic Reagent Facility of NIV has contributed towards supplying diagnostic kits to various laboratories across India. A total of 5049 kits were supplied in 2013-14 for detection of Japanese encephalitis, Dengue and Chikungunya viruses.

Basic Research

Basic research in multidisciplinary areas included pathogenesis and replication of HEV, pathogenesis of Avian Influenza and mutation based studies to investigate the NTPase activity of the helicase domain in hepatitis E virus replicon.

Investigation of Hepatitis A

Hepatitis A (HA) amongst children in an orphanage in Pune was investigated in August 2013. Of the 76 serum samples 19 were anti-HAV IgM positive while 20/76 stool samples were HAV RNA positive confirming Hepatitis A to be the etiological agent.

Immunogenetic studies in dengue

The Immunogenetic studies in dengue revealed that the T allele of rs3775291 may be associated with decreased risk of DHF. The R/R genotype of *FCGR2A* R131H and G/G genotype of *CCL2* -2518 polymorphisms were associated with thrombocytopenia in dengue infections.

Bioinformatics

Under the Bioinformatics the phylogenetic studies carried out for arboviruses. Molecular docking and dynamics studies helped understand the molecular basis of enhanced viral fitness induced by secondary (compensatory) mutations in hemagglutinin (HA)

protein of oseltamivir resistant post 2007-08 seasonal influenza H1N1 strains. Mathematical modeling studies on transmission of viral epidemics has been initiated.

Activities of Regional Centres

The NIV Bangalore Unit has contributed to diagnosis of Dengue, Chikungunya and Japanese Encephalitis and surveillance of Acute Flaccid Paralysis (AFP) cases from southern states. Major contributions include: (i) investigations on Hand, foot and mouth disease (HFMD) in Bangalore, (ii) Measles at PHC- Chitradurga district, Karnataka, and (iii) Foundation stone for the New Campus of NIV Bangalore Unit has been laid.

The NIV Gorakhpur Unit has contributed in diagnostic services for encephalitis and AES.

The NIV Kerala Unit has contributed to routine diagnostic services related to arboviruses.



Foundation stone laying ceremony for NIV Bangalore Unit.



First batch of trainees and dignitaries after inauguration of Diagnostic Virology Laboratory at MCC campus, Pune, a DHR initiative.

CENTRE FOR RESEARCH IN MEDICAL ENTOMOLOGY, MADURAI

JAPANESE ENCEPHALITIS

JE virus activity in Cuddalore district, Tamil Nadu

To monitor the vector abundance and JE virus activity in the endemic villages of Cuddalore district, Tamil Nadu, mosquitoes were collected during dusk hours from Kodikkalam (Sirumangalam PHC), Eraiyur (Sirumangalam PHC) & SS. Puram (Pennadam PHC) of Cuddalore district from April 2013 to March 2014. The overall vector abundance had increased (PMH 117.38) during the compared to last year (PMH 63.80). A total of 15,494 mosquitoes belonging to 18 species and six genera were sampled utilizing 132 man-hours. Among the culicine mosquitoes collected, *Cx. tritaeniorhynchus* was the dominant species (PMH 85.14), followed by *Cx. gelidus* (23.33) and *Cx. vishnui* (1.30). When dusk sampling was made, the Dusk Index (DI) of *Cx. vishnui* subgroup reached the peak in December 2013 (73.08), along with that of *Cx. tritaeniorhynchus* (75.40). When the density was compared between *Cx. tritaeniorhynchus* and *Cx. gelidus* it was found to be four-fold high than in the preceding year (19:85).

Among the 292 pools, 194 pools of *Cx. tritaeniorhynchus* (9,155 specimens), 21 pools of *Cx. vishnui* (381 specimen), 64 pools of *Cx. gelidus* (2,512 specimen), 5 pools of *Cx. fuscocephala* (31 specimens), 2 pools of *Cx. quinquefasciatus* (12 specimens), 1 pool of *Ma. annulifera* (5 specimens), 3 pools of *An. subpictus* (41 specimens) and 2 pools of *An. peditaeniatus* (21 specimens) were subjected to virus assaying. Of these 5 pools were found positive during 2013, viz. 2 pools from *Cx. tritaeniorhynchus* and 3 pools of *Cx. gelidus*.

JE virus transmission dynamics in Tirunelveli district, Tamil Nadu

Cx. tritaeniorhynchus was the dominant species in all the study villages. JE virus was detected from ten species comprising 28 positive pools,

viz., *Cx. tritaeniorhynchus* (10), *An. subpictus* (7), *Cx. infula* (2), *Mansonia annulifera* (2), *Ma. uniformis* (2), *Cx. bitaeniorhynchus* (1), *Cx. quinquefasciatus* (1), *An. pallidus* (1) *An. barbirostris* (1) and *Armegeeres subalbatus* (1). JEV infection was high in Ariyanayagipuram (13), followed by Senthimangalam (8), Kuthalaperi (4) and Magiladi (3). Peak JEV infection was noticed in the month of September (7) followed by April (6), January (5), November (4), December (3), July (2) and June (1). The % positive of school children for JE HI antibodies were found higher in the post-monsoon season.

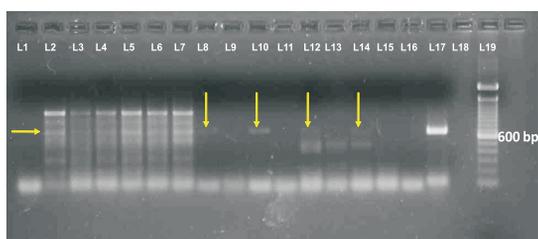
Development of RS-GIS based Model to Forecast JE Vector Abundance and Transmission risk

The study was carried out jointly by CRME and VCRC, in JE endemic areas in two districts, viz., Cuddalore in Tamil Nadu and Bellary in Karnataka, respectively. Three villages, one each from three different PHCs in each district were selected, where paddy cultivation is in practice. Vector abundance (immature density and adult density) and paddy growth stages were monitored for a period of 30 months. Satellite data (RISAT-1) corresponding to the different stages of paddy growth was obtained from National Remote Sensing Centre, Hyderabad, using which the backscatter coefficient (σ^0) was derived. The abundance of JE vector, *Cx. tritaeniorhynchus* peaked when the paddy was at its heading stage and dipped when the crop reached the maturing stage. A significant correlation was observed between paddy growth and adult vector density in the study sites (Cuddalore: $r = 77$, $P < 0.003$).

Research cum intervention on JE/AES in Gorakhpur

Five blocks in Gorakhpur district, eastern Uttar Pradesh have been selected for the study viz., Campierganj, Chargaawan, Bhathat, Khorabar and Belghat where intervention for vector control is to be implemented and one block viz., Majhgawa in Deoria district as control for comparison. In each block, five villages have been selected for collecting baseline information on vector bionomics.

Results



L1, – Negative control
 L2 to L7 – RNA of lab. Infected mosquitoes (dilutions of 1:40, 1:80, 1:120, 1:160, 1:200, 1:240).
 L8, L10 – GKP89 ([ELISA (+)ve; *Cx. vishnui*] [dilutions of 1:60, 1:100])
 L12 – GKP207 (*Cx. quinquefasciatus*)
 L14 – GKP349 (*Cx. fuscocephala*)
 L17 – Positive control (VCRC, Puducherry)

Plate. 1.

The per man-hour density of the JE vector *Cx. tritaeniorhynchus* showed an increase from July/August, reaching peak densities (35.8 to 42.9) during the months of September, October and November in all the blocks, *i.e.*, Khorabar, Bhatahut and Chargawan, being maximum in Chargawan and lowest in Khorabar blocks during and following monsoon months (Fig. 1).

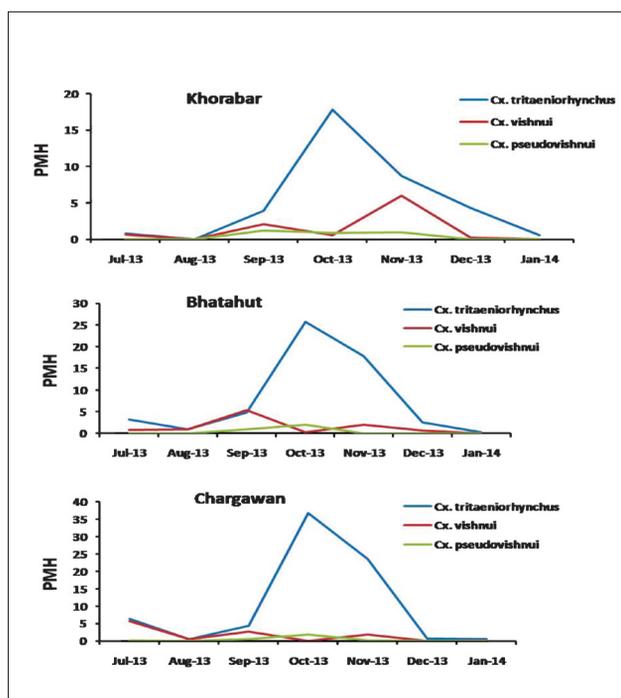


Fig. 1. Monthly change in per man hour density DC.

The Blood meal index for humans, bovine and pig samples were 0.005, 0.942 and 0.003 respectively. Blood meal analysis has shown that the JE vector *Cx. tritaeniorhynchus* predominantly feeds on cattle. For JE virus detection in mosquitoes, altogether 1160 pools were made from different

vector mosquitoes sampled during dusk collections from three blocks of Gorakhpur. Of the 157 pools tested so far, 15 were positive for JEV (12 by ELISA & 3 by RT-PCR).

LYMPHATIC FILARIASIS

Impact of vector control for augmentation of MDA to eliminate lymphatic filariasis in south India

An operational study was undertaken in the lymphatic filariasis endemic Tirukoilur taluk, Villupuram district, Tamil Nadu to assess the effectiveness of MDA done in eliminating lymphatic filariasis (LF). Mass blood survey was carried out in 10% of the population of each village in the 3 PHCs namely Ariyur, Edaiyur and T. Kunnathur. Based on the Mf prevalence and socio-ecological characteristics, thirty six villages (out of 100 villages) were selected as index villages, in order to implement vector control and to carry out longitudinal monitoring of vector and human infection parameters. These villages were grouped into three strategies namely MDA alone villages, MDA+EPS villages and MDA+EPS+PIC villages and each group comprised 12 villages. MDA was implemented through the local state health department in all the 36 index villages. MDA alone villages were considered as control village where mass drug administration alone implemented without any Vector Control (VC) strategies.

A total of 24 villages were selected for VC. In 12 villages (MDA+EPS arm), the cesspits and unused wells were modified /cleaned and applied with expanded polystyrene beads. In the other 12 villages (MDA+EPS+PIC) deltamethrin impregnated curtains were fixed in all the households of the 12 villages in addition to the application of expanded polystyrene beads in modified cesspits and larvivorous fishes (*Gambusia affinis*) in unused wells, as an integrated vector control strategy. The implementations of vector control in the 24 VC villages were carried out by the village community members with the active supervision of selected village volunteers and local public health personnel. The villagers were mobilized with various health education campaigns to motivate them to actively participate in the VC programme.

A new concept, *VCCom* (Vector Control through Community) was enunciated by forming an *Elephant Brigade*, defined as group of students motivated to spread the message of LF elimination by vector control through community participation in 24 VC villages. *VCCom* is the movement to eliminate LF, the *Elephant Brigade* is a tool to implement *VCCom*. With the support of community members, volunteers, local administrators and local health staff vector control strategies implemented effectively.

Impact of lymphatic filariasis vector control

During the period microfilaraemia and antigenaemia survey was carried out in 36 villages. Antigenaemia was screened using ICT cards. Overall AGP was 8.27% in three arms. In the final year of the project, *i.e.* in 2013 (one year post MDA 2012), 5564 persons were screened in the 3 arms. Overall MFP was 0.58%. The reductions as compared to the pre-VC were 84, 75 and 90% in the 3 respective arms. A total of 1164 children (2-10 years old) screened for antigenaemia by ICT test kit (BINAX NOW Filariasis ICT cards Lot No: 0588248; Expiry: January 2014). AGP was 2.53% in MDA alone, 1.43% in MDA+EPS and 1.91% in MDA+EPS+PIC arm. The AGP reductions were 70, 89 and 79% in the three arms (Figs. 2 and 3).

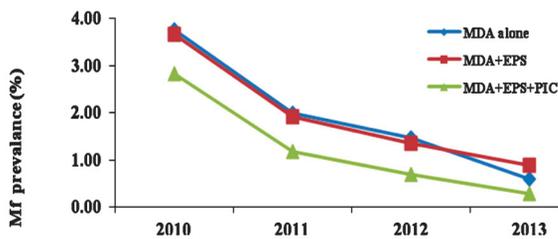


Fig. 2. Microfilaraemia prevalence in the 36 selected villages (All age groups)

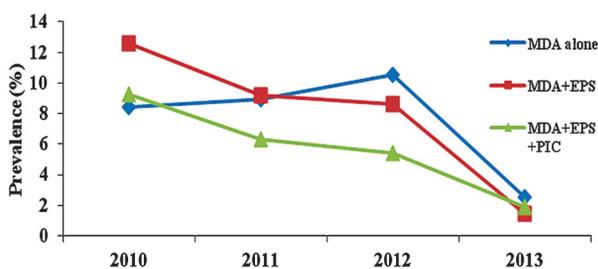


Fig. 3. Antigenaemia prevalence in the 36 selected villages (2-10 years age group)

The impact of vector control on entomological indices was carried out in all 36 index villages by collecting adult mosquitoes resting in human dwellings. The adult mosquitoes were identified laboratory. The female *Cx. quinquefasciatus* was dissected for determining the infection and infectivity status. The parity of the mosquitoes dissected was also determined.

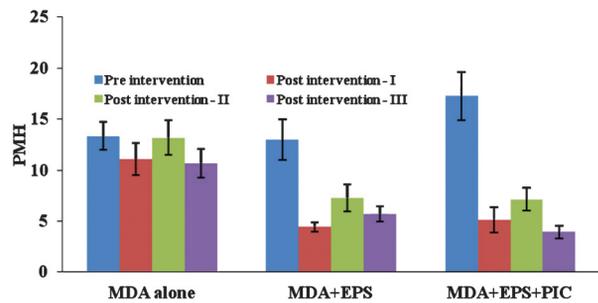


Fig. 4. Impact of vector control of the density of *Culex quinquefasciatus* in three strategy arms

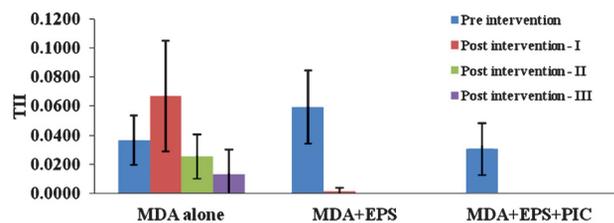


Fig. 5. Impact of vector control on Transmission intensity index in three strategy arms

During the year 2013, PMH density during post-VC III was 10.68, 5.17 and 3.95 in the three respective arms (Fig. 4). Significant reduction ($P < 0.05$) of PMH density was found in both VC arms, *viz.* 56% in MDA+EPS and 77% in MDA+EPS+PIC arm. Few infective vector mosquitoes were collected from MDA alone villages and the infectivity rate was 0.02%. In both the VC arms, there was nil infectivity. The TII was 0.0132 in MDA alone arm and nil in both the VC arms (Fig. 5). The reduction in transmission was 64% in MDA alone arm and 100% in the two VC arms.

The parasitological, entomological and sociological parameters have shown that the community based vector control programmes may reduce mf load and transmission intensity indices.

DENGUE

The burden and economic cost of dengue in India: A study at major teaching hospitals and nearby ambulatory institutions

A multi-centric national level study was conducted to understand the true burden of dengue infection and to analyse its impact in terms of economic cost of dengue illness in India. For this study, the country was organized into five regions based on geography and socio-cultural diversity, *i.e.*, North, South, East, West and Central India. There are two major teaching hospitals from each region, a totally ten hospitals were selected. Between 2006 and 2012 India reported an annual average of 20,474 dengue cases. Although dengue has been notifiable since 1996, regional comparisons suggest that reported numbers substantially underrepresent the full impact of the disease. Adjustment for underreporting from a case study in Madurai district and an expert Delphi panel yielded an annual average of 5,778,406 clinically diagnosed dengue cases between 2006 and 2012, or 282 times the reported number per year. The total direct annual medical cost was Rs. 32880 (or US\$548) million. Ambulatory settings treated 67% of cases representing 18% of costs, whereas 33% of cases were hospitalized, comprising 82% of costs. Eighty percent of expenditures went to private facilities. Including non-medical and indirect costs based on other dengue-endemic countries raises the economic cost to ₹ 60.11 (or \$ 1.11) billion, or ₹ 52.8 (or \$0.88) per capita. The economic and disease burden of dengue in India is substantially more than captured by officially reported cases, and increased control measures merit serious consideration.

VECTOR CONTROL

Field bio-efficacy evaluation of DRDO Defender Net against vectors of Japanese encephalitis and lymphatic filariasis

The CRME has evaluated the DRDO LLIN, called Defendernet, against the vectors of lymphatic

filariasis (LF) and Japanese encephalitis (JE) in rural areas of Tirukovilur and Vridhachalam, respectively and collected the following baseline data from three villages, to assess the efficacy of LLINs on disease vectors; the persistence of the insecticide on the nets by chemical assay; the bio-efficacy of LLINs relation to number of washes; the impact of LLINs on disease prevalence (LF and JE); and the social acceptability and collateral benefits

The studies were conducted in the area where vector mosquitoes are in high density and area is endemic for LF and JE. In the Tirukoilur block where LF is endemic, one village is intended for distribution of the treated nets (Thathanur), second one for plain nets (Paradapattu) and third one was control village (Veeranampattu). Similarly in JE endemic Virudhachalam area, the selected villages grouped into Insecticide treated village (G. Kudikadu), Plain net control village (Kollathankurichi) and Control village (Nanthapadi). The above three villages were selected based on topographical similarity and vector abundance.

Census was conducted prior to intervention in the selected experimental and control villages and houses would be numbered for the distribution of the treated net and plain net. This is to estimate the number of nets actually required for the trial.

During the baseline period, the entomological data, parasitological data and sociological data were collected. Defender Net distribution was initiated in the intervention villages from Tirukoilur block (Thathanur & Paradapattu) and Virudhachalam block (G.Kudikadu & Kollathankurichi) (Table1). Informed consent form in vernacular language Tamil was prepared and the defender net distribution was distributed to the community members. The impact of vector control was monitored by entomological indices, parasitological indices and sociological indices.

Table 1. Study area details in Tirukoilur and Vridhachalam block DRDO study

Tirukoilur block (Villupuram)			Vridhachalam (Cuddalore)		
Name of the village	No. of households	Population	Name of the village	No. of households	Population
Thathanur	107	951	G. Kudikadu	118	480
Paradapattu	151	685	Kollathankurichi	130	519
Veeranampattu	101	902	Nanthapadi	154	645

The entomological monitoring was carried out in three study villages of defender net project from April 2013 to March 2014. During the indoor resting collection only one adult female *Cx. quinquefasciatus* mosquito was collected and per man hour density range was recorded minimum 0.00 to maximum 20.3. In Thathanur village which is the defender net village where the lowest per man hour density was 0.00 recorded in October 2013 and highest density was 8.33 in December 2013. Whereas in Paradapattu village (Plain net village), maximum density was 18.0 during the monsoon period (November and December 2013) and minimum was nil during summer period. But in control village Veeranampattu, maximum density was 20.3 (Fig. 6).

The Highest parity status recorded in plain net village Paradapattu when compare to control village. Whereas in defender net village the parity trend was lowered when compare to other villages. The infection rate, infectivity rate and transmission intensity index was observed nil in the index villages (Fig. 7).

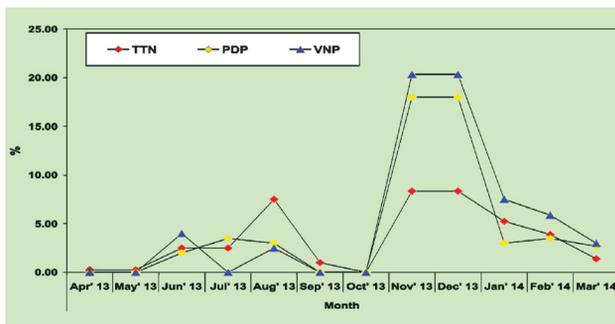


Fig. 6. PMH density of *Cx. quinquefasciatus* during indoor resting collection in the post intervention period (Apr'13- Mar'14).

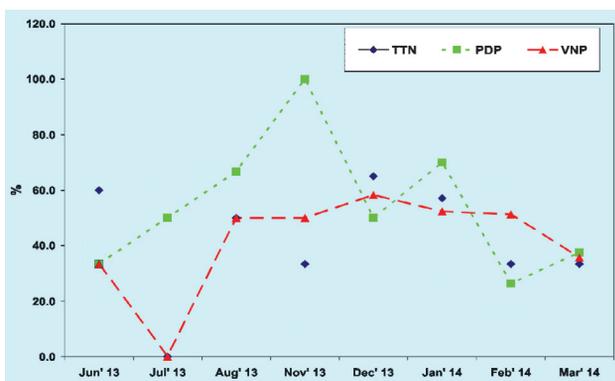


Fig. 7. Parity status of lymphatic filariasis vector mosquito *Cx. quinquefasciatus* during the post net distribution period (Apr'13- Mar'14).

Cone Bioassay was carried out to assess the susceptibility of lymphatic filariasis vector mosquitoes against Defender net and plain net in the respective study village Thathanur and Paradapattu in the Tirukoilur block. A total of five households were randomly selected and 25 mosquitoes (each household's five mosquitoes) exposed to the DEFENDER net. The exposure time was 20 minutes and also one day observation was done. Around 28% (7) mosquito's knockdown within twenty minutes (28%) and after 24 hours overall mortality was 88%. Similar test was carried out in plain net village also, where no mortality was recorded. No mosquito was knockdown in the control households.

A total of 754 mosquitoes comprising of 12 species 5 genera were collected utilizing 31 man hours had been spent in G. Kudikadu. The PMH density was calculated, among *Culicines* the *Cx. tritaeniorhynchus* was found to be dominant with PMH was 19.26 followed by 0.74 of *Cx. vishnui*, 0.71 of *Cx. gelidus*, 0.19 of *Cx. fuscocephala*, 0.58 of *Cx. quinquefasciatus*. Among *Anophelines*, *An. subpictus* was found to be dominant with 1.45, 0.65 of *An. peditaeniatus*. PMH density of the *Cx. tritaeniorhynchus* was decreased by nearly 2 folds during the post treatment period compared with pre treatment (33.58 to 19.26) (Fig. 8).

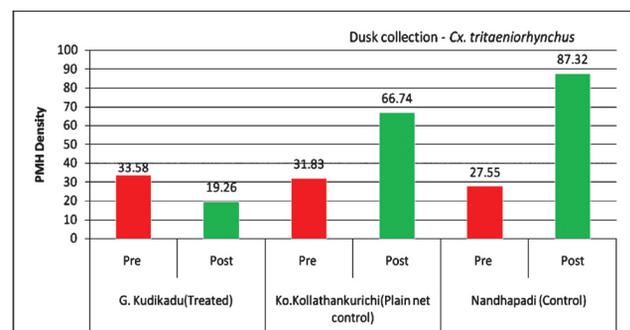


Fig. 8. PMH density of *Cx. tritaeniorhynchus*, the principal JE vector during dusk hours with comparison between Pre (Dec'12- Mar'13) and post (Apr'13- Mar'14) net distribution period.

A total of 2617 mosquitoes comprising of 14 species 6 genera were collected utilizing 31 man hours had been spent in Ko. Kollathankurichi. The PMH density was calculated, among *Culicines* the *Cx. tritaeniorhynchus* was found to be dominant with PMH was 66.74 followed by 1.39 of *Cx. vishnui*, 6.74 of *Cx. gelidus*. Per man hour density (PMH) of *Cx. tritaeniorhynchus* was 2

folds increased from 31.83 to 66.74 compared with pre and post net distribution period. A total of 2707 mosquitoes comprising of 16 species from 6 genera were collected by utilizing 31 man hours in three index villages in Nandhapadi. Among culicines the *Cx. tritaeniorhynchus* was found to be dominant with 87.32 followed by 1.10 of *Cx. vishnui*, 1.03 of *Cx. Gelidus*. Among Anophelines, *An. subpictus* was found to be dominant with 4.42, 4.29 of *An. Peditaeniatus*. Per man hour density (PMH) of *Cx. tritaeniorhynchus* was 3 folds increased from 27.55 to 87.32 compared with pre and post net distribution period.

Indoor Resting Collection was done in index villages of Virudhachalam block. In G. Kudikadu (Treated) during the post net distribution period (April to March 2014), a total of 340 (106 male 234 female) mosquito specimens belongs to 6 different species were captured. *An subpictus* was dominant mosquitoes (female PMH 8.65). PMH density of the *An. subpictus* was calculated and decreased from 12.50 to 8.63 compared with pre and post treatment period.

In Ko-kollathan kurichi (no net control) mosquito specimens belongs to five different species were captured. *An. subpictus* was dominant mosquitoes (female PMH 17.79) followed by *Cx. quinquefasciatus* 1.71. PMH density of the *An. subpictus* was calculated and it was escalating trend from 13.67 to 17.79. In Nandhapadi mosquito specimens belongs to six different species were captured. *An subpictus* was dominant mosquitoes (20.25) followed by *Cx. quinquefasciatus* 2.95, and *Cx. tritaeniorhynchus* was 1.1. PMH density of the *An. subpictus* increased from 8.33 to 20.25 comparing with pre and post net distribution period (Fig. 9).

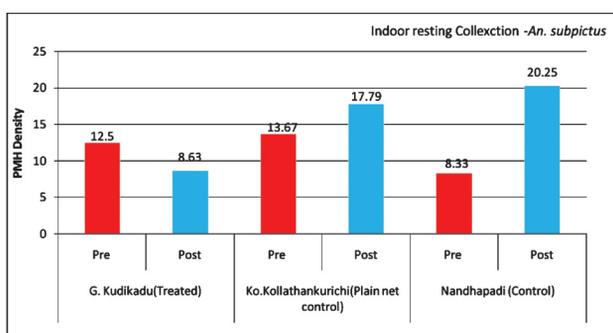


Fig. 9. PMH density of *An. subpictus* in Indoor resting collection with comparison between Pre (Jan '13-Mar '13) and Post (Apr '13-Mar '14) net distribution period.

JE vector mosquitoes collected during the survey were pooled and preserved in liquid nitrogen for JEV detection /isolation. The mosquitoes collected till September 2013 was tested for JE virus antigen and all were found negative.

Cone bio assay was carried out in G. Kudikadu (Treated) and Ko. Kollathankurichi (Plain net control) villages. In each village 5 replicates and 50 mosquitoes (10 in each replicate) were used and exposure time was observed in 3 minutes for knock down and mortality was observed after 24 hours. Knock down was observed after 3 minutes and kept in same, mortality was observed in after 24 hours. 46% of knockdown and 38% of mortality was observed in treated village of G. Kudikadu. None found Knockdown and mortality in Ko. Kollathankurichi (Placebo).

The percent knockdown of mosquitoes in cone bioassays was 92% in Vridhachalam samples and 84% in Cuddalore samples. The percent mortality 24 hours post-exposure was 96 and 88 percent in Vridhachalam and Cuddalore. In WHO cone bioassay the field samples of Defender Net from Vridhachalam and Cuddalore caused $\geq 80\%$ mortality or $\geq 95\%$ KD against *Aedes albopictus* mosquitoes and thus the samples meet the criteria of efficacy as per WHO guidelines.

Monitoring susceptibility status of vectors of various arthropod-borne viral diseases against different insecticides

Susceptibility status of vector species of *Ae. aegypti*, *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* species were determined by exposing fully fed adult females for 30 and 60 minutes against various compounds of organo carbamate (Prpoxor 0.1%), Organochlorine (DDT 4%, Dieldrin 0.4%, Dieldrin 4%), Organophosphate (Malathion 5%, Fenitrothion 1%) and Pyrethroid (Deltamethrin 0.05%, Permethrin 0.75%, Lambdacyhalothrin 0.05%, Cyfluthrin 0.15%, Etophenprox 0.5%). Hundred percent mortality was obtained in the exposure of *Ae. aegypti* against Malathion 5% whereas 48% mortality against Dieldrin 0.4% for the same time of exposure. However, one hour exposure resulted 100 percent mortality against Malathion 5%, Deltamethrin 0.05%, Dieldrin 0.4%, Dieldrin 4% respectively. This indicates that

Ae. aegypti mosquitoes are susceptible for all kinds of compounds. JE vector, *Cx. tritaeniorhynchus*, was found to be more susceptible against Malathion 0.5%, but developed resistance against Fenitrothion 1%. The reason might be that Fenitrothion utility is more in agriculture rather than public health in the past several decades. The heterogeneity of the population of *Ae. aegypti* and *Cx. tritaeniorhynchus* was found to be more susceptible for both Malathion and Dieldrin. Bancroftian vector species *Cx. quinquefasciatus* susceptibility status for one hour exposure resulted that the species found to be more susceptible against Dieldrin 4% and Permethrin 0.75% than other compounds

TAXONOMY AND BIODIVERSITY

Mosquito fauna survey was carried out in nine hill ranges, viz., Nagercoil, Tirunelveli, KMTR, Srivilliputtur, Palani, Aanaimalai, Theni, Sathyamangalam & Gudalur hills of Western Ghats. A total of 9767 mosquito specimens belonging to 82 species comes under 21 genera and 19 subgenera were recorded in 9 different hill ranges. *Aedimorphus stenoetrus* & *Culex (Eumelanomyia) iphis* were addition to CRME Museum which is collected from Palani hills (Kodikanal). Dengue prevalence in Tribal Population survey was carried out in five hill ranges of Western Ghats (Palani hills, Anaimalai, Theni, Sathyamangalam and Gudalur). Dengue/ Chikungunya vector (*Aedes albopictus*) was more found breeding predominantly in and around Tribal area studied. In pre-DDT era, *Anopheles fluviatilis* was more dominant species for foot hill human malaria in Western Ghats, but recent surveys shows that *Anopheles culicifacies* (rural malaria vector) was more abundant (80%) when compared to *Anopheles fluviatilis* (20%). Studies on mosquito taxonomy and systematic on emphasis on revision of FBI Monographs on Indian mosquito fauna at present inventory in India enlisted a total of 403 species under 50 genera & 43 subgenera.

NATIONAL AIDS RESEARCH INSTITUTE, PUNE

Development of Cohort of Long Term Non Progressors (LTNPs)

HIV is a chronic infection wherein the rate of progression to symptomatic stage (average 7-10 years) varies in different individuals. A few of HIV

infected individuals do not progress to symptomatic disease in spite of the HIV infection for more than 7-8 years, indicating that they been able to achieve good control over virus multiplication. Studies in this population, also known as ‘Long Term Non Progressors’, might throw some light on the mechanisms controlling HIV multiplication in these individuals. To study the association of HIV-specific responses such as neutralizing antibodies and cytotoxic T- cell response, NARI decided to develop a comprehensive program focusing on LTNP and began to identify and follow a cohort of LTNPs systematically in these individuals since 2010 (Fig. 1). Functionality of immune responses (both innate and acquired) and their genetic makeup in relation to various restricting genes will be correlated clinically.

The 21 true LTNPs were found to have a median CD4 count of 840 cells/mm³ and a median viral load of 3538 copies /ml. Studies on innate immune mechanisms to understand their role in maintaining LTNP status are ongoing.

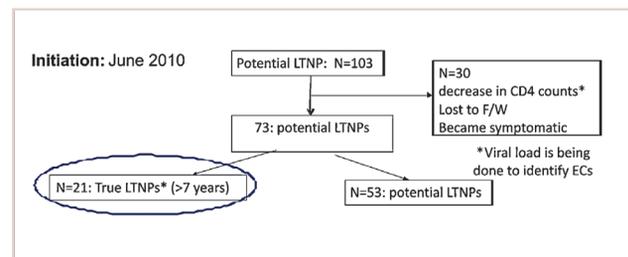


Fig. 1. Enrollment and follow up details of the LTNP cohort

Innate immune factors: The Natural Killer (NK) cells were found functionally competent (cytotoxic potential as compared with the NK cells from ‘progressors’ and ‘recently infected persons’ with a high viral load set point.

During the year, the functionality of NK cells was analyzed in terms of cytokine and chemokine secretion. It was observed that the IFN- γ and RANTES secreting ‘cytotoxic’ and ‘regulatory’ NK cells were present in LTNPs and the persons with recent HIV infection with low viral load set point indicating the importance of these cells in controlling virus multiplication and disease progression (Fig. 2).

The plasmacytoid dendritic cells, (pDC - an important innate cell having anti-viral capacity),

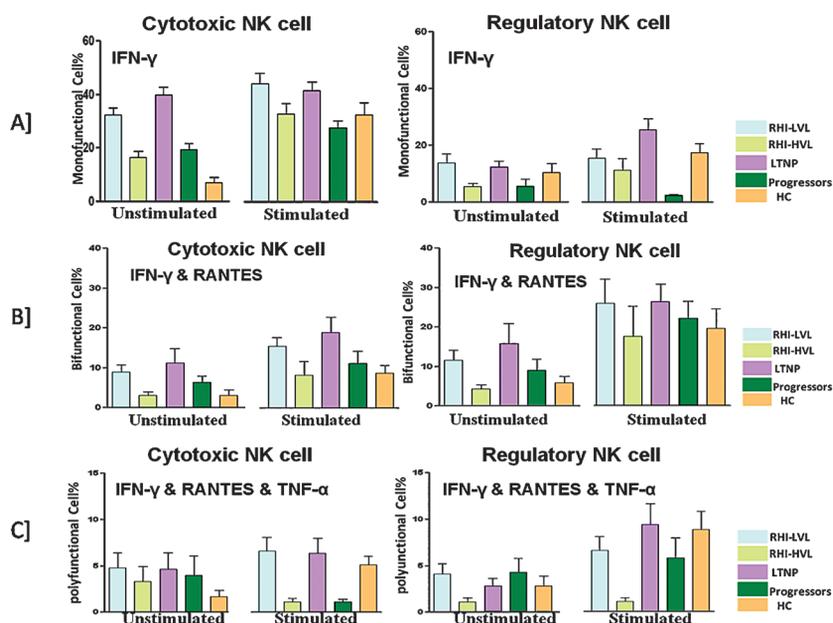


Fig.2. IFN- γ , RANTES and TNF- α secreting cytotoxic and regulatory NK cells in Recent HIV infection with low or high viral load set point, LTNP, progressors and healthy controls. The % cells secreting cytokine/s (Y axis) from both unstimulated (constitutive secretion) and stimulated cells (x-axis) have been shown.

Legend: A. Monofunctional cells secreting of only one cytokine (IFN- γ) by both, B. Bifunctional cells showing secretion of both IFN- γ and RANTES. C. Polyfunctional cells showing secretion of IFN- γ , RANTES and TNF- α . The blue bar indicates

were significantly reduced in recent HIV infection, LTNPs and also in patients successfully controlled on ART. However, the myeloid dendritic cells (mDCs), the cells that participate in development of adaptive immune response were found to revert to normal quantity only in LTNPs and patients on successful ART. This observation indicated that the pDC function once compromised never comes back to normal; however the reversion of mDCs might be one of the factors having a positive influence on slowing the rate of disease progression.

The LTNPs also showed reduced immune activation as compared to progressors.

HIV-specific immune response: LTNPs showed transient neutralizing antibody response against HIV as compared to the natural progressors and responses showed lower magnitude and breadth. The HIV-specific cytotoxic T cell (CTL) response was also found to be narrow in breadth and lower in magnitude and mostly against Gag and Env epitopes. The analysis of the epitopes exclusively recognized by LTNPs is underway.

In a study to understand the characteristics of the antibodies that mediate antibody dependant cell mediated cytotoxicity (ADCC), the preliminary

analysis showed that the LTNPs had higher percentage of antibodies mediating viral inhibition (ADCVI) as compared to the progressors.

Host genetic factors: The analysis of HLA types of the 21 LTNPs revealed that the Indian LTNPs do not show presence of the protective allele HLA B*57. This observation indicated that the Indian LTNPs might be having different genetic makeup than the Caucasian LTNPs.

First report of anal HPV prevalence among HIV infected women in India

In a cross sectional study on increased incidence of ano-rectal malignancies the prevalence and determinants of anal HPV among Indian women has been reported.

This first report of anal HPV by genotype among HIV-infected women in India identified a significant prevalence of oncogenic (9.2%) and non-oncogenic (4.1%) genotypes [overall prevalence =14%]. The data contextualise the reported raised incidence of ano-rectal malignancies among HIV infected in Pune. The study provides preliminary evidence for ano-genital HPV/cytologic screening among women living with HIV (WLHIV) in India as they begin to live longer due to improved ART access.

Improving cervical cancer prevention among HIV-infected women using novel HPV based Biomarker Assays

In view of the high prevalence of carcinogenic HPV DNA among HIV-infected women, a study is being undertaken to evaluate the clinical performance of two novel low-cost biomarker assays (i) immunocyto staining by p16INK4a/Ki-67 and (ii) E6/E7 mRNA for detection of histologically-confirmed cervical intraepithelial neoplasia grade 2/3 or more severe (>CIN2 and >CIN3) among HIV-infected women. A total of 292/400 participants have been enrolled. HPV DNA testing results indicates an overall HPV positivity of 49/160 (31.6%), and high risk/oncogenic HPV positivity of 37/160 (23.9%) has reported.

HIV AND TUBERCULOSIS RESEARCH

Comparison of phenotypic and functional characteristics of *M. tuberculosis* specific T cells in active and latent tuberculosis

Transition from latent to active TB is likely to be dependent on loss of immune control and escalating mycobacterial load, resulting in altered phenotype of cells of immune system. This study was designed for characterizing phenotype and functionality of TB specific memory T cells in patients with active and latent TB. Twenty participants from each of three groups: 1. active sputum positive pulmonary TB, 2. healthy household contacts of active TB patients and 3. community controls with latent TB infection have been enrolled in the study.

Preliminary data show that household contacts have a predominant Th17 type of response and their Chemokine receptor expression pattern also showed significantly higher frequency of CCR6 (Th17 type chemokine receptor) along with higher frequency of CXCR3 (Th1 type chemokine receptor) expressing T cells in contacts as compared to patients with active TB, indicating protective role of Th17 response in the contacts.

Molecular probing of the *M. Tuberculosis* isolates with respect to their resistance pattern and conservation pattern of new drug targets

This ongoing study is profiling various genes of *M. tuberculosis* isolates for their sequence conservation patterns. These genes are the targets of currently

used drugs and a few for newly predicted drug targets. The initial analysis showed a few genes with high frequency of mutations (as found in *gyrA* and *rpoB*) whereas a few remain conserved (as observed in case of *rrs* and *inhA*). The sequencing is ongoing and results are to be verified with phenotyping assays. This may provide the information about the better drugs targets and also may provide the directions for new drug development pipeline.

Rapid detection of anti-TB drug resistance in *M. tuberculosis* isolates from HIV infected and uninfected tuberculosis patients in Pune, India

The conventional methods used are time consuming and cumbersome. In a study the performance of rapid drug resistance detection techniques namely MGIT 960 system, MODS assay, Genexpert MTB/ RIF and Line Probe Assay for detecting the MDR TB was evaluated. During the period 95 *M. tuberculosis* isolates were tested for anti -TB drug resistance by MGIT 960, MODS assay and Genexpert MTB/ RIF. Of these, 51 isolates were from HIV positive and 44 from HIV Negative TB patients.

The preliminary results indicated 100% agreement between MGIT 960 and MODS assay for the detection of MDR TB (resistance to isoniazid and rifampicin). However, Genexpert MTB/RIF that detects only rifampicin resistance detected additional two rifampicin resistance MTB strains, which were not detected by other methods.

HIV PATHOGENESES AND HOST FACTORS

Gut flora and HIV infection

There has been an enormous amount of research in the area of metagenomics analyses with the advent and progress of next generation sequencing. However, there has been less focus in the area of infectious diseases specifically HIV. In a pilot study total DNA isolated from fecal samples obtained from different HIV infected individuals and control population was used. Using this DNA, a well known and a well conserved region of 16S rRNA gene was amplified and used to profile the microbial

communities in the samples. Preliminary findings show that certain samples show association between specific microbial communities with HIV disease staging. For instance, one sample is dominated by those which microbial genera which are responsible for provoking inflammation whereas one sample in control group has shown the presence of microbial communities which keeps the inflammation in check.

The role of miRNAs in the pathogenesis of HIV/AIDS and their utility as biomarkers of disease progression and therapy failure

This study was undertaken to validate miR-150 and miR-146b as biomarkers of HIV disease progression and therapy. In a cross sectional study a total of 225 participants belonging to five different categories were enrolled-50/50 healthy controls, 35/50 HIV infected asymptomatic individuals, 32/50 ART naïve HIV infected symptomatic individuals, 50/50 HIV infected on ART for at least 12 months and 13/25 those failing on ART. The follow-up of HIV infected individuals on ART for over 7 years and those initiated on ART (10 each) is ongoing every 3 months.

Role of HSV-2 in HIV-1/ HSV-2 Co-Infection

To identify a suitable T cell line model for studying HIV-1 and HSV-2 interactions, HIV-1 replication in three T-cell lines (CEMccr5, PM-1 and MOLT4/R5) in the presence and absence of HSV-2 was studied. The influence of HSV-2 was studied on HIV-1 viral loads in the cell lines tested was in the order of CEMccr5 > PM-1 > Molt4/R5. The data suggests that the most suitable T cell line for studying HIV-1/HSV-2 co infection is CEMccr5 cell line.

CLINICAL CARE AND PREVENTION

‘Early ART’ as a prevention method: Attitudes and practices of Indian HIV physicians for prevention of transmission in HIV serodiscordant couples

Acceptance and implementation of the WHO’s recommendation is critical for successful prevention in HIV discordant couples (HDC) setting in India’s heterosexually-driven epidemic. A survey was done on HIV-Health Care Physicians (H-HCP) from 13 Indian states to study current ‘treatment-as-prevention’ (TasP) attitudes and practices for the HDC.

Very high awareness and strong acceptance for Early ART as Treatment as Prevention for HDC were reported by Indian physicians. Private-sector physicians have already begun implementing WHO-2013 guidelines. However, a gap was noted in belief and implementation and also identified certain educational needs for good attitudes and practices(Figs 3 & 4).

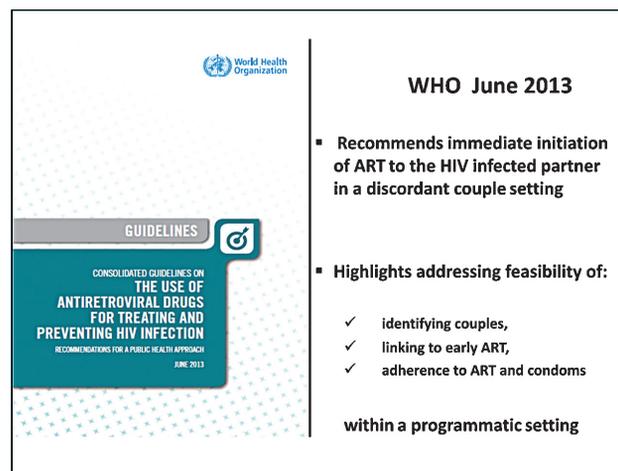


Fig. 3. Physician awareness of HPTN 052 study and impact on practices.

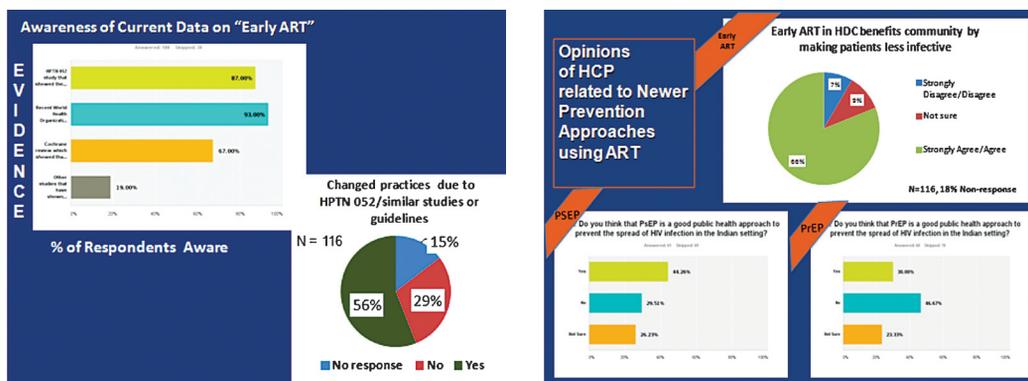


Fig. 4. Opinions of health care providers related to newer prevention approaches using ART.

Community engagement for JES and AES prevention and control: Research cum Intervention (RCI)

To understand the community beliefs, practices and problems a qualitative data was collected from a JES/AES infected area at Gorakhpur (U.P.). The preliminary analysis of 36 in-depth interviews highlights gaps in prevention program, capacity building of local health care providers and lack of ownership in the community for preventive health.

The information education and communication program for JES/AES is ongoing; however the didactic form of education does not seem to have made an impact. The concept for hygiene did not seem to include water, sanitation or food among community members. The local community prefers to go to the local health care provider to be able to access treatment fast as the disease is very serious, although the local provider has limited knowledge of treatment.

Lack of ownership of health care was evident which might be a challenge for taking practical step towards health care as the village leaders were indifferent.

To understand prevention and explore barriers for women: A multi-stakeholder perspective on vaginal microbicides and other NPTs

A total 219 in-depth/key informant interviews were conducted among HIV discordant and concordant couples, high risk women, men having sex with men and other stakeholders in high prevalence districts of Pune, Chennai and Belgaum to explore the willingness to use new prevention technologies such as vaccine, microbicides and pre exposure prophylaxis.

Preliminary analysis showed that women initiated options are preferred. Respondents stressed that location and time of 'risk' would contextualize the choice of NPT.

Cognitive aspects in HIV infected children before and after ART initiation

A study was conducted to assess cognitive functioning of school-aged, perinatally acquired HIV infected, ART naïve children attending NARI clinic. Fifty children between 6 and 12 years of age

were administered the Indian Child Intelligence Test (ICIT) after clinical evaluation. Age and gender matched HIV uninfected children (n=50) were tested as controls. The information on adaptive behaviour was obtained from the care giver and a brief interview with the child using Vineland Social Maturity Scale (VSMS). There was no significant difference in the overall cognitive abilities in HIV infected children using ICIT as compared to HIV uninfected children at baseline.

Development of a culturally-tailored scale to measure domestic violence among married women in Pune, India

The study aimed at developing a culturally tailored domestic violence (DV) scale for Indian women. The formative phase led to emergence of 63 DV items that were typical of Indian situations and which were developed into Indian Family Violence Control scale (IFVCS). A total of 630 interviews of this scale were administered in face-to-face, individual interviews in a random sample of married women from 16 wards in Pune Municipal Corporation between April and July 2013.

The psychometric properties and cultural specificity of the IFVCS render it an effective tool for measuring DV among married women in India. They speak to its capacity for enhancing understanding of DV epidemiology in HIV high-risk and HIV-positive populations, and for evaluating the effectiveness of future dual HIV/DV prevention programs and interventions targeted for HIV-infected populations in India.

ONGOING PROJECTS

Multicenter study of options for second-line effective combination therapy (SELECT)

This phase III, dual-arm, open-label, randomized, non-inferiority study aims at determining whether the combination of LPV/r + RAL is associated with virologic efficacy that is non-inferior to that achieved with LPV/r + best-available NRTIs by 48 weeks of follow-up.

A total of 14 participants (8 males and 6 females) who were failing on first-line antiretroviral therapy were enrolled in the study between December 2012 and Feb, 2013; of which 8 were on Arm A.

The median CD4 in Arm A and Arm B was 106 cells/mm³ and 149 cells/cu mm, respectively. The median plasma viral load (Log₁₀) was 4.5 cps/ml. All the participants have completed 48 weeks follow-up. Four participants developed severe hypertriglyceridemia that required medical intervention and one developed cancer cervix. One of the study participants had detectable viral load suggestive of treatment failure.

Genetic basis of azole resistance in clinical *Candida albicans* isolates

The azoles, particularly Fluconazole, remain among the most common antifungal drugs, however their increased use for both treatment and prophylaxis has led to resistance. A total of 113 clinical *C. albicans* isolates are being tested for mutations in genes (ERG11 and ERG3) encoding the drug target enzyme and expression patterns of genes encoding efflux pumps drug target site mutations.

The overall results of indicates that alterations in drug target sites may not play a major role in azole resistance in clinical *C. albicans* and leads to the speculation that drug efflux may be the predominant mode of azole resistance in clinical *C. albicans* and we are currently studying the efflux mechanisms.

Evaluation of sensitivity of in-house drug resistance assay using the dried blood spots (DBS) from HIV infected patients with or without ART.

As part of the validation study for the in-house assay for HIVDR genotyping using Dried Blood Spots as specimens, 25 clinical specimens with wide range of viral loads (1x10³-1x10⁷copies/ml) were tested to validate the analytical sensitivity of the assay. All clinical specimens confirmed acceptance criteria for sensitivity as per the WHO protocol *i.e.* more than half of the replicates should show amplification at a viral load of >2000 cp/ml. The study findings were reported to WHO to complete the accreditation of the in-house assay.

Study on gag mutations associated with resistance to protease inhibitors in Indian HIV-1 subtype C

An analysis of Gag cleavage site substitution among the patients failing on PI based ART

treatment and ART naïve HIV-1 subtype C infected individuals was done to find out natural variations and association of gag CS substitutions among ART naïve and the patients failing on PI based ART treatment. Compared to HXB2 reference sequence, High rate of natural variation at cleavage sites p2/NC, TFP/p6pol and p6pol/PR were observed among India HIV-1 subtype C sequences. The study showed an association of V128I (p17/p24), S368C/N (p24/p2), A431V (NC/p1) and L449V/P (p1/p6gag) cleavage site substitutions with Indian HIV-1 subtype C infected patients failing on PI based ART regimen.

Studies on *in-vitro* replication fitness of antiretroviral drug resistant strains of the Indian HIV-1 Clade C

Drug resistant mutant viruses harboring mutant RT gene from the patient plasma virus were generated and assessed for the single cycle and multiple cycle replication fitness. Two experimental vectors with Indian HIV-1 Clade C genetic backbone were also constructed for use in the Real Time PCR based growth competition assay. This subtype C specific assay which is undergoing further evaluation would be a useful tool for studying the replication fitness of drug resistant viruses of the Indian HIV-1 Clade C and to generate India relevant knowledge to inform treatment strategies.

PRODUCT DEVELOPMENT AND TESTING

Services provided for *in vitro* anti-HIV1 testing to Indian researchers

During the year, anti-HIV testing was carried out for four RT inhibitors referred by an university, three plant derived entry inhibitors and four anti-microbial peptides referred by pharmaceutical companies and none was found to have promising anti-HIV activity. Additionally, we received requests for testing 187 compounds of synthetic/herbal/microbial origin that have been referred to ICMR for approval.

Identification of anti-HIV leads from plant sources and determination of mechanism of Action

The extracts of the two previously identified lead plants *Terminalia paniculata* (Tp) and *Polygonum*

glabrum (Pg) were prepared and tested for anti-HIV activity. It was observed that acetone extract (TI = 51.16 & 62.73; 61.06 & 62.25) and methanol extract (TI= 57.50 & 61.24; 81.30 & 59.90) of *T. paniculata* and residual part of acetone extract (TI= 41.85 & 46.64; 63.95 & 86.42) of *P. glabrum* showed promising anti-HIV1 activity against cell associated HIV1_{UG070} and HIV1_{VB59} and cell free HIV1_{UG070} and HIV1_{VB59} respectively in TZM-bl assay. Isolation of compounds from these lead extracts is in progress.

Anti-HIV activity of lead 4-Thiazolidinones RT inhibitors against HIV-1 drug resistant isolates

In earlier studies, four Thiazolidinones as lead reverse transcriptase (RT) inhibitors were identified. During the year, these four lead RT inhibitors were tested against a panel of ten Nevirapine resistant isolates. Based on the inhibition obtained against drug resistant strains, the lead NCEs can be graded as per the following order - S009-1912 > S009-1908 > S009-1909 > S009-1911. The inhibitors showed differential response against different drug resistant strains. The data confirms that Thiazolidin-4-one analogues could serve as potential lead for further development of novel and potent NNRTIs.

CONTRIBUTION TO THE NATIONAL PROGRAMME

National AIDS Control Organization supported -early infant diagnosis (EID) Programme

NARI undertakes diagnosis of HIV-1 infection among HIV exposed infants and children using DBS from 33 districts of Maharashtra under DAC-Early Infant Diagnosis program. This year, 185/1930 infants (9.5%) were found to be HIV positive on DBS. Whole blood samples confirmation was received from 155/185 infants of which 125 (6.5% overall) were confirmed HIV positive.

HIV Sentinel Surveillance (HSS)

As a Regional Institute (RI), NARI supervises HSS in five Indian states of and National Integrated Biological and Behavioural Surveillance (N-IBBS) Gujarat, Maharashtra, Goa and Karnataka for core and bridge populations. During 2013-2014, data for the 13th HSS round ANC attendees was finalized and resulted in the publication of the Technical

Brief 2012-13 by DAC. Mean HIV prevalence among antenatal clinic attendees in Gujarat (0.5) and Maharashtra (0.4) was higher than national average (0.35), while combined prevalence for the region was 0.33% (range: 0.0 to 0.5).

Integrated Biological and Behavioural Surveillance (IBBS): Western Indian Region

NARI implemented IBBA surveys between 2005 and 2010. The technical expertise has now been translated to the national IBBS survey planned by DAC. NARI will play critical role in implementing national IBBS in Western region. Pre Surveillance Assessment (PSA) completed in August 2013 gave important direction to IBBS survey for which NARI participated in core preparatory activities.

National AIDS Control Organization supported – PMC-NARI ART Centre (ARTC)

Retention of Antiretroviral (ARV) Naïve Patients registered in HIV Care in NARI ART Centre

Retention in HIV care ensures delivery of services like secondary prevention, timely initiation of treatment, support and care on a regular basis. Analysis of ARV naïve HIV infected adults patients registered between January 2011 and March 2012 in HIV care (pre ART) revealed that higher CD4 count and illiteracy were significantly associated with lower retention in pre-ART care.

Report on Transfer Out Patients Receiving Antiretroviral Therapy at NARI ART Centre

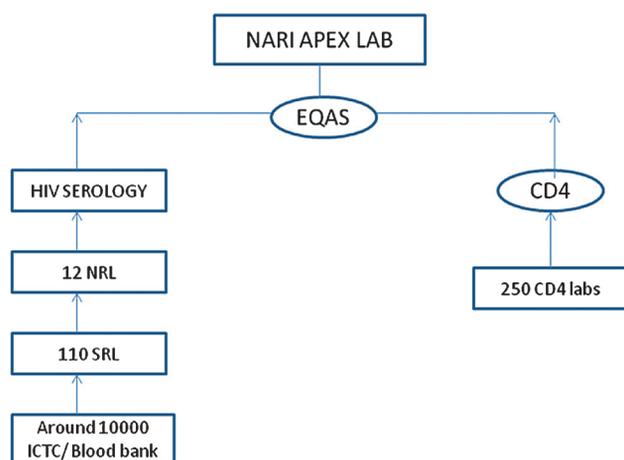
The analysis of NARI ARTC data (Jan2006 - July 2013) suggests that significant number of transfer out patients were lost in the HIV treatment system. On a national level, this group adds significantly to the burden of lost to follow-up patients. It is important to strengthen programmatic systems for tracking 'transfer-out' patients to seal this important leak in the cascade of HIV care.

Evaluation of "Intensified TB Case finding" Component of HIV-TB Collaborative Activities.

Evaluation of Intensified TB Case Finding (ICF) and knowledge of ART center patients about TB revealed the need to improve ICF by regular training of staff and local/ external supervision at ARTC.

Quality Control (QC) Testing Provided to HIV Testing Laboratories in the National Program

To monitor the performance of the more than 10,000 HIV and 250 CD4 testing laboratories in India, NARI has been identified as an apex laboratory and it runs External Quality Assurance (EQA) programme for HIV serology and CD4 count estimation. Test panels with predefined criteria are sent to all laboratories and their performance is assessed. For HIV serology, > 90% centres were successful this year and for CD4 count estimations. 236 centres participated.



A number of workshops were arranged for HIV serology (at least 2 workshops at each NRL and SRLs) and CD4 testing (2 workshops each at 13 training center all over the country) for the technicians carrying out respective tests. The reasons for unsuccessful participations were primarily non participation due to unavailability of technicians or kits, transcriptional error and the quality of kits in some cases. Number of unsuccessful Cyflow and FACSCalibur centers showed significant reduction this year indicating performance improve.

Extramural Research

ANTI MICROBIAL RESISTANCE

To strengthen the surveillance of antimicrobial resistance in India, a National Anti Microbial Resistance Surveillance Network (AMRSN) has been initiated to enable compilation of National data of AMR. The surveillance network has six Nodal centers (NCs) which are focusing on six different pathogenic groups: (i) Diarrhoeagenic bacterial organisms: CMC, Vellore, (ii) Enteric fever pathogens: AIIMS, New Delhi, (iii) Enterobacteriaceae causing

sepsis: PGIMER, Chandigarh, (iv) Gram negative non-fermenters: CMC, Vellore, (v) Gram positives including MRSA: JIPMER, Puducherry, (vi) Fungal infections: PGIMER, Chandigarh. ICMR has identified 24 Medical Colleges to act as Regional Centers (RCs) to work with National Centers.

DIAGNOSTICS

Indigenous H1N1 Diagnostics Kit Development and Evaluation

The ICMR/DHR has been coordinating the development and evaluation of indigenous kits for Influenza A (H1N1) pandemic (2009) in India. External validation / 3rd party evaluations of micro PCR Diagnostic kits manufactured by Bigtec Pvt. Ltd., Bengaluru have been completed. The kit has undergone a series of validations / re-validations done at 4 centres viz NIV (Pune), KMC (Manipal), NIMHANS (Bengaluru) and NCDC (Delhi), and reviewed by Expert Group / Consultative Group on H1N1 Diagnostics.

The sensitivity of the Bigtec microPCR kit for the detection of Influenza A (H1N1) is 83.3% while, the specificity is 96.8% when compared with the CDC protocol for PCR assay.

The Company (Bigtec Pvt. Ltd. Bengaluru) will bring down the cost of their micro PCR kit to less than 50% of the current cost for patient testing. The kit is recommended to be used in public health especially at low level / low volume / peripheral labs viz Medical Colleges, CHCs, MRHRUs etc.

DIARRHOEAL DISEASES

A study conducted at the Indian Institute of Science, Bangalore on Structural and Biological studies on Rotavirus enterotoxin and Enteroviral protein 3A has revealed that NSP4 proteins from different strains differ in their conformation, trypsin resistance, Thioflavin-T binding and the diarrhoea inducing activity did not correlate with any of the properties. This explained the basis for diarrheagenic and non-diarrheagenic nature of NSP4 proteins from different rotavirus strains.

In a project, the Punjab University, Chandigarh studied the epidemiology of multidrug resistant (MDR) *Salmonella enteric* serovar *Typhi* strains at molecular level. The repetitive

extragenic palindrome elements – PCR (REP-PCR) fingerprinting to carryout enterobacterial repetitive intergenic consensus elements – PCR (ERIC-PCR) fingerprinting and performed PCR-amplification of 16S-23S intergenic spacer region (PCR-ribotyping). In this study re-emergence of sensitivity to traditional first-line antibiotics such as chloramphenicol and cotrimoxazole was observed. The present study creates a paramount baseline for rationalizing the judicious use of quinolones and re-examine the use of chloramphenicol and cotrimoxazole.

In a study at the Post Graduate Institute of Medical Education & Research, Chandigarh enteroaggregative *Escherichia coli* (EAEC) (wild type & plasmid cured) induced IL-8 secretion from human intestinal epithelial cell line (INT- 407) cells was assessed. It was observed that infection of INT-407 cells with EAEC-T8/ EAEC-O42 could induce activation of NF- κ B as well as AP-1. It has been found that EAEC could activate all the three MAPKs in INT-407 cells.

A study has been completed on structural and functional analysis of a pathogenicity island in strains from human and animals with special reference to its pathogenicity

In a study at Indian Veterinary Research Institute, Eastern Regional Station, Kolkata a total of 22 genes of the locus of enterocyte effacement (LEE) Pathogenicity Island were searched in 19 human and 71 cattle Shiga toxigenic *Escherichia coli* (STEC) isolates. Of these, *cesD* was found positive in 8 (42.1%) isolates followed by *espH* [5 (26.3%)], *escD*&*espB* [4 (21.05%) each], *escF* [3 (15.8%)], *eae*, *espA*&*espF* [2 (10.5%) each], and *orf1*, *EscJ/sep-Z*, *escN*, *sepQ*, *orf4*, *sepL*&*espD* [1(10.5%) each] in the human isolates.

A study has been completed on the genetic diversity of Fluoroquinolone resistant *Salmonella entericaserovarTyphi* and *Paratyphi A* at Safdarjung Hospital, New Delhi. Role of efflux pumps and OMP was studied using PA β N inhibitor and SDS-PAGE respectively. VNTR was performed using five VNTR primers. FQ resistance was wide-spread (75% in *S.Typhi* and 100% in *Paratyphi A*) among *Salmonella* causing enteric fever with majority of the strains showing low level resistance.

HIV/AIDS

A project on tuberculosis with and without HIV co-infection was completed at Himalayan Institute of Medical Sciences, Dehradun . It aimed to study the nature of immune response to ESAT-6 generated by particular sub-populations of T-cells and macrophages. The study revealed that as compared to healthy household contacts, HIV negative TB patients recorded significantly elevated secretion of IFN γ following stimulation of both CD4 and CD8 cells with rESAT-6. Significant over-expression of IFN γ , at both the protein and mRNA level was observed, following stimulation of CD8 cells of TB-HIV co-infected patients with rESAT-6.

In a study at the Post Graduate Institute of Medical Education & Research, Chandigarh. The study aimed to evaluate the diagnostic utility of synthetic N-terminal *M. avium* KatG (KatG-N) on the basis of serum antibody ELISA for the identification of HIV patients with MAC bacteremia. It showed that first40 amino acids of *M. avium* KatG protein (KatG-N) are specific to MAC and can be exploited using the cost-effective techniques based on katG-N gene amplification or KatG-N protein serodiagnosis as the newly developed *M. avium* katG-N PCR is a single step DNA amplification system that is successful in the rapid and accurate differentiation of MAC from *M. tuberculosis*. It seems that a significant proportion of Indian HIV patients have disseminated MAC bacteremia even at CD4+ T cell counts exceeding 100 cells/ μ l, and mixed infection with *M. tuberculosis* MAC is not uncommon.

A study on immunological correlates of immune reconstitution inflammatory syndrome (IRIS) in subjects with HIV-1/Tuberculosis co-infection after initiating highly active anti- retroviral therapy was completed at Y.R. Gaitonde Centre for AIDS Research and Education (YRG CARE), Chennai. The study showed, that the higher responses of IFN- γ in the IRIS group than in the non-IRIS group, possibly indicates the roles of effector cytokines in the immunopathology of TB associated IRIS.

The Regional Institute of Medical Sciences, Imphal studied the sero-prevalence of HIV and STIs among injecting drug users (IDUs) female sex workers and epidemiology of HIV/STIs in them.

The study carried out in four districts of Manipur. The results showed that the number of HIV positive cases is maximum amongst Female Injecting Drug Users and Commercial Sex Workers. The number of co-infection of HIV and HCV is 59 and that of HCV+VDRL and HIV +HCV+VDRL is 12 and 6 respectively. A total of 5.5% of the clients had first sexual exposure before the age of 18. 100% of the respondents had multiple sexual partners and received money for sex and 12% of them had never used condoms. 99.5, 48 and 53.5% of the respondents have knowledge of HIV, HIV/HCV and HBV/HCV respectively.

A study at the PGIMER, Chandigarh aimed to devise a simple and cost effective rapid diagnostic test for the intensive screening for TB in HIV+ patients based on the antibody response to these antigens - The analysis of all the microbiological assays indicated 5 out of 175 i.e. (2.8%) asymptomatic HIV+ patient culture positivity in sputum /urine samples. Based on molecular analysis it was observed that overall, 26% of asymptomatic HIV+ patients and 100% of HIV+TB+ patients showed multiplex PCR positivity with sputum, urine and blood samples. The results indicated that there are number of HIV+ patients who remain asymptomatic for TB for a long time but can be identified by using a battery of investigations much before the appearance of clinical symptoms.

A study was completed at Blue Peter Research Centre, Hyderabad to identify immunologic markers that can detect persons at greatest risk of progression of latent to TB. The study determined if NK cell responses to mycobacterium tuberculosis (M.TB) are reduced in HIV+ persons with Latent TB infection (LTBI). It also identified D4GDI, as a novel product of Tregs that inhibits *M. tuberculosis* growth in macrophages.

In a study the National Brain Research Centre, Manesar investigated clade specific effect of HIV-1 Tat B and C protein with cocaine induced alterations in properties of human brain derived neural progenitor cells. It was demonstrated that the presence of Cocaine along with HIV-1 Tat attenuates the human neural progenitor cell (hNPCs) proliferation and ability to form new neurons. The study has direct clinical relevance with HIV/AIDS patients that are also drug abusers.

A multicentric task force study on Establishment for *Neisseria gonorrhoeae* surveillance in India was completed at the AIIMS, Delhi, Regional Institute of Medical Sciences, Imphal, JJ Group of Hospitals, Mumbai, Vardhman Mahavir Medical College & Safdarjung Hospital, Delhi, Government Medical College, Srinagar and Madurai Medical College, Madurai. The study has generated quality controlled data that can be pooled and disseminated to sexually transmitted infection (STIs) managers of the country. A total of 16 isolates (7.9%) exhibited decreased susceptibility to Extended Spectrum Cephalosporins (ESCs). Emergence of resistance to azithromycin was also observed in about 3% isolates. Additionally, characterization of ciprofloxacin resistance was undertaken by studying mutations in the *gyrA* and *parC* genes where novel mutations (e.g., G85D in *parC*) was observed. Standardization of NG-MAST (*N. gonorrhoeae*-multi antigen sequence typing) has been done. A much diversified *N. gonorrhoeae* population and a very high number of unique STs (e.g., 9785, 9786) have been observed which could be because of local emergence of new STIs and/or import of strains from abroad. The same have been submitted to *Neisseria gonorrhoeae* Multi Antigen Sequence Typing (NG-AST) Website.

IMMUNOLOGY

Vision Foundation, Sankara Nethralaya, Chennai studied the influence and immunogenicity of human amniotic membrane (HAM) on the cultured corneal epithelial cells compared with other scaffolds like chitosan- chondritinsulphate (Ch:CS), chitosan-poly vinyl alcohol (Ch:PVA), fish scale collagen (FSC), contact lens and cell culture plate. They also investigated the spectrum of antimicrobial peptides, which are expressed constitutively in the cultured corneal cells. This study confirms human amniotic membrane to be the best scaffold in corneal cell transplantation.

A study conducted at Centre for DNA Fingerprinting and Diagnostics, Hyderabad, demonstrated that *M. tuberculosis* heat shock protein 60 (Mtbhsp60, Cpn60.1, Rv3417c) interacts with both TLR2 and TLR4 receptors, but its interaction with TLR2 leads to clathrin-dependent endocytosis resulting in an increased production of interleukin (IL)-10 and activated p38 Mitogen-activated protein kinases

(MAPK). Blockage of TLR2-mediated endocytosis inhibited IL-10 production but induced production of tumor necrosis factor (TNF)- α and activated ERK 1/2. In contrast, upon interaction with TLR4, Mtbhsp60 remained predominantly localized on the cell-surface due to poorer endocytosis of the protein that led to decreased IL-10 production and p38 MAPK activation. The data suggest that cellular localization of Mtbhsp60 upon interaction with TLRs dictates the type of polarization in the innate immune responses in macrophages.

A study was conducted at Christian Medical College, Vellore with aim to sequence the HIV-1 RT, protease, integrase [IN] gene from plasma of HIV-1 infected individuals and assess the frequency of mutations that leading to drug resistance namely, protease inhibitor (PI), nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside RT inhibitor (NNRTI) and integrase inhibitor (INI) in both treatment naïve HIV-1 infected population and individuals showing ART failure. It was observed that the transmitted drug resistance for PI inhibitor in the study population is 3.12% while that for the NNRTI and IN is 3.1% and 16.6% respectively. None of the treatment naïve individuals in the study population had mutation that can lead to NRTI drug resistance. Study also showed no significant increase in the frequency of mutations that can lead to transmittable drug resistance in the treatment naïve HIV infected south Indian population as compared to earlier published data from same population.

Taking lead from an earlier study conducted by National Institute for Research in Reproductive Health (NIRRH), Mumbai indicating Nisin as a naturally occurring antimicrobial peptide (AMP), as a promising vaginal microbicide. Since this product is intended for frequent use by women, therefore, the same investigators conducted a 3 yrs study to evaluate its effect on the vaginal mucosal innate immunity. Toll-like receptors such as TLR-7, TLR-9, NOD2 and RIG-1 were found to be expressed in human cervicovaginal epithelial cells (CVECs) (End1/E6E7). The study concluded that Nisin the AMP, plays an important role in vaginal innate immunity and attenuates TLR ligands induced cellular immune responses *in vitro*.

A study was undertaken at NIV, Pune to understand the immunopathogenesis associated with HEV infection that may help in an indigenous prospective vaccine development/treatment strategy. The elevated frequencies of true and effector Treg cells along with rise in IL-10 in acute hepatitis E patients suggested that Treg cells might be playing a pivotal role in HEV infection, elevated levels of IL-10 & TGF- β in acute patients and TGF- β in the recovered individuals might be responsible for the generation of new Tregs and for the elevation of Foxp3 at the m-RNA level. It was also noticed that the higher allele frequencies of HLA-DRB1*11 in the control population compared to the hepatitis E patients from Western India indicated its protective advantage towards HEV infection.

An immunological study conducted at Sher-i-Kashmir Institute of Medical Sciences, Srinagar, wherein the relationship between the levels of serum sIL-2R, sCD30, MICA and anti-HLA I & II antibodies with the rejection episodes in renal allograft recipients was investigated. The study reported that serum sIL-2R concentrations showed significant correlation with the acute rejection episodes in the renal allograft recipients. However, the data does not support the idea of serum sCD30 being used as a potential marker for the renal allograft rejection.

MYCOBACTERIAL DISEASES

Leprosy

A study on affected and treated persons in the slums of Kolkata was completed at GRCALTES, Kolkata. It revealed that handicapped women, old patients with reduced mobility tightly gripped by poverty and ignorance pray for an ongoing programme of basic social service support that meets their incidental health needs, services like clean water supply, food support and shelter.

A study carried out at CODWEL Nireekshana, Hyderabad evaluated the diagnostic value of high-resolution Ultrasonography (USG) and colour Doppler (CD) to assess the peripheral nerve trunks in leprosy patients with reactions. The results have established that Ultrasonography can be used as an additional tool for the diagnosis of leprosy and is especially useful in the diagnosis of pure neural leprosy. USG when combined with colour Doppler

studies is useful in identifying reaction and neuritis in the nerve and can point to the need to initiate corticosteroid therapy. USG in leprosy also has prognostic value in interpreting the effectiveness of the reaction treatment and also to assess the extent of nerve damage by measuring CSA and endoneural blood flow.

The Institute of Postgraduate Medical Education & Research & Seth Sukhlal Karnani Memorial Hospital, Kolkata studied the early detection of MDR – Leprosy by PCR based molecular technique analysis of the gene involved in resistance to drugs used in treatment of leprosy and characterization of mutation patterns. The study has demonstrated that the Multiplex PCR developed in PI's laboratory would help in early diagnosis of leprosy (untreated, treated, relapsed, reinfection, treatment failure) cases. It also demonstrated that the change in molecular pattern in the genes responsible for drug resistance may play a major role in the failure of Multi Drug Therapy (MDT).

The study on Clinicopathologic determinants in leprosy type 1 reaction (TIR) was completed at National Institute of Pathology (ICMR), Safdarjung hospital, New Delhi. The study aimed to define and validate key clinical parameters for early diagnosis of leprosy reversal reaction and evaluate clinicopathological discordance. Increased expression of CXCL10 has been demonstrated in serum of patients from type 1 reaction. Though the study does show a trend of increased expression at mRNA and protein level in type 1 reactions, additional studies are required before CXCL10 and its receptor CXCR3 can be assumed to be predictors of an impending type 1 reaction.

A longitudinal cohort study at the Blue Peter Research Centre, (Lepra India), Hyderabad and The Leprosy Mission Shahadara community hospital, Shahadara, Delhi showed that the household contacts with homozygous AA genotype are at higher risk for developing leprosy since the genotype frequencies of household contacts are almost similar to that of leprosy patients. The results suggested the potential use of Ag85A as a stimulant in *in vitro* tests with IL-17 and IL-23.

A study on evaluation of the bactericidal activity of new drugs and drug combinations in mice infected with *Mycobacterium leprae*

was completed at Karigari, Vellore district. The combination of Moxifloxacin with Clarithromycin and Minocycline showed clearing of organisms at Day 6 which was confirmed at Week 4 and Week 8. There is no significant difference in the results of Rifapentine (RFP) and Moxifloxacin given alone. The Moxifloaxcin combination appeared superior to WHO Multi Drug Therapy (MDT) at Week 4. In Rifampicin (RFP) – Sensitive strain 1, standard WHO MDT shows complete clearance of organisms at 104, 103, 102 dilutions by 24 wks. Moxifloxacin alone showed similar results but Moxifloxacin in combination with Clartithromycin and Minocycline showed one positive footpad at 24 wks at 102 dilution. Under subinoculation into Tr mice, WHO MDT gives good results. The combinations with RFP appear superior to those with RMP. Under subinoculation into Tr mice, combinations including Rifapentine appear to give better results.

A study on mechanism of regulation of T Cell responses in leprosy was completed at National Jalma Institute of Leprosy and other Mycobacterial Diseases, Agra. The results showed non responsiveness of BL/LL patients as there is no effect of anti-notch antibody on lymphoproliferation in these patients. Higher STAT6 expression and IL-4 in BL/LL patients to *M. leprae* antigen suggested Th2 type of response in these patients. Highest cAMP response element-binding (CREB) transcription factor expression was observed in healthy individuals which correlates with the IFN γ expression in these individuals. Increased expression of STAT4 and STAT6 transcription factors by CD4 cells was noted in response to Multilocus Sequence Analysis (MLSA) in BL/LL after 6 months of treatment suggesting both Th1/Th2 type of response in these patients after treatment.

A study on Current Knowledge, Attitude and Practices (KAP) about Leprosy among leprosy patients, their family members, service providers and general population: a comparative study between high prevalent & low prevalent districts of West Bengal was completed at Greater Calcutta leprosy treatment and health education scheme (GRECALTES), Kolkata. This cross sectional study conducted in 3 high prevalent and 3 low prevalent districts of West Bengal revealed that community

still have adverse belief and attitudes towards leprosy. KAP of low endemic districts were found much less (64% unaware) as that of high endemic one and more proportion of MB cases (35%) warranting stringent IEC at community level with appropriate channels of communication. A high proportion of patients with disability were found with 42.16% in high prevalence and 76% in low prevalence area. The study identified priority areas like prevention & appropriate management of the disability that needed urgent and timely attention.

A study on Role of Foxp3+ Regulatory T Cells in Polarized Immunity among leprosy patients was completed at All India Institute of Medical Sciences, New Delhi. The study aimed to study the role of regulatory T cells in polarized forms of leprosy and to phenotype these T cells in the peripheral blood of leprosy patients and also to identify them at the disease sites. The increased frequency of Treg cells and heightened levels of suppressive cytokines like IL-10 produced by these cells indicate that they suppress the effector cells function through contact independent manner leading to immune compensation of the host. Elevated levels of negative regulatory molecules like PD-1 and its ligands like PDL-1 suggests its role in host immune compensation in lepromatous cases of leprosy.

A study on Gene Expression Profiling in Multicase Leprosy Families: the Susceptibility Gene Expression through General Proteomics Approach was completed at Blue Peter Research Centre, (Lepra India), Hyderabad. The study aimed to define the affected and unaffected multi cases in terms of the polymorphisms observed among Indians in the regions implicated in disease vulnerability *i.e.* that these cases are distinct or otherwise. The susceptibility for disease was associated to genotypes for both leprosy and household contacts.

A study on Methods to Enhance Voluntary Early Reporting (Ever) at Primary Health Center (PHC) & Other Integrated Settings was completed at The Leprosy Mission, Purulia, West Bengal. The results indicated that active interventions like local private practitioners awareness in school children, self detection and healthy contact examination by patients enable early detection.

A study on Development of a Population Based Leprosy Registry (PBLR) & New Case Detection Strategies (NCDS) to Eradicate Leprosy in Urban Areas was completed at The Leprosy Mission Hospital, Shahadara, Delhi. The study aimed to identify and test new case detection strategies in a variety of urban settings, develop a population-based leprosy registry and recommend effective IEC materials to promote early reporting and regularity. A number of leprosy patients were detected by different methods. Surveys proved to be difficult as communities did not extend full cooperation either during house to house survey or healthy contact survey. Awareness booths were found to be one of the more effective and innovative methods of cases detection.

A study completed at The Leprosy Mission, NOIDA. The study aimed to determine the extent and factors associated with non adherence to MDT, to develop and test innovative community-based or patient-driven strategies, to promote successful completion of MDT, to evaluate the interventions and formulate recommendations/ operational guidelines. Out of a total of 3579 new cases registered in the four centres, 1944 are defaulters *i.e.* 54.3% which center wise varies from 66% to 44%. Defaulter rate among total MB (55.7%) varies from that of PB (50.6%). Defaulter rates among MB show comparatively a higher trend than that of PB in all the 4 centres. The results showed that disability has diverse influence on MDT compliance of the patients in different centres, probably depending on the local cultural values. The study also highlights the counseling needs of the patients to avoid defaulting.

Tuberculosis

A study on comparing the efficacy of Yoga and pulmonary rehabilitation on dyspnoea, muscle strength, inflammatory markers and quality of life was completed at the All India Institute of Medical Sciences, New Delhi. It was found that Yoga is simple, cost effective and acceptable method to improve dyspnoea and quality of life in COPD.

A study on molecular analysis of MDR and XDR strains of *Mycobacterium tuberculosis* was completed at National Institute of Tuberculosis & Respiratory Diseases and All India Institute of Medical Sciences, Delhi. The study aimed to

evaluate the population structure among multidrug resistant *M. tuberculosis* strains isolated from TB patients in various regions of Delhi state using spoligotyping. The data obtained shows an almost similar population structure of MDR strains in different regions of Delhi. SIT 26 and SIT 1 were responsible for approximately 50% of burden of MDR-TB in Delhi, suggesting an enhanced ability of these strains to transmit.

A study on understanding the mechanism of action of Histidinol dehydrogenase from *M. tuberculosis* was completed at National Institute of Immunology, Delhi. The study aimed to make expression construct of Histidinol Dehydrogenase (HD, HisD) of *M. tuberculosis* in various expression vectors using recombinant DNA technology. The study has resulted in standardization of a protocol to over-express milligram quantities of three (HisB, HisD and HisC), of the 11 enzymes involved in making histidine, in *M. smegmatis* expression system, understanding their kinetics, and elucidation of the 3D structures of HisB and HisC may aid in designing small molecule inhibitors against these enzymes.

A study on the effect of physiotherapy in preventing and reducing the chest infection and other chest disease in tea garden workers in West Bengal was completed at All India Institute of Medical Sciences, Delhi. The study aimed to evaluate the effects of physiotherapy in preventing and reducing chest infections and other related chest diseases in the Tea Garden workers of West Bengal. Out of 450 workers, 150 were tea leave pluckers with chest complaints (Group A), 150 were tea leave pluckers without chest complaints (Group B), 100 were workers, who were not involved in tea leave plucking job, but working in and around Tea Garden (Group C) and 50 were tea leave pluckers were without chest pain (Group D i.e control group). Intervention showed significant improvement in all the groups as compared to control group.

A study completed at the All India Institute of Medical Sciences, Delhi aimed to identify the signature immune deficit that tightly correlates with the development of MDR-TB and treatment failure cases among HIV+ persons. There was increased frequency of regulatory T cells among HIV-TB co-infected patients. High Treg cell frequency

tightly correlated with significantly reduced *M. tuberculosis* specific IFN- γ response. This suggests that the suppressed state of immunity may lead to development of multi-drug resistance among HIV-TB co infected patients.

A study on drug resistance among sputum positive tuberculosis patients in Rayagada district of Odisha was completed at Regional Medical Research Centre, Bhubaneswar. The study aimed to estimate the prevalence of drug resistance among sputum positive tuberculosis patients in Rayagada district, Odisha. This study provided the much needed drug resistance information during the start of PMDT services at Odisha with 57% tribal population after a gap of 10 years from the first study in Mayurbhanj district in 2000-2001. The low prevalence of drug resistance observed in this area is a good indicator of RNTCP programme being implemented in the Rayagada district. The study helped to establish the RMRC Tuberculosis laboratory as RNTCP approved Tuberculosis Drug Susceptibility Testing Laboratory for Conventional Culture based DST and led to recognition of RMRC, Bhubaneswar Tuberculosis laboratory as National Reference Laboratory for Tuberculosis by Central TB Division, Ministry of Health and Family Welfare, Govt. of India.

A study completed at the All India Institute of Medical Sciences, Delhi has revealed that chronic obstructive pulmonary disease (COPD) is associated with increased inflammatory activity compared to smokers without COPD and non smoking healthy persons. The study also showed that COPD *per se* is associated with increased systemic inflammatory response. The etiological agent for COPD, *i.e.* tobacco or non-tobacco related causes, however, does not seem to be an important factor in affecting the degree of systemic inflammation.

A study on clinical and molecular characterization of multi drug resistant (MDR) isolates of *M. tuberculosis* complex from extrapulmonary tuberculosis (EPTB) cases in two tertiary care hospitals in north India was completed at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. The aim of the study was to know the prevalence of *M. tuberculosis* complex, anti-tuberculosis drug resistance patterns, molecular characterization of MDR and its epidemiological

pattern among EPTB cases in Northern India. About 70.2% of the isolates were identified as *M. tuberculosis* complex by NAP Test and 100% agreement with IS6110-PCR. It was observed that 59.6% (210) isolates were identified as fully susceptible strains and 10.5, 14.2, 8.5, 7.1 and 12.7% were single drug resistant, two drug resistant, three drug resistant, four drug resistant and MDR strains. The study indicated that the prevalence of MDR-TB (12.7%) among EPTB has been increasing and an alarming level in Northern India.

A study on functional analysis of Mce4A and Mce1A proteins of *M. tuberculosis*: role in cholesterol transport and phago-lysosome fusion inside macrophages was completed at the VP Chest Institute, Delhi. The study aimed to explore the possible cholesterol binding activity of the purified recombinant Mce (Mce1A and Mce4A) proteins of *M. tuberculosis*. The study concluded that mce4A gene product may be manipulating the host calcium concentration for a continuous supply of cholesterol as well as inhibiting phagolysosome fusion by affecting the intracellular calcium levels.

A study on S110 gene variants in defining susceptibility to tuberculosis in North Indians was completed at the VP Chest Institute, University of Delhi. In this study SP110 variants were probed in pulmonary (PTB) and lymph node tuberculosis (LNTB) cases to explore their role in controlling susceptibility to *M. tuberculosis* infection in north Indians. Twenty-four SP110 variants in about 210 north Indian tuberculosis cases and 140 ethnicity matched controls were genotyped. No significant association was observed between SP110 variants and PTB. Evidence of association of SP110 variants was found with LNTB, a form of extra-pulmonary tuberculosis at three loci namely rs6436915, rs1427294 and rs1346311. The results provided evidence that SP110 may be a risk determinant locus in LNTB while confirming a doubtful role of SP110 in PTB in north Indians. The present report for the first time has shown association of SP110 variants with lymph node TB.

The study on drug resistance profiling and molecular typing of *M. tuberculosis* isolates from different community settings in North Delhi was completed at VP Chest Institute, Delhi. The present

study validated the use of repetitive elements for molecular typing of *M. tuberculosis* in an Indian setting, and further underlines the usefulness of MIRU-VNTRs for discriminating low IS6110 copy isolates, which accounted for more than 1/9th of the strains in the present study. Spoligotyping, revealed 49 SIT patterns. Of these, 7 SITs were newly created which suggests a possible introduction of new genotypes due to casual contacts and/or increased international travel. The findings also revealed that there was transmission of infection between members of the same family, however, reemphasizes the importance of including education of patients for effective TB control. No increased incidence of improper treatment or cure of the patients under study was found.

A study on investigative workup of smear negative cases of Tuberculosis in HIV patients and antibiogram of Mycobacterial isolates was completed at JSS Medical College, Mysore. In this study on TB in HIV patients, antibiogram of mycobacterial isolates was carried out. Out of 162 patients having features of TB and HIV reactive, 76 mycobacterial isolates were obtained. Of these 69 were typical and 7 were atypical. Out of these 5 were MDR and 26 resistant to atleast single drug. Antibiogram of these isolates when compared showed that the MDR rate [5(6.6%), 2 (6.7%), 4 (8%)] did not differ much. But resistance to atleast single drug was 26 (34.2%) in HIV reactive and AFB smear negative patients, 3(10%) in HIV reactive and AFB smear positive patients and 8 (16%) in HIV negative and AFB smear positive patients. The study concluded that culture of specimens for Mycobacteria is more sensitive in detecting more TB cases in HIV reactive patients. Antibiotic susceptibility tests showed that HIV does not in any way influence the drug susceptibility pattern.

NATIONAL CULTURE COLLECTION OF PATHOGENIC FUNGI

The ICMR has funded the establishment of National Culture Collection of Pathogenic Fungi at Department of Medical Microbiology, PGIMER, Chandigarh from 2009-2014 with aim to preserve, identify and supply of medically important fungi to different Institutes and researchers. The website of the culture collection (www.nccpf.com) is functional since last year. At present the details of 2604

fungal strains maintained in collection have been catalogued and available in public domain. The facility received 4,215 isolates from 128 referrals across the country and few from the neighbouring countries for identification and preservation. Since inception more than 100 rare fungi causing human infections have been identified to help managing such patients and to understand the ecology of those fungi (the list of those rare fungi is given below). Few fungi were reported for the first time to cause human infections like *Thamnotylum lucknowense*, *Rhizopus homothallicus*, etc. The facility provided 390 standard fungal strains to 41 different centres for teaching and research purpose. The facility is also running EQAS (External Quality assurance scheme) in medical Mycology. At present 52 Centres from India and neighbouring countries are enrolled in the EQAS program.

OTHER MICROBIAL INFECTIONS

The ICMR) and the Public Health Foundation India (PHFI) with the London School of Hygiene & Tropical Medicine (LSHTM) organized a workshop on “Pathogen Diversity : Exploiting Pathogen Genetics for New Control Strategies”. The scientific deliberations led to recommendations to take initiatives on (i) methods of pathogen identification, (ii) challenges for vaccine, drug and diagnostic development and (iii) surveillance and monitoring.

A study was carried out to assess the applicability of quantitative PCR assay for the differentiation between *Pneumocystis jirovecii* pneumonia (PCP) and colonization among immunocompromised patients. The qPCR assay exhibited high accuracy to distinguish between definite PCP and colonized PCP patients. In another study carried out in Aravind Eye Hospital, Coimbatore, multiplex PCR was used to facilitate early diagnosis of *Fusarium* and *Aspergillus* spp. on corneal scrapings.

A study carried out at the All India Institute of Medical Sciences, evaluated the Roche Cobas Taqman PCR with that of in house reverse transcriptase PCR (IHRT-PCR) and found that IHRT-PCR gave better results in detection of *Chlamydia trachomatis* infections. This test facilitates timely diagnosis of *C. trachomatis* which is associated with many cases of infertility in women in India.

A study carried out at All India Institute of Medical Sciences, Delhi attempted Multilocus sequence typing (MLST) on the prevalent strains of *Clostridium difficile* and *Clostridium perfringens* causing antibiotic associated diarrhea. The MLST has been found as a promising new technique to determine genetic relatedness of organisms where as Pulsed field gel electrophoresis (PFGE) is difficult and requires expertise.

A study carried out in Uttar Pradesh highlighted the need to carry out extensive research on *L. monocytogenes* with special reference to its habitat such as water, food and clinical samples in order to understand its further transmission route in the environment and resistance to antibiotics. Isolates of *L. monocytogenes* recovered from clinical and environmental samples belonging to 4b, 4d, 4e or 1/2c, 3c or 1/2a, 3a or 1/2b, 3b, 7 serogroups exhibited multiple antibiotic resistance and showed the presence of variable virulence genes. Most of the isolates were resistant to the ampicillin, sulphamethoxazole/trimethoprim and rifampicin and sensitive to ciprofloxacin, streptomycin, chloramphenicol and ceftiofur.

The SGPGI, Lucknow studied the differential cytokin expression in patients with brain abscess and concluded that Th1 cytokines are significantly elevated in patients with Gram positive and mixed infections. Patients with Gram negative infections showed high levels of regulatory cytokines. These can be used as potential biomarkers of infections in brain.

- One hundred and thirty two *Staphylococcus epidermidis* isolates from various device related infections such as endophthalmitis following intra ocular lens (IOL) implantation, IV catheter related sepsis and orthopedic implant infections were studied for slime production, biotyping, antibiotic sensitivity and mec A & ica AB positivity by the recommended procedures. icaAB and mec A were found to be the two important virulence markers of *S. epidermidis* in implant infections. Slime production was an essential component for the development of biofilms as evidenced by inhibition of adherence by slime antibody.

PROJECTS IN NORTH EAST REGION

In a study at the National Institute of Malaria Research, Field Unit at Guwahati to map malaria risks and prioritizing interventions in Tripura state. Out of 15 different anopheline mosquito species, *An. minimus* and *An. baimaii* were found to be abundant and were observed to be fully susceptible to DDT. Molecular identification techniques confirmed that prevalent *An. minimus* is indeed *An. minimus sensu-stricto* (species A) and *An. baimaii* is the only species of the *An. dirus* complex that is abundant in northeast India. The study established that DDT is still effective and should remain the insecticide of choice for control of mosquito vector populations specific to Tripura.

The Govt. of Assam initiated an adult Japanese encephalitis vaccination programme on a pilot basis in Sivasagar district of Assam. A study carried out by the Regional Medical Research Centre, Dibrugarh, Assam evaluated safety and immunogenicity of single dose of live attenuated JE vaccine SA 14-14-2 in adults. The study documented safety of the vaccine in both seropositive and seronegative adult population. The study also showed immunogenicity of the vaccine in seronegative adults (86% of the 90 seronegative individuals were seroprotected) with fourfold rise to about 60% level in seronegatives and moderately seropositive adults. In view of safety and sustained protection, immunization with this vaccine may be extended to all populations at risk in the JE endemic areas.

National Institute of Malaria Research, Delhi studied the biodiversity of anopheline fauna in 26 districts of North-Eastern States of India. Six surveys, three in pre-monsoon and other three in post-monsoon season were carried out in 8 districts of Assam; 2 districts of Meghalaya, 2 districts of Manipur and 4 districts of Sikkim. The result of these surveys clearly indicated lot of ecological changes in the study area over the time. The species like *An. crawfordi*, *An. jeyporiensis*, *An. pallidus* and *An. ramsayi*, etc. which were recorded earlier were absent during these surveys. It has also been observed that *An. minimus* and *An. dirus* which were the main vector species for north eastern states were gradually reducing in number whereas other vector species viz. *An. culicifacies* and *An. fluviatilis* were established and found in higher density. Apart from

An. theobaldi, *An. pseudojamesii*, *An. hyrcanus*, *An. pseudowillimori*, *An. ramsayi* which were not found in the present survey, some new species like *An. vagus*, *An. nivipes*, *An. subpictus*, *An. barbirostris* and *An. jamesii* were introduced to the environment due to accelerated developmental activities in each district of Assam, Meghalaya, Manipur and Sikkim. *An. culicifacies* is incriminated as main vector in North-Eastern states of India.

A study was carried out at Agartala Medical College, Agartala, Tripura in collaboration with Institute of Postgraduate Medical Education & Research, Kolkata to assess the prevalence of Hepatitis B virus infection in different population groups of Tripura. The study revealed the presence of 3 distinct HBV genotypes D, C and A among the chronic carriers of Tripura of which the genotype D is most prevalent. Five sub genotypes of HBV/D, D1-D5 were found to be present in Tripura and strikingly, for the first time, the presence of D4 was detected, which has not been reported so far from any other part of this country.

REPOSITORIES

National Gastrointestinal Tract Pathogenic Repository at NICED, Kolkata : The Gastrointestinal Tract Pathogens Repository (GTPR) as established at National Institute of Cholera and Enteric Diseases (NICED), Kolkata has developed a webpage (www.gtpr.org.in) which has been linked to ICMR HQ webpage (www.icmr.nic.in). The main activity of GTPR includes identification, phenotypic and genetic characterization and also archiving of gastrointestinal pathogens of bacterial origin. At present, 169 strains (*Vibrio cholerae*, diarrhoeagenic *Escherichia coli* and *Salmonella* spp.) have been displayed on the webpage and these strains are ready for sharing for retrospective analysis and can also be used for imparting hands-on training on isolation and identification of pathogenic strains at various medical colleges and other research organizations. Archiving of other enteropathogens including *Shigella* spp., *Aeromonas* spp, and *Helicobacter pylori* strains will also be made. Strains of *V. cholerae*, *Salmonella* spp. diarrhoeagenic *E. coli* have been provided to five different Institutes located at Bhabnagar, Gujrat; Salt Lake, Kolkata; Bhubaneswar, Odisha; Panampilly, Kochi and Jadavpur, Kolkata.

Malaria Parasite Bank at NIMR , New Delhi

The Malaria Parasite Bank (MPB) functioning as a National Resource facility is involved in the collection of field isolates and has variety of human and non-human plasmodia. A total of 56 isolates including 16 *P.vivax* and 40 *P.falciparum* were collected during 2013-14 from Chhattisgarh, Karnataka and Jharkhand states. All these parasites are cryopreserved in liquid nitrogen at Malaria Parasite Bank which is National Facility of country. Till now a total of 1309 isolates of human malaria parasites (*P. falciparum.*, *P. vivax.* and *P. malariae*) were collected and preserved in parasite bank. A total of 80 major research organizations/universities were utilizing the facility of malaria parasite bank.

TRANSLATIONAL RESEARCH

An improved and novel process for yielding Thrombinase, a blood clot dissolving enzyme (fibrinolytic enzyme) from a *Bacillus sphaericus* (Strain No. NRRL B18949) was developed at Vector Control Research Centre, Puducherry. The improved method resulted in the decrease in the number of steps and time involved in purification of Thrombinase and a marginal increase in the yield. The scaled up process further reduced the fermentation time significantly.

The Vector Control Research Centre, Puducherry has carried out a study on “an aqueous suspension formulation of *Bacillus subtilis* subsp. *subtilis* (VCRC B471) for use against the pupal stages of mosquitoes”. This medium was found to enhance the production of the metabolite by 5 times when compared to the conventional medium. Maximum production of metabolite was observed by 72 hrs (2.2g/lit). The metabolite was found to be safe to non-target organisms found in association with mosquito immature in aquatic habitats. The invention has been submitted to the ICMR for filing of patent.

TRIBAL HEALTH

A study on genetic diversity of HLA in the two tribal groups of north Madhya Pradesh: association with pulmonary Tuberculosis was completed at Jiwaji University, Gwalior and All India Institute of Medical Sciences, New Delhi. The study aimed

to determine the HLA class I and class II allelic distribution at the DNA level in patients with pulmonary tuberculosis and correlate the same with pulmonary tuberculosis. The three locus HLA-A-B-DR haplotype analysis revealed significantly increased frequency of A*24-B*40-DRB1*15 haplotype among patients than controls. However, none of the other studied class I and class II alleles were found associated with TB in Sahariya tribe. In the absence of statistically acceptable number of Bhil TB patients, a comparison of Sahariya and Bhil controls was made, which showed significantly high frequency of HLA-A*01, HLA-B*35, HLA-B*52, HLA-DRB1*07 and HLA-DRB1*10 in Bhils, while for HLA-A*02, HLA-B *15, HLA-B*40, it is vice versa. These allelic variations seem to reflect distinct genetic differences in resistance or susceptibility to *M. tuberculosis* in Sahariya and Bhil.

A study on genetic polymorphism in *M. tuberculosis* isolates from Tuberculosis patients from Sahariya Tribe of North Central India” was carried out at Jiwaji University, Gwalior and National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra with the objectives of identification of mycobacterial strains from pulmonary TB patients of Sahariya tribe of Madhya Pradesh. Drug sensitivity profile of *M. tuberculosis* isolates from Sahariya tribe as well as non-tribal population revealed a high percentage (N=19, 15%) of multi drug resistant tuberculosis in tribal community, whereas, only 6 isolates were found to have MDR-TB among non-tribal population. Identification of *M. tuberculosis* isolates through spoligotyping and MIRU-VNTR typing grouped 178 isolates in 7 clusters, whereas, 42 isolates were found to be orphan, not present in international database. East African Indian (EAI3_IND) and Central Asian (CAS1_Delhi) strains were observed as major spoligotypes among both the populations.

A study on molecular characterization of diurnally subperiodic *Wuchereria bancrofti* causing Lymphatic Filariasis among the tribes of Nancowry, Andaman & Nicobar islands was completed at Regional Medical Research Centre, Port Blair. A total of 602 individuals were screened from the total population of 2869, accounting for 21% of the population. Out of 11 villages surveyed, 5 villages

were observed to have nil Mf in the individuals screened. The age wise prevalence showed that more microfilaremic cases are observed in the age group of 41 – 50 years.

A study on etiology of diarrhea in three tribal districts of Orissa was completed at the Regional Medical Research Centre, Bhubaneswar. The objectives of the study were phenotypic characterization of common enteric bacteria and to find the correlation between clinical isolates of *Vibrio cholerae* by different molecular techniques for detection of biotypes (tcpA-clasical/ EL TOR) serotype (oi/OI39) virulence (ctxA) and regulatory genes (toxR). The *V. cholerae* isolated from diarrhoea patients of four blocks were uniformly sensitive to tetracycline, ofloxacin, doxycycline and resistant to ampicillin, nalidixic acid, furazolidone, streptomycin, erythromycin, co-trimoxazole and polymixin-B. The *Shigella* spp. were uniformly sensitive to tetracycline, azithromycin, streptomycin. The *V. cholerae* were isolated during rainy and post rainy seasons, whereas *Shigella* spp. were more isolated during winter and summer months.

A study on evaluation of Biomarkers to assess malaria severity due to both *Plasmodium vivax* and *P. falciparum* was completed at RMRCT, Jabalpur. Coma, hemolysis, abnormal bleeding, respiratory distress, acute renal failure and multiple complications were significantly associated with mortality. Cytokines were tested in 300 samples. In a pool of 72 CM cases (25 died) combinational biomarkers (IL-1ra, sICAM-1 and MIF) were 88% sensitive and 70% specific to predict bad prognosis of the patients at admission. Analysis of axonal damage marker (Tau) revealed significantly higher percent detection (indicates neuronal damage) in all three severe malaria groups compared to mild and moderately severe groups.

The RMRC, Port Blair studied the indigenous plants, seaweeds and sponges traditionally used by the tribal community of Andaman & Nicobar Islands. Extracts of 36 plants were studied for anti-microbial activity and 21 were found to show anti-microbial activity. Fifteen marine sponge extracts and six seaweed extracts were also tested and seven sponge extracts and all the six seaweed extracts were found to show anti-microbial activity. Six plant extracts and five extracts from marine sponges

(direct or secondary metabolites from associated bacteria) showed activity against malaria parasite.

VECTOR BORNE DISEASES & SCIENCE FORUM

Bio-ecology of *Aedes aegypti* and *Ae. albopictus* vector of dengue and chikungunya in Andaman & Nicobar islands with special reference to its invasion into human habitation was studied at Regional Medical Research Center (ICMR), Port Blair, Andaman and Nicobar Islands. The study aimed to map the distribution of *Ae. aegypti* and *Ae. albopictus*, study their larval ecology and assess their status as vectors. The study was carried out in the three tehsils of South Andaman district, Port Blair, Ferrargunj and Little Andaman. In the 2422 households inspected for *Aedes* breeding, in 747 households 1021 containers out of 17314 examined were found to have *Aedes* breeding. 42 different types of manmade, natural and artificial water habitats were encountered. 28 species of mosquitoes were identified from the pupae collected. The pupa indices were 251.6, 132.1 and 198.6 respectively in Port Blair, Ferrargunj and in Little Andaman Tehsils. In Port Blair & Ferrargunj tehsils both *Ae. aegypti* and *Ae. albopictus* were found with the predominance of *Ae. albopictus*, while in Little Andaman *Ae. aegypti* was totally absent. The predominant container types supporting breeding were plastic drums followed by cement tanks, metal drums, tree holes, coconut shells, plastic cans, tin cans followed by discarded tires, plastic containers, plastic buckets, metal buckets, metal pots, flower vases, leaf axils etc. *Ae. aegypti* breeding was found more indoors than outdoors. Tehsil-wise analysis identified plastic drums as the predominant containers supporting *Ae. aegypti* breeding in Port Blair, and cement tanks in Ferrargunj, Tree holes formed the predominant breeding habitat to support *Ae. albopictus* breeding in Little Andaman Tehsil and Ferrargunj Tehsil. This study has shown that *Ae. albopictus* is exhibiting the phenomenon of invasion into human inhabitation in South Andaman district. 186 pools of *Ae. aegypti*, and 174 pools of *Ae. albopictus*, tested were negative for chikungunya and dengue virus infections.

Studies on *Aedes* mosquitoes to determine vectors of Dengue and Chikungunya virus in Alappuzha and Kottayam districts of Kerala was carried out

by the Kerala Field Unit of the National Institute of virology, Alappuzha, Kerala. The objective of the study was to identify/stratify the most prevalent and productive larval breeding habitats of *Ae. albopictus* and *Ae. aegypti*, and study their distribution in wet and dry seasons and to detect viruses by cell culture and RT-PCR. In Kottayam 484 (13.21%) containers were positive out of 3921 containers searched in 889 houses. During dry season 28% of houses and 11% wet containers were positive, while in the wet season 38.67% houses and 14.76% wet containers were positive. The pupa index was found to be 395 during the month of July. The overall indices were higher in wet season than the dry season. In Alappuzha district, a total of 413 (11.08%) containers were found positive for larvae out of 3770 containers searched in 1010 houses. During the dry season 24% of houses and 11.17% wet containers were positive, while in wet season 29.72% houses and 12.09% wet containers were positive. The two most pupal productive containers were discarded plastic waste contributing to 26.33%, and plastic sheet to 30.82% of the total pupae collected. A total of 243 pools were assayed by RT-PCR, 5 (12.7%) pools of *Ae. albopictus* was found positives for Dengue virus type-2 (2%) virus in Kottayam district and none of the mosquitoes pools were in Alappuzha district. The three most productive containers cement tank, discarded plastic waste, and plastic sheet yielded 80% of total pupae. Targeting these most common containers would bring about a significant reduction in pupae production.

A study was carried out at Field Unit of the National Institute of Malaria Research (ICMR), Guwahati, Assam to ascertain the seasonal abundance of *Aedes albopictus* and *Ae. aegypti* in Guwahati metropolis and in its suburban settlements and to characterize species-specific breeding habitats in various ecotypes. Surveys were conducted in various localities of Guwahati city (urban) and Sonapur block (semi-urban) during January 2013 till October 2013. Based on monthly breeding surveys, it was observed that 82% (2481/3013) of the containers searched were dry during winter season (January–March), and those recorded wet were devoid of mosquito breeding. During months of rainfall (April–October), 32% (1872/5793) of containers searched were wet of which good

number was positive for *Aedes* mosquito breeding. *Ae. albopictus* was observed breeding in tyres, cut bamboo stumps, tin/plastic containers, flower vases and leaf axils both in urban and semi-urban areas. However, it was the predominant mosquito species in semi-urban areas breeding preferentially in flower vases, cut bamboo stumps and leaf axils. *Ae. aegypti* instead was the most common species in urban areas breeding predominantly in discarded tyres; other breeding sources included tin/plastic containers. It was observed that both *Ae. aegypti* and *Ae. albopictus* were resistant to DDT (4%), but fully susceptible to malathion (5%). However, both these species exhibited varied response to pyrethroids (deltamethrin and permethrin).

Mapping of *Ae. aegypti* and *Ae. albopictus* mosquito distribution in Pune and identifying breeding sites using entomological (larval & pupal) indices was carried out at the National Institute of Virology, Pune. The objectives of the study were mapping of *Ae. aegypti* distribution in Pune, detection of subspecies in *Aedes* group, and detection and isolation of dengue/chikungunya viruses. The study was conducted in Pune District, which includes Pune Municipal Corporation (PMC), Pimpri-Chinchwad Municipal Corporation (PCMC) and the associated rural areas. Vector breeding sites were geo-referenced using hand-held Global Positioning System (GPS) recorder. *Ae. aegypti* breeding was encountered in all the dengue virus affected areas. Species that encountered were *Ae. aegypti*, *Ae. albopictus*, *Ae. vittatus*, *Ae. walbus* and *Ae. novalbopictus* (a member of *Ae. albopictus* sub-group). The survey revealed that *Ae. aegypti* breeding was predominant in outdoor breeding situations, and plastic containers (drum Shape) with 200-300 liters volume capacity were the principal breeding habitat.

Study on immunological determinants at the lesional site of patients with post Kala azar dermal leishmaniasis was carried out at the Institute of Postgraduate Medical Education & Research, Kolkata. The objectives of the study were to evaluate the status of T cell subsets at the lesional sites in patients with PKDL at disease presentation and after completion of treatment and correlate status of cytokines within T cell subsets (including T regulatory cells) at the lesional site in patients

with PKDL at disease presentation and after completion of treatment. The levels of IgM and IgG in polymorphic PKDL cases were higher than in macular PKDL cases, while significant curtailment in levels of IgM and IgG following the treatment was evident only in polymorphic PKDL cases. The dermal infiltrate comprised primarily of CD8, CD68 and CD20 cells which correlated with parasite burden and disease duration. There was a notable near absence of CD4 lymphocytes concomitant with decreased CD1a Langerhans cells. Treatment significantly decreased the proportion of CD8, CD68 and CD20 cells along with an increment in CD1a cells.

A study was carried out at Vector Control Research Centre (ICMR), Puducherry to establish a national network of researchers and programme managers interested in the genotyping of *Wuchereria bancrofti* and *Brugia malayi* prevailing in different endemic areas and to determine the frequency of alleles of different genes (B tubulin, Alt2 and ITS2 region of rDNA) among *W. bancrofti* parasite populations in different parts of the country. A National Network for genotyping of major lymphatic filarial parasite, *W. bancrofti* has been established involving RMRCs (Bhubaneswar and Dibrugarh), CRME Madurai, Anna University, Chennai and MGIMS Sewagram, with NVBDCP and VCRC as coordinating Centres. Genotyping of *W. bancrofti*, based on 29 bp tandem repeat sequence of Alt2 gene revealed the occurrence of two to three sub-populations, in different endemic regions of India. This network will be useful for monitoring the genetic divergence of the parasite due to long term chemotherapy programme (GPELF - Global Programme for Elimination of Lymphatic Filariasis) launched in the country, as well as globally.

VIRAL DISEASES

Laboratory Containment of Wild Polioviruses

Phase I of the activities under the Laboratory Containment of Wild Polioviruses in India (laboratory survey and preparation of the inventory) was completed. The final national Inventory of laboratories storing wild poliovirus and/or potentially infectious materials includes 50 institutions. One laboratory has wild poliovirus material and the remaining 49 store potentially

wild virus infected materials. A total of 71469 organizations were listed from 35 States and Union Territories which included 27091 diagnostic pathology labs; 14818 labs situated in hospitals/dispensaries, 4351 in clinics/dispensaries, 3312 institutes/colleges, 19812 in PHCs/CHCs/health posts, 315 in research centres, 652 in pharma companies. 1118 records were found not relevant to biomedical laboratories survey. There were 1072 laboratories with -200C or lower temperature freezers. Laboratories/organizations storing vaccines, reagents and/or human blood/serum were classified as no risk laboratories. 251 laboratories were investigated further to understand the risk. Based on telephonic inquiry, website check and publications 111 labs were found to not store any poliovirus or potentially infectious material. Information of the remaining 140 laboratories was validated by site visits. 63 laboratories were validated by NPSP and 85 by independent experts invited by the task force. 8 laboratories got validated by NPSP as well as independent experts. Report of completion of first phase of the Lab Containment activity was submitted to the South East Asia Regional Certification Committee for Polio Eradication and India was declared free of polio. Phase II activities have been initiated.

Acute Encephalitis Syndrome (AES)

A Research cum Intervention project on AES/JE was launched for prevention, case management and rehabilitation measures for prevention and control of JE/AES in Gorakhpur. This programme has been recommended by a Group of Ministers under the Chairmanship of Health and Family Welfare Minister with active involvement of various Ministries viz: Health and Family Welfare, Social Justice and Empowerment, Rural Development, Urban Development, Drinking Water Supply and Sanitation and Women and Child Development. ICMR initiated the project on 1st November 2012 involving seven ICMR Institutes viz: NIV, Pune and its field unit at Gorakhpur, VCRC, Puducherry, CRME, Madurai, EVRC, Mumbai, NIE, Chennai, NIMR Field unit at Bengaluru and NARI, Pune in 5 highly endemic blocks of Gorakhpur for AES/JE (Bhathat, Khorabar, Chargaon, Belghat and Campierganj) with one control block with low incidence of JE/AES in Deoria (Mazhgaon block).

The salient achievements are as follows:

- A baseline data on vector ecology and bionomics has been generated. *Cx. Tritaeniorhynchus* was found in abundance in the month of March and then from July to October in 2013. Based on this data, now vector control interventions will be implemented.
- The JE vaccination conducted in 5 districts of eastern UP ranged from 36% in Kushinagar to 66 % in Gorakhpur. The main reason for non vaccination at all sites has been found to be the unawareness of the mother about the JE vaccine (70%).
- During the month of Nov 2013 *Gambusia* was released in 78 wells and 54 ponds but in December the fish was detected only in 18 wells and 12 ponds. 20,000 *Gambusia* fish were relocated from ponds which were drying and 3700 *Guppies* were released in wells. In all the wells where fish were not found were filled with garbage. The State Govt has been suggested to cover these wells with the slabs for prevention of mosquito breeding.
- The modules for socio behavioral studies have been formulated. The study will focus on hygiene practices in selected 5 blocks and increase in awareness of community towards improvement of personal hygiene, consumption of safe drinking water and to improve compliance by sensitizing the mothers especially towards the benefits of the vaccine.

A study was completed at the Maulana Azad Medical College, New Delhi. The study focused on detection of serum level of IL-8 in DF/DHF patients and its correlation with different dengue virus serotypes. Higher IL-8 levels were observed in dengue patients than healthy controls. Among dengue patients, IL-8 levels were significantly higher in DHF patients as compared to dengue fever patients. These results suggest that IL-8 might play a vital role in the pathogenesis of dengue infection. During the study, Den-1 and Den-2 serotypes were detected, with predominance of Den-1.

In a study at the Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. The

samples of urine and plasma of 50 renal transplant recipients and their donors were examined for the presence of BKV-virus. The result showed that early BKV infection in kidney transplant recipients is usually of donor origin and recipients who are at high risk of BKV infection and nephropathy based on donor and recipient characteristics should be monitored closely and considered for judicious reduction of immunosuppressive regimens.

A study conducted at the Post Graduate Institute of Medical Education & Research, Chandigarh aimed at investigating the role of toll like receptors (TLR 2, 3, 4, 7 and 8) and Hepatitis E virus (HEV) viral load in the pathogenesis of acute and fulminant HEV infection. A total of 748 clinically suspected viral hepatitis patients were enrolled in the study of which 117 (15.64%) were found to be positive for anti-HEV IgM and/or HEV RNA. A total of 80 patients fulfilling the inclusion criteria were further stratified based on clinical features and biochemical parameters into acute self limiting viral hepatitis (AVH) and acute liver failure (ALF). It was found that the low CMI response, lower level of TLR3 expression, IFN γ production, and low viral load play an important role in the fulminant course of the disease.

In a study at the Sankara Nethralya, Chennai a total of 180 peripheral blood specimens were collected from clinically diagnosed patients of infectious mononucleosis and subjected to EBV-VCA ELISA for the detection of antibodies to Epstein Barr viral capsid antigen and PCRs targeting EBNA1 and VCA genomes. 84/180 samples (47%) were positive for EBNA1 gene and 89/180 (50%) samples were positive for EBV-VCA gene. In addition, 70 peripheral blood specimens were collected from patients with liver and kidney transplant. PCR targeting EBNA1, VCA genomes and real time PCR were performed. Among the 34/70 samples (48%) were positive for EBNA1, VCA genomes. A total of 10 aqueous chamber aspirates (AC TAP's) were collected from patients suspected with Serpiginous Choroiditis (OS) and PCR for VCA and EBNA1 gene were performed. All 10 (100%) were tested positive for VCA PCR. Nested PCR targeting the Epstein Barr Nuclear Antigen 1 gene and Viral Capsid Antigen gene was successfully standardized

and optimized for detection of Epstein Barr Virus from clinical specimens.

A study conducted at the All India Institute of Medical Sciences, New Delhi aimed to develop a single step multiplex real time polymerase chain reaction (PCR) assay for detection of A, B, C and E hepatitis viral related nucleic acids in sera from infected patients. The PCR was standardized to detect A, B, C, and E hepatitis viruses in serum using variables including annealing temperature, extension temperature, MgCl₂ and primer concentrations. The conserved regions of all viral genomes were used as targets for amplification. The novel assay was found to be a fast, sensitive, specific and reproducible system for detection of A, B, C and E hepatitis in serum.

In a study at the Post Graduate Institute of Medical Education & Research, Chandigarh. the viral load of enterovirus and adenovirus in patients with dilated cardiomyopathy (DCM) were determined and the viral load with coxsackievirus adenovirus receptor (CAR) expression were also correlated in these patients. A significantly high adenoviral load ($p < 0.05$) and CAR expression ($p < 0.05$) were observed in DCM cases as compared to controls. Whereas EV load showed no significant difference.

A study conducted at the Regional Medical Research Centre, Bhubaneswar aimed to screen human cases and selected mosquito species from defined areas of Odisha State for the detection of chikungunya

virus infections by serologic and molecular tests and nucleotide sequencing of the entire E1 genomic region for phylogenetic analysis. Chikungunya virus was identified in 70% of cases by both IgM and RT-PCR, thereby indicating the efficacy of molecular methods in detection of CHIKV at an early stage of the epidemic. Phylogenetic analyses of E1 and E2 genes revealed the circulation of ECSA genotype (IOL strains) in the affected areas. Structural modelling revealed that E2 gene of CHIKV was composed of three domains and the major adaptive mutations were detected in domain B of CHIKV, which can thus modulate binding of CHIKV to host cells. Several adaptive mutations were reported in E1 and E2 genes.

Network of Viral Diagnostic Laboratories

Fifteen Viral Diagnostic Labs (VDLs) of different categories (Grades I, II and III) were established by ICMR from 2010-2013. However in the year 2013, the scheme proposed by the Deptt. of Health Research on “*Establishing a network of laboratories for managing epidemics and natural calamities*” was approved by the CCEA. Under the scheme, a total of 10 Regional labs, 30 State level labs and 120 Medical College level labs will be established during the 12th Plan period. All the labs will be established in Govt. Medical Colleges only. The scheme has already been implemented w.e.f. July 2013. As per the guidelines of the scheme and the budget allocated to DHR for 2013- 14, a total of 2 Regional Labs, 4 State Level Labs and 8 Medical College Level Labs have been set up.

REPRODUCTIVE HEALTH

National Institute for Research in Reproductive Health (NIRRH) is a premier research institute of the Indian Council of Medical Research (ICMR) situated in Mumbai. The Institute is affiliated to the University of Mumbai which awards degrees to the M.Sc. and Ph.D students in the areas of Biotechnology, Life Sciences, Biochemistry and Applied Biology. The Institute is a WHO Collaborating Center for Research and Training in Reproductive Health.

Intramural Research

NATIONAL INSTITUTE FOR RESEARCH IN REPRODUCTIVE HEALTH, MUMBAI

EXPANDING CONTRACEPTIVE CHOICES

Identification and Characterization of Sperm Antigens Using Multifaceted Approach

The presence of Liprin $\alpha 3$ as an acrosomal protein on sperm; was found to be co-localized with Leucocyte Antigen Related (LAR) and Rab Interacting Molecule (RIM). LAR is trans-membrane protein tyrosine phosphatase. It interacts with extracellular ligands and regulates cell function. Its presence on sperm and co-existence with Liprin $\alpha 3$ suggests its role in acrosome reaction. Further, it is demonstrated that LAR extracellular ligands are present on the surface of mouse cumulus cells and can activate acrosome reaction *in vitro*.

Development of Anti-fertility Vaccines with Sperm Proteins

Synthetic Peptides of 80kDa Human Sperm Antigen (80 kDa HSA) and Human Seminal Plasma Inhibin (hSPI) have been found to be potential candidates

for the development of an anti-fertility vaccine. Active immunization of normal fertile male bonnet monkeys with synthetic Peptide1 using Muramyl Dipeptide (MDP) as an adjuvant emulsified with squalene and arlacel elicited anti-peptide antibody titer in male bonnet monkeys and these animals failed to impregnate normal fertile females. The fertility was regained following decline in antibody titer.

Gender Equity-Focused, Male-Centered Family Planning for Rural India

The study aims to enhance young couple's contraceptive knowledge and acceptance of spacing methods through gender equity focused family planning interventions delivered by the village health care providers (local doctors practicing majorly Homeopathy). In the intervention area, out of 469 couples, a total of 428 men participated in the first session, 365 men participated in second session and 247 couples participated in third session. Till date, out of 1081 couples, a total of 117 (10.8%) couples have participated in 18 month follow-up survey.

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

The study aims to understand the effectiveness of an intervention to improve contraceptive use among couples where women report an unmet need for contraception and also have experienced domestic violence. The study was conducted in two slum communities namely, Tunga Village and Kajupada in Mumbai covered under Health Post of Municipal Corporation of Greater Mumbai (MCGM). A total of 1136 of currently married women; aged 18-39 years having at least one child and reporting

unmet need of contraception were selected using systematic random sampling procedure.

Baseline data show the mean age of the women was 26.5 (± 4.4) years. Majority of them were literate (84.3%) and Hindu (65.3%). About 16.9% women experienced either verbal/emotional or physical violence in the past 12 months preceding the survey. The project is ongoing.

INFERTILITY

FEMALE INFERTILITY

Polycystic Ovary Syndrome

Genetic Analysis of Polycystic Ovary Syndrome (PCOS) with Special Emphasis on Genes involve in Insulin Resistance

In the reporting year, association of PON1 with PCOS and its related traits was investigated. PON1 exerts a cardio-protective effect by preventing the oxidative modification of Low Density Lipoprotein (LDL) and subsequent atherosclerosis. Polymorphisms in both promoter and coding regions have been studied as they have been reported to be involved in regulation of enzyme activity and concentration. The L55M coding region polymorphism showed association with PCOS susceptibility. Carriers of the polymorphic genotype displayed lowered paraoxonase activity whereas Q192R and promoter polymorphisms showed no association with PCOS. The study is being extended in larger sample size.

Molecular Signature of Human Follicular Fluid in PCOS by Proteomics Approach

The quantitative proteomic analysis of human follicular fluid (FF) from women with PCOS and healthy women undergoing in vitro fertilization showed differential expression of 186 proteins. 770 proteins have been identified in the FF which is the largest number of proteins identified in FF till date. Several vital proteins that are indispensable for follicular growth were found to be altered in PCOS. Among these, EGF like growth factor was found to be down regulated in PCOS. This has been validated by ELISA. EGF like growth factors perform important function of traversing LH signal from mural to cumulus granulosa cells, which then induces transcription of several genes of cumulus oocyte complex (COC) matrix, required for COC

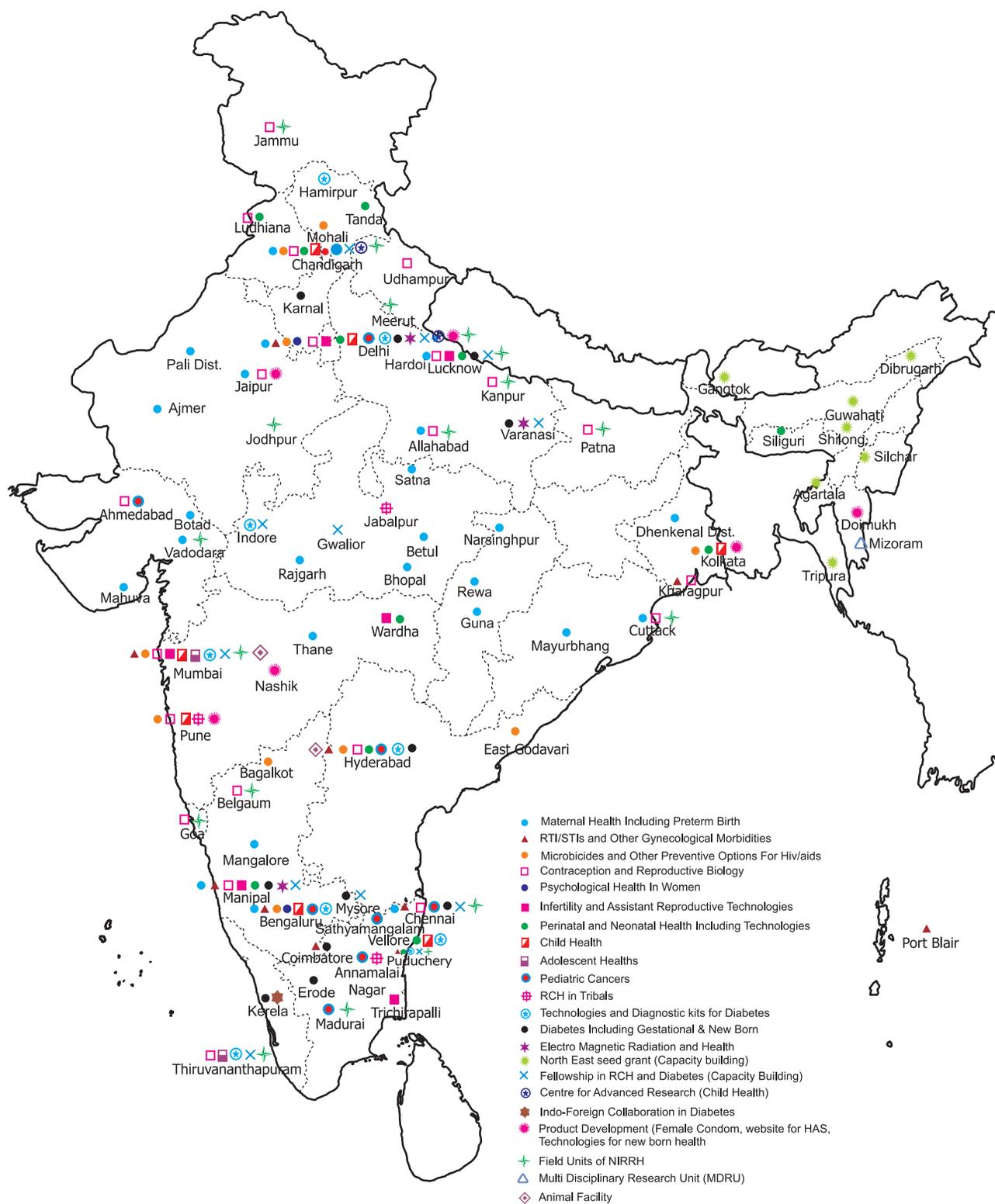
matrix expansion. Another COC matrix protein, tumor necrosis factor alpha induced protein 6, whose expression is induced by EGF like growth factor, was also found to be down regulated in PCOS FF. This further confirmed by real time PCR in granulosa cells. This indicates a defect in COC matrix function in PCOS. Further studies are ongoing to understand the mechanism of COC matrix functioning defects in PCOS.

The Study of Anti-apoptotic and Pro-apoptotic Molecules in the Development of Cystic Follicles using an Estrogen Treated Mouse Model

PCOS is characterized by anovulation and accumulation of multiple cysts arrested in preantral stage. Follicular growth, maturation and atresia depend upon the balance between the pro and anti-apoptotic molecules. To investigate, whether the follicular cysts formation in PCOS is due to any alteration in apoptotic mechanism, a murine model of polycystic ovary was developed. Investigations were carried out to study expression of both the extrinsic pathway and intrinsic apoptotic pathway molecules in cystic ovary. A detailed analysis showed that as cystogenesis progresses, granulosa cells of cystic follicles undergo apoptosis via mitochondrial pathway using JC-1. A marked increase in LH-FSH values were observed in estradiol treated group whereas no significant change was observed in vehicle control group. Studies will be continued to investigate the ultrastructure of the cystic ovary in estrogenized mouse model.

Autoimmune Markers for Diagnosis of Endometriosis

A non-invasive test for early diagnosis of endometriosis have developed. A multicenter study has been initiated for validation of the peptide ELISA based test to detect antibodies reactive to epitopes of Tropomyosin 3 (TPM3), Stomatin like Protein 2 (SLP2) and Tropomodulin 3 (TMOD3) proteins. Total study participants recruited so far in Mumbai, Kolkata, Goa and Nagpur centres were: Group I: Endometriosis (n=330); Group II: Disease Control (n= 118) and Healthy Controls (n=175). From our observations, sera from women with endometriosis showed higher mean reactivity against all 11 peptides as compared to disease controls and healthy controls. More than 40% of endometriosis



MAJOR ICMR RESEARCH PROJECTS IN REPRODUCTIVE AND CHILD HEALTH

patients showed increased reactivity for SLP2 and TMOD3 peptide using cocktail ELISA. More than 90% overlap was observed amongst endometriosis patients that show increased reactivity against SLP2 and TMOD3 cocktail ELISA.

Deciphering the Functional Significance of Heat Shock Protein 90 beta (HSP90 β) in Ovarian Autoimmunity

Ovarian autoimmunity accounts for up to 1% cases of female infertility, of which 47% cases show the presence of auto-antibodies to Heat Shock Protein (HSP90). Antibodies to HSP90 had earlier generated in mouse model, by immunizing it with a peptide EP6, the most immune-dominant epitope of HSP90, and established its detrimental effect on ovarian and reproductive functioning. In order to understand the patho-physiological mechanism, alteration in the immune physiology in the splenic and ovarian cell population were investigated in the immunized mice.

Effect of Dexamethasone Treatment of Autoimmune Premature Ovarian Failure

Autoimmune premature ovarian insufficiency (aPOI) has been under investigation for several years with a lack of defined treatment regimen for such patients. Barring a few anecdotal reports there has been a dearth of information on the use of glucocorticoids such as dexamethasone for such cases. Studies have been ongoing in the area of aPOI using a mouse model in our lab which has led to the identification of several antigens. Further investigations were undertaken to assess the effect of corticosteroid dosage on antibody response. Mice were immunized with three immune-dominant peptides from a single antigen, heat shock protein 70 (HSPA5/GRP78). Histology shows induction of premature ovarian failure (POF) with significant increase in polyovular follicles-hallmark of POF. Serum samples were tested against rat ovary lysates by Western blot analysis. The animals were treated with two doses of dexamethasone: 15mg/kg and 24mg/kg twice. Weekly serum samples were analyzed for reactivity to ovarian antigens. Work is ongoing to assess effect of immunization and dexamethasone treatment by Western blot analysis.

Proteomics Based Studies to Identify the Proteins in the Endometrial Secretomes of Humans and Rats

During the reporting year, attempts were made to investigate the mechanism by which an excess of extracellular HMGB1 leads to pregnancy failure. It was found that the levels of inflammation associated molecules such as nuclear factor kappa b (NF κ β) and interleukin 6 (IL-6) are increased in the uterine horn exposed to an excess of HMGB1. The increase in NF κ β localization was restricted to the luminal epithelial compartment, whereas IL-6 upregulation was observed in all three endometrial compartments, luminal epithelium and glandular epithelium andstroma. Further HMGB1 levels were found to be low during the embryo attachment in pregnant rats. Thus the studies highlighted the relevance of extracellular HMGB1 in pregnancy.

The Factors of Relevance in Endometrial Adhesiveness to Embryonic Cells

Adhesiveness of endometrial epithelium (EE) to embryo is known to be of paramount significance in the initiation of pregnancy. It was considered worthwhile to employ a proteomics based approach to explore the "adhesiveness" of the EE. During the reporting year, attempts were made to develop the surfactomes of two human EE cell lines, known for their differential adhesiveness to embryonic cells. Analysis of the 2D surfactomes of RL95-2 (exhibiting higher adhesiveness to JAr, an embryonic cell line) and HEC-1A (exhibiting lesser adhesiveness to JAr cells) identified 55 differentially abundant proteins. Of these, ten proteins were identified by Matrix-Assisted Laser Desorption Ionization Time-of-Flight in tandem or Liquid Chromatography Mass Spectrometry in tandem. Tubulin beta 2C (TUBB2C), ADAMTS and elongation factor beta were found more abundant on the cell surface of HEC-1A whereas HSP27, HSPA9, gp96, calreticulin (CRT), Tapasin-ERP57, protein disulfide-isomerase and β actin were more abundant on RL95-2 cell surface.

Functional genomic studies carried out by various groups have demonstrated significant alterations in the expression of cell adhesion molecules (CAMs) in the endometrium during the receptive phase. However, considering that in addition to

differential expression, differential localization of CAMs on the cell surface can be one of the major determinants of endometrial receptivity. In the wake of this, studies were undertaken to investigate the role of Rab11A (a recycling GTPase) in the trafficking of CAMs (integrin α V, β 3 and E-cadherin) to the endometrial cell surface. It has been demonstrated that over-expression of Rab11A (wild type and dominant negative mutant DNA) in Ishikawa (endometrial epithelial cell line) leads to decreased localization of integrin α V on the apical cell surface. To corroborate this, stable cell clones expressing RAB11 mutants have been generated. These clones are being investigated for their attachment and adhesion potentials.

Collectins and other Immune Related Proteins in Immunoregulation during Pregnancy Maintenance

Placental collectins could be relevant in maintenance of pregnancy. Earlier studies have confirmed presence of the three collectins namely SP-A, SP-D and MBL in human and murine placenta with intense staining of term human placenta syncytiotrophoblast for SP-A, SP-D and MBL, of inter villous space for SP-A and MBL, of stroma for SP-D and MBL protein. Exogenous treatment of the human term placental tissue explants of women undergoing caesarean section with recombinant human SP-D showed increased production of pro-inflammatory cytokines IL-1 α , IL-1 β , TNF- α , IL-6, IL-8 and MCP-1 implicating SP-D in the induction of pro-inflammatory immune status in the process of parturition.

Decidual tissues from the two groups of women undergoing normal delivery (labor, n=5) and women undergoing elective C-section (no labor, n=5) were analyzed for differential localization of the SP-A, SP-D and MBL during labor. SP-A, SP-D and MBL were localized in both stromal cells (cytoplasmic) and matrix compartments of term decidua of both the groups. The data revealed a significant increase in SP-D and MBL and a decrease in SP-A in decidual tissues ($p < 0.05$) of 'spontaneous labor' group compared to 'no labor' group. Importantly, significantly elevated levels, of MMP9 cleaved SP-D product (25kDa), were observed in term placentae of the spontaneous labor group. These observations suggest that dynamics

of collectins is similar across the fetomaternal interface and collectins may be integral to decidual-placental cross talk during parturition.

Investigating the Role of Immune Cells and Mesenchymal Stem Cells (MSC) in the Pathogenesis of Endometriosis

Mesenchymal stem cells isolated from endometriotic and endometrial biopsies for MSC markers such as CD90, CD146, CD9, PDGF R β , and stemness related markers such as OCT-4, Sox15 and Nanog were isolated and characterized. MSCs from endometriotic tissue (ESCs) showed significantly up-regulated expression of certain TLRs and also up regulation in cytokines and growth factors suggesting that pro-inflammatory endometriotic MSCs could be contributing to the chronic inflammation observed in endometriosis.

Human endometrial MSCs were transfected with sflt-1 (VEGF soluble receptor that inhibits angiogenesis) carrying Adenoviral vector (86% sflt-1 positive cells) and were administered to a SCID mouse model of endometriosis. Control groups were administered with viral vector alone or vehicle (PBS) alone. A significant reduction was observed in the size of endometriotic lesions formed in the treatment group as compared to control groups and Real time PCR analysis showed significant reduction in expression of transcripts for VEGF, VEGFR, FLK1 and MMPs 3 and 9 in the treatment group lesions as compared to control groups. The results suggested that sflt-1 gene transfected endometrial stem cells could be a potential therapeutic strategy for endometriosis.

Role of homeobox Gene HOXA10 in Endometrial Decidualization

The aim of this study was to decipher functions of HOXA10 in the adult primate endometrium. Based on the microarray analysis of decidual cells that were knocked down for HOXA10, it was demonstrated that HOXA10 has multiple targets in decidual cells and affects pathways involved in actin remodelling, immune modulation and trophoblast invasion. In vitro studies revealed that loss of HOXA10 in the decidual cells stimulates trophoblast invasion by a cell non-autonomous mechanism involving STAT1. It has been demonstrated that in vivo, levels of HOXA10 drop significantly in the decidua around

the time of trophoblast invasion. There seems to be a very strong spatial and temporal control of HOXA10 regulation in the developing decidua to dictate trophoblast invasion.

Experiments with transgenic mice expressing a HOXA10 shRNA and to investigate the involvement of HOXA10 in endometriosis *in vivo* are underway.

Role of RAGE (Receptor for Advanced Glycation End product) in Endometrial Physiology

Earlier studies demonstrated hormonal regulation of endometrial RAGE and one of its ligands i.e. HMGB1 in rats. These proteins were found to be expressed in higher levels in the proliferating endometrium. *In vitro* experiments also demonstrated a dose-dependent effect of recombinant HMGB1 on the proliferation of Ishikawa cells (an endometrial epithelial cancer cells). Further its dose dependent effect was also evident on wound healing behavior of Ishikawa cells. Studies will be conducted to explore the effect of another ligand i.e. AGE (advanced glycation end product) on endometrial cell proliferation and migration.

Development of a Non-human Primate Model for Endometrial Hyperplasia

The aim of the present study was to develop a primate model for endometrial hyperplasia. Ovariectomized marmosets were treated with 5 mg estrogen pellet, replaced after 3 month interval and this treatment was continued for one year. Uteri were found to be larger in estrogen treated ovariectomized marmosets, compared to control marmosets. Glandular epithelial and stromal cells showed signs of excessive proliferation and histological features similar to human of estrogen treated ovariectomized marmosets endometrial hyperplasia. Studies are in progress to examine whether the endometrium of these animals exhibits similar molecular characteristics, as reported in human hyperplastic endometrium.

MALE INFERTILITY

Pathways and Molecular Mechanisms Regulating Sperm Motility

A differential proteomic analysis of the phosphoproteins in asthenozoosperm vis-à-vis

normal sperm revealed that carbohydrate and energy metabolism, cAMP mediated PKA signaling, PI3K /AKT signaling and pathway regulating actin based motility by Rho were keys to the regulation of sperm motility. Alpha tubulin isoforms TUBA 3C, 3E, 4A and 8 were amongst those differentially expressed in asthenozoospermatozoa. A deeper insight into the testis specific isoforms TUBA3C, 4A and 8 revealed that the protein as well as the transcripts for the same was differentially expressed in asthenozoosperm. Mechanism of the differential expression of the acetylatable isoforms 3C and 4A and their role in motility was further investigated. Studies investigating the alpha tubulin specific deacetylase, HDAC6 revealed that HDAC6 is present on the sperm flagella where it colocalizes with acetyl α tubulin and is catalytically active; pharmacological inhibition of HDAC6 increases α tubulin acetylation and abrogates sperm motility. It is proposed that HDAC6 and not tubulin acetylation is the key determinant of sperm axonemal microtubule stability and that microtubule dynamicity is a prerequisite for sperm motility.

Molecular Characterization of Human Sperm Progesterone Receptor

The purpose of this study was to determine the progesterone mediated signaling mechanisms in spermatozoa that regulate sperm motility. In the reporting year NIRRH demonstrated the involvement of PI3K pathway in regulation of sperm motility by progesterone. It has been shown that progesterone activates PI3K/AKT pathway and requires the calcium channel CatSper. Inhibition of AKT impedes progesterone mediated increase in forward progressive motility and hyper activation without affecting acrosome reaction. AKT was found to be significantly enriched in the progesterone driven sperm phosphoproteome. These results for the first time demonstrate the involvement of the PI3K pathway in regulation of progesterone mediated sperm functions.

Deciphering the Role of Lim Homeodomain gene *Lhx2* in Gonadal Development

The aim of the study was to investigate the role of *Lhx2* in gonad differentiation. Previous studies in our laboratory have shown that loss of *Lhx2* during mouse embryonic development leads

to disturbances in vascular patterning. Ectopic vascularization was observed in gonads of Lhx2 ^{-/-} XX embryos. Using gonad recombination assays, it has been demonstrated that defective vascularization is not due to a flaw in the endothelial cells but as a result of loss of Lhx2 in the developing gonads. Two key genes R-spondin1 and Wnt4 (required to suppress vascularization in XX gonads) are down regulated in gonads of Lhx2^{-/-} XX mice, suggesting masculinization of the XX gonads in absence of Lhx2. Other genes involved in male and female developmental pathways to determine how loss of Lhx2 regulates formation of gonads are being looked at.

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

Studies have shown that only MBL-A showed stage specific expression during spermatogenic cycle in murine testes. During early stages (I to VI) and in the late stages (IX to XII), MBL was localized to apical portion of sertoli cells. MBL localized to round spermatids in the middle stages (VII-VIII). The presence of collectins on spermatid tail might be important in establishment of immune tolerance at the male ejaculatory duct and their presence in sertoli cell apical portion may be important in the clearance of apoptotic germ cells. Studies showed that recombinant fragment of human SP-D (rhSP-D,) bound with murine testicular cells (Sertoli cells, Leydig cells and peritubular myoid cells isolated with more than 90% homogeneity) in a calcium dependent and dose dependent manner. Sertoli cells showed maximal affinity towards rhSP-D. Importantly, the transcript levels of immunosuppressive molecules viz. TGF- β , IL-10 and serpin3n and percentage of F4/80⁺ macrophages and MHC II⁺ cells were significantly increased while a decrease in the percentage of CD11c⁺dendritic cells in the testis of SP-D gene deficient mice (SP-D^{-/-}) mice indicating the importance of SP-D in testicular immunoregulation.

The Role of DAZ Gene Copies in Infertile Males: It's Impact on Fertilization and Embryo Quality

Our recent meta-analysis of published literature has shown that deletions of the Y chromosome (referred

to as Yq microdeletions) occur in ~6% of infertile men, a frequency lower than that observed in the West. This prompted speculation that there should be other genetic defects on the Y chromosome that may be responsible for defective sperm production in these men. A small cluster on the Yq (termed as AZFc cluster) that is home to four copies of the DAZ gene and known to be essential for spermatogenesis was focused upon. Three types of rearrangements viz gr/gr, b1/b3 and b2/b3 that delete only two of the four DAZ copies were identified. Of these, gr/gr rearrangements are found to be at a higher prevalence in azoospermic men as compared to oligozoospermic and normozoospermic men. This cluster is being further detailed to delineate how loss of individual genes within this locus contributes to 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 trans-membrane conductance regulator gene (CFTR). Studies have been carried out to identify the spectrum and frequency of CFTR gene mutations in Indian infertile males with CBAVD. A total of 50 males with CBAVD, their female partners and 20 healthy controls were recruited. Out of 50 CBAVD patients, 5 patients were diagnosed with renal anomalies. The sequencing analysis of CFTR gene in Indian CBAVD males resulted in identification of several mutations and variants. The most common polymorphism in exon 10 *i.e.* M470V was reported in 19 CBAVD males (38%). The other coding SNPs c.2694 T>G, c.4521G>A and c.4002A>G were also reported in Indian CBAVD males. Further, 22 novel and 9 previously reported intronic variants were found.

Design of Constructs Using Testis Specific Promoters and Generation of Transgenic Infertile Mice

The main goal of this study was to identify the regulatory elements modulating c-kit gene expression in mouse spermatogonial stem cells (SSCs). During

the current year, using MAPPER 2.0 & MatInspector online prediction software, we demonstrated the binding of retinoic acid receptor gamma (RXR γ), Sp1 transcription factors to the motifs of c-kit promoter region. These results were confirmed *in vitro* by electro-mobility shift assay (EMSA) using c18-4 cell line. Studies on the mutational analysis of RXR γ , Sp1 and Ets revealed the involvement of these transcriptional factors in the modulation of c-kit gene expression in primary SSCs. Chromatin immune-precipitation (ChIP) assay was employed to validate the presence of these transcription factors in c18-4 cells and SSCs by FACS.

Studies to Elucidate the Molecular Mechanism of Estrogen Action in Spermatogenesis

Estradiol, traditionally a female hormone, plays a very crucial role in spermatogenesis and male fertility. Estrogen administration or deprivation can have adverse effects on male fertility. This has been further strengthened by the presence of estrogen receptors α and β (ERs) in the male reproductive tract. Studies are being conducted to elucidate the molecular mechanism of estrogen action during spermatogenesis through both its receptor using receptor specific agonist and antagonist.

Studies using estrogen receptor β (ER β) specific agonist (DPN) and antagonist (PHTPP), have demonstrated a significant increase in pre- and post-implantation embryo loss after 60 days of DPN treatment and a concomitant decrease in litter size. However, a significant increase in only pre-implantation loss was observed after 60 days of PHTPP treatment. The levels of the FSH, LH and estradiol were unaffected at most of the doses of

DPN and PHTPP, hence the anti-fertility effects observed after the treatments could be attributed to disrupted estrogen signaling through ER β . These results further underline the important role played by ER β in male fertility and the disruption of estrogen signaling through it would potentially lead to decreased fertility.

Assessment of Services for Prevention and Management of Infertility in the Primary Health Care System in India

The study was a non-interventional, descriptive, cross sectional survey of the representative sample of selected health facilities from six zones (North, South, East, West, Central and North East) in the country. The districts chosen for the survey had high prevalence of infertility as per DLHS-3.

The findings indicate that 80% of Gynecologists and 67% of Medical Officers reported that infertility is a problem in their locality and 5-6% of people visiting their facilities came with difficulty in conceiving. A basic service like semen examination was available at only 6% of PHCs and 21% of CHCs but, this test was mostly being performed on post vasectomy cases and not used to diagnose infertility. Other advanced techniques like diagnostic laparoscopy, transvaginal sonography were often used for indications other than infertility. None of the facilities had any IEC material on infertility and there were hardly any awareness programs conducted in the community. Services related to prevention of infertility under other national health programs on TB, RTI/STI and safe deliveries were available at majority of facilities but services for safe abortion were not available at majority of CHCs and PHCs (Fig. 1-3).

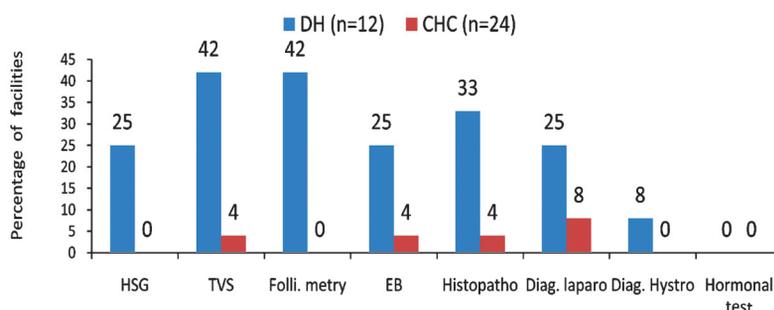


Fig. 1. Percentage of facilities offering basic services relevant to infertility at DH, CHC and PHC.

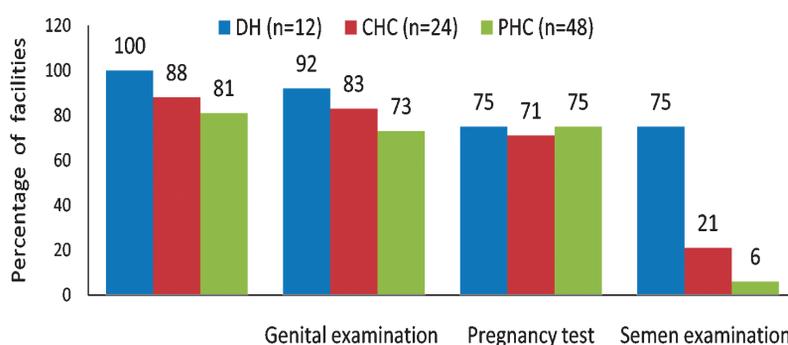


Fig. 2. Percentage of facilities providing advanced services relevant for diagnosis of infertility at DH and CH. Hystero-salpingography; Trans-vaginal sonography; Folliculometry; Endometrial biopsy; Histopathology of tissues; Diagnostic laparoscopy; Diagnostic hysteroscopy.

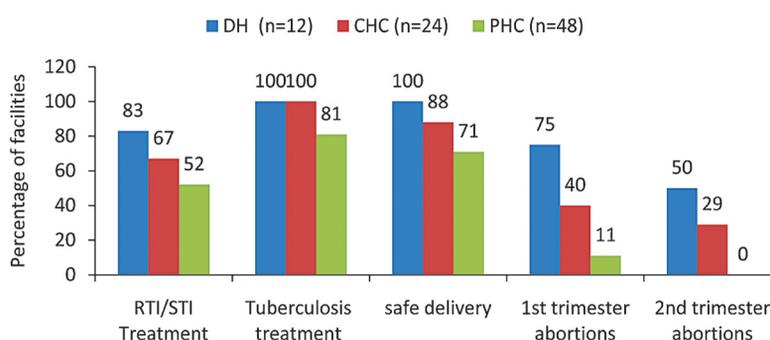


Fig. 3. Availability of services which help in prevention of infertility.

A Consultation cum Dissemination meeting was organized at the institute on 23rd May 2013 which was attended by Secretary (DHR), Ministry of Health Govt of India, officials from State and Central Governments,

ICMR, NIHF, IIPS, TISS, relevant faculties from Medical Colleges, WHO, UNFPA, international NGOs in India and other national stakeholders. Both the group of investigators and experts who had worked on development of guidelines and this study also participated. The experts recommended the service delivery interventions came out with the recommendations on strategies for incorporation of infertility services in the National program.

RTI/ STIS/HIV/ MICROBICIDES

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

Studies were carried out to determine the effect of SsALF24 on the autophagy response of the host during infection. Immunofluorescence studies revealed that when VK2/E6E7 cells are treated with

autophagy inducers such rapamycin or LPS, there is formation of LC3-positive puncti-like structures. These structures are indicative of autophagosome formation and were significantly increased when the cells were treated with LPS as compared to the untreated controls. Interestingly, when SsALF24 treated VK2/E6E7 cells were stimulated with LPS, the number of autophagosomal puncti were decreased. These results are being confirmed using more number of samples (Fig.4).

Development of a Multistrain Probiotic Lactobacillus Formulation Effective against Reproductive Tract Infections

Probiotic Lactobacilli have been demonstrated to be a safe, reliable, topical effective treatment to reduce recurrence of urogenital infections, to restore the disrupted ecological environment and to overcome the secondary effects of antibiotics.

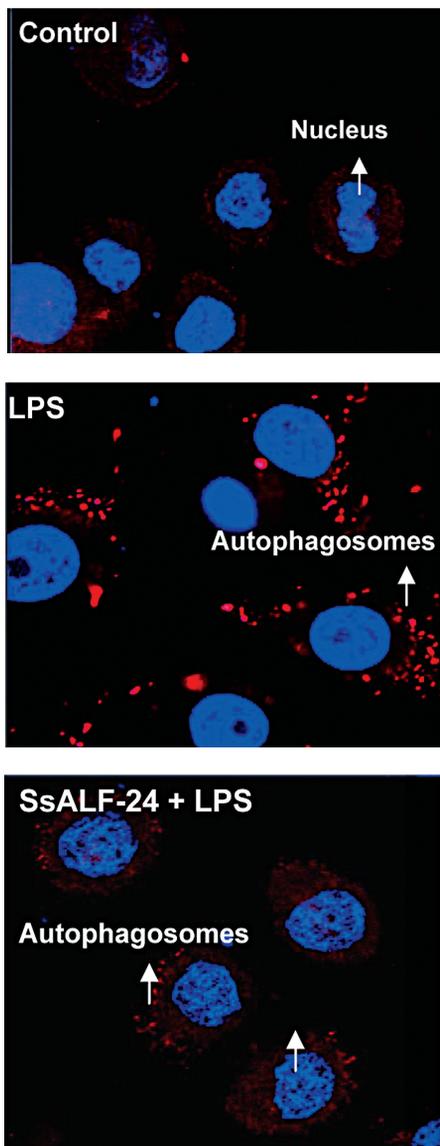


Fig. 4. Effect of SsALF-24 on LPS-induced autophagosome formation.

Hence, selected strain of probiotics should be resistant to the administered antibiotics and thus could be given concomitantly. Different species of lactobacillus were evaluated for their susceptibility to antibiotics like metronidazole, doxycycline and clindamycin by in-vitro broth dilution assay. Our *in-vitro* study shows that growth of all Lactobacillus was inhibited by clindamycin. Hence probiotics need to be administered post clindamycin treatment to avoid risk of infection and possibility of recurrence. Most lactobacillus species were unaffected by Doxycycline, whereas, metronidazole affects growth of *L.acidophilus*, *L.delbrueckii*, *L.johnsonii* and *L.salivarius* (MIC<125 ug/ml) whereas no effect on *L.gasseri*, *L.jensenii* and *L.reuteri* observed.

Hence augmentation of metronidazole with resistant Lactobacillus as probiotics would have an added advantage for control of anaerobes. Preliminary studies in 25 women using gram staining, microscopy and culture studies to investigate bacterial morphotypes indicated the presence of one or two lactobacillus species in each individual samples. The identification of these species is in progress.

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

An *In silico* designed peptide, HbAHP-25, with a potential to bind gp120, was synthesized. Anti-HIV activity of this peptide was evaluated in presence of seminal plasma and vaginal fluid. The site of gp120 to which HbAHP-25 binds was identified using different monoclonal antibodies against gp120. Activity of HbAHP-25 on cell-cell fusion was also determined using TZM-bl and HL2/3 cells. Effect of HbAHP-25 on tight junction proteins was determined using immunofluorescence. Immunological characterization of this peptide is currently under progress.

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

The role of microRNA let-7f in modulating innate immune responses of human endocervical epithelial cells (End1/E6E7) has been reported earlier. Histone deacetylase-2 (HDAC2) as a novel target was identified for let-7f. Inhibition of HDACs by trichostatin A (TSA) reversed CpG-ODN (ligand of TLR9) induced tolerance. Polarized End1/E6E7 cells responded to apical stimulation with ligands of TLR9 (CpG-ODN) and RIG-I {Poly(I:C)LL}, without compromising End1/E6E7 cell integrity. The results suggest that factors present in the spent medium of End1/E6E7 significantly reduced the secretion of pro-inflammatory cytokines (TNF α , IL-2, GM-CSF, IFN γ) in LPS-induced human primary monocyte derived macrophages (MDMs) and DC: T cell co-cultures. Spent medium obtained from End1/E6E7 cells after induction with ligands of TLR9 and RIG-I restored secretion of these cytokines spent medium also induced phagocytosis and chemotaxis of U937 cells. Anti-inflammatory effect of spent medium of End1/E6E7 cells was observed to be TGF- β dependent. The results

indicate that End1/E6E7 cells play an indispensable role in modulating anti-viral immune responses at the female lower genital tract.

Approaches for Controlling Biofilm formation by *Gardnerella vaginalis*

Studies were carried out to determine the effect of two commonly used antibiotics for the treatment of BV, namely metronidazole and clindamycin on biofilm formation by different strains of *G. vaginalis*. Considerable variability was observed in the action of the two antibiotics. Though both clindamycin and metronidazole prevented biofilm formation, only metronidazole disrupted established biofilms of some *G. vaginalis* strains and none of the antibiotics possessed the ability to disrupt as well as prevent biofilm formation by all the tested strains. The observed variations in the action of the antibiotics on biofilms may underlie their failure to prevent recurrent BV. Under the targeted peptide based approach we sought to examine the effect of two rationally designed peptides derived from the sequence of a protein involved in biofilm formation. Both these peptides did not affect the viability of planktonic cells and studies on their action on the biofilms are ongoing.

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

Integrin $\alpha_4\beta_7$ ($\alpha_4\beta_7$), a gut homing addressin, has been recently reported to function as a receptor for the HIV envelope glycoprotein gp120. In view of the importance of integrin $\alpha_4\beta_7$ in mucosal transmission of HIV, a new project was initiated in the reporting year to investigate some of the downstream events resulting from the interaction of this integrin with HIV-1 gp120 and to determine their contribution towards HIV pathogenesis. Studies carried out so far with CD4⁺ T cells have revealed that treatment with HIV-1 gp120 results in an altered phosphorylation status of a number of intracellular kinases as well as concomitant activation of LFA-1, a key component involved in virological synapse formation. Further these events were found to be mediated by integrin $\alpha_4\beta_7$ and thereby suggest the involvement of gp120 mediated integrin $\alpha_4\beta_7$ dependent signaling in the cell-to-cell transmission of HIV-1.

Investigations on Role of SP-D (a C-type collagenous lectin) in Vaginal Innate Immunity and its Potential as a Vaginal Microbicide

The role of an innate immunity protein, human surfactant protein D, secreted by vaginal epithelial cells in host defense against sexual transmission of HIV is being investigated. In earlier studies, a gel formulation of a recombinant fragment of human surfactant protein D (rhSP-D) showed binding to gp120 of HIV-1 and inhibited replication of three clinical strains and one lab strain of HIV-1 subtype C in human monocyte derived macrophages, TZMbl reporter cell line, T cell line (Jurkat J6), monocytic cell line (U937). The formulation significantly downregulated expression of various HIV-1 co-receptors on PBMCs and had no adverse effect on the viability of vaginal epithelial cells (VK2) and viability of four Indian clinical isolates of vaginal lactobacilli. Further, HIV-1 challenged U937 cells and Jurkat T cells, showed reduced kinase activation (p38, Erk1/2 and AKT) and pro-inflammatory cytokine production in presence of rhSP-D.

Studies confirmed the interaction of cell surface expressed gp120 (on HL2/3 cells) with SP-D using a FITC labeled polyclonal antibody raised against gp120. *In silico* studies using PatchDock revealed that in the best docked complex, SP-D (CRD) interacts with the glycans at Asn234 and Asn276 and various residues in the known CD4 binding regions namely C2, C4 and C5 domains of gp120 (critical for binding of neutralizing antibodies) and predicted that SP-D may inhibit gp120-CD4 interaction.

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

Immunoregulation is an essential component of implantation and successful outcome of pregnancy. In our earlier studies, Surfactant protein D (SP-D) was detected in the glandular as well as luminal epithelium in the murine uterus. A significant increase of SP-D transcripts in the estrogen treated ovariectomized mice suggested that SP-D is hormonally regulated and may have an important role in the endometrial biology. SP-D transcript expression decreased manifold during early pregnancy.

In silico analysis of the mouse SP-D gene promoter region showed presence of two Estrogen responsive elements (ERE) and one progesterone responsive element (PRE). SP-D^{-/-} mice exhibited an altered estrus cycling with an elongated metestrus (2.85 ± 0.14 days) and diestrus phase (3.65 ± 0.18 days) as compared to the wild type mice (metestrus: 0.69 ± 0.03 and diestrus: 1.75 ± 0.08). The pre-implantation loss (PIL) in the SP-D^{-/-} mice (40.24 ± 10.71) was significantly higher than the wild type mice (14.35 ± 10.40). There was no significant difference in the post-implantation loss (POL) while the litter size in the SP-D^{-/-} mice (6.66 ± 1.25) was significantly lower than the wild type mice (9.8 ± 1). In the LPS induced embryo implantation loss model, wild type mice showed more than 50% embryo loss whereas the SP-D^{-/-} females were found to be resistant to embryo loss induced by LPS. The observations in the SP-D^{-/-} mice, suggest that these mice may have a skewed uterine Th1/Th2 cytokine ratio with a Th2 bias and SP-D plausibly regulates the uterine Th1:Th2 cytokine levels during embryo implantation.

Association of Host Immunogenetic Factors with HIV Transmission

NK cells have anti HIV activity mediated through killer cell immunoglobulin-like receptors (KIRs). The study on HIV discordant couples revealed significantly higher frequencies (91.4% vs. 72.3%, $P=0.006$) of activating KIR3DS1 gene in HIV seronegative (HSNs) spouses, while HIV seropositive (HSPs) had inhibitory gene KIR2DL5 (74.6% vs. 51.0%, $P<0.03$). Activating gene KIR 2DS1 was associated with viral load control in HSPs. Sixty KIR genotypes were observed, 16 of them reported for the first time in Indian population. Twenty unique genotypes were reported for the first time. Presence of exclusive genotypes either in HSPs (N=22, 11 unique genotypes) or in HSNs (n=27, 9 unique genotypes) indicates their possible association either with HIV infection or with protection.

Data on opportunistic infections in 185 HIV positive individuals revealed 48.06% with OIs, significantly ($p=0.01$) high in 31-40 years age group. Tuberculosis (TB) was the most common OI (68.8%). More (53.7%) spouse/children of HIV positive individuals without OIs were HIV-1 positive.

HIV-1 gp120 Induced Gene Expression Signatures in Vaginal Epithelial Cells

HIV1 the envelope protein (gp120) has been shown to bind human Mannose Receptor (hMR) on vaginal epithelial cells. Following HIV binding and entry into vaginal epithelial cells the virus is transmitted to distal cells. Further studies on the host pathogen interaction by microarray analysis of CD4 independent interaction of gp120 with hMR on vaginal epithelial cells demonstrated alteration in gene expression profile of these cells. The functional implication of such an association was studied by annotation clustering and gene ontology analysis of differentially expressed genes. About 29 genes were found to be upregulated such as CXCL1, CXCL2, TNF MMP7 and TRIM22, while about 11 genes were found to be down regulated such as IL18R, CASP8 and TRIM. Altered gene expression was validated by real-time PCR. Gene expression signatures induced by gp120 seem to modify the vaginal milieu and may facilitate establishment of primary infection.

Genotypic Characterization of HIV-1 C Variants in PBMCs and Urogenital cells

Presence of distinct HIV variants in different cells and secretions has been detected due to poor proof reading activity of HIV reverse transcriptase enzyme. Distinct variants have also been detected in blood and urogenital cells and secretions of the same individual which may influence the sexual transmission of HIV including viral binding and entry into the urogenital cells and secretions and may also provide information regarding therapeutic interventions such as microbicides and anti-retroviral drug therapy. The study demonstrated that the translated amino acid sequences of C2 - V3 region of env gene of HIV-1 C in PBMCs and urogenital cells showed the presence of distinct variants in PBMCs and urogenital cells of the same individual. Further using N-Glycosite and Geno2pheno [Co receptor] 1.2 programs it was observed that these variants were found to have different N linked glycosylation (NLG) sites and variation of affinity co receptors in same individual. Additionally viral load in blood and urogenital secretions did not show correlation in same individual and showed variation in response ART.

Immune Dysregulation in HIV Pathogenesis

CD4+regulatoryTcells(Treg)playvitalrolesinT-cell homeostasis and the control of immunopathology associated with persistent pathogens. Although the association of circulating Treg with HIV-1 clade B has been reported its role pathogenesis of other clades and duly (HIV-1 and HIV-2) infected individuals is not fully understood. Thus, the present study aims to evaluate the role of these cells in HIV-1 (Clade C), HIV-2 and HIV-1/HIV-2 (dually) infected individuals using multiparametric flow cytometry. Preliminary data from 10 each from healthy individuals and HIV-1 infected individuals and 9 HIV-2 infected individuals on anti-retroviral therapy (ART) has been generated with respect to successful identification by multi-parametric flow cytometry of CD4+CD25(IL-2 receptor)+CD127(IL-7 receptor)-FOXP3+ Treg cells. It was observed that levels of CD4+CD25+CD127- T reg cells were elevated in HIV-1 infected individuals compared to healthy controls and HIV-2 infected individuals. These observations suggest distinct pathogenesis mechanisms for HIV-1 and HIV-2 infected individuals with respect to IL-2 and IL-7 homeostasis in HIV infection that in the case of HIV-1 occurs even during ART where viral load is significantly reduced or absent. These results imply that ART by itself in the absence of immunomodulatory therapy would be insufficient to correct functional impairments in T-cell immunity in HIV-1 infected individuals.

Immunogenicity of Indigenous Recombinant Anti HIV Vaccine

Vaccines against HIV have undergone human trials and some of them found to be safe but with poor or moderate immunogenicity and efficacy. Semliki Forest virus (SFV), an alphavirus is a promising vaccine vector for generating effective immune responses against HIV-1. Furthermore it is safe as it is non-pathogenic in humans and does not have pre-existing immunity. It has a broad host range and produces high levels of transient gene expression. RNA replication is cytoplasmic and hence there is no risk of chromosomal integration. In the present study, HIV1 C gag, env and polRT genes were individually cloned in SFV Vector. The replicon RNA constructs thus obtained individually as well as in combination elicited in-vitro and in-vivo cell mediated and humoral immune

responses. Further these constructs were then co-transfected with SFV 2 helper RNA to obtain viral like particles (VRP) VRP also showed expression of respective proteins in vitro. Both individual and in combination the recombinant constructs were found to generate HIV-1C specific immune responses in Balb/C mice. HIV-1 Env-specific and Gag-specific humoral responses were elicited by rSFV constructs as detected by gp120 ELISA and p24 Gag ELISA respectively. Clearly detectable Env-specific, potent Gag-specific and moderate HIV Pol-specific cellular responses were detected by IFN-gamma ELISPOT assay. Overall, the viral like particles demonstrated greater immunogenicity both ELISPOT responses against HIV antigens as well as in terms of higher titers of specific binding antibodies to HIV-1 Env and Gag proteins.

MENOPAUSE AND OSTEOPOROSIS

Osteoporosis is an important public health issue with increase in the ageing population and there is a need to develop indigenous assays for assessment of bone health. Serum C-terminal cross-linked telopeptide of type I collagen, N-terminal pro-peptide of type I collagen, Osteocalcin and Pyridinium crosslinks (PD/DPD) are among the sensitive bone turnover markers used for precise assessment of bone health. These markers are measured individually by assays that are time consuming and require substantial sample volume. A multianalyte ELISA array technology for bone markers is currently under development for diagnostic applications. Towards this end we have generated epitope specific antisera to the four markers, purification and biotinylation of these antibodies was carried out with a view to develop sandwich ELISAs which is on-going. Later the assays will be transformed into an array format and validated.

The protein profiles of monocytes (MO) were explored from pre- and postmenopausal women with high versus low BMD using a 4-plex iTRAQ technique. In MO from premenopausal women, 4 differentially expressed proteins with respect to low and high BMD were identified. In contrast, 27 differentially expressed proteins were identified in MO from postmenopausal women with high versus low BMD conditions. Heat shock protein 27 (HSPB1) was the common protein up-regulated in low BMD condition in pre- as well as postmenopausal women.

MATERNAL AND CHILD HEALTH

A Prospective Observational Study of "Near Miss" Obstetric Events and Maternal Deaths in Tertiary Hospitals of Mumbai

Maternal near-miss refers to a woman who nearly died but survived a complication that occurred during pregnancy, childbirth or within 42 days of termination of pregnancy. Over the past decade, there has been an increasing interest internationally in studying near misses, or severe morbidity, to complement traditional audit of maternal mortality. Review of these cases has the potential to highlight the deficiencies as well as the positive elements in the provision of obstetric services in any health system. The overall objective is to review the near miss cases and maternal deaths which would provide feedback for the improvement of maternity services in the selected health facilities and similar tertiary hospitals.

The study is being conducted in two tertiary hospitals Lokmanya Tilak Municipal General Hospital (LTMGH) and Sir J. J Group of Hospitals (JJH) in Mumbai.

Data obtained from both the tertiary centres for the period September 2012 –August 2013 is as follows:

EVENTS	LTMGH	JJH	TOTAL
Obstetric Admissions	15032	4144	19176
Deliveries	12736	2495	15234
Abortions	1175	269	1444
Ectopic	136	50	186
Maternal deaths	75	19	94

The total near miss cases for the period September 2012 –August 2013 was 884 (689 in LTMGH & 198 in JJH). The total maternal deaths reviewed for the same period were 94 (75 in LTMGH & 19 in JJH). Data collection is completed and data analysis is ongoing.

Socio-behavioural Aspects of Smokeless Tobacco with Implications on Reproductive Health of Low Income Married Women in Mumbai Slum Communities

This study explores the smokeless tobacco use patterns, beliefs and perceptions on reproductive

health of women in a low income community of Mankhurd in Mumbai. The results indicate the prevalence of SLT use in the study area is 31% among women aged 19-40 years. Age at initiation ranges from 5 years to current age at the time of interview (range: 5–40yrs), with a little less than 40% reporting to start before the age of 15 years. About 18.3% of women have reported initiation of SLT use during first pregnancy. Out of the total SLT users (n =409), 36.2% were poly SLT users (using two or more types of SLT in a day). Every woman in the study has 5 or more people known to them, apart from their husband who uses one or more forms of smokeless tobacco. Thirty-eight percent of current users have reported trying to quit SLT use in the past 12 months. Findings suggest that women do recognize a number of risks to infants but need correct information about harmful effects of SLT use during pregnancy or infancy. This emphasizes the need for preventive programmes during ante natal care period both at the clinic and community.

The findings were shared with the participants in five different locations of the study area and suggestions for intervention were sought from them as well as pan shop owners and community based NGOs. This was followed by a scientific dissemination of the findings and a national consultation meeting at the institute. Experts working in the field of smokeless tobacco participated and contributed towards discussions. The recommendations of the meeting were widely disseminated to various national and state government departments as well as concerned NGOs.

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. The aim of this pilot study was to know the burden of preterm birth in this group and to study the risk factors associated with preterm birth among women conceived by ART in Mumbai. Objectives of the study were to investigate the (i) clinic based incidence of preterm birth among pregnancies conceived by ART in Mumbai (ii) association of risk factors (related to causes of infertility like endometriosis, Polycystic Ovary Syndrome, stage of embryo transfer, cervico-vaginal infections and other risk

factors) in this group (iii) neonatal outcome in the study participants (iv) To identify risk reduction strategies to prevent preterm birth in this group. Since September 2012, 80 participants recruited against a target of 400 for the study from different fertility clinics.

District Level Household and Facility Survey-4 (DLHS-4): Clinical, Anthropometric and Biochemical (CAB) tests in Maharashtra and Goa

In response to invitation from National Institute of Health and Family Welfare (NIHFW) and Ministry of Health & Family Welfare (MOH&FW), Government of India, District Level Household and Facility Survey IV (DLHS IV) was conducted. Main objective of the survey was to provide Reproductive and Child Health (RCH) database at district level in India. The survey data will be useful for the Central and State Governments for evaluation, monitoring and planning strategies. The field survey agency being International Institute for Population Studies (IIPS). National Institute for Research in Reproductive Health (NIRRH) completed the hemoglobin estimation of anonymized blood samples collected by field staff hired by IIPS. Blood samples were received from 16 districts of Maharashtra and 2 districts of Goa. Hemoglobin estimation was done for 79935 blood samples. The blood samples were in the form of dry blood spot. NIRRH also did the training of field staffs for anthropometric (weight and height) and blood pressure measurements including the monitoring. Project completed and final report submitted to NIHFW.

Lactogenic Properties of Indigenous Medicinal Plants

One in two children is malnourished in tribal areas of India. There are several reasons responsible for this malnutrition. One of the most important reasons is less than optimal consumption of mother's milk by infant. Breastfeeding is the best method for reducing the rate of infant's malnutrition. Many traditional practices have been utilized for increasing milk quantity during lactation by the ancestors. However, scientific validation of use of traditional practices is not known completely. Present studies are based on identification of galactagogues from

herbal remedies of plants such as *Pennisetum americanum* (L.) Leeke and *Trigonella foenum-graecum* Linn using Holtzman rats. Female rats that received oral doses of aqueous extract of these plants during their first lactation. The measurement of milk production during lactation was achieved by measuring pup weights during suckling period. About 4.19 and 2.38 fold more milk was produced in experimental group of animals those received 400 mg of Bajra and 200 mg of Methi seed extract respectively than compared to controls. Pup weight gain and weight of mother rats of extract treated groups were significantly higher than control group ($P < 0.01$ and $P < 0.001$). Additionally protein and carbohydrate content of mammary gland tissue were also significantly more (i.e. 35 to 39% and 48 to 81% respectively) than control group of animals. In addition, the mammary gland tissues of experimental group showed obvious lobulo-alveolar development with milk secretion. This study demonstrates that the aqueous extract of these plants can stimulate milk production in the female rats and could consequently have the properties claimed for inducing greater milk production in lactating mothers, as well as in cattle's etc.

ADOLESCENT HEALTH SERVICES

Strengthening the ARSH Services as per the National Standards of ARSH Implementation Guide in Karjat Block of Raigad District (Quality Assessment of Adolescent Friendly Health Services)

NIRRH has been providing technical and monitoring support to Govt. of Maharashtra in Karjat block of Raigad district since 2009 to operate and sustain the eight established ARSH clinics known as "Maitri" at government health facilities. These clinics meet the sexual and reproductive health needs of adolescents.

Financial decentralization enabled the clinics to plan and conduct most of the ARSH programs as per the guidelines received from the state. NIRRH played a lead role in strengthening ARSH linkages with schools and enabling them to play a proactive role in generating awareness and improving referral linkages. Nodal school teachers and principals of secondary schools were oriented and trained on ARSH program. Directives were given from

education department to initiate ARSH activities in all secondary schools of the block by incorporating ARSH issues during the school extra-curricular activities as such as debate/ speech/ essay writing competition etc including installation of a question box. Self-explanatory VCDs for adolescent boys and girls regarding growing up issues in Marathi were made available to all secondary schools to assist teachers to conduct adolescent health programs. The Matri Clinic CD was also given to all schools to sensitize parents about the program.

To improve demand for services in the community, an infotainment camp was organized in coordination with a number of NGOs and academicians on issues like Nutrition, HIV/AIDS, Substance Abuse, Superstition, Spirituality and Cancer. A total of 400 adolescents from Karjat, Mohili, Kadav and Neral attended this advocacy camp. Mobile helpline service for adolescents was retained during the year however the uptake of this services by adolescents was very poor. There was a number of operational feasibility issues linked to this poor response.

The attendance of adolescents at the centers remained approximately same with 7263 in the last year to 7271 during the current year. The project is currently in the concluding phase and Govt of Maharashtra has agreed to sustain these clinics by allotting specific budget to these clinics in the forth- coming State Program Implementation Plans (PIPs). NIRRH would continue to provide technical guidance whenever required in future (Fig.5).

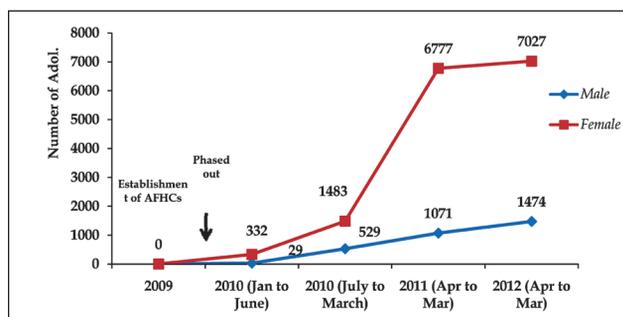


Fig. 5. Attendance of Adolescents at AFHCs.

The quality and coverage assessment of services is an ongoing activity every year. The assessment during the current year depicted a downfall in overall score from 75% in 2011-12 to 69% in 2012-13 mainly due to the unavailability of certain ARSH related guidelines and protocols and poor

documentation of outreach activities. The overall score for all standards clearly show that the AFHS intervention has substantially contributed in improving the program effectiveness in all the health facilities overtime. In view of this, NIRRH continued its mentoring and supervisory activity during the current year and also tried to assist the services to responsibly handle the sustenance of ARSH program.

Capacity Building of Volunteer Adolescent Peer Leaders for Promoting Effective Reduction in Early Marriage and Early Pregnancy among Rural Unmarried Adolescents in Maharashtra

The objective of this study was to develop and implement a pilot, volunteer community-based peer led program to delay age at marriage and prevent early pregnancies among rural unmarried adolescents in Bhivandi taluka of Thane District of Maharashtra. Survey data was collected using mobile devices (PDA handhelds) from unmarried girls aged 14-16 years (n=113), unmarried boys aged 16-20 years (n=116), and their parents (n=227 mothers and 203 fathers).

During the year 2013, meeting was held for two days with scientific experts working in the area of adolescent health to finalize the curriculum for a pilot peer leader training program. A list of 40 boys and girls in the age group of 14-20 years and willing to be part of the study was prepared to introduce them as peer leaders under the program. Results indicate that almost all boys and girls were aware about legal age at marriage for a boy and girl. Awareness about contraceptive methods among girls was low as compared to boys. Negative effect of using contraception early in marriage was fear of infertility.

Post intervention results suggest that girls and boys preferred to choose their life partner on their own. The interventions have greatly facilitated in developing understanding on major aspects of reproductive health issues and gender equity. The IEC programs, Provision of IEC material in group setting, peer leader training and interventions with participants helped to initiate a dialogue on these issues and helped them to overcome their misunderstandings. The information provided was shared with friends and relatives but not with

the parents because they felt shy to talk to them. Community leaders and parents felt that such educative programs should be conducted in schools, colleges, and at community level with parents. Peers felt that intervention program helped them to increase their confidence level.

REPRODUCTIVE CANCERS

Hormonal Regulation of Human Telomerase and its Possible Implications in the Pathogenesis of Prostate Cancer

Studies were undertaken to determine whether the expression of ZEB2 is regulated by AR in androgen-independent prostate cancer cells also. Forced expression of AR led to a decrease in the levels of ZEB2, both at transcript and protein levels in androgen-independent prostate cancer cell lines (DU145 and PC3). Further, a decrease in ZEB2 expression led to a decline in invasiveness and migration of PC3 and DU145 cells. Conversely, an increase in the expression of E-cadherin (epithelial marker) was observed. Thus the studies demonstrated negative regulation of ZEB2 expression by AR in androgen-independent cell lines. Observations support a notion which underscores the benefits of maintenance of AR levels, to a certain extent, in prostate cancer therapeutics.

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

Efforts were directed to determine whether the cell surface bound estrogen binding proteins/ receptors are encoded by genes which encode the conventional nuclear estrogen receptors (α and β). Towards this, immunolocalization studies were carried out to detect these proteins on the cell surface of LNCaP cells, silenced for the expression of conventional ER α /ER β . A decrease was observed in the cell surface localization of estrogen binding proteins/ receptors in these cells. Thus cell surface bound estrogen binding proteins/ receptors appear to be the products of genes which encode the conventional nuclear estrogen receptors (α and β).

Further, few proteins have been identified, which are modulated in response to the activation of cell surface estrogen binding proteins/receptors. Two dimensional polyacrylamide gel electrophoresis

(2D-PAGE) coupled with MALDI-TOF-TOF revealed altered phosphorylation of key cytoskeletal molecules, like actin and cytokeratin 8 in response to stimulation with E-BSA conjugate. Studies are in progress to further elucidate the function of these cell surface estrogen binding proteins/receptors in prostate cancer cells.

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

Analysis of the High-risk Human Papillomavirus (HPV) 16 E6 variants in cervical cancer cases revealed the presence of three novel variants, besides European T350G (50%), European prototype (40.3%) and the North-American (4.8%) variants. Among the three novel variants, two were nonsynonymous. This study highlighted significant association of more than one type of HPV infection with the European T350G variant, while the European prototype variant was associated significantly with HPV 16 alone ($p < 0.05$, C.I. 1.2-13.6).

Cytotoxic T lymphocyte associated molecule-4 (CTLA-4) gene expression is associated with inhibition of T cell mediated immune response. Our study in cervical cancer revealed significant association of specific polymorphisms in CTLA-4 gene (+49 AA) with cervical cancer. Distribution of CTLA-4 -318 C/T genotype is also reported for the first time in Indian population.

Enhancing Knowledge and Promoting Health Seeking Behaviour of Couples on STIs and Cervical Cancer in Urban Slums

The objective of the study was to assess the knowledge, attitude, behaviour and practices about STIs and cervical cancer among eligible couples and plan appropriate intervention towards increasing the health seeking behaviour. The project was completed on 31st March 2013. Endline data was collected from 1025 couples from control area and 1013 couples from intervention area during August 2012 to March 2013. To sustain the programme, Pap smear camps once in a month were continued during this period.

Results of endline survey indicates significant increase in awareness on sexually transmitted

infections (STIs), cervical cancer and Pap smear test among husbands and wives in the intervention area with respect to control area. Inter personal communication to increase the awareness about Pap smear test among both husbands and wives during post intervention by project and health post staff; group meetings and educational programme were the contributing factors.

During Jan-Mar 2013, three Pap smear camps were organized by NIRRH and a total of 147 women had undergone screening. The Pap smear findings revealed that 64 percent smears were associated with inflammatory reactive changes. About 48% smears were tested positive for infections. Human Paplioma Virus (HPV) infection was found to be common (38.8%), followed by bacterial vaginitis (29.9%), fungal infection (19%) and *Chlamydia Trachomatis* (12.9%). Multiple infections were detected in 29.3% women. Women with positive result of Pap smear test were referred to Municipal Maternity home for treatment.

The programme was further sustained by Dr. Babasaheb Ambedkar Maternity Home, Vikhroli under vicinity of Municipal Corporation of Greater Mumbai (MCGM) from Apr.-Dec. 2013. During this period 45 Pap smears were collected on Gynecology OPD days (i.e every Tuesday). These pap smears were processed and screened by Laboratory Technician and Pathologist of Rajawadi Hospital (MCGM). Technical assistance and supervision was provided by NIRRH. The Pap smear findings revealed that 84.4 percent smears were associated with inflammatory reactive changes and 2.2% mild atypia. About 64.4% smears were positive for infections. Bacterial Vaginitis was found in 48.9% followed by *Chlamydia Trachomatis* in 8.9% and Human Paplioma Virus (HPV) in 6.6%. Women needing further management and treatment were referred to maternity home.

STRUCTURAL BIOLOGY AND BIOINFORMATICS

Functional Significance of Novel Mutations in Follicle Stimulating Hormone Receptor Gene

Follicle Stimulating hormone (FSH) acts through its receptor and is essential for ovarian folliculogenesis. Mutations in FSH receptor (FSHR) gene have been

reported to cause reproductive abnormalities. One of the novel mutations identified by our group in infertile women was detected in extracellular loop 2 of FSHR. *In-vitro* studies were carried out to understand the functional significance of this residue on receptor activity. Results suggest that the mutation affects cell surface expression of FSHR and also hormone induced signaling.

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

Understanding the molecular interaction of FSH with its receptor is a pre-requisite for designing molecules having FSH-agonistic or antagonistic activity. Contribution of amino acid residues from the extracellular loops (ELs) of FSHR in hormone-receptor interaction was investigated by employing site directed mutagenesis approach. Results indicate that the Leu⁵⁰¹Phe mutation in EL2 was responsible for lower internalization and impaired cAMP production the mutant as compared to wild type FSHR. In case of EL3, the combined effect of the Lys⁵⁸⁹Asn and Ala⁵⁹⁰Ser mutations was found to be responsible for the increased internalization of FSH-FSHR complex.

Studies on the Binding Specificities of Gonadotropins and their Receptors

The interaction of gonadotropin receptors with their cognate hormones leads to steroidogenesis in males and folliculogenesis in females and hence their activity is crucial for reproduction. A combination of *in silico* methods such as pharmacophore-shape similarity and fragment-based approach was used for rational design of peptidomimetics that display good affinity and specificity for human Follicle Stimulating Hormone Receptor (hFSHR). Docking studies revealed that the designed peptidomimetics can mimic the native hFSHR-FSH interactions. The designed peptidomimetics also displayed higher binding affinity to hFSHR as compared to two reported hFSHR antagonists.

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

New world primate monkey specific FSH is required to improve superovulation protocols and

also to develop homologous assays for measuring serum FSH in these species which are used as animal models in various biomedical studies. In the current study *Callithrix jacchus* FSH was cloned and expressed in yeast *Pichia pastoris* expression system. The recombinant protein obtained was characterized further and it demonstrates biological activity when tested *in vitro*.

Understanding the Role of CRISP-3 in Prostate Cancer

Cystein-Rich Secretory Protein 3 (CRISP-3) is upregulated in prostate cancer. CRISP-3 was knocked down in LNCaP cells using shRNA which affected their invasion ability. Global gene expression profiling of the CRISP-3 knockdown and vector transfected clones identified PSA and SPINK1 to be down regulated upon CRISP-3 knockdown. PSA is an androgen regulated gene and its levels are routinely tested for prostate cancer detection as well as prognosis. Higher levels of SPINK1 have been associated with adverse prognosis and SPINK1 knockdown has been reported to attenuate invasion. The effect of CRISP-3 knockdown on cell invasion may partly result due to down regulated SPINK1. CRISP-3, PSA and SPINK-1 appear to be co-regulated genes and estimating levels of all three proteins in the serum may be of a better prognostic potential. In order to study the regulation of CRISP-3 expression, DNA fragments of differing lengths upstream of *CRISP-3* gene were cloned in luciferase reporter vector and transfected into CRISP-3 expressing and non-expressing cell lines. Region imparting strong promoter activity has been identified.

Studies to Understand the Tertiary Structure-Dependence on Antimicrobial Activity of 2 Novel Peptides

Template-based analogs/mutants of a known helical, antimicrobial peptide, BMAP 28 (1-18) were designed to understand the role of tertiary

structures in antimicrobial activity. The peptides were modeled to adopt helical conformations and MD simulations were performed to assess and compare the helical stabilities of the wild type and mutant (increased similarity with template) peptides during the course of simulation. The mutants exhibited higher helical content and stability as indicated by the root mean square deviation (RMSD), root mean square fluctuation (RMSF) and DSSP analysis of the trajectories generated by MD simulations.

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

A database of experimentally elucidated structures of antimicrobial peptides (AMPs) was developed to study the secondary structural features that influence the antimicrobial activity of AMPs. The sequence information present in CAMP database (developed by our group in 2010) has also been updated. The database is manually curated and currently holds 6756 sequences and 682 three dimensional structures of antimicrobial peptides. Sequence and structure analysis tools are also available in the database for the benefit of researchers. The fully functional database can be accessed online at <http://www.camp.bicnirrh.res.in/>.

STEM CELL BIOLOGY

Preclinical Studies with Pancreatic and Tri-Potent Cardiac Progenitors Obtained from In-house Derived Embryonic Stem Cell Lines

Well characterized animal models for diabetes in mice and myocardial infarcts in rabbits by inflicting cryo-injury have been established. Pancreatic islets were encapsulated in an immunosolatory device and transplanted in diabetic mouse to study alleviation of disease symptoms. Similarly methodology to prepare a cell sheet which will be used as a support for cardiac progenitors was prepared (Fig 6).

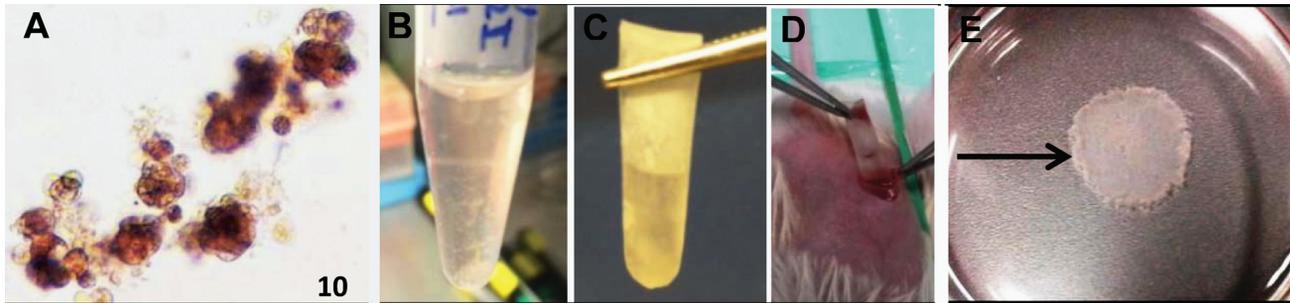


Fig. 6. (A) DTZ stained mouse pancreatic islets (B-D) Large number of islets are isolated, packed in an immuno-isolatory capsule (indigenously prepared and provided by Dr Prabha Nair, Shri Chitra) and transplanted into a diabetic mice (E) A 'patch' prepared by culturing rabbit adipose tissue mesenchymal cells in a thermo-sensitive dish. A fully confluent culture of cells shrinks and floats as a 'patch' when the culture dish is brought to room temperature.

VSELs in mammalian gonads

We earlier reported that PMSG treatment induces neo-oogenesis and primordial follicle assembly in adult mammalian ovary. Stem cells, collected by gentle scraping of sheep ovarian surface epithelium, were cultured for 3 and 15h with and without FSH. FSH treatment resulted in clonal expansion of stem cells and germ cell nest formation and this effect was mediated via alternatively spliced FSH receptor transcript R3, expressed on the stem cells rather than the canonical FSH R1 transcript which is expressed on the granulosa cells. These results suggest novel action of FSH via FSH-R3 on ovarian stem cells and challenge the existing paradigms of ovarian biology that states that initial follicle growth is gonadotropin independent and also that a female is born with fixed number of eggs.

Ovarian cortical tissue is cryopreserved as a source of primordial follicles for cancer patients prior to therapy – with an aim to achieve biological parenthood later on in life. In addition to primordial follicles, we show that ovarian cortical tissue is an excellent source of stem cells which are increased in numbers after FSH treatment and also retain the ability to differentiate into oocyte-like structures *in vitro*.

Similar to testicular VSELs reported last year, mouse ovarian VSELs also survive oncotherapy and culture of intact, chemoablated ovary in presence of FSH (10mIU/ml) results in increased ovary surface epithelium proliferation formation of cellular aggregates (germ cell nests from the surviving VSELs) that incorporate BrdU and express germ cell (MVH) and pre-meiotic

(STRA8) markers. Culture of busulphan treated mouse ovary surface epithelial cells resulted in spontaneous differentiation into GDF-9 and MVH positive oocyte-like structures. PMSG treatment resulted in increased percentage of SCA-1+/Lin-/CD45- VSELs in the chemoablated ovaries. These results provide evidence that VSELs, which survive chemotherapy, are modulated by FSH and retain ability to undergo oocyte-specific differentiation. Results have implications for women who undergo premature ovarian failure because of oncotherapy.

Thus the various studies undertaken on the novel stem cell biology in adult mammalian gonads has provided insights into various processes including cancer and will have translational relevance.

NATIONAL CENTER FOR PRECLINICAL REPRODUCTIVE AND GENETIC TOXICOLOGY

Exposure of Endocrine Disrupter, Bisphenol A (BPA) in Neonatal Rats: Epigenetic Effects on Male Germ Line

Studies were initiated to determine the effects of neonatal exposure to BPA on histone acetylation pattern in testis. A significant increase in hyperacetylation of histone 4 was observed in BPA treated group as compared to control, while down regulation was observed in Class I and II histone deacetylases (HDACs) as compared to control. These results indicated that the neonatal BPA exposure leads to aberrant histone acetylation pattern in testis. Further studies are underway to understand the association between altered histone acetylation pattern and apoptosis upon BPA exposure.

Genotoxic and Mutagenic Activity of Bisphenol A, an Endocrine Disruptor and Understand its Mechanism of Action

Exposure to BPA in adult rats for six consecutive days at NOAEL dose (5.0 mg) and even at 10 µg dose led to a significant increase in clastogenic activity in bone marrow cells and DNA fragmentation in blood lymphocytes. In order to understand the mechanism of its action, the animals were administered with 10 µg and 5.0 mg mg/kgbw of BPA. The levels of various antioxidants such as reduced glutathione, superoxide dismutase, catalase and lipid peroxidation were estimated. BPA treatment caused a significant decrease in the levels of reduced glutathione, superoxide dismutase, catalase and an increase in the lipid peroxidation in bone marrow cells, blood lymphocytes, plasma, testis and epididymis tissue as compared to control animals. The results obtained in the present study demonstrate that oxidative stress could be one of the possible mechanisms for genotoxic activity of BPA.

Effects of Oxytocin Exposure on Reproduction in Adult Male and Female Rat

Earlier studies showed formation of urinary calculi following oxytocin treatment. To confirm the formation of urinary calculi, 90 day repeated dose exposure was undertaken in Holtzman rat, Wistar rat and Swiss albino mice. All these studies did not show any significant effect on feed consumption, weekly body weight, hematological and serum biochemical parameters. However, the urinary calculi were observed in all the above studies. In Holtzman rat, on termination of the study, 4 animals showed presence of urinary calculi in urinary bladder whereas in wistar rats 8 animals from treatment groups showed the presence of calculi in urinary bladder and two animals each from low dose (0.4 ng/kg bw) and mid dose (4 ng/kg bw) group of Swiss albino mice calculi was observed. Effect of Oxytocin on differential gene expression was evaluated using microarray approach. This study revealed that 5370 genes were differentially regulated in treated groups as compared to control. These were found to be involved in various molecular and cellular processes such as glycolysis, hormone biosynthesis, cytoskeleton regulation etc. Validation of few of the genes by quantitative real time PCR is underway.

Effect of Bisphenol-A, an Endocrine Disruptor on Spermatogenesis in Common Marmoset at Cellular and Molecular Level

Ultrastructure of the marmoset testis treated with BPA (25 µM) showed thickening of basement membrane, cytoplasmic vacuolation in Sertoli cells and degeneration of developing spermatid in comparison with vehicle treated control. Further studies are in progress.

SEXUAL AND REPRODUCTIVE HEALTH – HIV LINKAGES

Strengthening Linkages between HIV (ICTC/PPTCT) and Family Planning Services for Prevention of Unwanted Pregnancies Among Women Living with HIV/AIDS

The PPTCT program in India focuses on Prong 3 (Provision of Nevirapine to pregnant infected mothers) and reports quote that it reaches only 32% of pregnant mothers who need it. Preventing unintended pregnancies among HIV positive women (Prong 2) could help reduce the burden on Prong 3. To improve use of dual protection and prevent unintended pregnancies among women infected with HIV, an operational research study was implemented in two randomly selected tertiary hospitals in Mumbai (supported by ICMR). The main intervention in this experimental control study was linking ICTC/PPTCT with family planning services by capacity building of providers (counseling and testing medical eligibility to use methods), provision of IEC material, referral slip, maintaining MIS and testing the acceptability of dual methods among 300 women infected with HIV attending ICTC/PPTCT.

Sixty percent of the referred participants reached Family planning centres. Statistically significant improvement in knowledge about contraception and dual protection, three times increased acceptance of dual methods (Fig. 7), significant increase in consistent use of condoms and lesser numbers of unwanted pregnancies and births were observed in the experimental as compared to control group. IUD was the most accepted method in the experimental group (Fig.8).

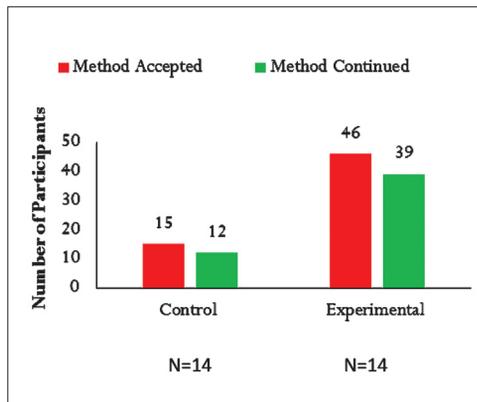


Fig. 7. Dual methods accepted.

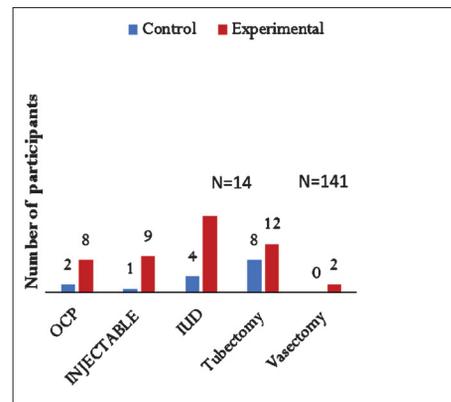


Fig. 8. Types of methods accepted along with condom.

The cumulative failure rate of contraception in the experimental group reduced resulting in preventing 12 women from risk of getting pregnant in first year of use compared to control. The study demonstrated the feasibility to establish this linkage and improved dual method use among participants. Since providers use different criteria to decide on eligibility to use family planning methods by PLHIV, especially CD4 counts, there is a need to formulate standard guidelines. Repeated reminders through follow-up are needed to motivate PLHIV to use Family planning methods. This is feasible by involving ART centers and incorporating this mandate in the follow-up agenda of ORWs undertaken for postpartum women. The study recommends testing these results in larger settings at district level to generate evidence for expanding the current mandate of PPTCT to focus on prong 2 and improve use of dual methods.

GENETIC RESEARCH CENTER, MUMBAI

Genetic Research Center (GRC) is one of the major centers of which is working mainly on inborn errors of metabolism, sex reversal disorders and mental retardation, because of the high mortality and morbidity associated with these diseases

Molecular Diagnosis of Inborn Errors of Metabolism

Inborn errors of metabolism are genetic disorders due to lack of a specific enzyme leading to abnormal accumulation of substrate or deficiency of product. They are responsible for considerable mortality and morbidity in childhood. The focus has been on identifying the molecular basis of

following groups of disorders- Inborn errors of metabolism (Lysosomal Storage Disorders and Organic acidemias).

Till date, 152 patients of lysosomal storage disorders have been included in the study. The number of patients group wise were as follows- Tay Sachs disease (TSD) (57), Sandhoff disease (SD) (27), GM1 gangliosidosis (7), Niemann Pick (3), MaroteauxLamy syndrome (5), Morquio syndrome (5), metachromatic leukodystrophy (15), Pompe disease (12), Hunter disease (4), and Krabbe disease (5). Thirty different mutations were identified in HEXA gene in patients of TSD, 20 were missense, 5 splice site variants and 5 frameshift mutations. Twelve different mutations were observed in HEXB gene in SD patients (7 frameshift, 3 splice site, 1 missense and 1 nonsense). Novel mutations were also identified in ARSA, GAA, IDUS genes. Ten cases with adrenoleukodystrophy were identified and novel mutations were identified in ABCD1 gene. A total of fifty patients from the organic academia group were studied. The number of patients in the organic academia group were as follows – glutaricaciduria type 1 (GA-1) (20), maple syrup urine disease (15), HMG CoA lyase deficiency (4), propionic academia (5), tyrosinemia (6). Novel mutations were identified in the genes – GCDH, BCKDHA, HMGCL and PCCB.

Mutational Analysis of in Indian Cases of Rett Syndrome

Rett syndrome (RTT) is a severe neuro developmental disorder characterized by the progressive loss of intellectual functioning, fine and gross motor skills and communicative abilities, deceleration of head growth, and the development

of stereotypic hand movements, occurring after a period of normal development. The classic form of RTT involves mutation in MECP2 while the involvement of CDKL5 and FOXP1 genes has been identified in atypical RTT phenotype. FOXP1 gene encodes for a fork-head box protein G1, a transcription factor acting primarily as transcriptional repressor through DNA binding in the embryonic telencephalon as well as a number of other neurodevelopmental processes. In this report we have described the molecular analysis of FOXP1 gene in Indian patients with Rett syndrome. FOXP1 gene mutation analysis was done in a cohort of 34 MECP2/CDKL5 mutation negative RTT patients. We have identified a novel mutation (p. D263VfsX190) in FOXP1 gene in a patient with congenital variant of Rett syndrome. This mutation resulted into a frameshift, thereby causing an alteration in the reading frames of the entire coding sequence downstream of the mutation. The start position of the frameshift (Asp263) and amino acid towards the carboxyl terminal end of the protein was found to be well conserved across species using multiple sequence alignment. Since the mutation is located at forkhead binding domain, the resultant mutation disrupts the secondary structure of the protein making it non-functional. This is the first report from India showing mutation in FOXP1 gene in Rett syndrome.

Molecular Aberration Studies in Sex Reversed Patients with Disorder of Sex Development

Determination of sex is the result of cascade of molecular events that cause undifferentiated bipotential gonad to develop as a testis or an ovary. A series of genes such as SRY, steroidogenic factor-1 (SF1), AR, SRD5 α , Desert hedgehog (DHH) etc., have been reported to have a significant role in development of sex in the fetus and secondary sexual characteristics at the time of puberty. Recently, mitogen activated protein kinase kinase kinase 1 (MAP3K1) gene was found to be associated with 46, XY disorders of sex development (DSD). Our study focused to identify mutations in MAP3K1 gene in the cohort of 10 Indian patients with 46,XY DSD including one family with two affected sisters. These patients were already screened for SRY, SF1 and DHH gene, but no mutation was observed in any of these genes. The entire coding regions of

MAP3K1 were amplified and sequenced using the gene specific primers. Sequence analysis of MAP3K1 gene has revealed four variants including one missense, two silent and one deletion mutation. The missense mutation p.D806N was observed in four patients with hypospadias. Two patients showed the presence of silent mutation p.Q1028Q present in exon 14. Another silent mutation p.T428T was observed in a patient with gonadal dysgenesis. We have also observed one deletion mutation p. 942insT present in two patients. The pathogenicity of the missense mutation p.D806N was carried out using in-silico approach. Sequence homology analysis has revealed that the aspartate at 806 was found to be well-conserved across species, indicated the importance of this residue. The score for polyphen analysis of this mutation was found to be 0.999 indicating to be pathogenic mutation. Since, p.D806N mutation was found to be important residue; it might contribute to sexual development. We have reported the presence of mutations/polymorphism in MAP3K1 gene. All the mutations were found to be polymorphism upon comparing to single nucleotide polymorphism database. However, in-silico analysis of the missense mutation revealed to be a pathogenic mutation.

Extramural Research

FERTILITY REGULATION

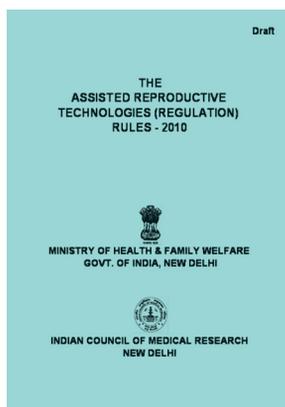
Phase-III Clinical Trial with an Intravasal Injectable Male Contraceptive – RISUG® : The Phase-III Clinical Trial is going on at five centers in the country and few more centres are in process of inclusion. A total of 166 subjects have received RISUG injection and they have been followed up for efficacy and safety. No side effects have been reported in any of the subjects post RISUG injection. All subjects are maintaining the clinical efficacy of the drug except two subjects who received RISUG injection three months ago but are showing severe oligospermia. A new prototype of RISUG Syringe which was developed and approved by the Monitoring Committee is now being used by the participating centers for enrolling new subjects. Simultaneously the gamma radiation facility at Central Glass and Ceramic Research Institute, Kolkata, which is being used in RISUG drug development, is in process of up-gradation. The

ICMR is in process of providing financial support to up-grade the facility in a project mode.

Evaluation of a progesterone vaginal ring as a new contraceptive option in India

The safety efficacy component of the study was initiated in December 2011 and till date 789 women have been enrolled in the study (PVR-459, Cu T 330). A total of 459 and 330 women were enrolled until 8th April, 2013 for PVR and IUD respectively and they were followed up for 3796 and 3191 woman-months of PVR and IUD use respectively. The continuation rate in PVR users was observed to be 83.2, 70.6, 62.8 and 58.5 % at 3, 6, 9, and 12 months of use respectively and with CuT 380A IUD the continuation rate was observed to be 94.1, 89.7, 84.6 and 80.3 at 3, 6, 9 and 12 months of use respectively. No serious adverse event has been reported till date. The study is ongoing and new enrolments are on hold and would be restarted after meeting all the stipulations laid out by the CDSCO/DCGI in India which includes registration of Institutional ethics committee, Insurance cover and compensation, audio-video recording etc.

The Assisted Reproductive Technology (Regulations) Bill – 2013



The Cabinet note along with the proposed draft ART (Regulation) Bill were circulated to all the concerned Ministries and Departments of Govt. of India for their comments by 16th July 2013. Based on these discussions and recommendations the Draft Bill and Cabinet note was revised and sent to DHR along with ICMR's clarifications to all the comments of all the concerned Ministries and Departments. After the approval of the DHR, MOHFW the revised Cabinet note along with draft ART Bill, comments of various Ministries

& Departments of Govt. Of India and clarification of ICMR to these comments will again be sent to the Ministry of Law and Justices for taking further necessary action in this matter.

National Registry of ART Clinics and Banks in India: Currently more than 1331 ART Clinics and Banks have been identified in the country. Out of these, 696 ART Clinics and 120 Banks have confirmed their contact details (total 816) and remaining are in the process of confirmation. Out of 816 ART clinics, 696 ART clinics have submitted their duly completed proforma. Nearly 237 ART clinics have been found in compliance with the provisions of the proposed ART (Regulation) Bill therefore, these 237 ART clinics have been enrolled under the National Registry of ICMR.

As per the provision of the draft Assisted Reproductive Technology (Regulation) Bill there is a provision of establishing an institution called "ART Bank" hence steps have been initiated to develop National guidelines for accreditation, supervision and regulation of ART banks in India. The draft National Guidelines for ART Banks have been developed describing categories of ART Banks, minimum infrastructure facilities required at ART Bank, minimum trained manpower and procedures being under taken at these ART Banks, procedures and steps for screening of gamete donors, surrogate mothers, social, ethical & legal provisions of gamete donors and surrogates, format for necessary consent forms, etc. After the approval of the drafting committee and the DG, ICMR the document will be made available to the public.

Effect of Non-ionizing Electro Magnetic Field (EMF) on Human Health: To study the effect of EMF on Neurological, Reproductive, Cardiovascular, ENT, Cancer and other vital parameters, ICMR is conducting a long term prospective cohort multi-centric study at AIIMS, New Delhi. To collect and record laboratory and clinical examinations data from various disciplines 24 proforma have been developed in consultation with the investigators which were approved by the Expert Committee. After completing exclusion and inclusion criteria and following a web based mechanism around 1500 healthy volunteers have been enrolled under the study and out of that around 700 are being followed for clinical and laboratory

examination. The data received is under validation and analysis.

MATERNAL HEALTH

Engagement of AYUSH practitioners (APs) in the Public Health System to provide SBA services: Shortage of skilled birth attendants (SBA) is one of the determinants of maternal mortality in India. To combat this shortage, innovative task shifting strategies of engaging providers of the Ayurveda and Homeopathy system of medicine called AYUSH practitioners (APs), to provide SBA services was explored through a qualitative and quantitative study conducted in two districts each of the states of Rajasthan, Maharashtra and Odisha.

Acceptability and integration was highest in Maharashtra and least in Odisha. This could be attributed to the fact that APs have been employed at peripheral health centres in Maharashtra for more than a decade and are well accepted in the health system. Whereas in the other two states, appointment of APs is a recent intervention under the NRHM and would take time to get accepted. In Maharashtra, the role definition was clearer and APs were at par with their counterpart medical officers with respect to their responsibilities and position in the health system. Legal and regulatory sanctions were identified as an important barrier by both APs and program managers.

Results of this study were **translated into policy change**. APs working in government facilities have now been permitted by the MOH&FW to conduct deliveries and use certain allopathic drugs after appropriate training.

Reducing maternal mortality through evidence based intrapartum and postpartum care:

The Government of India has issued evidence-based guidelines for maternity care providers and also provides training for their implementation. However, uptake and implementation of the guidelines remain a challenge. A qualitative survey was done among 125 providers including doctors, nurses, LHVs, ANMs to explore the barriers faced by the providers in adhering to GoI guidelines. The key factors which emerged from the analysis were Awareness, knowledge and training of providers.

The **key recommendations** of this study which would be useful for policy makers and programme managers for improving maternal and neonatal health are:

1. The GoI guidelines need to be disseminated widely; continuous interaction of mentors and providers needs to be promoted to incorporate the suggestions of SBAs to eliminate implementation barriers.
2. Effective change management using novel strategies, encouraging self-learning, supportive supervision might help to motivate providers to adopt evidence based practices and eliminate harmful or ineffective conventional practices.
3. Medical colleges should play a greater role in pre-service and in-service SBA training and facility based maternal and perinatal outcome review.
4. Appropriate staff deployment and task shifting mechanisms are needed at all levels.
5. Greater coordination between civil works department and facility management committee is suggested to prioritize and resolve infrastructural issues. Active participation of Rogi Kalyan Samitis should be encouraged to improve facilities and services.

Modulation of Cytokine Production in women with recurrent pregnancy loss:

The success or failure of pregnancy depends on the balance of pro-inflammatory and anti-inflammatory cytokines on the conceptus. It is understood that dydrogesterone inhibits the production of the Th1 cytokines (TNF- α , IFN- γ) from lymphocytes and up-regulates the production of the Th2 cytokines (IL-4, IL-10), inducing a Th1 to Th2 cytokine shift. A placebo controlled trial was done to study the effect of dydrogesterone on cytokine modulation in women with recurrent pregnancy loss. Occurrence of another abortion after 3 consecutive abortions was significantly higher in women with RPL (16.76%) as compared to healthy pregnant controls (3.45%). Risk of occurrence of miscarriage after three abortions in placebo group (16.7%) was higher as compared to dydrogesterone group (6.8%) was RR=0.21, (95%CI=0.09-0.48). The percentage change in the serum level of IL-10 was found to be significantly higher in women who miscarried in RPL group as compared to healthy pregnant

control ($p < 0.05$). Mean gestational age at delivery increased significantly in dydrogesterone group (38.01 ± 1.96 weeks) as compared to placebo group (37.23 ± 2.41 weeks).

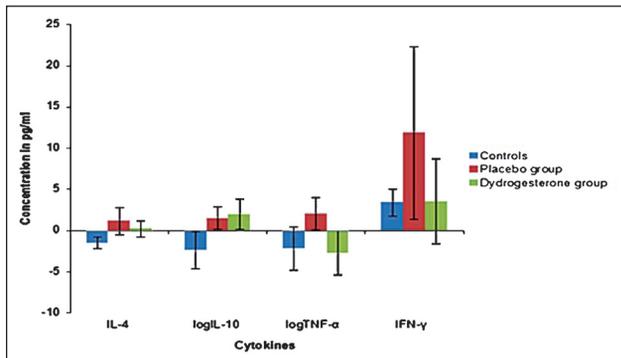


Fig. 1. Percentage change of cytokines (Mean \pm SE) in women who had miscarriage in different groups.

Data indicate reduction in abortion rate and increase in gestational age at delivery in the dydrogesterone group as compared to the placebo group. However, the effect of dydrogesterone does not appear to be through modulation of cytokines.

Promoting HIV prevention by understanding fertility desires among HIV-positive women and men in India: Expanded access to ART and concomitant improvements in life expectancy and perceived quality of life among individuals on ART has brought to the fore issues related to the sexual and reproductive health and rights (SRHR) of people living with HIV/AIDS (PLHIV). A qualitative research study was undertaken in Bangalore, Chennai and Mumbai to understand the fertility desires and contraceptive practices of married people living with HIV (PLHIV); examining the role of antiretroviral therapy and individual and relationship factors in shaping desires and practices; and identifying ways that preventive counseling and care services can effectively address PLHIV's needs.

A total of 54 and 38 qualitative interviews were conducted with PLHA and health care providers, respectively, and 439 survey interviews were completed. It was found that most subjects had no childbearing intentions.

An Epidemiological study and development of a novel endometrium specific biomarker in Female Genital Tuberculosis: Tuberculosis is a

major public health problem and female genital tuberculosis (FGTB) comprises of 10% of cases with pulmonary tuberculosis. Currently, there are no known specific biomarkers for FGTB hence this study was done to estimate the proportion of FGTB; evaluate the efficiency of PCR based techniques its diagnosis and develop a novel endometrium-specific biomarker for female genital tuberculosis using Suppression Subtractive Hybridization (SSH) technique. An endometrial specific biomarker based on differentially expressed genes in uninfected and mycobacterium infected human endometrial cell lines was developed using SSH method to construct subtracted cDNA libraries and the libraries were screened for differential expressed genes. Three sets of primers for PCR amplification of three genes (HSP 65, CFP 10 & ESAT6) were used. By AFB culturing, two patients were positive for the presence of *Mycobacterium tuberculosis* and one patient was found to have Non Tuberculous Mycobacterial (NTM) infection.

Development of a novel endometrium-specific biomarker by studying the differential gene expression: Ishikawa cell lines were infected with *M. tuberculosis* H37Rv. Subtracted cDNA library was constructed from the infected cells harvested 24 hours post infection. Around 218 clones containing the cDNA inserts were obtained. The clones containing the cDNA inserts were screened by sequencing the cDNA inserts. Subsequently, the sequences were subjected to BLAST analysis using NCBI BLAST search tool. Out of 218 clones, 106 different gene sequences were obtained and rest of the gene sequences were repeated. The gene sequences identified were categorized based on their functions. From the above mentioned gene sequences, the genes related to ubiquitination were studied for its gene expression profile at different time intervals of incubation after infection.

Validation Ubiquitin pathway related genes using Quantitative Real Time PCR method: Ishikawa cell lines were infected with *M. tuberculosis* H37Rv for 4 hours and then harvested at 0 HPI (Hours Post Infection), 6 HPI, 15 HPI and 24 HPI. Total RNA was isolated from the harvested cells and cDNA was synthesized. Real time PCR was carried out for the genes - MKRN1, RNFT1, ZFP91, COPS5, NDFIP2, UBE2F, PSMD13, PSMB6

Upregulation of *mkrn1* and *cops5* at 24 Hours Post Infection:

The Makorin ring finger protein 1 (*mkrn1*) showed an initial down-regulation in the gene expression to 0.5 folds at 0HPI and did not show any considerable change in the gene expression from 6HPI to 15 HPI when compared to the control whereas at 24 HPI, the infected cells showed an up-regulation of 3 folds than the control. *cops5* (COP9 constitutive photomorphogenic homolog subunit 5) showed an under-expression of 0.6 folds at 0 HPI and subsequently showed an increased expression from 6 HPI to 15 HPI with a remarkable over-expression to 3 folds at 24 HPI (Fig. 2).

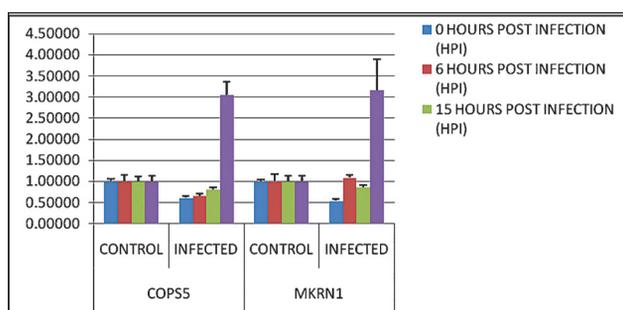


Fig. 2. Comparison of gene expression profiles of Ub ligases in Ishikawa cell lines upon *M. tb* infection: Gene expression of *mkrn1* and *cops5* showing up regulation at 24 Hours Post Infection.

Downregulation of *rnft1* and *zfp91* at 24 Hours Post Infection:

Ring finger protein, transmembrane 1 (*rnft1*) and *zfp91* (Zinc Finger protein 91) showed no considerable change in gene expression at 0HPI and 6HPI when compared to the control. *rnft1* and *zfp91* showed an upregulation of 2 and 3.4 folds respectively upon infection at 15 HPI and then down regulated to 0.6 folds and 0.5 folds respectively at 24 HPI (Fig. 3).

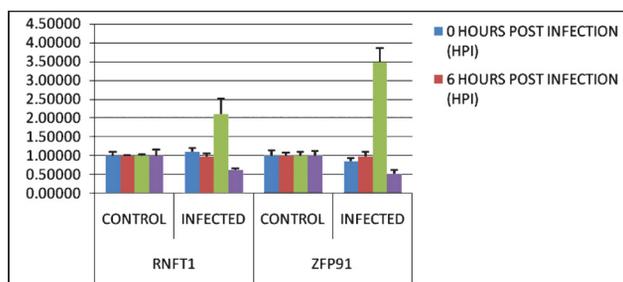


Fig. 3. Comparison of gene expression profiles of Ub ligases in Ishikawa cell lines upon *M. tb* infection: Gene expression of *rnft1* and *zfp91*.

Down regulation of *psmd13* and *psmb6* at 24 Hours Post Infection:

psmd13 (proteasome (prosome, macropain) 26S subunit, non-ATPase,

13) and *psmb6* (proteasome (prosome, macropain) subunit, beta type, 6) are the proteosomal subunits which degrades the ubiquitinated protein. *psmd13* and *psmb6* showed downregulation at 0HPI (0.8 folds) and 6 HPI (0.6 folds). Both the genes have showed an upregulation of 1.6 and 1.2 folds at 15 HPI respectively whereas at 24 HPI downregulation to 0.6 folds was observed in both the genes (Fig. 4).

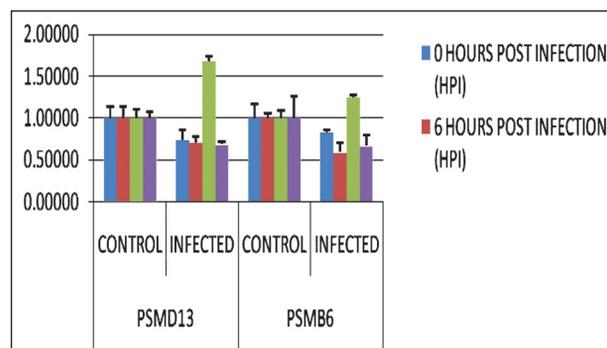


Fig. 4. Comparison of gene expression profiles of proteasome subunits in Ishikawa cell lines upon *M. tb* infection.

Downregulation of *ube2f* and *ndfip2* at 24 Hours Post Infection:

ube2f (ubiquitin-conjugating enzyme E2F) and *ndfip2* (Nedd4 family interacting protein 2) are other genes involved in the ubiquitination pathway. *ube2f* binds to Ub for further transfer by Ub ligase to protein of interest and *ndfip2* activates HECT- domain containing Ub ligases. *ube2f* showed a mild downregulation at 0 HPI and 6 HPI. *ndfip2* showed an underexpression of 0.5 and 0.6 folds at 0 and 6 HPI. Both the genes showed a upregulation of 1.4 and 1.9 folds respectively at 15 HPI further downregulated to 0.5 and 0.4 folds respectively at 24 HPI (Fig. 5).

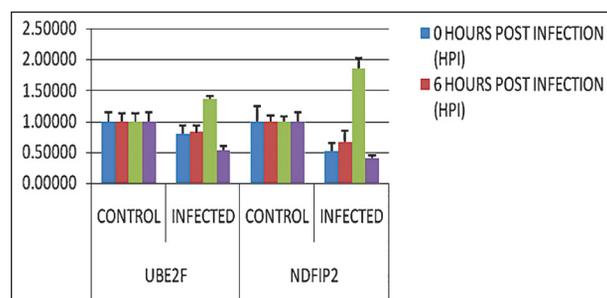


Fig. 5. Comparison of gene expression profiles of other Ub pathway genes in Ishikawa cell lines upon *M. tb* infection.

The above data clearly shows that there is a modulation in the expression of ubiquitination pathway related genes during the infection of Ishikawa cell lines with

M. tuberculosis. However, there were insufficient cases of FGTB to enable clinical validation using ubiquitination pathway genes. It was suggested that this system may be used to study a different model of tuberculosis such as Pulmonary Tuberculosis as well as other infective diseases.

Health care seeking behaviour among Extra pulmonary TB (EPTB) including Female Genital Tuberculosis (FGTB) patients under RNTCP in and around Pondicherry was studied among 831 female TB patients. Most FGTB patients belonged the age group of 21 to 40 years (93.9%), nearly 75.9% were taking treatment under Category I under the RNTCP and 16.9% were on category II treatment. About 58% were diagnosed with pulmonary TB and 35% with EPTB. On comparison between symptoms of FGTB and the final diagnosis of FGTB, the symptoms of ‘infertility’ and ‘white discharge’ were found to be statistically significant ($p < 0.001$). Table 1 shows the predictive value of common symptom combinations for FGTB.

Reproductive morbidity among adolescent girls (10-19 years) in the primary setting:

A cross sectional survey of Anganwadi centres in Thiruvananthapuram district of Kerala was undertaken to screen adolescent girls ($n=3932$) for self-reported symptoms of reproductive morbidity using Teenage Screening Questionnaire –Trivandrum (TSQ-T). Common self-reported reproductive morbidities were menstrual problems (36%), reproductive tract infections (8.4%), urinary tract infection (2.3%) and polycystic ovaries (6%). The results of the study indicate that specialized approaches must be implemented in RCH program under NRHM to motivate adolescent girls for menstrual problems and RTI screening. Teenage

Screening Questionnaire –Trivandrum (TSQ-T) is a feasible tool to detect reproductive morbidities in community settings.

CHILD HEALTH

Role of non-health system stakeholders in the management of Government run child health programmes through village health sanitation and nutrition committees: A situational analysis from Gujarat and Jharkhand: It was found that non-health stakeholders were not aware of various health programs and did not play any role in the supervision of the health services except for some PRI members. Role played by NGOs in the committees was found to be minimal. The roles played by the VHSNC members and non-health stakeholders are seen as extension of their routine functions rather than a dedicated function. Devolution of power is low as they are not empowered and sensitized towards their role of supervision and demand generation of health services.

Vitamin D Supplementation for Severe Pneumonia in Under-Five Children: A Double Blind, Randomized Placebo Controlled Trial: Acute respiratory infections (ARI), pneumonia, are a major cause of deaths in under-five children. Association between vitamin D deficiency and risk of pneumonia has been observed. A study is ongoing to evaluate the efficacy of single mega-dose of vitamin D supplementation for prevention of severe pneumonia and prevention of episodes of pneumonia in next six months. A total of 206 cases (out of projected 323) have been enrolled till 31st March 2014. Out of 206 cases enrolled, 189 (91.75%) were available for estimation of time to resolution of severe pneumonia (follow up loss 17

Table 1: Clinical symptoms and diagnosis of FGTB (PCR test positive) among participants

Combination of symptoms present among ‘FGTB suspects’	PCR positive (n=73)	PCR negative (n=125)	Predictive value of symptoms in PCR positive (FGTB) patients
White discharge, menstrual disorders, lower back pain and burning urination	35 (47.9%)	67 (52.8%)	35/102= 34%
Infertility, post coital bleed, blood stained discharge	21 (28.8%)	39 (31.2%)	21/60=35%
Infertility, white discharge, lower back pain	5 (6.9%)	17 (13.6%)	5/22= 23%
Infertility with dysmenorrhoea	12 (16.4%)	2 (1.6%)	12/14=86%
Total	73 (100%)	125 (100%)	73/298=25%

cases 8.25%). Recurrence of pneumonia in next six months is 197 (95.6%), of these, 143 cases have completed 6 months follow-up; the rest are in various stages of follow-up. Study is in progress. Code will be broken at the end of enrollment.

A Community-based Perinatal Death Audit in the Urban Slums of Ludhiana: A cross-sectional study is being carried out in 91 urban slums of Ludhiana to investigate the causes of perinatal deaths in the population, to quantify their contribution to the overall perinatal mortality and suggest improvement measures. Till now, a total of 1000 perinatal deaths have been audited, of which 697 were still births (SB) and 301 early neonatal deaths (END). There were 17 maternal deaths in this population. Around one fifth (217/998) deliveries took place at home. Gestational age at death was 7 months or less in 25.6% cases; in 82% delivery was normal. Around half (595/1000) of the perinatal deaths had a medical certificate of death but cause of death could be ascertained in only 97 of 1000 perinatal deaths. In 72.4% of cases, the death was not registered. According to ICD-10 Classification, major causes of deaths were birth asphyxia (75%), prematurity (22.7%), meconium aspiration (10.7%), and IUGR 5.6%. Study is ongoing.

Status of procurement, distribution and management system of zinc dispersible tablets (ZDTs), IFA tablet/liquid and Vitamin A (VA) supplements in Uttar Pradesh, Maharashtra, Himachal Pradesh, Kerala and Rajasthan states of India: This study was undertaken to assess the status of procurement, distribution and management system of zinc dispersible tablets, IFA tablet/liquid and VA supplements in Uttar Pradesh, Maharashtra, Himachal Pradesh, Kerala and Rajasthan states of India. The data has been collected at the National level and in 5 selected states in the country. A low priority was accorded to all the three National Programmes namely a) National Anaemia Control Programme, b) National Programme for Prevention of Nutritional Blindness due to VA deficiency, and c) Zinc linked with ORS under National Diarrhoea Disease Control Programme at all the levels i.e. National, State, District and Block levels. It was found that the procurement and distribution of micronutrient supplement was irregular. There were limited knowledge on how and when to use ZDTs(except in Kerala state).

Surveillance of infection in neonates: Infections are major causes of mortality in neonates. There is lack of data on etiological agents causing sepsis in the newborns in the community and their antibiogram. Neonates with either intramural or extramural delivery with signs and symptoms of probable sepsis are being enrolled. A total of 1417 cases of suspected sepsis were enrolled out of which 255(18.0%) were culture positive. Major gram positive organisms identified were Staphylococcus aureus, and gram negative organisms were Klebsiella, E.coli and others. Study is in progress.

Effect of early physical therapy intervention on singleton preterm VLBW infants at high risk of developing motor developmental delay - a RCT: The infants with decreasing gestational age and birth weight are at high risk of developing motor developmental delay. The evidence of physical therapy interventions as part of development care interventions in Indian context is not been ascertained till date.

A RCT with the primary objective to ascertain the effect of early physical therapy intervention on motor developmental outcomes was carried out in the NICU of North Bengal Medical College Hospital. The intervention protocol included: tactile, proprioceptive, visual, vestibular and auditory stimulation. Multi - modal sensory stimulation, facilitation of righting / equilibrium reactions of the body and movement patterns using neuro-developmental therapy techniques at post discharge from NICU.

The experimental group showed statistically significant differences in motor and mental developmental outcomes when compared to control group. There were also significant differences in weight and height gain at four months corrected age in the experimental groups compared to the control group; however no significant differences were noted in head circumference and Ponderal index at four months corrected age. Thus physical therapy intervention on singleton preterm very low birth weight infants proved to be beneficial in terms of improved motor and mental neuro-developmental outcomes. The study provides policy implications of embedding multi-disciplinary interventional approach in NICU to preterm very low birth infants in improving neuro-developmental outcomes.

Centre for Advanced Research (CAR) on Evidence Based Child Health: The CAR at PGIMER has conducted 7 capacity building short courses /protocol development workshops during the reporting period. The short courses trained more than 435 participants in critical appraisal of various clinical studies and introduced them with the concepts of Evidence Based Child Health. The Advanced Centre provided **literature support** (full-text articles both electronic and manual) to persons developing protocols or working on systematic reviews. The following six reviews have been completed and two systematic reviews completed by the CAR have been published in reputed journals

- Management of neonatal sepsis with particular reference to Intramuscular Gentamicin
- Efficacy of pneumococcal Vaccines for childhood mortality.
- Efficacies of *Haemophilus influenzae* type b conjugate vaccines for childhood mortality.
- Invasive *Haemophilus influenzae* Type B disease burden in Indian children under 5 years of age: Systematic Review
- Efficacy and effectiveness of rotavirus vaccine against diarrhoea: a systematic review and meta-analysis.
- Implementation strategy for public health interventions like immunization coverage.

Solar powered baby/infant radiant warmer was installed at neonatal intensive care unit at Lok Nayak Hospital, New Delhi

The solar radiant warmer is working well without any technical problems (Fig. 6). Total electricity cost saved by using one solar power radiant warmer =Rs1,19,770.10/year. The energy consumed from grid all through the year was 0.72 % and the energy consumed from solar plant during this period was 99.28% . Thus the solar panel (Fig. 7) is enough for one radiant warmer. Cost of Panel of 1.2 kWp for one radiant warmer is Rs 2,25,000/- while the electricity saved is Rs. 1,19,770/-. Therefore, approximately net expenditure saved is Rs1,05,300/year. Using subsidized panel can decrease the cost further.



Fig. 6. solar powered radiant warmer.



Fig. 7. Solar panel on roof top of hospital.

Fabrication of Solar Powered portable culture incubator

Two solar powered portable culture incubators have been fabricated (Fig 3). The design allows the field workers to carry the incubators conveniently to transport samples from sub district level to institutions where culture facilities exist.



Fig. 8. Solar powered portable culture incubators.



Fig. 9. Solar panel fixed on roof of vehicle.

The solar module/panel is attached at a slant on top of the transport vehicle and the incubator can be charged using the battery of the transport vehicle (Fig. 9). Four samples were collected from Palwal General Hospital, Faridabad district for testing transportation in the solar powered incubator-3 throat swabs from children and on sample of pus from drainage tubes. These were smeared on the culture plates (Fig. 10) and then kept in the incubator's culture compartment (Fig. 11). Throughout the journey the temperature was maintained between 37.0 ° C -37.5° C during transport. On reaching the destination the portable culture incubator was disconnected from the solar module and the portable culture incubator and was carried to Department of Microbiology. The temperature at the time of handing the reports was 37.0 ° C. The reports were obtained and dispatched to civil Surgeon for treatment.



Fig. 10. Samples smeared on culture plates.



Fig. 11. Culture compartment of incubator.

National Retinoblastoma (RB) registry programme: Retinoblastoma is a rare cancer of childhood. It is curable when diagnosed early. There were 2596 cases enrolled into the registry from Jan 1, 2009 to September, 2013. The survival

of 2596 cases of RB enrolled in the RB registry in India is being analyzed.

Age-Related Blood Lymphocyte Subsets in Healthy Indian Children: A Multicentric Study was done to measure the age-related blood lymphocyte subsets and establish the normal ranges among healthy infants and children.

Pharmacokinetics of anti tuberculosis drugs in children: impact of age, nutritional status and HIV infection. This is the first study to report on the pharmacokinetics of RMP, INH and PZA in HIV-uninfected and HIV-infected children with TB being treated with short-course, intermittent regimens, and also the first to relate drug levels with treatment outcomes. Results indicated that age, HIV infection and INH acetylator status influenced drug pharmacokinetics, and that these could in turn influence treatment outcome. Future recommendations for anti-TB treatment in children should consider age, HIV infection, INH acetylator status and nutritional status in order to achieve optimal treatment outcomes.

Effects of malnutrition and HIV infection on pharmacokinetics of anti-tubercular drugs in children: This study was done to find out the serum drug levels of rifampicin, isoniazid and ethambutol at 2 hours after the administration of the medication in children with tuberculosis. In view of similar data from other studies and also the recommendations of the WHO and National consultation on diagnosis and treatment of pediatric TB by the Central TB division, it will be appropriate to study the pharmacokinetics of the anti tubercular drugs using the new recommended doses.

Role of zinc supplementation in children with cystic fibrosis (CF): randomized controlled trial. A double blind randomized controlled study was carried out to detect the effect of 30 mg daily zinc supplementation on the need for and duration of antibiotic administration. A total of 40 children with CF (20 in each group) between 5-18 years of age were enrolled and 37 (19 in zinc group and 18 in placebo group) children completed 12 months follow up. Base line characteristics of patients in two groups were comparable. On an average the need for antibiotics in children were similar (54.7 ± 58.9 days versus 61.52 ± 62.56 days) in both groups.

There was no significant difference in FEV1, side effects of medications and pseudomonas infection in the two groups.

Outcome of very low birth weight (VLBW) babies and economic burden of intensive care on families of VLBW (≤ 1250 grams) babies during hospital stay: This is an observational descriptive study of all VLBW babies (< 1250 grams) born during one year period and subsequently being followed up till 2 years corrected age. A total of 341 cases were enrolled and short term outcome was analyzed. A total of 164 babies were discharged alive who were in various stages of follow up. The growth, neurodevelopment, vision, hearing, speech and psychological assessments are being done as per protocol. The loss of follow up at various stages was between 15-28%.

RCT to compare the efficacy and safety of mycophenolate mofetil versus levamisole in children with frequently relapsing Nephrotic syndrome : This project is evaluating in relapsing and resistant Nephrotic syndrome the efficacy and safety of Mycophenolate Mofetil and Levamisole . 53 patients (45 boys) have been randomized since initiation of study, including 17 with steroid dependence.

Role of innate immune cells in the pathogenesis of Juvenile idiopathic Arthritis –Enthesitis related arthritis: The mean frequency of gamma-delta T cells was higher in patients (9.5+4) as compared to healthy controls (4.1+1.5; $p<0.01$). No difference was observed in frequency of NK and NKT cells. However NK cells had reduced expression of perforin in patients (51=16% vs 77+8.4; $p<0.01$) and increased expression of KIR3DL (34+8.2 Vs 14+0.5; $p< 0.01$) as compared to controls. Monocytes having CD16 expression were higher in patients (9+3.2 vs 5+0.9; $p<0.001$) and MFI for CD1d was higher in patients (60+25 Vs 26+8; $p<0.01$) as compared to controls. In paired samples, the frequency of monocytes, NK, NKT cells was similar. KIR3DL and perforin expression on NK was reduced in SFMC as compared to PB

Higher frequency of CD14+CD16+ monocytes and lower frequency of perforin+ NK cells suggests that the cytokine producing innate cells are increased and these may contribute to damage in JIA.

DIABETES RESEARCH

Diabetes Genetic Susceptibility in the Asian Indian Population: The study identified an association between several genetic variants with early-onset type 2 diabetes. In the first stage, 100 cases and 100 controls were selected for sequence analysis identified of approximately 16,000 variants. In the second-stage of the project 199 SNPs were for validation of the initial finding, their association with early onset T2DM. Of the various genetic variants, four achieved a highly significant association. Two of the variants were novel and will benefit from additional analysis, to the variants have been described previously for Diabetes management and drug discovery. These results suggest that many more variants remain to be discovered in a larger study. This project has resulted in the identification of 15 loci on the genome, to be associated with early onset type 2 diabetes in India.

Development of blood out growth Endothelial cell and nano- fibre Scaffold synergy in wound healing: The role of endothelial cells on polylactic acid-chitosan-collagen nanofibrous scaffolds was explored and three dimensional lyophilized as well as two dimensional electrospun scaffolds were developed as potential substrates for blood outgrowth endothelial cells (BOECs) attachment, viability and functionality. Electrospun scaffolds showed the best viability and functionality for BOECs. Moreover this study also showed that fibrin coatings also have good potential for improving endothelial cell performance in these scaffolds. BOEC cells would release nitric oxide and promote healing. The specialty of the scaffold is that it is made up of nano and micro scale fibers on which the cells are very viable. These wound healing badges are especially useful for chronic hard-to-heal wounds, such as diabetic wounds. Further translational studies are required to prove the concept in vivo.

Photo stimulatory and photo inhibitory effect of low energy laser therapy in diabetic wound healing dynamics-A preclinical study: Significant increase in the hydroxyproline content and histopathological changes was observed in study group of 7 J/cm² as compared to 10 J/cm². The healing actions seem to be due to increased

collagen deposition as well as better alignment and maturation. In conclusion the biochemical analysis and histopathological study suggested that correct laser dose with photo stimulation effect facilitates the tissue repair process by accelerating collagen production in diabetic wound healing.

Genetic Molecular And Biochemical Studies of Post Transplant Diabetes Mellitus following Treatment with immunosuppressive Drugs:

The calcineurin inhibitors (CIs), cyclosporine (CsA) and tacrolimus (Tac), are immunosuppressive drugs used to prevent rejection in patients with organ transplant. Both drugs have a narrow therapeutic range and show highly variable pharmacokinetics. This study determined the role of gene polymorphisms involved in drug absorption and metabolism, ie ABCB1C3435T and CYP3A5A6986G, with respect to inter-individual variability in CsA/Tac levels, in a cohort of renal transplant recipients. Genotypes were assessed by PCR-RFLP in 201 renal transplant cases on immunosuppressive therapy for more than three months. ABCB1TT exhibited high CsA and Tac levels($1033.97\pm 284.37\text{ng/dl}$; $8.57\pm 3.86\text{ng/dl}$) and blood C2levels/dose ratio(5.40 ± 1.76 ; 2.92 ± 1.70) when compared to CC and CT genotypes. Similarly the CYP3A5GG genotype was associated with poor metabolism and showed increased CsA/Tac C2levels($1171\pm 319.02\text{ng/dl}$; $9.85\pm 3.71\text{ng/dl}$) as well as levels/dose ratio(5.78 ± 1.68 ; 3.36 ± 1.51). This suggests that CsA/Tac blood levels are regulated by these two genes. When gene-gene interaction was evaluated it was observed that ABCB1TT and CYP3A5GG genotypes showed the highest blood levels of Tac, however, in case of CsACYP3A5GG genotype is responsible for higher blood levels, irrespective of the ABCB1 genotype. These individuals would require a lower CsA/Tac dose to maintain therapeutic levels. Determination of these genotypes before renal transplant may alert clinicians and enable them to manage immune-suppression effectively and prevent complications.

Study of factors influencing host defence against infection in Diabetic foot with special reference to vitamin D deficiency: Vitamin D (1α , 25 dihydroxyvitamin D3) has been shown to inhibit IL-12 secretion, thus promoting Th2 response with increased production of Th2 cytokines IL-4, IL-6,

IL-10. Th2 cells primarily play a role in response to extracellular pathogens (most bacteria & parasites). Therefore, it is speculated that vitamin D3 supplementation can lower the risk of foot infection in diabetic patients by enhancing the immune cells response against infection.

Association of obstructive sleep apnea (OSA) with metabolic syndrome, insulin resistance and TNF- α , IL-6 and ACE gene polymorphisms—

Community-based Case-Control Study: Study explored TNF- α , IL-6 and ACE gene polymorphism in patients with OSA and metabolic syndrome. All parameters except hypertension, diabetes, systolic and diastolic blood pressures were significantly different between apneic and non-apneic groups. In the multivariable analysis of OSA as an outcome variable and age, BMI, gender, HOMA-IR, metabolic syndrome, fat % and neck circumference as independent predictors no association of OSA with metabolic syndrome and insulin resistance was found. Increased age, BMI, neck circumference and male gender as independent predictors of OSA, the GA genotype of TNF- α (-308G/A) polymorphism was also found to be independent predictor of OSA in the multivariable logistic analysis.

Development of N-BNP assay for screening of cardiovascular disease in patients with Type II diabetes mellitus:

Study developed HPLC method for the estimation of N-BNP in the blood. N-BNP is a biomarker for the detection of cardiac dysfunction. The results of this study show that N-BNP levels are significantly high in patients with type 2 diabetes. The assay will help in screening of the patients with type 2 diabetes for cardiac dysfunction.

Evaluation of indigenous non-invasive blood glucose monitoring technologies:

The mathematical model has predicted blood glucose value using salivary parameters with 75% accuracy and average error of ± 8 mg/dL. The concept has been validated on subjects and specificity has been confirmed. The electronic system developed has to be designed with programmable software in the form of a watch which can also monitor the glucose level as well as metabolism.

Association of Oxidative stress pathway gene Poly-Morphisms with type 2 Diabetes and related complications: The activity levels of

SOD and GST were significantly lower in T2DM patients than in healthy subjects whereas, that of catalase was higher in T2DM patients than in healthy subjects. Analysis of data also showed that enzyme activity levels decreased with increasing age both in normal and T2DM conditions. Only GST level showed significant decrease in diabetic males while both GST and SOD decreased in diabetic females. *GSTM1* (-/-) and *GSTP1* 'AG' genotype individuals may be at risk of developing T2DM. *GSTP1* genotypes (I/I, I/V) have shown highly significant association with FBS, PPBS and lipid profile in T2DM patients when compared with controls. In catalase -21 C/T gene polymorphism CT genotype have shown highly significant association with FBS, PPBS and lipid profile in T2DM patients when compared with controls.

Development of low glycaemic noodles from sweet potato and low calorie sago from cassava as anti-diabetic foods: The study led to the development of half a dozen spaghetti products having high nutritional value coupled with low glycaemic index (proven through animal studies only presently), which could prove to be a boon to the diabetic patients. Resistant starch rich banana starch, Annealed cassava starch (RS content of 28.6%) and NUTRIOSE, a commercial RS rich source were excellent additives to lower the glycaemic index of sweet potato spaghetti. Techniques were also developed to reduce the digestibility of sago (sabudana), using RS enhanced cassava starch. Sago is consumed on a wide scale on certain festive occasions in certain States of India and is reported to lead to type 2 diabetes.

Role of advanced glycation end products (AGE)-mediated apoptosis, gene expression and polymorphism of AGE- receptor in the development of micro- and macro-vascular complications of diabetes mellitus: Effects of Curcumin and Resveratrol: The study elucidated the role of AGE accumulation, RAGE expression and RAGE gene polymorphism in the development of diabetic complications and found that the blood level of AGE may be used as a marker for predicting the possibility of vascular complications in T2DM

patients. Diabetic patients having C allele of -429T/C are more prone to develop macro vascular complications whereas an allele of -374T/A confer protection towards macro vascular complications and minor allele of Gly82Ser polymorphism are more prone to develop micro vascular complications. Aminoguanidine (inhibitor of AGE formation), N-acetylcystein, resveratrol and curcumin (antioxidants) exert ameliorating effect towards AGE mediated toxicity effectively and may be considered as potential candidate for anti-AGE therapy.

Non invasive Photo plethysmographic Based Glucometer for mass Screening of Diabetes: On the principles of Photo Plethysmography, a working model was created and a study was initiated among 1135 subjects in the process categories of calibration, fine tuning and validation. The Lab VIEW and Micro controller based models find suitable for the working range from 70mg/dl to 360mg/dl with effective transfer characteristics in the step size of 5mg/dl with good gender discrimination. The performance of the Glucometer is analyzed through Clarke Error Grid, Parkes Error Grid and Bland-Altman Plot against the standard clinical method. It is observed that there is a good agreement between all the three methods. Finally the system is evolved with 2% average error in terms of volts and glucose level.

Development of cost effective glucose bio-sensor for clinical diagnostics: A visible color gradient proportional to increasing Glucose concentration achieved partially in this project, at concept level it was found that glucose can be detected using Paper test strips using glucose oxidase immobilized on Fe₃O₄-CH nanocomposite, which is showing high sensitivity (9.3µA mg/dl cm⁻²) and response time of 5s.

Fellowships:

To promote research among young scientists, a total of 70 fellowships were supported. The main areas were reproductive health (51%), maternal health (10%), child health (6%) and diabetes (33%).

NUTRITION

The ICMR continues to adopt multi-pronged and multi-disciplined approach for advancement of nutrition research in the country. Specifically, pragmatic approaches were evolved by integrating laboratory and hospital based research with community participation in tackling nutritional problems confronted by different regions of the country. Salient features of various programmes undertaken by the ICMR in the area of nutrition during the year 2013-14 are summarized below.

Intramural Research

NATIONAL INSTITUTE OF NUTRITION, HYDERABAD

COMMUNITY STUDIES

A rapid nutrition assessment among tribal families of Attappady, Palakkad District, Kerala

Following media reports about unusual number of infant deaths among tribal families in the Attappady Block of Palakkad District in Kerala in 2013, a rapid nutrition assessment was conducted in May 2013, among children and mothers to ascertain the cause of infant deaths. The food and nutrient intake of the tribal population was lower than the recommended levels. The extent of deficit in micronutrients such as iron, vitamin A, riboflavin and free folic acid was higher. The prevalence of underweight, stunting and wasting among <5 year children was higher compared to other tribes as well as their rural counterparts (Fig.1). The study showed high maternal undernutrition (48% had BMI<18.5). During one year preceding the survey, 10 abortions/still births, 11 neonatal deaths and three infant deaths were reported. The major cause of stillbirths and abortions was pregnancy induced hypertension (PIH) and diabetes, followed

by premature delivery and accidental death. For neonatal deaths, the major cause of death was PIH, premature delivery, and ante-partum haemorrhage, obstructed labour and congenital anomaly. Lapses in healthcare delivery system were identified and reported for corrective measures.

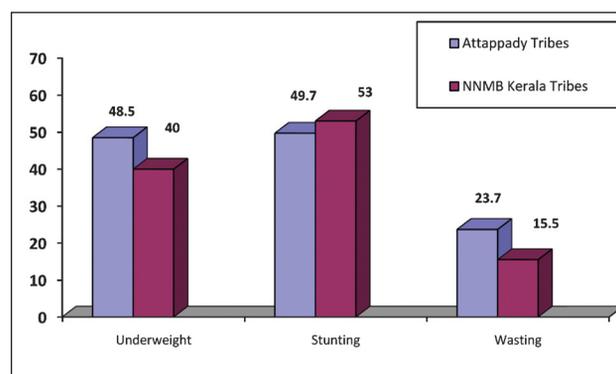


Fig. 1. Prevalence (%) of undernutrition (<Median - 2SD) among <5 year children according to SD classification.

Assessment of new skim milk based ready to eat supplementary food products for their sensory properties and acceptability

Two new skim milk based ready to eat supplementary food products with different cereal, pulse, skim milk, sugar and fat ratios and fortified with micronutrients (Product 1 - 50:05:10:25:10 ; and Product 2 - 55:05:10:20:10), were developed by AP Foods Ltd., for management of undernutrition among young children. These were assessed for their sensory properties and acceptability in comparison to the currently existing soy based ready to eat supplementary food (Modified Therapeutic Food, MTF - 45:20:00:25:10) of the ICDS-WDCD, Government of Andhra Pradesh. The mean sensory scores given by Mother's Panel for all the sensory attributes including overall palatability was significantly higher for Product 1 followed by Product 2 as compared to MTF. Based on 3-criteria

priori set for product, Product 1 appears to be more acceptable suggesting its long term adherence over a 14-day period. It is recommended that the product should be supplied weekly in individual sealed package and service providers need to impart education to caregivers on handling, usage and storage of the product. Accordingly, the Govt. of Andhra Pradesh is supplying the food for more than 20 lakh children daily.

Nutrition status of children in Meghalaya

The Government of Meghalaya is contemplating to develop State Nutrition Policy and develop plan of action for implementation of district level strategy, to improve the nutritional status of children. Therefore, a study was carried out in all the seven districts of the State. Only 37% of the deliveries were institutional and rest of them (63%) took place at home and 11% of the babies were low birth weight (< 2.5 kg). Half of the newborns were breastfed within an hour of delivery and none of the infants had nutrition deficiencies. The overall prevalence of underweight, stunting, wasting was 21, 44 and 5% respectively, which were lower than the national figures (Fig.2).

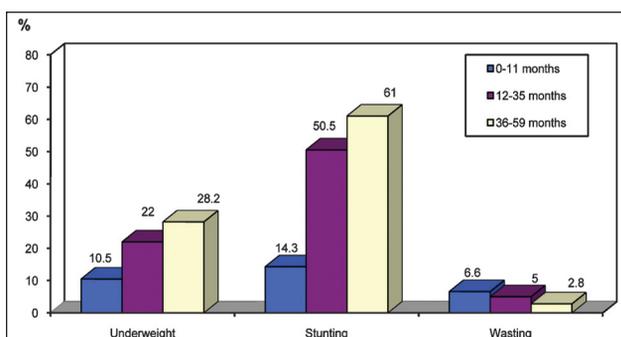


Fig. 2. Prevalence (%) of undernutrition (<Median-2SD) among <5 yr children by age.

CLINICAL STUDIES AND MICROBIOLOGY

Study on low levels of vitamin D in pregnant women

A study hypothesised that low levels of vitamin D would be associated with impaired regulatory T cell function and increased B cells with CD23/CD21 expression, resulting in higher risk of asthma/allergy. It explored Treg cell function, CD23/CD21 expression and VDR expression in pregnant women with vitamin D deficiency. The results indicated that of the total T cell population,

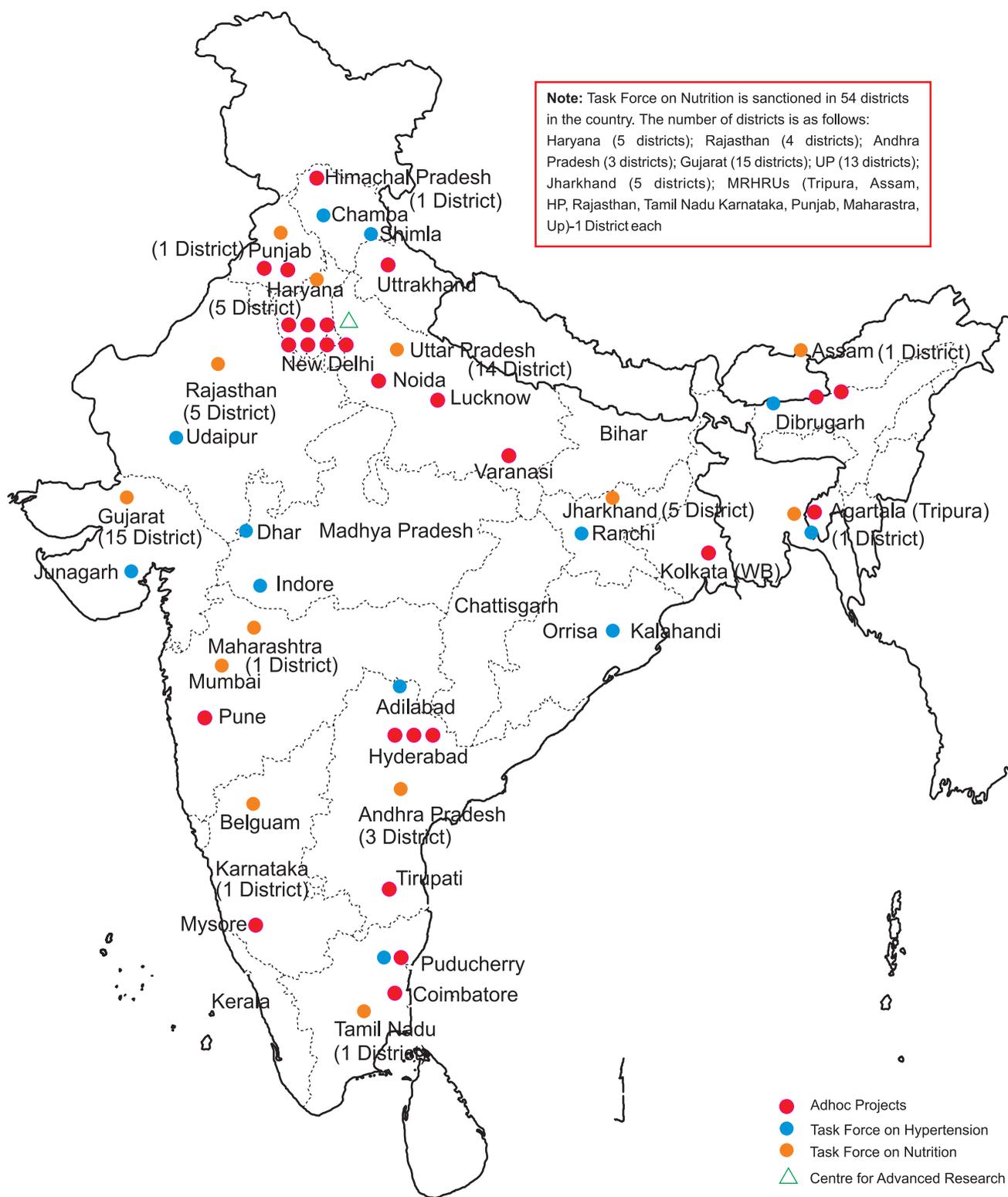
the proportion of the regulatory T cell population (CD4/CD25/CD127-/FOXP3) was significantly lower in vitamin D deficient subjects compared to sufficient and insufficient subjects. Of the total B cells (CD19) population, the proportion of B cell with CD23 expression levels was significantly higher in vitamin D deficient subjects compared to vitamin D sufficient and insufficient. B cells with CD21 expression levels were also significantly higher in vitamin D deficient subjects compared to vitamin D sufficient and insufficient subjects. VDR and FOXP3 gene expressions were down regulated and CD23 and CD21 genes were up regulated in the placenta of vitamin D deficient pregnant women. These results show impaired regulatory T cell function and increased IgE receptors expression in cord and maternal blood and placenta of vitamin D deficient pregnant women.

Foetal immune programming with probiotic and prebiotic (synbiotic) supplementation in pregnant dams

Considering probiotics and prebiotics are important functional foods that have a role in immunomodulation, a study explored foetal immune programming with probiotic and prebiotic (synbiotic) supplementation in pregnant dams. Cell mediated immunity showed intergenerational effect with synbiotic supplementation and this improvement in pups (F1 generation) was associated with *Bifidobacterial* colonization. In contrast, the antibody response to Hep-B surface antigen showed intergenerational effect in pups (F1 generation) born to probiotic supplementation suggesting foetal immune programming with probiotic supplementation.

Development of tools to identify and map IgE binding epitopes, sequencing and transcriptome analysis of brinjal or eggplant (*Solanum melongena* L.) fruit

As part of a study on development of tools to identify and map IgE binding epitopes, sequencing and transcriptome analysis of brinjal or eggplant (*Solanum melongena* L.) fruit was done to identify allergenic genes and proteins. From the total RNA, unwanted RNAs (rRNA, tRNA and other small RNAs) were removed and qualified brinjal fruit mRNAs (qualified by Qubit Analyzer) were used



MAJOR ICMR RESEARCH PROJECTS IN NUTRITION

to pair end library preparation and were sequenced on Illumina Hiseq 2500. There were a total of 89,763,638 raw reads in brinjal fruit. Of them, 149224 (Level 3 assembly) were found to be clean reads after filtering. On analyzing the assembly length distribution of brinjal fruit transcripts, most of the sequences were between 100-500 and few sequences were 3000-4000 nucleotides length and the highest sequence length was 10795 (1 sequence) (Fig.3). Of the 149224 sequences identified from brinjal fruit by transcriptome analysis, 6804 sequences were annotated and were found to be functional genes. Of the 6804 sequences, the exact functions were identified for 1053 sequences by mapping. From the total sequences, 72625 sequences had Basic Local Alignment Search Tool (BLAST) hits and were matching with existing databases. However, 68742 sequences were newly identified and did not have any Blast hits with existing databases (Fig.4).

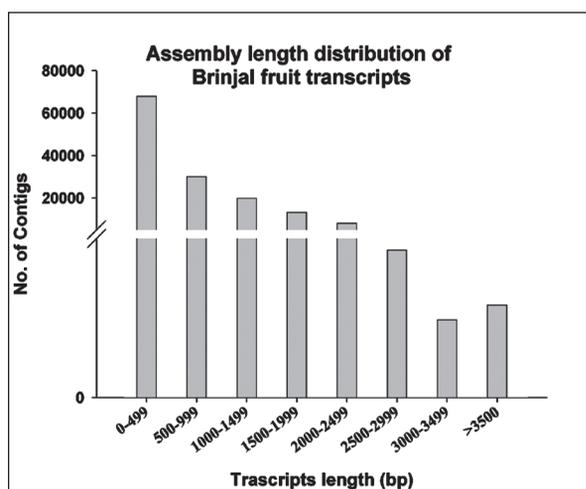


Fig 3. Brinjal assembly length distribution.

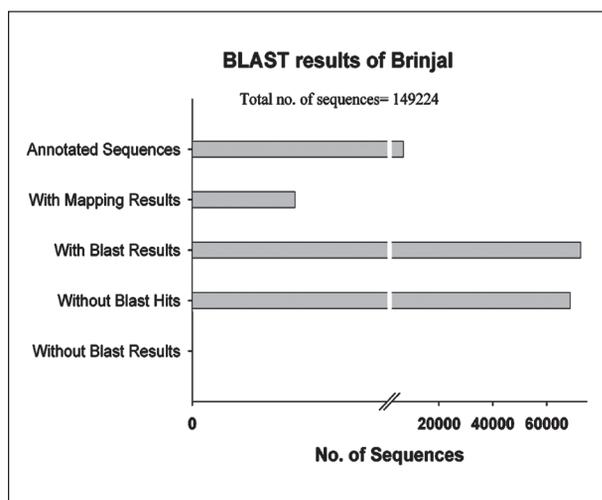


Fig 4. Brinjal BLAST statistics.

MICRONUTRIENT RESEARCH

Project Grow-Smart, a randomized controlled interventional trial among infants and pre-schoolers

Project Grow-Smart, a randomized controlled interventional trial, was carried out among infants and pre-schoolers in 26 villages of four State administrative blocks (*mandals*) from Nalgonda District of Andhra Pradesh (now in Telangana). The baseline data were analyzed for estimating anaemia prevalence and severity and to characterize maternal and child factors associated with anaemia in infants (N= 518) and preschoolers (N=326), in preparation for designing an effective anaemia control program. There was high prevalence of anaemia in infants (66.4 %) and preschoolers (47.8%). Determinants of anaemia in infants were male gender, maternal anaemia, low infant ferritin and high c-reactive protein. Maternal anaemia, maternal low education, child age, high c-reactive protein and soluble transferrin receptor were significant risk factors for anaemia in preschoolers.

Biochemical characterization of low molecular weight (5kF LMW) human milk whey fractions

Biochemical characterization of low molecular weight (5kF LMW) human milk whey fractions that have been found to reduce, solubilize and enhance Caco-2 cell uptake of ferric iron in intestinal cells were investigated. The salient findings were - heat denaturation inhibited the ferric iron reduction but not solubilisation; zinc inhibited both ferric iron solubilization and reduction; the NMR spectroscopy of purified LMW human milk factor identified the compound as citric acid; citrate lyase treatment inhibited ferric iron solubilization and reduction. The findings demonstrated that citric acid present in human milk solubilizes the ferric iron which could be reduced by other heat labile components leading to increased uptake in intestinal cells.

ENDOCRINOLOGY AND METABOLISM

Role of antenatal and perinatal magnesium deficiency in foetal programming for neuro musculoskeletal development in the rat offspring

It was observed that in the embryos/offspring of the Mg restricted WNIN rat dams, the brain

development was impaired albeit transiently (but not at birth). This appears to negate any role of impaired brain / neuronal development in the changes observed in their body composition in later life. The differential gene expression of Leptin, adiponectin and 11 β HSD1 and the corresponding change in DNA methylation in the embryos and at later time points appear to suggest that maternal Mg restriction induced changes in the body composition of the offspring may be programmed right during the intra uterine growth. The findings suggest that maternal Mg restriction induced alteration of promoter DNA methylation could be a mechanism underlying changes in the expression of relevant genes responsible for adiposity and associated stress.

Role of changes in neuronal metabolism and neurochemical profile in reduced longevity in WNIN/Ob rat

In order to assess the possible role of changes in neuronal metabolism and neurochemical profile in reduced longevity in WNIN/Ob rat, the volumetric changes in the brain of WNIN/Ob obese rats vs that of normal rats has been deciphered by MRI. The volumetric analysis of the brain MRI images did not show any significant differences between the age matched obese and control rats in various brain regions analyzed. This is in line with the previous observations that wet brain weights were comparable between WNIN and WNIN / Ob rats of comparable age.

Role of epigenetics in the maternal vitamin B12 restriction induced changes in the C57BL/6 mouse offspring.

It was observed that chronic dietary vitamin B12 restriction per se induced anxiety in C57BL/6 mice compared to controls. Behaviour experiments reflected development of anxiety in the vitamin B12 restricted F0 mice. Chronic, maternal vitamin B12 restriction significantly increased body fat % in C57BL/6 mice offspring, but had no significant effects on BMC or BMD. Offspring of vitamin B12 restricted mice had significantly higher plasma Hcy and cortisol than the controls. Rehabilitation from weaning did not correct the changes due to maternal vitamin B12 deficiency but rehabilitation from parturition corrected the changes partially.

Offspring born to vitamin B12 restricted mice also reflected development of anxiety at six months of age and rehabilitation from weaning did not prevent this.

Role of dietary phytate-mineral interactions in suppressing colon cancer

It was observed that the body weights were comparable among the groups although feeding phytate along with azoxymethane [but not azoxymethane (AOM) alone] decreased food intake in rats. Serum Zn concentration was lower in AOM treated group than controls, but in colon they were significantly higher than in controls. Plasma thiobarbituric acid reactive substance (TBARs) levels were higher and activities of superoxide dismutase (SOD) and catalase were lower in AOM group than control. Incidence of aberrant crypt foci (ACF), an identifiable, early marker for the onset of colon cancer was there in all AOM treated but not in any control animals. Preliminary findings indicate protective effect of phytate against AOM induced colon cancer.

Effects of obesity and obesity induced Type -2 diabetes

Studies on the effects of obesity and obesity induced Type -2 diabetes on advanced aging phenomenon showed a significant increase in the body weights of WNIN/Gr-Ob rats fed high sucrose diet at three and nine months of feeding, but their BMI, LBM%, FFM% and fat% were comparable in general to those fed starch diet. Adiposity index was also not different between WNIN/Gr-Ob rats fed high sucrose compared to WNIN-GrOb rats and WNIN-Ob/Ob rats fed starch diet.

Studies on anti diabetic effect of fenugreek seeds

In an attempt to delineate the antidiabetic effect of 4-HIL, the anti diabetic effect of fenugreek seeds (which contain both fenu fibre and 4-HIL) and only fenufibre (the fibre component of the fenugreek seeds) is being assessed and the difference between the two will be considered to be the effect due to 4-HIL. Fenu fiber supplementation for 45 days to freshly diagnosed T2D patients significantly reduced fasting plasma glucose levels but not fasting plasma insulin levels. However, HOMA IR, an index of

insulin resistance, was significantly decreased on fenofibrate supplementation. In line with these findings, fenofibrate supplementation for 45 days significantly decreased the AUC glucose during the oral glucose tolerance test with no significant decrease in AUC insulin. Although plasma HDL levels were significantly increased on fenofibrate supplementation for 45 days, total cholesterol and triglyceride levels were not affected.

Studies on muscle wasting in vitamin D deficient rats

Muscle wasting seen in vitamin D deficient rats was observed to be due to increased muscle protein breakdown despite comparable food intake with vitamin D sufficient rats. The coordinated up regulation of enzyme activities, gene and protein expression of various components of the ubiquitin-proteasome pathway suggests a major role for this pathway in vitamin D deficiency induced muscle wasting. The study also demonstrated that calcium alone in the absence of vitamin D can partially correct most of the muscle changes due to vitamin D deficiency. Interestingly, vitamin D deficiency reduced body adiposity in the rat model as evident from decreased fat mass and altered expression of adipogenic genes.

Efficacy of polyphenol-rich dietary ingredients as proteasome inhibitors and their role as anticancer agents.

It was observed that *Murraya koenigii* leaf extract induced cell death in a panel of human cancer cell lines and was observed to be associated with decrease in the proteasome activity

OCULAR BIOCHEMISTRY

Procyanidin-B2 (PCB2) inhibits *in vivo* formation of advanced glycation endproducts (AGE)

It has been shown that procyanidin-B2 (PCB2) as the active component of cinnamon, inhibits *in vivo* formation of advanced glycation endproducts (AGE) which in turn ameliorated renal changes in diabetic rats. Feeding of diabetic rats with PCB2 prevented glycation mediated RBC-IgG cross-links and HbA1c accumulation. Interestingly, PCB2

prevented the AGE mediated loss of expression of glomerular podocyte proteins-nephrin and podocin (Fig.5). Finally PCB2 also prevented proteinuria in diabetic rats.

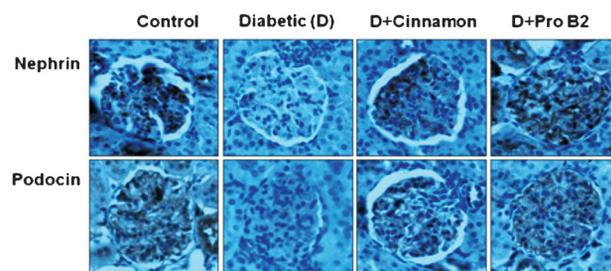


Fig. 5. Immunohistochemical analysis of glomerular expression of nephrin and podocin in control and diabetic rats treated with either cinnamon or PCB2.

Effects of soluble lutein and soluble curcumin on lipid peroxidation

Lutein and zeaxanthin are present in macula and lens of the human eye to protect from oxidative damage and reduce the risk of age related macular degenerations and cataracts. Previously, it was shown that lutein (1%) and curcumin (0.01%) in the diet delayed but did not prevent cataract in diabetic rats. Bioavailability is a major issue for the success of clinical utilization of compounds such as lutein and curcumin. Now, it has been demonstrated that soluble lutein and soluble curcumin are more effective in delaying diabetic cataract compared to their respective regular formulations at the same dose (Fig.6). Increased bioavailability of soluble formulations might explain the observed biological effects.

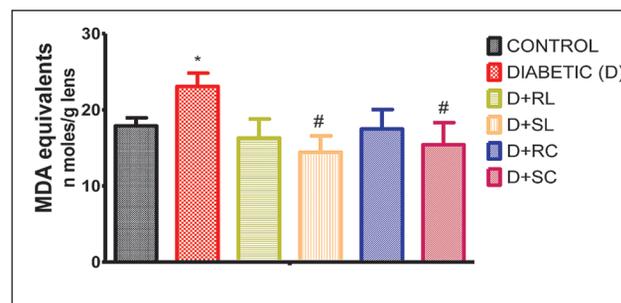


Fig. 6. Effects of soluble lutein and soluble curcumin on lipid peroxidation. Control (control rats); D (diabetic rats); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein); D+RC (diabetic+regular curcumin); D+SC (diabetic+soluble curcumin).

STEM CELL RESEARCH

Assessing the viability and functions of macro-encapsulated islets implanted in non-immune-suppressed non-human primates

The study demonstrated that islets remain viable and functional for 12 months in partially pancreatectomized non-immuno suppressed NHPs undergoing autologous and allogenic implantation. The results also indicate that subcutaneous implantation of macroencapsulated islets are minimally invasive and has potential for transplantation without immunosuppression (Figs 7 and 8).

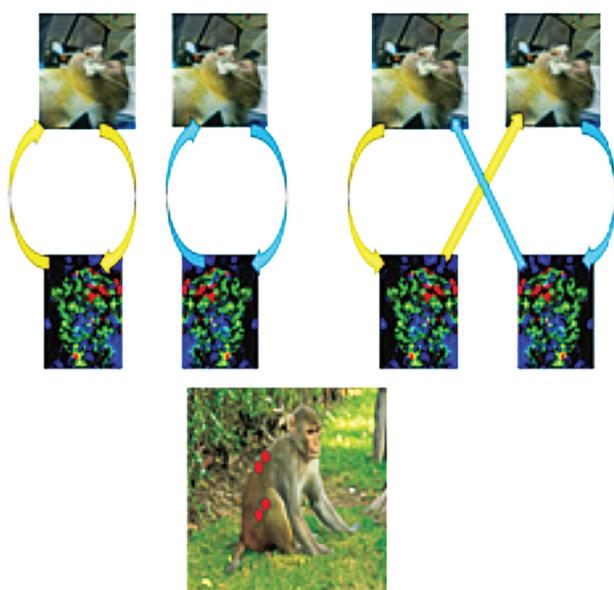


Fig. 7. Transplantation of theracytes containing islets

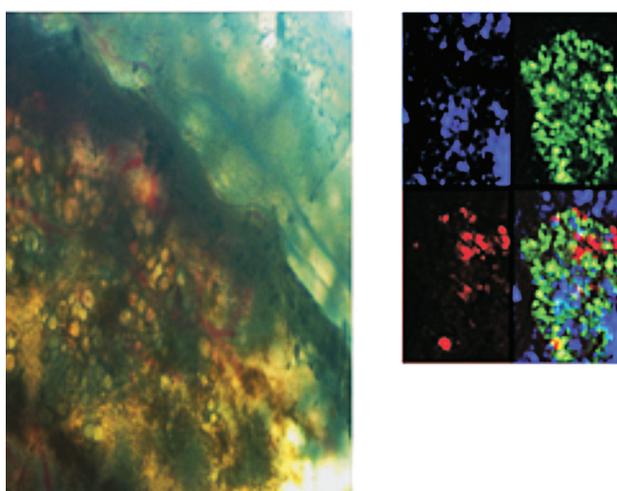


Fig. 8. Retrieved Islets (6M)–

MOLECULAR BIOLOGY

It was hypothesized that high levels of free fatty acids, as happens during obesity, can induce ER stress in the SVF components of the adipose tissue which in turn can affect insulin stimulated glucose uptake in the neighbouring adipocytes involved in glucose homeostasis. In contrast to the popular notion that ER stress leads to development of inflammation presumably via the activation of JNK pathway, suggest that the vice versa *i.e.* inflammation can also lead to development of ER stress. However, the exact molecular mechanisms by which pro-inflammatory cytokines can induce ER stress remains to be understood.

In another study, obesity is characterized by chronic low grade inflammation due to the infiltration of immune cells like macrophages into the adipose tissue and the proinflammatory cytokines produced by the resulting interactions. In addition to macrophages, infiltrated T cells can also induce inflammation in the adipose tissue and contribute to the development of insulin resistance. It was observed that T cells can induce lipolysis in mature 3T3-L1 adipocytes and pharmacological inhibitors of JNK (SP600125) and NF- κ B (PDTC) ameliorate T cell-induced insulin resistance and lipolysis. When co-cultured with 3T3-L1 adipocytes, T cells can inhibit the differentiation of 3T3-L1 pre-adipocytes into mature adipocytes by modulating various adipose specific genes like CEBP/ α , PPAR γ , FASN, SREBP and SCD1, which are critical in adipose tissue differentiation and fatty acid synthesis and processing. It appears that, once infiltrated into the adipose tissue, T cells can elicit vast arrays of inflammatory insults to the adipocytes and impair normal adipocyte functions. Therefore, blocking T cell migration into the adipose tissue during obesity may help prevent obesity-associated insulin resistance.

BIostatistics and Bio-Informatics

- A meta-analysis of randomized, controlled iron fortified feeding trials that evaluated haemoglobin (Hb) concentration using meta-regression analysis indicated that the duration of the study was positively related to the effect size. The net pooled effect size after removing the confounders was 4.74 g/l. Association was

observed between intakes of iron fortified foods on Hb concentration in children less than 10 years. Iron fortified foods could be an effective strategy for reducing iron deficiency anemia in children.

- In another study, when evidence from parallel and cross-over randomized controlled trials were combined to assess the impact of iodine fortified foods on urinary iodine concentration (UIC) in children, meta-regression analysis indicated that dose of feeding was positively related to the effect size. There was an association between intakes of iodine fortified foods and UIC in children.

EXTENSION & TRAINING

A study was conducted to impart education on nutrition and physical activity through participatory approach and peer education using Oorja clubs (school nutrition clubs). After intervention, through “Oorja club concept” a significant improvement in knowledge related to nutrition and importance of physical activity was observed among the adolescent trainers.

FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE, HYDERABAD

Prevalence of fluorosis in Doda District of Jammu and Kashmir

A study was conducted on prevalence of fluorosis in Doda District of Jammu and Kashmir. The fluoride levels were the highest in the Golibagh followed by Malwas village resulting in high intake of fluoride. Dental fluorosis was more common in girls than boys. High level of urinary fluoride excretion was observed in affected individuals. There were also kidney, bone, vitamin D and liver related abnormalities in children exposed to fluoride.

Effect of excess nitric oxide in pathophysiology motor neuron degeneration in neurolathyrism

In order to assess the effect of excess nitric oxide in pathophysiology motor neuron degeneration in neurolathyrism, guinea pigs were fed lathyrus seeds with low and high toxin (ODAP). Low toxic variety had 100 mg/100 g of β -ODAP within 30

days of feeding there was 38% decrease in body weight of a low toxin group and 42% decrease in high toxin variety fed group. Three animals out of eight in low toxin group and five out of eight in high toxin group were affected with neurolathyrism after two months. (Fig. 9).

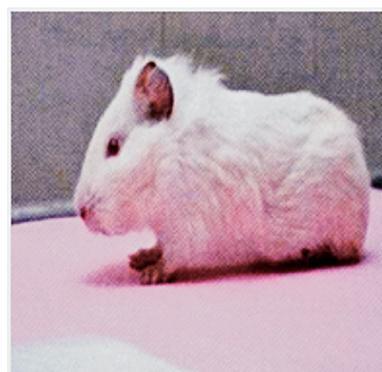


Fig. 9. Neurolathyrism affected guinea pig in high and low toxin groups and control guinea pig.

Thermal stability of oxytocin in milk

When the thermal stability of oxytocin in milk and digestive stability of oxytocin *in vitro* and *in vivo* was assessed, it was found that there was no thermal degradation of oxytocin by boiling. *In silico* digestion analysis revealed that pepsin, chymotrypsin and intestinal proteases possess specific proteolytic cleavage sites in oxytocin amino acid sequence while trypsin has no such sites. Pancreatin could significantly digest oxytocin due to serine proteases. The studies revealed that exogenous OT injections do not influence its content in milk and OT administered orally is rapidly digested during intestinal.

Aflatoxin analysis in rice and wheat in public distribution system (PDS) and non-PDS chains

A study was initiated to assess quality and safety of rice and wheat during their distribution in the public distribution system (PDS) chain. Aflatoxin analysis was carried out on non-PDS rice samples and rice products collected from retail and wholesale markets. In addition, 14 PDS rice and 25 non-PDS rice samples were collected from households during 2013. Out of the 25 non-PDS rice samples, aflatoxins (aflatoxin B1 and B2) were detected in nine samples of which majority were below the limit of detection (LOD) and only one sample had levels above the LOD at 3 µg/kg. PDS-rice samples also did not indicate presence of aflatoxins. Aflatoxins were detected in broken rice samples at levels ranging from 1.0-14 µg/kg. Other rice products analysed contained aflatoxins at negligible levels. Work is under progress to assess aflatoxin levels in rice grains stored at different time durations in Food Corporation of India (FCI) godowns.

Allergenicity potential of novel proteins expressed in genetically modified *Brassica juncea*

The assessment of thermal stability of recombinant Barnase (RNase) and Barstar (RNase inhibitor) proteins expressed in transgenic *Brassica juncea* was undertaken. Heat stability of Barnase and Barstar recombinant proteins was assessed. The enzyme activities of these proteins did not indicate significant changes upon heat treatment showing high heat stability of these proteins. These proteins were rapidly digested by pepsin in SGF and showed no significant amino acid sequence homology to known allergens. The potential for food allergy risk from these proteins is considered limited.

NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES, HYDERABAD

Studies on anti obesity properties of *Garcinia* species

The maximum activity against hyperlipidemia was exhibited at high dose level of *Garcinia indica* (5%). It reduced body fat significantly without any toxicity. Since this compound is antiglycating, it helps in correcting insulin resistance. The results of the present study indicate that further studies need

to be conducted to explore its mechanism of action so that a good antihyperlipidemic and antiglycating agent could be developed.

Dose and time dependent effects of *Mucuna pruriens*, linn ethanolic seed extract on stress related parameters in WNIN/GR-Ob rats

In this study, physical parameters like growth, food intake, feed efficiency ratio, activity was monitored for the experimental period of 45 days. Physiological parameters like body composition and bone mineral contents were measured. Biochemical parameters like cholesterol, triglycerides and stress markers like serum cortisol and L-dopamine were analyzed. Cortisol has many functions. It helps the body use sugar (glucose) and fat for energy (metabolism), and it helps the body manage to stress. By supplementing *Mucuna pruriens* which contains dopamine and improving environmental conditions the animals exhibited less stress levels.

- A study evaluated promising plant extracts and active constituents for anti-obese, anti-diabetic and hepato-protective properties in WNIN/GR-Ob rats. There was a significant reduction in the body weights and food intake of animals treated with the plant extracts. Body composition estimation by TOBEC revealed that, there was a significant increase in the lean body mass and reduction in the total body fat content in treated animals compared to controls. However, no significant changes were seen in bone mineral content and density. Hypoglycemia was prominent in *A. indica* extract treated animals compared to the other experimental animals. Lipid contents like cholesterol and triglycerides decreased significantly in all the treated animals. There was no toxicity observed in the Liver of animals treated with plant extracts. Varying degrees of degeneration of kidney tubules were observed in all the experimental animals except in controls and animals treated with *A. indica*.

Effect of *Piper nigrum* on obesity

Rats fed with high fat diet when treated with *Piper nigrum* have shown reduced physical parameters like food intake, growth. Significant increase was found in body composition parameters like lean body mass. Decrease in total body fat and % fat was observed in the HFD rats treated with piperine

extracts. DXA analysis revealed that there is a significant increase in bone mineral content and bone mineral density. Regional wise fat distribution analysis revealed that there is predominant deposition of fat at retroperitoneal region. The MRI analysis revealed that there is a significant reduction in the adipose tissue volumes in the whole body and regional adipose tissue volumes *i.e.* subcutaneous, thoracic, and retro peritoneal. This study can be explored further with respect to its mechanism of action to translation research.

Extramural Research

In the year 2013-14, approx. 10 new *ad hoc* studies, 15 ongoing studies and seven *ad hoc* studies were completed. Another approx. 25 *ad hoc* studies were under consideration during the period. The projects are supported from different parts of the country including the tribal and north-east regions. The area of research for various *ad hoc* projects varied from experimental studies like role of micronutrients in cancer stem cell metabolism and therapy; to clinical trials like vitamin D and calcium supplementation for improvement in skeletal muscle strength and observational studies like study of *Jenu Kuruba* tribe in Karnataka with special reference to diabetes and hypertension. During the period under report, fellowships were granted to approx. 19 research scholars from different parts of the country, besides 31 fellowship projects which were ongoing and 13 fellowships which were completed during the period.

Centre for promotion of nutrition research and training with special focus on North-East, tribal and inaccessible population: The laboratory at the Centre has carried out the analysis of over 11,000 samples for haemoglobin, glucose and lipid profile under the multicentre intervention study on hypertension. Further, around 1500 samples for various biochemical parameters like serum retinol, hsCRP, haemoglobin, vitamin B-12, folic acid, ferritin, urinary iodine, *etc.* have also been analyzed under various small collaborative projects with the aim to facilitate researchers/ students for their degrees and providing assistance to medical colleges/universities where adequate facilities for

research are not available. Two Ph.D and two M.Sc students have submitted their theses whereas two Ph.D and two M.Sc students are currently enrolled with the Centre. The registration of two more Ph.D students is under consideration.

SPECIAL ACTIVITIES

Interagency collaborations: Under the ICMR-ICAR-FSSAI Joint Committee for Research on Food Safety, five research projects have been completed on the extent of use of artificial ripening agents and oxytocin in fruits and vegetables, effect of oxytocin injection on vegetables, *in vivo* and *in vitro* digestibility of oxytocin and effect of oxytocin ingestion on reproductive health of animals. The findings of these studies will help address public concerns regarding safety of food being consumed. Various other interagency collaborative efforts include formulation of a Committee of Secretaries to address various issues related to development and marketing of GM foods and an interagency group on micronutrients involving various Departments to develop strategies to combat micronutrient deficiency in India following interdisciplinary approach.

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*’ has been initiated at 10 Centres across the country including seven in tribal belt.

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*” has been initiated in 50 districts of the country out of which 45 districts are high burden districts, where prevalence of underweight and anaemia was found to be high as per DLHS-2 survey and five districts are those where Model Rural Health Research Units (MRHRUs) of Department of Health Research have been established.

ENVIRONMENTAL AND OCCUPATIONAL HEALTH

ENVIRONMENTAL AND OCCUPATIONAL HEALTH

The National Institute of Occupational Health, Ahmedabad; the National Institute for Research in Environmental Health; and the Bhopal Memorial Hospital and Research Centre both located at Bhopal are pursuing research in the priority areas of occupational and environmental health relevant to the national needs. The highlights of the various activities undertaken by the ICMR in the area of occupational and environmental health during the year 2013-14 are given below.

Intramural Research

NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH, AHMEDABAD

EFFECT OF INDOOR AIR POLLUTION ON WOMEN AND CHILDREN IN INDIA

Indoor air pollution causes major health problem to women and children due to release of smoke from combustion of wood, dung cakes, crop residues and coal, as they spent more hours in home. To assess the overall exposure indoor air monitoring, medical examination of the subjects including Pulmonary Function Test and biological monitoring was done. A total of 580 questionnaires were filled up from the three study areas of Ahmedabad. Indoor air monitoring was carried out at 93 selected houses. It was done for 9 hrs to cover all the activities of the subjects during the day time including cooking. The average particle surface area concentration ranges from $126.666 \mu\text{m}^2/\text{cc}$ to $1084.868 \mu\text{m}^2/\text{cc}$. Average particle mass concentration of (PM_{10}), ($\text{PM}_{2.5}$), ($\text{RESP}/\text{PM}_{4}$), (PM_{10}), and ($\text{Total}/\text{PM}>10$) ranges

from $0.198 \text{ mg}/\text{m}^3$ to $0.653 \text{ mg}/\text{m}^3$, $0.202 \text{ mg}/\text{m}^3$, to $0.664 \text{ mg}/\text{m}^3$, $0.210 \text{ mg}/\text{m}^3$ to $0.678 \text{ mg}/\text{m}^3$, $0.253 \text{ mg}/\text{m}^3$ to $0.798 \text{ mg}/\text{m}^3$ and $0.328 \text{ mg}/\text{m}^3$ to $1.070 \text{ mg}/\text{m}^3$ respectively. Average particle count ranges from 3348 pt/cc to 190094 pt/cc. Average CO_2 and CO concentration ranges from 443 ppm to 710 ppm and 0 ppm to 4 ppm respectively. Average temperature ranges from 23.3°C to 33.2°C . Average Relative Humidity ranges from 17.8 % RH to 54.4 % RH.



In this study, the average age of 87% of women ranged from 21-35 years, while women in the age group 26 to 30 were highest in numbers among the interviewed, and more than 90% of interviewed women were married. More than 90% of interviewed women were literate with maximum (~75%) studied from primary to matric standard, almost 98% of interviewed women's family incomes were less than Rs. 10000. Nearly 97% of the women were living in nuclear family. Maximum of families (98%) were having single room with indoor kitchen without partition. More than 95% of the women were keeping their door and window open (> 6 hours a day).

Detailed medical examination of the selected subjects (61) and their children (103) below age 10 were carried out with reference to respiratory problems like presence of airway obstruction. The past medical histories of the subjects were also recorded on the questionnaire. Ventilatory Function Tests (VFT) of the screened subjects (61 non-working women) were carried out on Spirovit SP 10 spirometer. Kamat's regression equation was applied to calculate the predicted Forced Vital Capacity (FVC) for the adult subjects (61 women). In the absence of predicted equation for children, the VFT Data could not be evaluated. Out of 61 women, 60 had normal VFT, whereas obstructive impairment was observed in one woman. Further study is in progress.

Association of Adverse Neonatal/Perinatal Outcome with Biomass Fuel Use

The indoor air pollution resulting from biomass fuel combustion may cause chronic bronchitis, chronic obstructive pulmonary diseases and also increases the risk of other important child and adult health problems including low birth weight, perinatal mortality, asthma, and middle ear infection for children, and tuberculosis, nasopharyngeal and laryngeal cancer, and cataract in adults.



A hospital based case control study was initiated to compare cases of adverse neonatal/perinatal outcome with control subjects in relation to their fuel use characteristics. Significantly increased risk of 'low birth weight' (OR 1.78, 95% CI; 1.11-

3.64) and 'need of newborn to stay in neonatal care unit' (OR 1.82, 95% CI; 1.08-4.06) in the form of calculated odds ratio was observed in biomass fuel users after adjusting for age of mother, type of residence, age at marriage and haemoglobin level of the mother at last trimester. Increased risk was also observed in case of occurrence of lesser head circumference, neonatal death and less developed genitalia. Lower education level of mother was also observed to be a near significant contributor (OR 2.6, 95% CI; 0.91-6.25) of adverse neonatal outcome.

Identification of Occupational Health Risk Pollutants and their Metabolites Among Foundry Workers

In a study to identify the pollutants and their metabolites in shop floor of foundry, severe heat stress in the moulding and melting sections was observed. The respirable suspended particulate matter (RSPM) concentration was predominantly higher in shaking out ($3320 \pm 633 \mu\text{g}/\text{m}^3$), blasting ($2439 \pm 654 \mu\text{g}/\text{m}^3$), finishing sections ($3816 \pm 1797 \mu\text{g}/\text{m}^3$), compared to moulding ($1682 \pm 349 \mu\text{g}/\text{m}^3$), and melting sections ($1707 \pm 298 \mu\text{g}/\text{m}^3$) of the foundry. The distribution of emission particulate matter according to size fraction (PM_{1.0}, PM_{2.5}, PM_{4.0}, PM₁₀ and TSPM) was collected. The proportionate average dust concentration of PM_{1.0} was ~ 70%, PM_{2.5} was 75% of the total suspended particulate matter existed in the working area. The timeweight average concentration of nanoparticles (1 nm to 100 nm) which may get deposited in the tracheobronchial and alveolar regions of worker in finishing section ($1934 \mu\text{m}^2/\text{CC}$ in alveolar zone and $423.8 \mu\text{m}^2/\text{CC}$ in tracheobronchia zone) were high, and the PM_{1.0} was also found higher in this section. This may lead to adverse health effects among workers on the continuous inhalation of such particles. The average personal exposure of PAHs such as acenaphthylene and benzo[a]pyrene were 16.99 and $13.64 \mu\text{g}/\text{m}^3$, respectively. The mean concentration of urinary 1-OHP and total-PHE were significantly (<0.05) higher in foundry workers than the control subjects (Fig. 1).

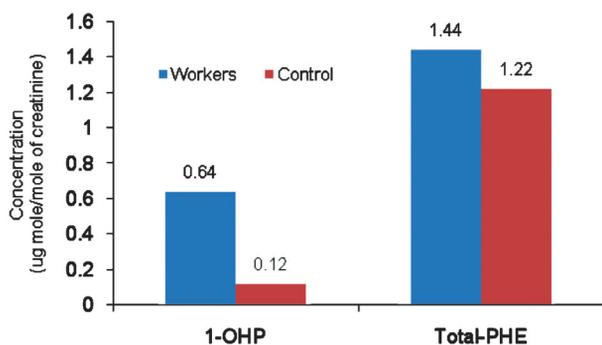


Fig. 1. Mean Urinary 1-Hydroxypyrene (1-OHP) and total hydroxyphenentherene concentration (µg mole/mole of creatinine)

The composition of fumes in the foundry workplace was found enriched with various heavy metals which varied not only with the kind of metal melted but also with the quality of scrap used and the type of melting process. The levels of heavy metals in urine of the exposed (N= 58) and control (N=26) subjects showed that heavy metals concentration in urine among exposed subjects were significantly higher (<0.05) than that of control (Table1).

The findings showed that the workers are systematically exposed to physical and chemical (both organic and inorganic) pollutants. These exposures may cause potential risk to workers. Special attention should be paid to adopt personal protective equipments, engineering control measures and continued awareness on safety.

An intervention study amongst dry salt workers of Gujarat

Dry salt workers involved in the process of crushing, grinding, milling, packaging and loading of salt are exposed to salt through their working environment. The salt particles are inhaled and absorbed in the airway surface epithelium, may result in hypertension of occupational origin. A total of 133 workers from three different salt industries were examined. The hypertension was not found

to be an occupational health problem in dry salt workers of the salt packaging industries. Other skin morbid conditions such as palm callosities, cracks, fissures, palmar and plantar keratoderma and ocular morbidities like pterygium are existing in this occupational group.

The intervention measures like provision of facemasks and hand gloves has not resulted in significant reduction in mean systolic and diastolic blood pressure. The permissible exposure for sodium chloride-respirable fraction is 5 mg/m³ (TWA- US OSHA). In none of the industries, the time weighted average (TWA) concentration of sodium chloride was found exceeded the value of 5 mg/m³.



Edible salt packaging machine



Palm callosities in salt worker workers

Balance and Stability in Manual Materials Handling Tasks

In a study different manual material handling (MMH) tasks like lifting-lowering, twisting-turning, holding etc in a simulated laboratory setup was investigated with the hypothesis that muscle activity may be

Group	N	Cu	Cr	Fe	Mn	Ni	Pb
Exposed	58	180±32.8*	397±49.4*	1756±234.3*	127±16.8*	760±117.7*	767±93.8*
Control	26	80±23.7	297±92.3	1506±429.3	82.5±29.8	405±130.9	524±162.8

influenced by changes in methods of MMH tasks and increase in grades of load. Eight healthy volunteers, having no history of motor problems, neurological disease, or vestibular impairment were recruited for the study. Static loads of three different grades of 4 kg, 8 kg and 12 kg were selected. The experimental set up included a 16-channel portable and Wi-Fi enabled pocket EMG machine for recording muscular activities during MMH task and recorded signals were analyzed off-line using MYOLAB software. Surface EMG was carried out on seven different muscles at the right side of the volunteer namely m. deltoid, m. upper trapezius, m. erector spinae, m. rectus femoris, m. semitendinosus hamstring, m. vastus medialis and m. tibialis anterior. The lifting-lowering task performed at individuals' knee, waist and chest level, where as twisting-turning and holding task was performed at individuals' waist level.



Results revealed that root mean square (RMS) values of muscles increased with increase in load from 4 kg to 8 kg, then from 8 kg to 12 kg. It may be due to rest pause given between each trail. When the load is increased to thrice of the initial value (4 kg to 12 kg) significant influence was found on upper extremity muscles (deltoid, upper trapazius and erector spine). However, the trend was dissimilar when load was increased by only 4 kgs (4 to 8 kg or 8 to 12 kg). The study also explained that the RMS values of deltoid, rectus femoris and semitendinosus hamstring muscle were also increased with the increase in height of handling load from knee to waist, then waist to chest for each three grades of load. Further, increasing load-holding time was resulted in decreased median frequency (MDF) values of the muscles. The decreasing trend of MDF values starts earlier with higher magnitude load as compared to lower one indicates muscles became fatigued earlier with higher grade of load. This suggests any MMH holding activity in endurance would manifest in fatiguing nature of muscles. Study on response pattern showed that overloading of muscles leading to fatigue and/

or injuries can be avoided by selection of appropriate magnitude of load with rest pause.

Assessment of Physiological and Metabolic Status and their Effects on the Morbidity Pattern of the Bus Drivers

A study conducted on 60 Bus drivers aimed to assess (i) acute and chronic morbidity pattern among bus drivers; (ii) their nutritional status (anthropometry), workload and energy expenditure during work; (iii) their physiological and metabolic status with reference to cardiovascular responses, pulmonary function, muscular-skeletal pain and discomfort and blood and urine biochemistry.



Average and Peak Working Heart Rate of bus driver during work showed the workload as 'Moderate' to 'heavy' categories, whereas the Energy Expenditure (Kcal/min) of bus driving was found to be under the category of 'Moderate' workload. A total of 25% bus drivers reported body pain and discomfort during the course. Among them, 73% drivers had complained of lower back pain followed by knee pain (40%). This may be attributed to the awkward posture wherein frequent twisting of trunk is required. Besides subjects have to apply pressure with leg muscles to operate clutch and brake frequently. Skin disease was reported in 15% bus drivers. This is a sign of lack of hygiene and working in a warm, moist environment without change of clothes for a prolonged period of time.

The Lipid profile of the drivers revealed mean and median of triglyceride and cholesterol within the reference interval, while these parameters were observed to be elevated in 30% and 12% subjects respectively. The HDLc values were found to be less than or equal to 35 mg/dl in 13% drivers and in two bus drivers it was less than 30 mg/dl *i.e.* less than the reference range, which may become

a causative potential risk factor for coronary heart disease.

Minimum and maximum values of plasma glucose were within the reference range. Mean and median of serum urea, creatinine, total protein and albumin were within the reference interval but 20% subjects had a serum creatinine level more than 1.2 mg/dl. Only two subjects showed the serum homocysteine level more than the upper limit of the reference interval value (15 μ mol/l).

The liver enzymes like ALT and AST were within the reference interval, whereas these values were found to be more than the reference range in 17% and 15% subjects, respectively. A total of 23% bus drivers had GGT values more than the upper reference limit, *i.e.*, 61 IU/l. In a few of the cases the Gamma GT value was almost double of the upper reference limit, which indicates increased in synthesis of GGT enzyme in the liver due to enzyme induction process. This may be due to chronic abuse of alcohol as 68% of the study subjects were alcohol user.

Environmental and Bio-monitoring of Women Workers Exposed to Pesticides in Tea Plantation with Special reference to DNA Damage

To assess the effect of pesticide exposure, the levels of cholinesterase enzyme and DNA damage in blood samples of women working in tea plantation were determined. A total of 100 subjects (exposed 88 and control 12) were included with the mean age 45yrs and 44 yrs respectively. They were working in the tea plantation sector from 15 to 48 years. The average duration of exposure was 260 days per year. The majority of the subjects (88%) never used the tobacco. None of the workers and control subjects had reported the smoking habit and alcohol consumption. Among the exposed subjects conditions like back pain (48%), neck pain (34%), headache (32%) and eye irritation (25%), tears (21%) were reported. None of the workers used personal protective equipment while working. The acetylcholinesterase (AChE) activities among workers and control subjects were estimated. No variation was observed between these two groups. The exposed workers had greater mean DNA tail length than the controls.



Utility of Personal Cooling Garment for use in Outdoor Hot Environment

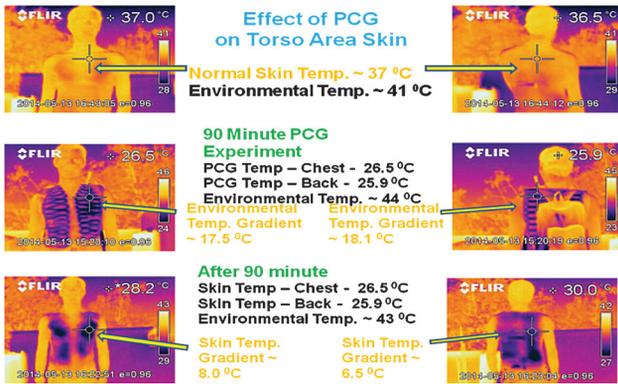
In India a large number of occupations involves the exposure to extreme heat which elevates of physiological responses such as body core temperature, skin temperature, heart rate, sweating, dehydration *etc.* with risk of various heat related injuries, decline in work efficiency, discomfort and fatigue, and even death. Therefore, NIOH developed low cost Personal Cooling Garment (PCG). PCG is a microclimate Auxiliary body Cooling System, comprises a jacket-like enclosure, creating an interior space for upper part (Torso) of the body. Ice chilled re-circulating water from the silicone tubing absorbs body heat by skin conduction and provides comfortable microclimate to the wearer.

Improvisation of PCG

- Earlier PCG developed by NIOH was electrically operated, now with rechargeable battery.
- Using silicone tubing for better heat absorption instead of PVC tubing.
- Modified ice container reservoir for flexibility.
- Flow control switch attached.
- Using 3 way flow – (Front right- Front left- Back) for immediate circulation of cold water
- Earlier PCG was tested in laboratories. Now it has been tested in field conditions like iron foundry and construction works, and results are encouraging.
- System can be made in any size and shape

Thirty subjects (24 - iron foundry and 14 - construction workers) were tested with PCG. Study indicated benefits of wearing PCG for use in hot environment by manifesting reductions in physiological indicators of heat strain like skin

temperature, heart rate, and sweat loss. Overall performance of PCG by subjective responses indicates applicability and efficacy in such hot environment.



Thermo-graphic image showing differential skin temperature gradient with PCG

Popularization of Redesigned Model of Cycle Rickshaw

To popularize the new model of cycle rickshaw, which was patented earlier, a few units have been fabricated. Two units each of redesigned cycle rickshaw have been sent to Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna and Regional Medical Research Centre (RMRC), Bhubaneswar. Meetings with Directors and Scientists of these Institutes were held, and conducted awareness programme and efficacy study on newly launched redesigned cycle rickshaw at the respective places.

Activities of NIOH-Poison Information Centre

The activities of the Institute’s Poison Information Centre include laboratory support, toxico-vigilance, provision of Information, teaching and training of health professionals and research. During the year, 421 cases of poisoning were referred to the Center for Laboratory Support and the Information on Toxicity. The poisoning agents included various groups: household products, organophosphate pesticides, other pesticides, industrial/environmental chemicals, drugs, compounds of plants or animal origin (natural toxins), miscellaneous, mixed and the unknowns. Among the poisoning agents, organophosphate pesticides was the most frequent

agent which included chlorpyrifos, monocrotophos, phorate and dimethoate.

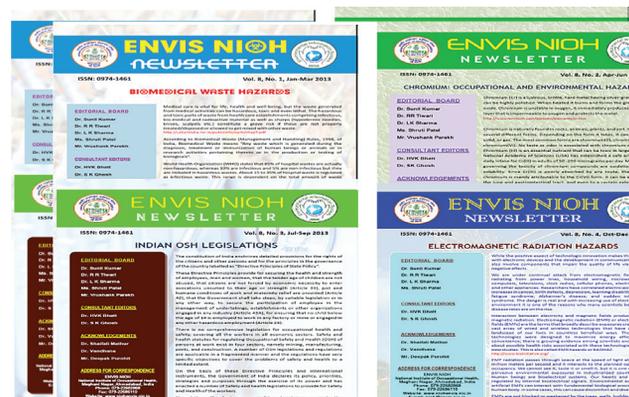
Development of NIOH Institutional Repository

Spreading knowledge in term of publications among the community is an important task of scientific institute. In view of this the NIOH has been developing an institutional repository of the occupational health research work carried out by the institute. A case study of the NIOH’s institutional repository namely “कल्पकोश” (*Kalpakosh*) has been reported in this article. Initially, the requirement of occupational health research has been studied and the research work related to occupational health is properly organized. The community and collections are defined based on available literature related to occupational health in the institute and it implemented on DSpace framework. The various navigation features are provided for user friendly exploration of research publication of the institute.

ENVIS-NIOH ACTIVITIES

Environmental Information System [ENVIS] Centre, housed at the NIOH is sponsored by Department of Environment, Ministry of Environment and Forests, Government of India, New Delhi.

The ENVIS centre at the NIOH is engaged in collection, collation, storage, retrieval and dissemination of Indian information related to occupational and environmental health. This information is then translated to create awareness about occupational health and safety through awareness programmes and improve working conditions through recommendations to decision makers.



NATIONAL INSTITUTE FOR RESEARCH IN ENVIRONMENTAL HEALTH, BHOPAL

The National Institute for Research in Environmental Health functioning from Kamla Nehru Hospital Building in the Gandhi Medical College Campus, Bhopal aims to address environmental health research issues in general with focused research on MIC affected population in the areas of (a) respiratory diseases, (b) eye related diseases, (c) cancers, (d) renal failure, (e) genetic disorders, (f) congenital disorders, (g) women related medical issues, (h) second generation children, and (i) mental health and other relevant aspects.

Intramural Research

Population based long term epidemiological studies on health effects of Bhopal toxic gas exposure

A long term population based epidemiological study on the health effects of the toxic gas is underway since 1985 (1985-1994 under the ambit of erstwhile BGDRC; 1996-2010 under the banner of Centre for Rehabilitation Studies, Government of MP; 2010 onwards under NIREH. Data of 46th and 47th rounds of surveys were analyzed.

During the 46th round of survey (Jan –June, 2013) a cohort of 16,815 gas exposed people from severely affected (5,052), moderately affected (6,574) and mildly affected (5189) areas and 5,244 unexposed people from control area was followed up. Any morbidity recorded in severely exposed areas was 21.5%, in moderately exposed areas 17.2%, in mildly exposed areas 18.1% and in control areas 8.0%. The respiratory morbidity rates remained high in the severely exposed areas (11.7%), moderately exposed areas (9.2%), and mildly exposed areas (11.1%) as compared to control areas (1.7%). The ophthalmic morbidities were 12.9% in severely exposed areas, 8.7% in moderately exposed areas, 10.9% in mildly exposed areas and 2.0% in control areas. The gastrointestinal morbidities in severely exposed areas (3.6%), moderately exposed areas (3.1%), and mildly exposed area (4.7%) were marginally higher than the control areas (0.7%).

A special drive was undertaken to trace out the migrated families of the original cohort resulting in

reopening of 679 more families. Thus, in this round a cohort of 19,763 gas exposed people (severely affected 6,605, moderately affected 6,991, mildly affected 6,167) and 5,528 unexposed people from the control area were followed up. The trend of various morbidities in 47th round, by and large, remained the same as that of 46th round with any morbidity ranging from 22.5 to 9.4%, respiratory morbidities from 11.3 to 1.9%, ophthalmic morbidities from 13.1 to 2.4% and gastrointestinal morbidities from 4.0 to 0.9% in the four areas.

Long term genetic effect(s) of Methyl Isocyanate (MIC) gas on Bhopal Population

Retrieval of the old data of six laboratories viz. Calcutta, Bangalore, Delhi, Varanasi, Lucknow and Bhopal which were studied in 1985 was completed. Of the 913 prescreened cases, records of only 795 individuals could be traced (326, 196, 16 and 257 from severely, moderately, mildly exposed and unexposed areas respectively). For the pilot study 100 cases each belonging to exposed and unexposed areas from the list of available prescreened subjects are planned to be selected and their 3 generation pedigree will be prepared.

Genetics and Epigenetics of Lung Function among the Victims of Bhopal Gas Disaster

From the NIREH database of 6,968 individuals, based on the availability and satisfying the inclusion criteria (gas victims of 40-70 years age whose PFT data done in 1990s), 466 individuals from exposed and 420 from unexposed areas have been identified. Further, from BMHRC database (between 2002-2008) 809 individuals have been shortlisted and their availability is being verified. After completion of final list barcodes will be generated for individuals and pilot study will be launched.

Cytogenetic Analysis in MIC Exposed Population and their Progeny

So far, 56 males and 27 females of various categories as per the protocol (exposed individuals, non-exposed individuals, 1st & 2nd generation progeny etc.) have been enrolled randomly from the registry of JNCHRC, Bhopal and biological samples from them have been collected. Laboratory techniques have been perfected.

To evaluate biochemical markers in cases of clinically stable stages of Chronic Obstructive Pulmonary Disease (COPD) in MIC affected population

For this pilot study 30 subjects (with COPD and no co-morbidities) have so far been identified as per the inclusion/exclusion criteria and their monitoring is being done routinely. More cases are being screened for increasing the desired sample size as there is a high possibility of dropouts due to stringent selection criteria. These markers would also be evaluated for their expression and quantification in induced sputum and serum.

Services Provided

Special Respiratory Clinic, being run at NIREH has benefitted about 300 severely ill COPD cases, identified at field level during epidemiological surveys.

Respiratory Physiotherapy Centre, started by NIREH and being catered by a Physiotherapist, has benefitted 30 cases of COPD.

Severely ill MIC affected patients identified during epidemiological surveys, who need emergency care/specialized care are being examined at doorstep through community based health services of NIREH and if needed, transported to BMHRC for investigation and treatment. Over 200 patients were benefitted under this programme.

Mental Health Training

Two mental health-training programmes covering about 20 medical officers from BMHRC and Sambhavana Trust, Bhopal were organized of 10 hours duration each. Videos of patients with various types of emotional problems among the survivors of gas disaster and standard WHO videos on the specific syndromes were used during the training. Detailed discussions were held to bring out the applicability of the approaches in the clinics. Role plays involving the participants were carried out to increase the clinical skills of the participants. Feed back and intensive interaction with the participants brought out the following felt needs on psychiatric knowledge and skills : (i) the diagnostic terms used are limited and non-specific, (ii) there is limited diagnostic skills, (iii) knowledge

of psychiatric medicines is limited, (iv) there is near absence of psychological interventional skills, (v) the knowledge of course and outcome of mental disorders and emotional problems associated with physical conditions is limited.

World Environment Day

World Environment Day was celebrated on 5th June, 2013 in the institute which was attended by staff members of NIREH, Kamla Nehru Hospital and Gandhi Medical College. On this occasion a poster presentation was organized among the staff members

NIREH Foundation Day

The fourth Foundation day of NIREH celebrated on 11th October, 2013. Dr. V.M. Katoch, Secretary, DHR & DG, ICMR was the Chief Guest. Dr. Manoj Pandey, Director I/C, NIREH, Dr. V. K. Vijyan, Advisor to DG, ICMR and Dr. D. K. Shukla, Head, Division of NCD, ICMR were the other dignitaries present on the occasion. The Second Technical Report on population based long term epidemiological studies was released by Dr. Katoch on this occasion.

Vigilance Awareness Week

Slogan writing, lecture session and poster presentation were organized by the Institute. during the observation of Vigilance Awareness week between 29th October-3rd November, 2013.

BHOPAL MEMORIAL HOSPITAL & RESEARCH CENTRE (BMHRC), BHOPAL

CLINICAL WORK

Clinical work at BMHRC involves treatment of patients in the OPD, investigations (pathology, radiology, and microbiology), procedures and surgeries, in-patient care and rehabilitation.

Out-Patient Department

In the out-patient department at BMHRC the total number of patients seen in the year in the OPD was 2,08,858. This includes new patients as well as follow-up patients. The departmentwise number of the patients is given in Fig. 1.

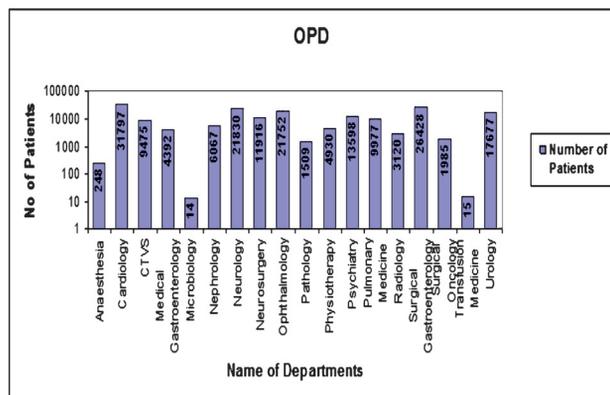


Fig. 1. Departmentwise number of the patients seen in the OPD

Diagnostic Investigations in BMHRC

During the year a team of doctors and technical staff in the departments of pathology, microbiology and radiology excel at every level and provide round the clock services to the patients. The huge number of tests being done does not affect their accuracy and attention to minute detail. The emphasis is laid on precision, expertise, accuracy and speedy reporting. There is excellent communication between the clinician and the dedicated doctors and paramedical staff in the laboratories. Regular inter-departmental academic activities enhance the accuracy of diagnosis and the subsequent result is a boon to the patient. Wherever a patient may be, whether in the ICUS, in the casualty, in a consultant's chamber, in the OPD or admitted in the ward, there is always someone to collect his samples, run through the tests and speedily report to the treating physician or surgeon who maybe waiting to take the next decisive step in the operation theatre. The number of investigations done at BMHRC during the year is illustrated in the Figs. 2-4.

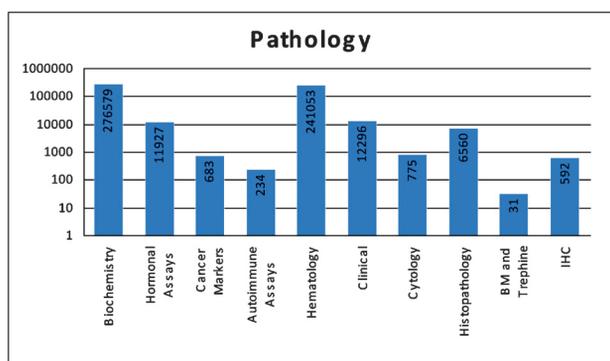


Fig. 2. Pathological investigations

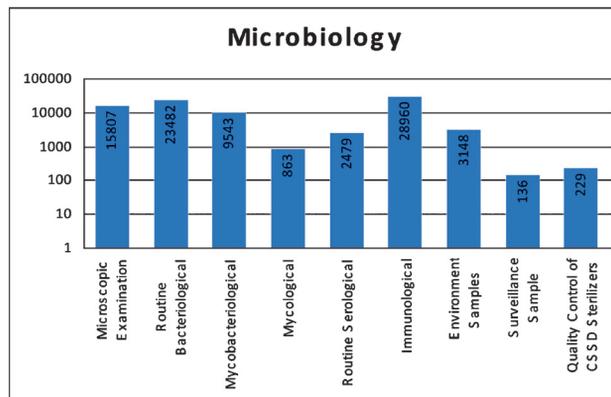


Fig. 3. Microbiological investigations.

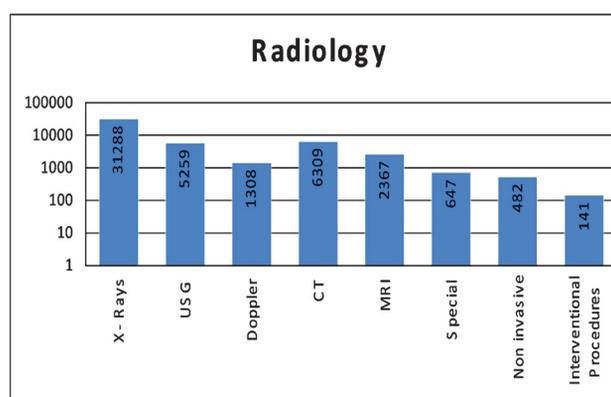


Fig. 4. Radiological investigations.

INPATIENT MANAGEMENT AT BMHRC

More than twelve thousand (12456) patients were admitted and managed at BMHRC during the year. The specialty wise distribution and the bed occupancy are given in Figs 5 & 6 respectively.

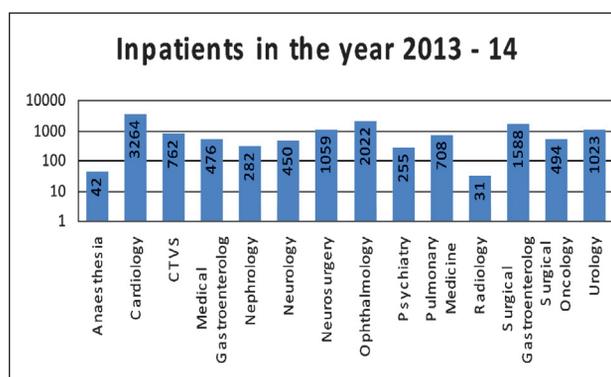


Fig. 5. The number of inpatients treated.

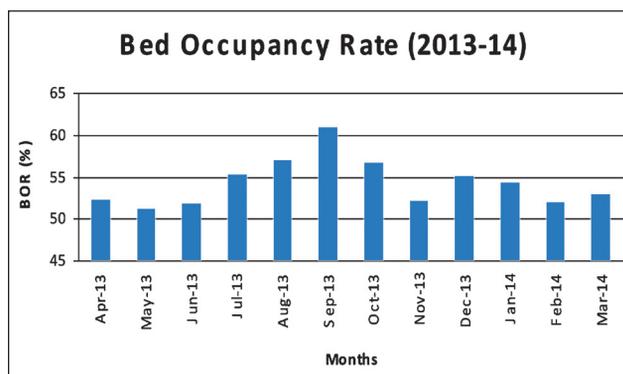


Fig. 6. Bed Occupancy Rate.

S No	Department	Surgeries	Procedures
1	Cardiology		14219
2	CTVS	582	
3	Medical Gastroenterology		2335
4	Nephrology		3541
5	Neurosurgery	596	
6	Ophthalmology	1999	36656
7	Psychiatry		12
8	Pulmonary Medicine		74
9	Surgical Oncology	141	
10	Neurosurgery	596	
11	Transfusion Medicine		182
12	Urology	2239	

CLINICAL WORK AT THE MINI UNITS (2013 TO 2014)

The door-step health care clinics *i.e.* the eight Mini Units of BMHRC recorded 4,67,922 patient visits by gas victims in the year 2013 - 2014 and a total number of 2,01,753 investigations were done including radiology (X-rays), pathological tests, eye refractions and ECGs. The numbers of patients visited and investigated at each Mini Unit are given in Tables 1-4.

S No	MINI UNITS	OPD
1.	Kainchi Chola	57763
2.	Station Bajaria	56700
3.	Chandbad	46964
4.	Teela Jamalpura	61602
5.	Ginnori	85546
6.	Jahangirabad	58701
7.	Karond	50270
8.	Bal Vihar	50376
	Total	4,67,922

Mini Units	APR 2013	MAY	JUNE	JUL	AUG	SEP	OCT	NOV	DEC	JAN, 2014	FEB	MAR
1	4465	4161	4221	5580	5270	5336	4877	5163	4930	4546	4440	4774
2	4690	4455	4259	5194	4644	5186	4913	4948	4798	4668	4172	4773
3	3707	3384	3215	4035	3931	4156	4017	4219	4021	3787	3661	4102
4	5268	4745	4476	5445	5175	5379	5201	5241	5155	5208	4955	5356
5	7631	7127	6705	8150	7346	7447	7255	6890	7022	6619	6361	6993
6	4982	4467	4450	5357	5126	5283	4820	4891	4795	4654	4629	5249
7	4043	3633	3545	4342	4245	4442	4312	4413	4354	4251	4070	4550
8	4243	3910	3554	4114	4185	4503	4365	4260	4217	4338	4099	4588

Table 4. Investigations at Mini Units

MINI UNITS	X -RAY	PATHOLOGICAL	ECG	Refraction
1	1441	15109	335	2433
2	463	26103	422	1368
3	748	16159	602	2072
4	793	14067	1163	4259
5	1389	31606	Nil	3829
6	319	21444	922	2337
7	Nil	29713	889	2205
8	918	16336	703	1606

Extramural Research

Multicentric Study on Prevalence of Chronic Kidney Disease in Adults in Urban Indian Population

The Task Force study aiming to evaluate the prevalence of chronic kidney disease in adults

in urban settings has been initiated. It is a cross sectional community based study envisaging to survey 2714 subjects above 18 years of age, selected through cluster sampling technique, from each participating centre through questionnaire, routine physical examination followed by estimation of urine protein and serum creatinine.

NON-COMMUNICABLE DISEASES

In the area of non-communicable diseases the ICMR's Institute of Cytology and Preventive Oncology, Noida continues to carry out research studies for prevention and early detection of cancer. The National Centre for Disease Informatics and Research, Bengaluru continues to focus on activities related to population based cancer registries, hospital based cancer registries, time trends in cancer incidence rates, software development, among others. Other research studies were carried out in the areas of oncology, diabetes, neurological sciences, obesity and metabolic syndrome, among others. Major highlights of various programmes undertaken by the ICMR in the area of non-communicable diseases during the year 2013-14 are given below.

Intramural Research

INSTITUTE OF CYTOLOGY AND PREVENTIVE ONCOLOGY, NOIDA

The Institute has mandate of cancer prevention as its main goal. This is being achieved by following different action lines keeping in mind the multistep process of cancer development. Main strategy to prevent cancer is to find out cancer causative factors like environmental, behavioral, genetic and their interaction, and managing such factors to prevent cancers. Another strategy is to detect cancer early by way of finding molecular genetic markers of cancer that has obvious role in cancer prevention by way of minimal treatment and management. The activities are directed towards (i) epidemiological studies and early cancer detection activities; (ii) genetic susceptibility studies; and (iii) genetic markers.

Screening for Cervical Cancer

The Institute has developed a magnifying device named "Magnivisualizer". Its suitability and user friendliness, in the hands of Gynecologists, Dentist and Para-medicals (nurses), was tested. The validation study was carried out at hospitals from Delhi, Meerut, Kolkata, Jaipur, Agartala, Noida and Chennai. The utility survey for both cervical and oral cavity examination revealed that 95 to 100% of the examiners were comfortable with the instrument as also with different parameters of the examination. The comparative performance of Magnivisualizer vs. Tungsten light for the examination of the cervix (20.5% vs. 12.7%) and oral cavity (24.8% vs. 16.5%) revealed that Magnivisualizer detected 1.5 to 1.6 times more lesions compared to tungsten light. Ninety per cent of the cervical lesions detected by Magnivisualizer were also detected positive by cytology (LSIL +). Ninety three per cent of oral lesions detected by Magnivisualizer were also detected by cytology (benign, dysplastic, or cancerous lesions). Magnivisualizer could differentiate oral lesions most efficiently (95%) vs. Tungsten light (44.3%).

A field trial of Magnivisualizer for the detection of pre-cancer and cancer lesions of oral cavity was also carried out. The results were encouraging. Specifically, Magnivisualizer can detect the pre-cancer lesions of oral cavity more than two fold as compared to torch. Visual sensitivity was increased by two fold and it was 80-90% compared to cytology. Also, Magnivisualizer may also be used conveniently by Dentist for detection of pre-cancer lesions of oral cavity in addition to detection of pre-cancerous lesions of uterine cervix. It is the enhancement of the scope of its use other than in case of cervical cancer for which it has been used.

After successful completion of these validation exercises, the Magnivisualizer was launched by the Ministry of Health and Family Welfare on December 23, 2013.

Molecular Screening for Cancer

The scientists are actively looking for specific molecular markers of malignant and premalignant lesions of cervix, oral cavity and other cancers. These studies intended to achieve : (i) Molecular mechanism of carcinogenesis that will help in planning cancer preventive strategies and treatment; (ii) Molecular markers for early detection of cancerous or precancerous lesions that will help in developing tests for the early detection of these lesions for better prevention and treatment; (iii) Genetic susceptibility studies will help in understanding the population at risk when exposed to particular environmental carcinogens, and when a particular gene is associated with cancer, a probable mechanism may be suggested ; and (iv) Role of various infectious agents like, HPV and hepatitis viruses, *Helicobacter pylori*, etc. in the process of carcinogenesis will help in prevention of cancer by using strategies to stop these infections.

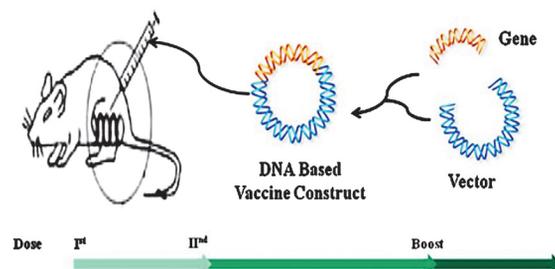
Preventive, Diagnostic and Therapeutic Studies

HPV DNA vaccine: The Institute has identified 16 major variations (variation more than 15% in compare to prototype) in HPV 16 L1 gene and one major variation in E6 gene. Change in amino acid level due to variant nucleotide is also determined. The effect of these variations on epitopes of HPV 16 L1 was predicted using bioinformatics tools. It was identified that some major variations decreased the binding score and others increased the binding score towards the MHC alleles. Their effect on immunogenicity was validated in mice model by administering HPV 16 variant DNA vaccine constructs. One variant showed a ~2.7 fold increased in the antibody titer in comparison to prototype HPV 16 where as others are not. Work is under progress to enhance its immunogenicity.

Genetic immunization

6±8-week-old BALB/c female mice were immunized with DNA vaccine constructs for generation of HPV 16 L1 antibody titer. The combine effect of full length L1 construct having

all the variations showed higher immune response (~2 folds higher antibody titer) in compared to the prototype where as V16 construct showed 2.7 times more than the reference construct. Work is under process to increase the immunogenicity by using different adjuvant.



Therapeutics

Telomerase molecule as a target for cancer therapy:

In addition to other targets, the Institute has examined the telomerase molecule to be used as a target for cancer therapy using *in vitro* model systems. The functional role of telomerase in cancers was probed by targeting telomerase RNA as a target, an integral component of telomerase enzyme complex. It was found that telomerase RNA component have non canonical functions and can be used as a target in management of cancer disease. This study provides proof of concept that telomerase can be used as a target in cancer therapy for better management.

Plant-based anti-cancer library for therapeutic drug discovery :

A central resource Naturally Occurring Plant-based Anti- cancer Compound-Activity-Target Database (NPACT, <http://crdd.osdd.net/raghava/npact/>) has been developed which gathers information related to experimentally validated plant derived natural compounds exhibiting anti-cancerous activity. It contains 1574 compound entries, and each record provides information on their structure, manually curated published data on *in vitro* and *in vivo* experiments, inhibitory values, properties (physical, elemental and topological), cancer types, cell lines, protein targets, commercial suppliers and drug likeness of compounds. NPACT database would facilitate drug discovery in the area of cancer.

Curcumin based EGFR inhibitors for lung cancer

: Molecular docking and molecular dynamics investigation has been carried with an ensemble of epidermal growth factor receptor tyrosine kinase (EGFR-TK) structures against a synthetically feasible library of curcumin analogs to discover potent EGFR inhibitors. It was demonstrated that 4-arylidene curcumin analogs may decrease growth of lung cancer cell lines.

DIAGNOSTICS**E2F as potential target in cervix cancer:**

Computational methods involving the combination of gene expression data from microarray experiments and promoter sequence analysis of a curated gene set involved in the cervical cancer causation has been utilized for identifying potential regulatory elements. Consensus predictions led to the identification of twelve TFs that might be crucial to the regulation of cervical cancer progression. Subsequently, TF enrichment and oncomine expression analysis suggested that the transcription factor E2F played an important role for the regulation of genes involved in cervical carcinogenesis. Our results suggest that E2F possesses diagnostic/prognostic value and can act as a potential drug target in cervical cancer.

MicroRNA based diagnostic marker :

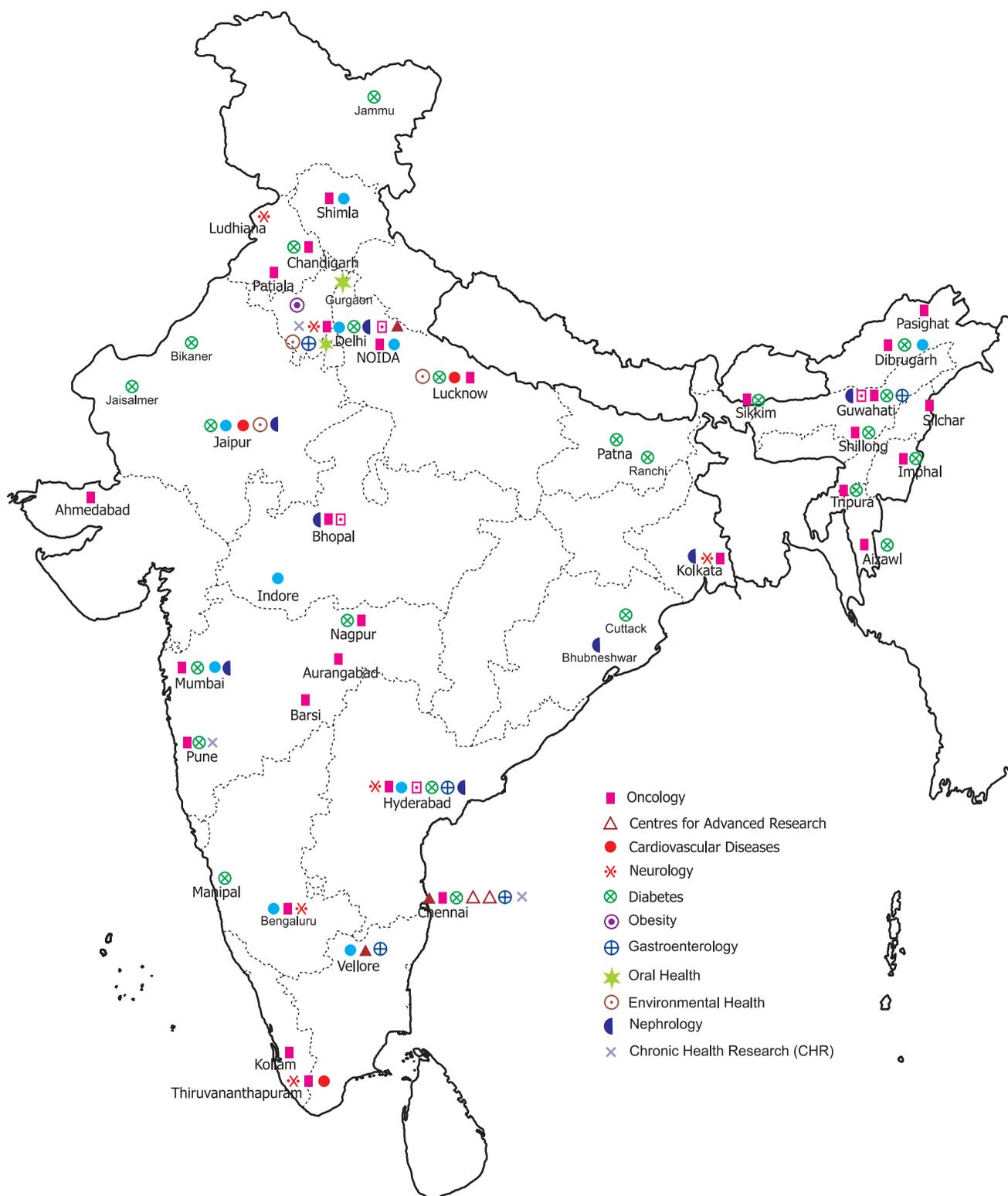
miRNAs reported to be dysregulated in cervical cancer were identified and their targets predicted. These targets were subsequently compared with previously curated gene dataset involved in cervical cancer. Then network properties (composed of degree, betweenness centrality, closeness centrality and clustering coefficient) of the putative, validated and human protein-protein interaction network were compared. Based on the topological properties, it was observed that the gene targets BIRC5 (survivin), HOXA1 and RARB were enriched in cervical cancer. Also, miRNA-mRNA network found that miR-203 and miR-30b could target these genes. The analysis indicated that BIRC5 may act as a therapeutic target and miR-203 demonstrated to be highly expressed in squamous cell carcinoma of cervix can be detected in serum and therefore would be valuable in early diagnosis of cervical cancer.

HPV Serology

The Institute has established ELISA test for HPV16 Antibodies. The SOP provided by Global Research Laboratory (GRL), Sweden is being followed. This test has its own merit since it is performed on blood samples which is easy to collect and can be done on younger age group where otherwise cervical scrap is impossible to take due to ethical reasons. We performed HPV serology proficiency study for testing of antibodies against HPV 16 and HPV 18 using ELISA with a panel of serum samples comprised of 90 human sera. We analyzed as per the SOP provided by GRL, Sweden.

Genetic Studies

Genetic susceptibility: These studies aim to find out the genotypes that makes individual more prone to develop cancer when exposed to endogenous and exogenous carcinogens. Glutathione transferase is one such big family of genes involved in phase II detoxification system. Out of many genes involved in Phase II detoxification two genes are of particular interest GSTM1 and GSTT1 because of complete absence of these genes in individuals, making them prone to defect in detoxification of certain chemicals. Because of substrate specificity we have observed that the absence of one of these genes makes an individual prone to develop certain cancers. We have shown that GSTT1 null individuals are more prone to develop oral cancer and GSTM1 null genotype in women makes them more prone to develop cervical cancer. We have shown that risk of esophageal cancer was approximately twice in individuals having homozygous GSTM1 and GSTT1 homozygous null genotypes in Assam and around three times in GSTT1 homozygous null genotypes in Delhi cases. In Delhi population GSTM1 seems to play a protective role for esophageal. A significant association of GSTM1 null genotype with esophageal cancer was observed in younger age groups in Assam and in Delhi association was observed in smokers with GSTT1 homozygous null individuals, and alcoholics having GSTM1 genotype. A significant association of GSTM1 null genotype in Assam was observed between esophageal cancer and fermented betel nut chewers only. Our study indicates that cancer development is not only due to endogenous and exogenous carcinogens, but depends on their



MAJOR ICMR RESEARCH PROJECTS IN NON-COMMUNICABLE DISEASES

interaction with genes that are involved in the detoxification of these carcinogens.

Tumor markers : The scientists at ICPO are trying to look for specific tumor markers for various cancers, especially cervical and breast, so that they can be used for early detection of cancer. Plasma IL-6 and IFN- γ is being studied for cervical cancer prognosis, BRCA1 mutation and its expression for breast cancer. Transcription factors like YY1, GSK3 β are being studied in cervical cancer.

NATIONAL CENTRE FOR DISEASE INFORMATICS AND RESEARCH, BENGALURU

Population Based Cancer Registries (PBCRs)

The report of 25 PBCRs (2009-2011) includes the data of four new PBCRs (Meghalaya, Nagaland, Tripura and Wardha) that have commenced working and included under the NCRP network since the last report for the years 2006-2008 was prepared and published during the period under report.

Software Development

A considerable progress has been made in software development both offline and online.

Offline: It includes (i) Importing from Hospital Based Cancer Registries(HBCR) software to PBCR Software - HBCR records from Hospital Based Cancer Registries Data Management (HBCRDM) with Pin codes for PBCR area exported to CSV from HBCRDM and imported to PBCRDM via importing software;(ii)Importing online PCA / HBCR data to Patiala PBCR - Patiala data entered online PCA/HBCR can be downloaded and then imported to offline Patiala PBCR database using an intermediate excel file; (iii) All Cause Mortality: Pending testing with Delhi, Sikkim and Bhopal All cause files; (iv) Import of additional information on cause of death (cancerous and non-cancerous) from all cause data received from registries. Additional statistical report on percentage after matches achieved after matching based on additional information imported; (v) Summary Table(single/consolidated yrs)

(a) Incidence numbers and rates, (b) Leading sites, (c) Basis of diagnosis, (d) Mortality numbers and

rates (e) Data quality and indices of reliability Tables are exportable to excel, word /pdf.; (vi) Sub-site specific Annexure – ongoing; (vii) Deployment of software - Pasihat PBCR; (viii) Ready generation of Time Trends in Incidence Rates - Developed programme to calculate AAR (Age Adjusted Rate) for each site of cancer (single / combined years) for Time Trends. Additional work done : Options for CR (Crude Rate), ASPR (Age Specific Rate), combination of age groups and Clinical Extent of the Disease Before Treatment for calculating Time Trends; (ix) Childhood Cancer Rates - Programme to calculate childhood rates for: (a) All the 10 broad types of childhood cancers together for single/combined years. (b) Each broad type of childhood cancer for any range of calendar years ; and (x) Integration of HBCR & PBCR - Done for testing at Centres at Bhopal, Bangalore, Kamrup and Dibrugarh PBCRs. Direct import of data from HBCRDM to PBCRDM in bulk where both applications are offline. This will import cases based on valid Pin codes provided by PBCRs. It does not import records having other Pin codes/doubtful cases.

Online software

- PBCR- Incidence data entry and basic quality check at data entry.
- District wise reporting for annexure tables for Mizoram and Manipur districts and State.
- Consistency error checks on the all the PBCR's data to improve quality.
- PBCR Hyderabad has commenced data entry since January 2014 as per laid out guidelines.

Hospital Based Cancer Registries (HBCRs)

The five year (2007-11) consolidated report of the seven HBCRs including the data of two new HBCRs viz. Dr. B. Borooah Cancer Institute, Guwahati and Postgraduate Institute of Medical Education and Research, Chandigarh was prepared and published during the period under report.. This report gives, among others, a comprehensive picture of the overall magnitude of cancers diagnosed at the respective Centres.

Time Trends in Cancer Incidence Rates 1982-2010

The report on “Trends in Incidence Rates of Cancer: 1982-2010” was prepared and published during the period under report. Among males, cancers of the tongue, mouth, colon, rectum, liver, lung, prostate, brain, non-Hodgkin’s lymphoma and lymphoid leukaemia have shown statistically significant increase in incidence rates. Some additional sites of cancer for males that have shown a rising trend and included in this report are gallbladder, pancreas, kidney and urinary bladder. In females, cancers of the gall bladder, lung, breast, corpus uteri, ovary, thyroid, brain, non-Hodgkin’s lymphoma and myeloid leukaemia have shown a statistically significant increase in AARs.

Patterns of Cancer Patient Care and Survival

The project on Patterns of Cancer Patient Care and Survival for three sites of cancer *viz.* cancer breast, cancer cervix and head & neck cancers is on-going and several Centres have been able to achieve 70% follow-up on the 2006-08 data. A detailed analysis is being carried out.

Development of an Atlas of Cancer in Punjab State

The project on “Development of an Atlas of Cancer in Punjab State” is an important ongoing activity. During the period under report, Punjab cancer website online and dynamic E-monitoring of data capture page which was developed for administration is speeded up. It contains participating Institutions activity online, summary of progress report – institution wise and details of status of data – district and institution wise *etc.* Dynamic output tables for listing of cases for district wise, centre wise and site wise have been programmed. Programme has been done for generating reports based on pooled districts / centres. Reports on Malwa region can also be generated dynamically. As of now there are 154 Centres that have registered (141 Centres from Punjab State, three from Chandigarh, two from Haryana State and one from Jammu & Kashmir, four from Delhi and three from Rajasthan). So far data on about 60,481 cancers have been received.

Hospital Based Cancer Registries Data Management (HBCR-DM) Software

Eighty three Centres have started using HBCR-DM software. These Centres have contributed data on 1,08,089 cancers so far. Twenty four new Centres have been registered under the project during the period under report. Many modifications and upgrades were done on the software so as to make it more user-friendly and powerful in terms of data quality checks.

Pathology Software

NCDIR-NCRP has developed an online Pathology Software Module which could be used for reporting of both malignant and non-malignant cases. This is a very user friendly module with features like uploading the microphotographs of slide, *etc.* As of now 79 institutions across the country have registered to use this module and seven Centres have started using the software. So far data on about 1,475 cancers has been received. Based on the feedback received from the Centres, this module has been revised into three formats. This revised module has been given access to 10 registries.

Radiotherapy Module

NCDIR-NCRP has developed an online Radiotherapy Software Module also. Using this module one can enter the identification information of the patient along with diagnostic details as well as treatment details and reports can be printed / saved in PDF. This module has been made into two formats *viz.* Basic and Cancer Registry Format. As of now two Centres have been given the online access of this module.

Setting up of Hospital Based Cancer Registries in Regional Cancer Centres (RCCs)

The project on Setting up of Hospital Based Cancer Registries in Regional Cancer Centres for the period under report has been initiated. Accordingly, a letter of request to initiate the action of setting up the HBCRs and patterns of care and survival studies on cancer breast, cancer cervix and head and neck cancers has been sent to all the RCCs.

The number of Centres under NCDIR-NCRP and data sets received are given in the Table.

Table. Cancer registration in India - 2014

	Centres	Data Sets
1. Population Based Cancer Registries	29	28
2. Hospital Based Cancer Registries including RCCs	30	20
3. POCSS (incl. five HBCRs)	21	39
4. Cancer Atlas NE	21	15
5. HBCR-DM-SW	65	47
6. Punjab Cancer Atlas	154	58
7. Pathology Software	79	16
Total	242	223

Development of Population Based Stroke Registry(PBSR)

Efforts are being made to initiate the process of development of PBSR under three Parts as follows:

Part I: A NCDs (Non-Communicable Diseases) magnitude monitoring mechanism for the country involving medical colleges: It aims to involve the Community Medicine department of all the medical colleges in India to develop a network to monitor the magnitude of NCDs. During the first phase, 30 Medical Colleges (Govt-18; Pvt-12) across India have been shortlisted representing 20 States and three UTs, and developed a research plan “A national model to monitor the magnitude of non-communicable diseases (diabetes, CVD, stroke, cancer) in India with an intention to examine the possibility of developing population based diabetes, CVD, stroke, and cancer registries involving medical colleges through primary health care setup” and sent this proposal for financial assistance.

Study on the Magnitude of Diabetes involving Medical Colleges – The Institute has also evolved a plan to involve rest of the 80 medical colleges through informatics to monitor the magnitude of diabetes across India. Since all the 106 medical colleges have agreed to send core details of all the diabetes cases attending the hospital it is achievable.

Part II: National Cardiovascular diseases project: With regard to cardiovascular diseases our

approaches are stratified into three levels, they are the magnitude approach, pattern of care approach, and EMR approach. At this juncture in time we are deferring EMR approach. A data capture format for heart failure has been developed for both magnitude approach and pattern of care approach.

Part III: Verification of completeness of data obtained through Punjab cancer atlas through a cross-sectional survey with the additional objective of knowing the magnitude of diabetes, CVD, and stroke in the Malwa region of Punjab (under NCRP): Other than that, to verify the completeness of data obtained through Punjab cancer atlas, it has been decided to conduct a cross-sectional survey of almost 1,20,000 individuals (around 10000 families) in four districts of Malwa region (Muksar, Batinda, Mansa, and Barnala) in Punjab. During the same survey it has been planned to get the estimate of magnitude of other three NCDs viz., diabetes, CVD, and stroke. This will be used to pilot the questionnaire designed to the project mentioned in Part I.

Translational Research under NCRP

Translational Research under NCRP which was started with the objective of finding out how the large volume of epidemiological data on cancer be suitably translated into research activities that would benefit the cancer patients and the society at large continued for the year 2013 also. The format for update of the work being carried out at NCRP for “Translational Research under NCRP” was sent periodically (every three months) to ICMR Hqrs.

Risk Factor studies –Stomach Cancer

As part of the Translation Research, the proposal for a multi centric hospital based epidemiological study to assess the risk factors of stomach cancer, especially the association of *Helicobacter pylori* and stomach cancer has been initiated. Ten PBCRs expressed interest to participate in this study viz. (1) Tata Memorial Hospital, Mumbai, (2) Cancer Institute, Chennai, (3) AIIMS, Bhopal, (4) Kidwai Institute of Oncology, Bangalore, (5) RCC, Trivandrum, (6) Gujarat Cancer and Research Institute, Ahmedabad, (7) Civil Hospital, Aizawl, (8) Sir Thutob Namgyal Memorial Referral Hospital, Gangtok, (9) Dr. B.Borooah Cancer Institute, Guwahati and (10) Assam Medical College Hospital, Dibrugarh.

Extramural Research

ONCOLOGY

Review of Cancer Management Guidelines

Under Task Force Project on review of cancer management guidelines, the consensus documents on management and treatment of various cancer sites are being formulated. Consensus documents on management of buccal mucosa cancer, colorectal cancer, stomach cancer, soft tissue sarcoma and gall bladder have been formulated and are being printed.

Indo-German Collaboration on Research in Oncology

Development of chimeric genetic vaccine against human papillomavirus type 16:

The project was aimed at identification of specific HPV 16 L1, E6, E7 gene variants prevalent in India, preparation of HPV 16 variant and chimeric gene constructs; characterization of these constructs by sequencing and *in vitro* expression; and evaluation of immunogenic efficacy of these constructs in mouse model. A total of 500 consecutive subjects comprising 300 histologically confirmed cervical cancer cases along with 200 control samples were collected from Lok Nayak Jai Prakash and Safdarjung hospitals, New Delhi. Out of these 300 cases, 278 (92.7%) were found infected with HPV, of which 273 (98%) samples were HPV 16 positive. In addition, only 4.5% (9/200) of healthy controls were found to be HPV positive, and all of them were infected with HPV type 16. A total of 16 major variations in full length L1 and one major variation in E6 gene were detected; however no major variation was found in E7 gene in cervical cancer cases. The investigators have also identified four major variations which may play important role in the immunogenicity against HPV by affecting the binding affinity of immunogenic peptide (epitope). The immunogenicity was tested *in vivo* using balb/c mice.

The influence of O-acetylated cell surface expressed sialoglycans angiogenesis of bone marrow associated leukemias

The study was aimed to assess if o-acetylation, either through masking sialoglycan binding sites

or creating new specific receptor molecules on endothelial cells is involved in regulation of homing and angiogenesis. Fifty confirmed cases of ALL before chemotherapy were enrolled. The investigators have observed significant decrease of total sialidase activity in the membrane fraction, and increase of total sialidase in cytosolic fraction, of several cell lines of B- and T-ALL lineages compared to normal PBMC. No significant difference was observed in total sialidase in lysosomal fraction, whereas a significant decrease of membrane bound sialidase (neu3) activity as well as Neu3 gene expression was observed. The three sialidases Neu2, Neu3 and Neu4 were significantly down regulated due to enhanced expression of GD3 synthase in ST8Sial1 transfection cells. GD3 synthase influenced the adherent property of human micro vascular endothelial cell (HMEC) to the extra cellular matrix. The role of O-acetylation of sialic acid on angiogenesis and effect of GD3 on cell cycle progression of pancreatic cancer was established and may lead scope of benefitting bone marrow associated leukemias.

Nano carriers for intracutaneous targeting in management of skin cancer and skin diseases

The study was aimed at synthesis of a novel cationic lipid, designing and characterization of cationic and non-ionic charged nano carriers system *viz.* liposomes, leciplex and invasomes. The novel cationic lipid (NCL) was successfully synthesized and characterized. Liposomes and leciplex with and without NCL were formulated successfully. Preliminary *in vitro* penetration study demonstrated enhanced penetration of leciplex system in deeper layer of skin. The systems investigated are scalable and there is good scope of its translation into medically feasible outcome.

INDO-INFORM COLLABORATION ON CANCER RESEARCH

Effect of cellular and immune response in mice and patients with acute promyelocytic leukemia treated with arsenic trioxide

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. A series of experiments to address the role of the immune response both as a prognosticator and potential target for intervention were undertaken. In a mouse model of leukemia the synergistic effect between all-trans retinoic acid (ATRA) and a DNA plasmid vaccine was demonstrated. A delay recovery of NK cells in patients treated with ATO but also demonstrated *in vitro* the 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 have significant clinical implications and potential for translation into the clinic. Through this project there has been successful transfer of the mouse model of leukemia and the use of DNA plasmid vaccine

ICMR-Indian National Diabetes Study (ICMR-INDIAB)-Phase II

The project was initiated at Andhra Pradesh, Karnataka, Punjab, Uttar Pradesh and Ahmedabad (Gujarat) at first instance with funding from Department of Health Research. Briefly, in Andhra Pradesh and Gujarat, the prevalence of diabetes in urban areas is double the rate found in rural areas. In Bihar, the prevalence of diabetes in urban area is three fold higher than rural areas. In Karnataka, the prevalence of diabetes in urban is more than double than the rates in rural areas. In Punjab, the prevalence of diabetes in urban is higher than the rates in rural areas. Of the five States studied, Andhra Pradesh has the highest prevalence of diabetes in urban areas (12.7%) and Punjab has the highest prevalence in rural areas (8.7%) with the most marked urban rural differences observed in Bihar.

ICMR-Indian National Diabetes Study (ICMR-INDIAB)-North East

The study is aimed to estimate prevalence of pre diabetes and diabetes among rural and urban population in north eastern region of the country. Four States have completed the survey. In Assam, the urban residents had significantly higher cholesterol, triglycerides, LDL cholesterol and cholesterol to HDL ratio compared to the rural. The study conducted in Assam State resulted in training of approximately 120 personnel at field

level besides capacity building. In Mizoram, the urban residents had significantly higher cholesterol, triglycerides, LDL cholesterol and cholesterol to HDL ratio compared to the rural. Overall weighted prevalence of diabetes was 5.7% and that of pre-diabetes was 5.8%. Data of other two States are being analysed.

Task Force on genetic analysis of MODY and neonatal diabetes in India

The project was aimed to screen genes for MODY and neonatal diabetes. The study population includes unrelated patients, diagnosed with non-insulin dependent diabetes before 25 yrs of age and also has a positive family history of diabetes from various parts of India. This also includes those clinically characterized as MODY and others who were clinically labeled as early onset type2 diabetes. The three novel variants and 11 known polymorphisms in *HNF1A* gene were identified. In addition, one novel variant (IVS5+4A>G) and three known polymorphisms were identified in *HNF4A* gene. During the year under report, five publications have been brought out in scholarly journals.

NEUROLOGICAL SCIENCES

Vascular Cognitive Impairment

A study on “Development and validation of a comprehensive clinical and neuropsychological test battery for use in the Indian context for patients with vascular cognitive impairment” has been initiated at Nizam’s Institute of Medical Sciences, Hyderabad; Apollo Gleneagles Hospital, Kolkata ; and All India Institute of Medical Sciences (AIIMS), New Delhi. The study aims to develop a comprehensive clinical and neuropsychological test battery for use in the Indian context for literate and illiterate population across five languages and to validate the Indian vascular cognitive impairment (VCI) protocol in patients with VCI across five geographic regions. Adaptation of neuropsychological test battery in the Indian context is being carried out. The selected neuropsychological tests for Indian context are being developed in Telugu, Hindi, Malayalam, Kannada and Bengali. The batteries are being developed for both illiterates and literates. These batteries will be validated in stroke cases and age

and gender matched controls with and without cardiovascular risk factors.

Selection and finalization of tests to be carried out for development of VCI test battery were carried out. In this process the tests which were expensive and had copyright problems were replaced with tests comparable tests in the public domain (with permissions from researchers/ developers). Indigenous tests were developed for the tests which are not in the public domain and these will be validated in the pilot study. English, Hindi, Telugu and Bengali versions of the case report form has been developed. Malayalam version of CRF is also being made. The battery will be validated in the pilot study.

Evaluation of development of neurosurgery skills by hands-on skills training and interactive virtual training modules (web based, tele-education and virtual simulation)

Neurosurgical procedures are complex and iatrogenic errors have direct impact on the consequences of surgery. Therefore there is a need of neurosurgical skills training. A joint collaborative project between AIIMS, New Delhi and Indian Institute of Technology (IIT), Delhi aims to formulate, standardize, validate and evaluate neurosurgery skills training modules based on hands-on skills training. Development, timely up-gradation and maintenance of an interactive virtual training platform (web based, tele-education and virtual evaluation) for trainee and trained neurosurgeons is being undertaken. The project will also evaluate and review the efficacy of neurosurgery skills training modules by experts based on hands-on skills, web based, tele-education and real time simulation interactive virtual training modules. This will help in building an evaluation and assessment system to grade the trainee neurosurgeons based on their surgical techniques.

Formulation of different hands on training modules on neuro-anatomy, neuro-endoscopy, high speed drilling, microsuturing, spine and spine instrumentation on the basis of round the year daily skills training sessions, short term skills training programs and workshops have been developed. Modules are categorized into task-based and procedure-based sub-modules for the purpose of

standardization and validation. Cadaver Anatomy Module (CAM), Video Demo Module (VDM), Synthetic Simulation Modules (SSM), Cadaver Neuro-Anatomy Dissection Module (CNADM), Cadaver Animal Tissue Module (CATM), Live Animal Tissue Module (LATM), *etc.* have been developed for different neuro-endoscopy, high speed drill, micro-suturing techniques and spinal instrumentation skill training modules. All skills training activities are recorded for self and expert evaluation. The sessions help in the development of eye-hand coordination, instrument-tissue manipulation, dexterity, speed and effectualness translating into steep learning curve. 'Neurosurgery Skills Training Workshops' are organized thrice a year for three days. Asynchronous education and training in neurosurgery is provided through website. The synchronous education consists of setting up Learning Management System (LMS) and Content Management System (CMS) based e-learning platform on a domain at the IIT Delhi server for Neurosurgery Education and Training School Learning Management System. The e-learning platform has a centralized user profile management, discussion forum, question answer forum and on-line courses for neurosurgery with 3-D contents. There were 61,046 views with 172 subscribers for neurosurgery educational operation, 3D animation and graphics based videos.

CHRONIC DISEASE HEALTH RESEARCH

The Task force project "Development of a model for strengthening of existing health system to address non communicable diseases in India" was conducted in Ballabgarh, Sunder Nagari and Sangam Vihar. A total of 45 Health Workers, 89 (CHWs) and 80 LHWs were trained under this project in NCD Prevention and Control. The project aimed at assessing the existing health system's ability to carry out NCD screening, prevention and control activities. The major conclusions of the study are:

- (i) Health workers after training in relevant measurements (waist circumference, blood pressure, weight, and height) can be used for NCD risk assessment.
- (ii) Opportunistic screening at primary and secondary level health facilities appears to be feasible and a cost effective strategy for early

case detection. Domiciliary approach has to contend with the fact of low compliance to referral to higher facilities.

- (iii) There was no impact of introduction of NCDs activities on other national programs or on workload of the workers at the implementation level seen in the project.
- (iv) ASHAs and lay volunteers can be trained in risk assessment and family and community education related to NCDs.
- (v) A strengthened PHC can serve a focal point for NCD Prevention and Control.

ICMR signed a MoU with the Global Alliance for Chronic Diseases (GACD) for undertaking research on chronic NCDs. A joint call for proposals on Implementation research for the prevention and control of type 2 diabetes was put out in October 2013. There were 26 Letter of Intent applications received and were reviewed by an Expert Group constituted by DG, ICMR. The Expert Group recommended four applications to be shortlisted for inviting them for submission of full proposals by July 2014.

Under the ICMR-Academy of Finland MoU, the first workshop on chronic NCDs was organized in Delhi in March 2014. Experts from both countries presented, discussed and recommended mutual areas of research cooperation in the areas of cardiovascular diseases, diabetes, obesity and metabolic syndrome.

ICMR is collaborating with the Central Reserve Police Force (CRPF) to address various health related issues of the forces. ICMR has provided them with inputs on malaria in the tribal, jungle and inaccessible areas of operation. National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore is being funded by DHR

to provide training and capacity building of the CRPF personnel to tackle mental and psychological health issues.

GASTROENTEROLOGY

A multi-site task force project “Prevalence of celiac disease in indigenous populations of southern, northern, and north-eastern parts of India and identification of reasons for difference in its prevalence” was completed at CMC Vellore, AIIMS, New Delhi and Guwahati Medical College, Guwahati. A sample size of about 22,000 subjects (both men and women) was screened (Table). Preliminary results indicate varied seroprevalence of anti-Ttg antibodies.

It was noted that in the Vellore study site and in the Delhi study site, the proportion of individuals with celiac disease was higher in the 21-40 (Delhi) and 31-50 (Vellore) age group compared to individuals over the age of 50 years, whereas in the Guwahati study site, the prevalence of celiac disease was proportionately distributed across all the age groups tested. Genotyping of individuals negative for celiac disease was done in all three study sites (247 in Vellore, 110 in Delhi and 226 in Guwahati). DQ2 and/or DQ8 genotypes were found in approximately one third of the population in each site – ranging from 31.42% in Guwahati to 36.43% in the Vellore site and 38.19% in the Delhi site. Interestingly, the DQ8 genotype and allelotype were more common in the Vellore study site compared to the other two sites, indicating regional differences in the frequency of the DQ8 determining alleles.

There was a gradient from Delhi to Guwahati to Vellore with 69.4, 54.9 and 12.5% respectively in the three sites showing changes consistent with celiac disease. This suggests that the presence of biopsy changes in serology positive patients

Table. Crude prevalence rate of antibodies to Transglutaminase across all three sites

Variables	Delhi	Guwahati	Vellore	P value
Number of participants	6209	8149	8750	
Aesku test positive, n (%)	556 (8.95)	637 (7.81)	513 (5.86)	
Aesku and Inova positive, n	76	70	12	<0.0001
Crude prevalence of celiac disease, %	1.26	0.85	0.13	

correlates positively with the population prevalence of celiac disease in the concerned geographic region (Figure).

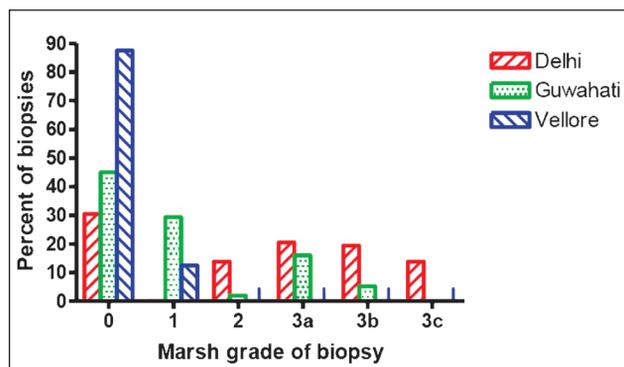


Fig. Marsh grade off biopsy in Delhi, Guwahati and Vellore sites.

OBESITY AND METABOLIC SYNDROME

Under the MoU of ICMR with the Canadian Institutes of Health Research (CIHR), proposals have been funded and are ongoing on the collaboration on childhood obesity.

Understanding the determinants of adiposity among newborns of Indian ancestry in Canada and India: The South Asian Birth Cohort

Among three birth cohorts *viz.* rural India, urban India, and S. Asian from urban Canada, this study aims to understand the effect of diverse environments on the development of adiposity among newborns and the growing offspring during the first three years of age. Rural pregnant women weighed lesser and had lower BMI at recruitment compared to the urban women. No significant differences in the anthropometric measurements were observed among the groups. The rural women had lower fat free mass as compared to the urban pregnant women

Disability

ICMR has established a Disability Research Group in January, 2013 to promote research on all types of disabilities whether caused by infectious organisms, trauma, metabolic problems as well other factors. To initiate this activity, the Core Group has recommended an action plan to be implemented by ICMR. One of the important recommendations of the Core Group is the setting up of National Disability & Rehabilitation Research Network (NDRRN) so that important national/regional institutes

under ICMR/GOI and some of the highly reputed national/regional medical colleges/institutions are linked electronically in order to undertake research in those areas with main goal of intervention to improve the outcome. Under the NDRRN, there is a provision of establishing Regional Coordinating Units in the Medical Colleges/Institutions, preferably located in Department of Physical Medicine and Rehabilitation, wherever existing, or other Departments like Orthopaedics or Neurology or whichever is locally important/feasible. It is proposed to establish a Coordination Unit/Centre at one of the Central Institutions, so that such activities could be coordinated and there should be a Central Unit to synergise the efforts.

ENVIRONMENT

Climate Change Project

Multicentric Collaborative study on impact of Global warming and ultra violet radiation (UVR) exposure on ocular health in India is ongoing at AIIMS New Delhi, National Physical Laboratory, New Delhi, Guwahati and Hyderabad to study the effect of environmental factors and UVA & B radiation on eye diseases. The field study was completed in 12 village clusters with a total population of 6057 in Assam. In these villages, 1430 individuals aged 40 years & above were interviewed and 1367 have undergone complete ophthalmic examination. Guwahati showed slight increase trend in long term changes in UVR during winter months (Nov.-March) and a slight decreasing trend during summer (April- October). The UV at Delhi is found to be the highest during May while the lowest during December, whereas at Guwahati, the UV peaks in the month of April and is lowest during December. No appreciable change was noticed in Chennai. In order to observe the actual variation of UV (300-385nm) and shortwave (310-2800 nm) on the surface at Delhi, the measurements were done using radiometer (CUV 4) and a pyrometer (CMP-21). Separate measurements at Delhi showed that of the total UV flux received at surface the UVA contribution is about 97-99% and the UVB flux constitutes only 1-3%.

Centre for Advanced Research on Environmental Health: Air Pollution

Subject recruitment for the M-C and adult cohorts is complete. A total of 1416 pregnant women were

enrolled after screening for eligibility and seeking an informed consent from 110 villages and 10 municipal zones for the M-C cohort. Currently 1285 women are active in the cohort with the loss-to follow up rates being maintained at < 10%. A total of 1283(women and men) were enrolled from the same households as the M-C cohort subjects or from the same village/zone for the adult cohort. Child health assessments are in progress in 921 infants (of the 1118 infants currently available after excluding abortions, intra-uterine demise, still births and neonatal deaths). Exposure dataset include household measurements of 24- hour concentrations of PM_{2.5}, CO and select air toxics as well as ambient measurements in all study villages. Gestational age, birth-weight and ARI assessments are being made concurrently on subjects of the M-C cohort. Micro-environmental area measurements for PM 2.5 have been completed in all the subject (1285) households in the M-C cohort. Micro-environmental area measurements for PM 2.5 have been completed in 885 subject (of the 1283 available) households in the adult cohort. Lung function and respiratory symptom assessments have been completed on 291 and 330 subjects respectively. Cord and maternal blood samples are being archived in a bio-repository and 129 samples have been archived of which roughly half have been processed for SNP and epigenetic analyses.

ORAL HEALTH

Dento-facial anomalies and congenital birth defects of face including cleft lip and palate

The approximate incidence of cleft lip and palate is around 1.4/1000 live births in India and for isolated cleft palate it is 0.3/1000 live births. An epidemiological study to collect data from different parts of India regarding distribution of dento-facial anomalies at three Centers viz.Center for Dental Education and Research, AIIMS, New Delhi, Safdarjung Hospital, New Delhi and Medanta

Hospital, Gurgaon was undertaken. 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 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) was noted.
- A significant percentage of cases required lip and nose revision surgeries (36% and 35% respectively)
- Fifty five present cases had a post-surgical oronasal fistula
- A large proportion (77.5%) of the operated UCLP cases had complex orthodontic treatment needs.
- A high proportion of patients had hearing defects (44.7%) and many of these also had concomitant tympanic membrane afflictions also (nearly 40 % cases), in one or both the ears.
- Around 50 percent (49.7%) cases had clinically relevant speech intelligibility problems.

Thus, it can be concluded that in the sample of cleft patients assessed in the project, the treatment needs were high. There seems an urgent need to devise strategies to improve the delivery of quality care with joint efforts of all experts and health care providers. It is important to note that the data are not representative of the outcome of the three Centers.

Chronic Kidney Disease

Project of chronic kidney disease (CKD) sanctioned at eight Centres is progressing. The Centres have completed the pilot study and the main study is due for initiation.

BASIC MEDICAL SCIENCES

During the year under report the intramural research in the field of basic medical sciences was carried out in the two permanent institutes of the ICMR namely, National Institute of Pathology (NIOP) at New Delhi and National Institute of Immunohaematology (NIIH), Mumbai, as also in various other centres. Extramural research was conducted in several areas such as biochemistry, cellular and molecular biology, haematology, nanomedicine, pharmacology, genetics, stem cell research, traditional medicine, biomedical ethics, etc.

Intramural Research

NATIONAL INSTITUTE OF PATHOLOGY, NEW DELHI

TUMOR BIOLOGY

Breast cancer

Studies on gene expression and hypermethylation profile in early (<45 years) and late onset (>55 years) breast cancers (BC) have identified discriminating molecular signatures associated with early onset cancers as well as various prognostic indicators of breast cancer in Indian women. The expression analysis has helped in identification of key pathways and biological process that are deregulated by the signatures including mitotic cell cycle, homeostasis, focal adhesion; ECM receptor interaction, AP1 transcription factor targets, aurora kinase signaling and hypoxia responsive transcription factor EPAS1 mediated signaling events. Specific gene patterns to ER/PR+ve, HER2+ve, TNBC and TPBC cancer subtypes have been identified.

To identify miRNA signatures associated with breast cancer stem cells and to understand their contribution for the response of cancer cells to chemotherapeutic agents, a study was done using

CD44 and CD24 markers to select cancer stem cells (CD44+/CD24-) by fluorescent activated cell sorting. Breast cancer stem cells were isolated from breast cancer cell lines MCF7 and MDA-MB-231, T47D NIPBC1 and NIPBC2 using FACS and CD44+/CD24- cells were separated from these cell lines and grown on non-adherent conditions.

Another study was aimed to identify sequence variations and chromosomal rearrangements of deregulated genes in early onset breast cancers. Exome sequencing has been done in 14 breast cancer patients and three controls. This study was undertaken to identify novel genes upregulated by androgen receptor (AR), upon DHT stimulation in breast cancer cell line MDA-MB-453. A total of 576 candidate genes having prominent role in cell cycle, apoptosis and cellular metabolism were randomly sourced from the Cell cycle gene database, Apoptosis gene database and Metabolic gene database. All 576 genes were scanned for the presence of an AR site and 75 novel genes were found to have AR binding sites (Fig. 1). All the novel genes were found out using the bioinformatics tool TF search. Apart from the scores of an AR binding site in the target gene, other factors given importance were relationship of the target to AR functions, distance of the AR binding site from the transcription start site for downsizing the 75 genes to 10 genes. Of the total 75 putative AR targeted genes, approximately 60% had a role in cell cycle, 27% in apoptosis and 13% were found to be involved in the process of metabolism. Finally, characterization of 10 putative AR targets was done using real time qPCR to show the genomic actions of these genes. Gene expression profiling of the AR targets was done in MDA-MB-453 breast cancer cell line in presence of DHT stimulation for 24 hr and vehicle control. It was observed that the genes involved in cell cycle were upregulated, whereas

all the genes, involved in the process of apoptosis were downregulated.

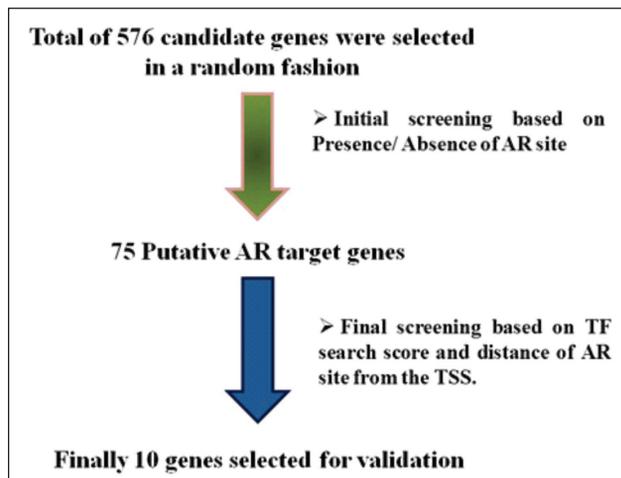


Fig. 1. Strategy for the identification of the novel androgen receptor (AR) regulated genes. Flow chart of the methodology followed in finding novel AR targets.

Polymorphisms in DNA repair and cell cycle genes contribute to increased breast cancer risk. Their association and interaction in relation to betel quid and tobacco chewing habits needs exhaustive multi-analytical investigation to explain BC predisposition due to DNA damage. Polymorphism in *TP53*-72Arg>Pro, *RAD51*-135G>C, *BRCA2*, and *CCND1*-G870A were examined in 204 BC cases and 217 controls from North-East Indian population. Betel quid chewing was identified as the predominant risk factor. *CCND*-AA and dominant model showed protection towards BC in BQC (betel-quid chewer) and NBQC (non betel-quid chewer). *TP53*-Pro/Pro genotype showed protection towards BC in NBQC. *RAD51*-C allele was associated with BC risk in BQC. Two BQC cases had *BRCA2* 8415G >T:K2729N mutation in exon18. Interaction diagram concurred the interactions between TP53 and RAD51 (1.32%) with independent effect (1.89%) of *CCND1* in NBQC. In classification and regression tree (CART) analysis, BQC with *CCND1* GG genotype were at risk followed by combination of BQC, *CCND1*, No-Smk, Alc. Risk was also observed in BQC, *CCND1*, No-Smk, Non-Alc, TP53 combination. NBQC group showed risk with combination of NBQC and TP53. These data indicate that common genetic variations in DNA repair and cell cycle genes contribute towards breast cancer

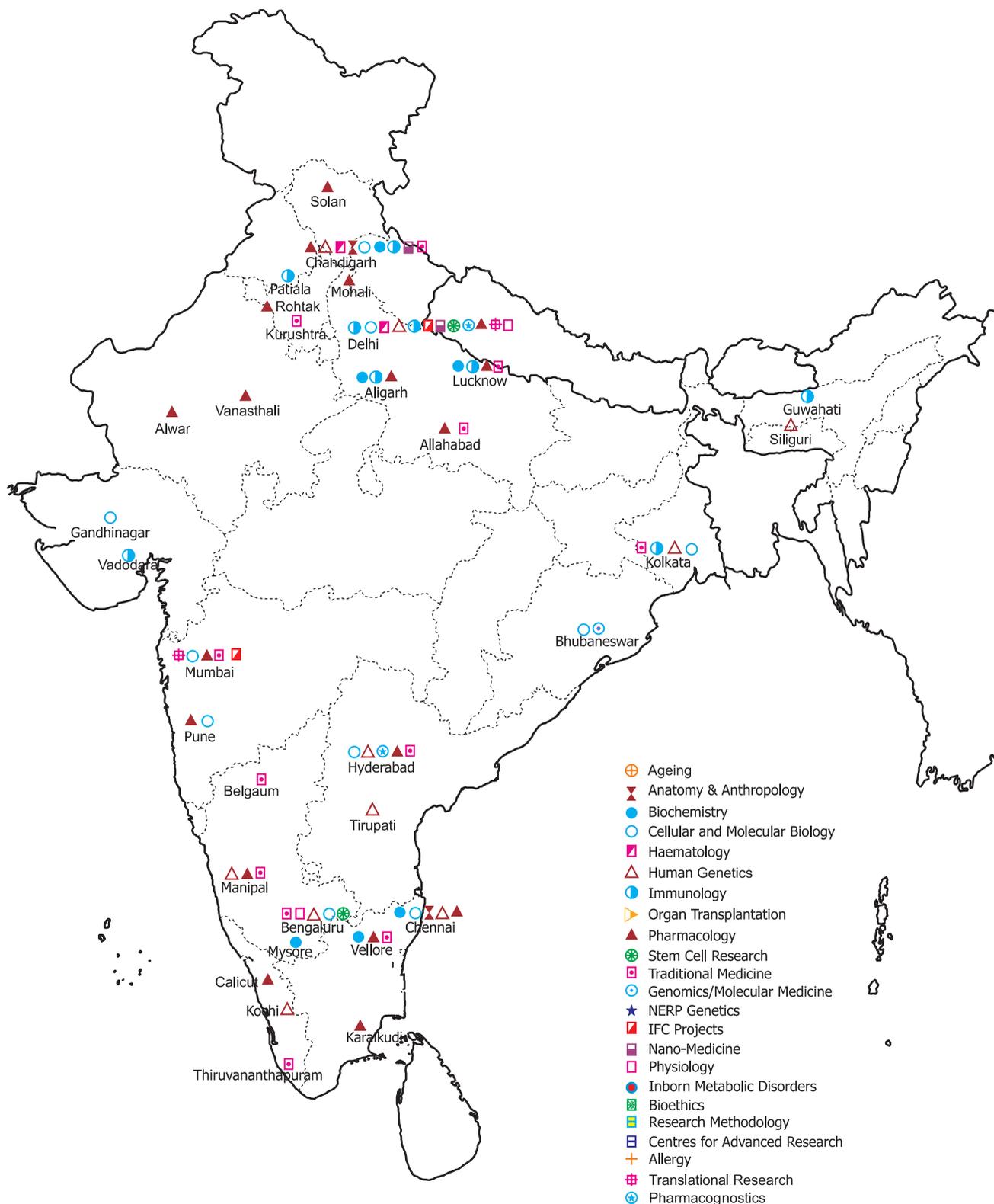
risk. In addition, unparallel predisposition was observed amongst BQC and NBQC breast cancer patients rendering dissimilar susceptibility towards breast cancer. BQC might be at an elevated risk for breast cancer attributable to betel quid carcinogens and minor roles of *BRCA2* mutation and C allele of *RAD51*. NBQC could be at slightly lower risk for breast cancer due to the protection offered by the Pro/Pro-TP53 form. *CCND1* polymorphism conferred protection irrespective of the betel quid chewing status.

Urinary Bladder Cancer

Urinary bladder cancer patients are susceptible to a high rate of recurrence (~70%). A study was aimed to identify tumour specific protein in urine of urothelial cancer patients to serve as post surgery surveillance markers. Two-dimensional (2D) gel electrophoresis for separation of complex protein samples coupled with mass spectrometry for protein identification was used to analyze protein expression patterns for paired tumour and normal sample types. Most of protein spots were present between the pH range 5 to 6. The number and intensity of spots were found to be higher in tumour tissue compared to normal tissue.

Prostate Cancer

To investigate molecular biomarkers *TMPRSS2*-*ERG* and *PCA3* in patients with prostate cancer, and to analyze their clinical relevance as a prognostic/diagnostic tool, patients scheduled for diagnostic Trans rectal ultrasound (TRUS) guided 12 core needle prostate biopsy, were screened for serum prostate specific antigen (PSA) and by digital rectal examination. Prostatic biopsies were collected from 54 patients, total RNA was extracted and cDNA synthesized. The presence of the *TMPRSS2*:*ERG* fusion transcript was assayed by Quantitative real time PCR (QRT-PCR) by using Taqman chemistry in 41 cDNA samples. Histopathology reports confirmed 25 cases as adenocarcinoma prostate (PCa) and 16 patients as benign prostatic hyperplasia (BPH). Among 41 samples analysed, 16 were found to be positive for *TMPRSS2*:*ERG* fusion and 25 were fusion negative. When the fusion analysis results were compared with the histopathology reports, all the 16 fusion positive samples were from prostate adenocarcinoma (PCa). All BPH samples were found to be fusion negative.



MAJOR ICMR RESEARCH PROJECTS IN BASIC MEDICAL SCIENCES

Cancers in North-East India

Nasopharyngeal carcinoma (NPC) has a remarkable racial and geographical distribution affecting southern China and South-East Asia and some regions in NE India. The aetiological factors of NPC include a complex interaction of genetic, viral, environmental and dietary factors. A study was conducted to find out if Epstein Barr viral (EBV) sequences in the tumour tissue could modulate host immunity or if some host immunogenetic factors could explain the high prevalence of nasopharyngeal carcinoma in different ethnic groups of North-eastern States.

Blood samples from newly diagnosed cases of NPC (n=120) and controls (n=100) were collected from two different centres in NE Region, *i.e.* Barooah Cancer Institute, Guwahati, Assam and Regional Institute of Medical Science, Imphal, Manipur. Study of 33 markers selected from the HLA region was done by fragment length analysis and data analysis was done using Gene Mapper software. Analysis of results showed a high association of HLA class I region with the occurrence of nasopharyngeal cancer. Expression of EBV RNA was found in 92% of NPC cases suggesting its significant role in NPC aetiopathogenesis. Further, to study the genes evolved in the innate immune response which act as key players and may be a driving force in the pathogenesis of NPC, the role of TNF- α , TNF- β , and HSP 70 polymorphism was studied in the NE part of India. The results showed association of TNF- β and HSP 70 gene polymorphism with occurrence of NPC. Thus, the association with HLA class III subregion in NPC pathogenesis is important since it is known to play an important role in the escape of tumour cells from host immune surveillance and may be responsible for decreased recognition and killing of cancer cells. The high incidence of NPC in NE India may be a cumulative outcome of interaction of environmental factors with genetic variations in HLA region.

Another study was undertaken to understand the contribution of Copy Number Variation (CNV) to aetiopathogenesis of oesophageal cancer in North-East region. Paired tumour tissue and blood samples (germ line DNA) from 15 patients were processed for Affymetrix® Genome-Wide Human SNP Array

6.0. A total of 93 altered genes were found, of which 43 genes were from amplified regions and 50 from deleted regions. To find out the cancer candidate gene form 93 genes, the combined bioinformatics analysis was done by using different tools (Fig. 2). Five cancer candidate genes (amplified region genes *COL11A1*, *FGF12*, *PAK1*, and deleted region genes *DLC1*, *NPHP4*) were selected for functional validation.

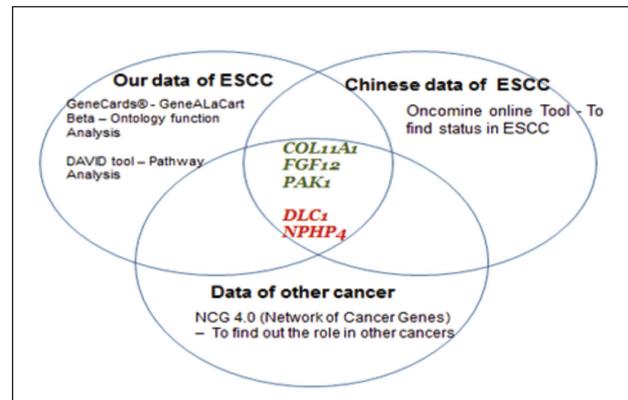


Fig. 2. Genes selected for functional validation.

To find out the suitable cell line for functional studies, *FGF12* gene was blasted in gene expression cell line database in cell line encyclopedia, where *FGF12* was highly expressed with 7.6 fold value in KYSE 410 oesophageal cancer cell line in comparison to other cell lines. KYSE 410 cell line is being maintained in a medium containing RPMI 1640+Ham's F12 (1:1) with 2% foetal bovine serum (FBS) at 37 °C with 5% CO₂. KYSE 410 cell line was transected using Lipofectamine® RNAiMAX Reagent followed by proliferation assay. GAPDH siRNA was used as a positive control. This assay showed more than 50% inhibition of cancerous cells after 48 hours of transfection, but no significant inhibition was found at 72 hour due to transient transfection.

Oesophageal cancer incidence is reported in high frequency in North-East India. The aetiology is different from other population in India due to wide variations in dietary habits or nutritional factors, tobacco/betel quid chewing and alcohol habits. Since DNA methylation, histone modification and miRNA-mediated epigenetic processes alter the gene expression, this study was undertaken to elucidate the epigenetic markers of oesophageal cancer risk in North-East Indian population.

divided into acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL). Natural killer (NK) cells have been implicated in defense against malignancies, especially leukemia. Because patients with leukemia and preleukemic disorders manifest low NK activity, it is possible that NK cell impairment may contribute to leukemogenesis. In view of this possibility, it is important to characterize the NK cell defect of leukemic patients and to design new approaches for its correction. Expression of CD56 was analyzed in AML (n=30), control (n=30) and ALL (n=30) by flowcytometry. It was found that ROS mediated expression of CD56 was higher in AML in comparison to ALL patient (Fig. 5).

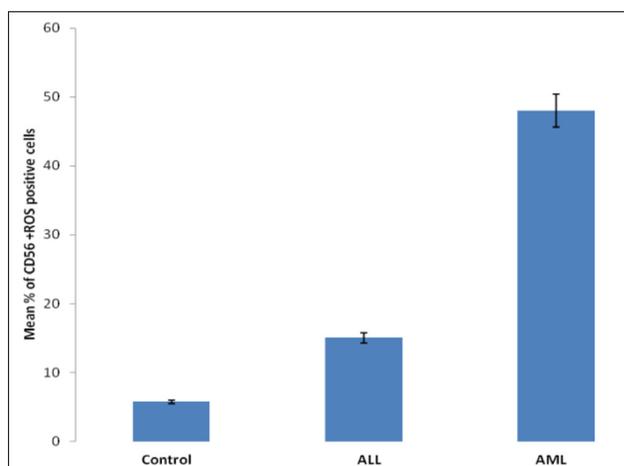


Fig. 5. NK cell status in acute leukaemia patient: CD56+ve NK cell were found higher in AML patient in comparison to ALL patients.

PATHOLOGY OF INFECTIOUS DISEASES

Leishmaniasis

Single nucleotide polymorphisms (SNPs) in miltefosine (MIL) resistant and sensitive parasites were evaluated and mRNA expression level of selected genes in clinical isolates of *Leishmania donovani* was evaluated. Sequence analysis of *LdMT* and *LdRos* genes revealed SNP, C1259→A resulting in substitution of Thr 420→Asn in the MIL resistant cell lines. Additionally, a novel SNP, T 527→A resulting in substitution of Val 176→Asp and in *LdMT* gene of resistant cell lines was observed. However, no point mutations were detected in case of *LdRos*.

Experimentally generated paromomycin (PMM) resistant *L. donovani* were used to understand

the mechanism of resistance and parasite biology. Increased membrane fluidity accompanied with decreased intracellular drug accumulation was found in the PMM resistant parasite. There was a marked increase in gene expression of ABC transporters (MDR1 & MRPA) and protein phosphatase 2A that evinced increased drug efflux. Further, PMM resistant parasite was more tolerant to the nitrosative stress and predicted a better survival capacity as indicated by resistance towards complement mediated lysis and increased stimulation of host interleukin (IL)-10.

Despite substantial efforts there is no licensed vaccine against human leishmaniasis. Live attenuated *Leishmania* parasite, generated by deletion of Centrin 1 (*LdCen1*^{-/-}) and p27 (*Ldp27*^{-/-}) gene has been found to be safe and protective in mice, hamster and dog model. We evaluated immune responses generated by *LdCen1*^{-/-} and *Ldp27*^{-/-} *Leishmania* parasite in comparison to the wild type in human PBMCs cells. *LdCen1*^{-/-} and *Ldp27*^{-/-} parasites elicited predominant Th1 response in PBMCs from visceral leishmaniasis and PKDL patients indicating that these would promote protective response leading to host resistance to infection.

A novel ubiquitin like (Ubl) system has been identified in *Leishmania* comprising an Ufm1-Uba5-Ufc1 ubiquitination like pathway. Another such Ubl:Ubiquitin related modifier-1 (Urm1) and its conjugation pathway in *L. donovani* has now been identified. *Leishmania* ubiquitin-related modifier -1 (*LdUrm1*) and its E1- like activating enzyme *LdUba4* were cloned, expressed and the corresponding polyclonal antibodies were raised. *LdUrm1* had characteristic C-terminal diglycine motif which is essential for *LdUrm1* to conjugate to its protein target molecules *in vivo*. *LdUba4* was established as E1 like enzyme for *LdUrm1*. Immunolocalization studies showed that the *LdUrm1* was localized at the anterior end near flagellar reservoir believed to be early endosomes, while *LdUba4* was localized to cytoplasm (Fig. 6). Mass spectrometry (MS) analysis revealed that *LdUrm1* was conjugated to *Leishmania* proteins that are majorly associated with early endosomal vesicular trafficking, cytoskeleton and cell division. Thus, *LdUrm1* is an important post-translational modifier in *Leishmania*.

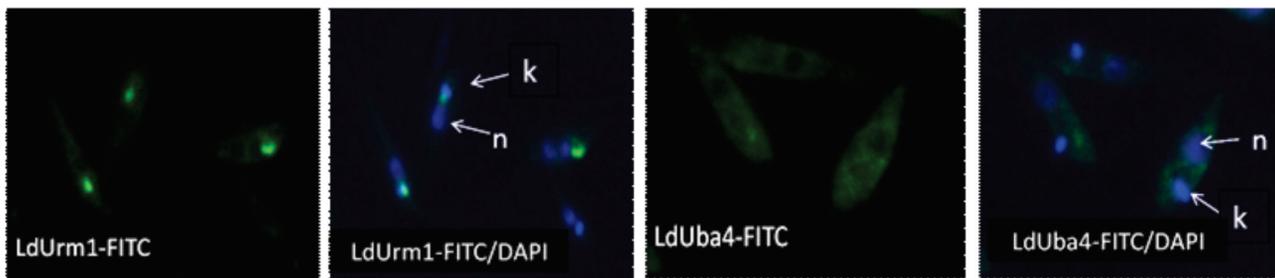


Fig. 6. Intracellular localization of LdUrm1 and LdUba4 in Leishmania promastigotes. LdUrm1/ LdUba4 was detected with anti-LdUrm1/ anti-LdUba4, respectively and visualized with FITC labelled anti-rabbit antibody. X100. k, kinetoplast; n, nucleus.

A new loop-mediated isothermal amplification (LAMP) assay based on 6 primers has been developed which detects multiple species of *Leishmania* with increased sensitivity and specificity. Addition of two extra primers, known as loop-primers, accelerates the product formation, thereby shortening the required reaction time to 30 min. The assay was positive in *L. donovani*, *L. tropica* and *L. major* spp. (Fig. 7).

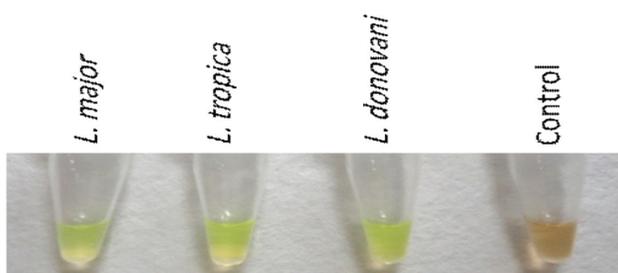


Fig. 7. Specificity of LAMP assay for different Leishmania spp. parasite DNA (10 ng). The assay was positive for *L. major*, *L. tropica* and *L. donovani* species of parasite.

Tuberculosis

The sequences specific to *Mycobacterium tuberculosis* identified were termed as “Signature Sequences (SS)”. These SS for their ability to discriminate *M. tuberculosis* DNA from a large number of other mycobacterial DNAs with high specificity were tested. A PCR based method using sputum and blood samples from clinically confirmed TB patients was developed (Fig. 8).

Human resistin (hRes) is a pro-inflammatory molecule that stimulates the synthesis and secretion of TNF- α , IL-6 and IL-12 from macrophages

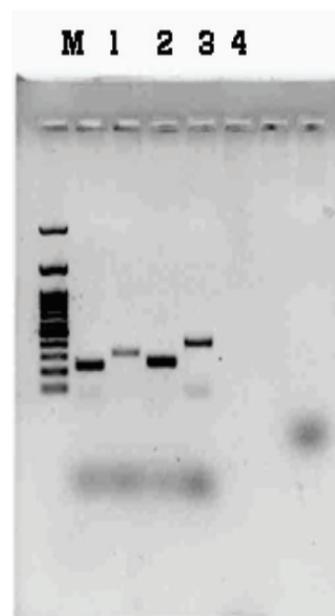


Fig. 8. PCR amplification of signature sequence from sputum of TB patients. Lanes 1,2,3 and 4 are SS 1,2,3 and 4, respectively.

through NF- κ B activated pathway. Resistin could be a surrogate marker for TB treatment in addition to its utility as an early prognostic biomarker for monitoring disease onset. It has been shown that human resistin has chaperone like activity implicating human resistin as a possible molecular link between cellular stress and inflammation during infections. Being a pro-inflammatory cytokine secreted by macrophages and a link between inflammation and unfolded protein response, raises important question about its role in mycobacterial pathogenesis. Infection induces cellular stress in macrophages, and during stress resistin is retained in the endoplasmic reticulum where it functions as chaperone (Fig. 9).

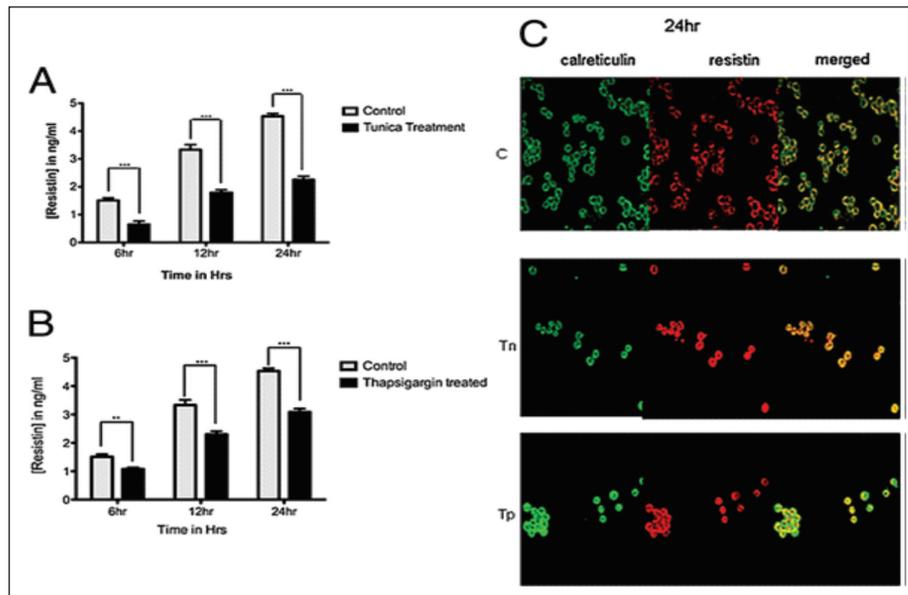


Fig. 9. Levels of secreted resistin were reduced under ER stress. U937 cells were treated with 5 µg/ml of tunicamycin (A) or thapsigargin (B) for different time points. (C) Colocalization of resistin and calreticulin in ER. Untreated U937 cells and those treated with 5 µg/ml of tunicamycin (Tn) or thapsigargin (Tp) for 24 h were processed for confocal microscopy using respective fluorescence-labelled antibodies. ER marker (calreticulin) is shown in green colour and resistin in red.

The acid rich PE/PPE protein family is exclusive to *Mycobacterium* and particularly abundant in pathogenic strains. This group of proteins is a potential source of antigenic variation and has critical roles in pathogenesis. Comparative ANCHOR analysis of PE/PPE family of H37Rv (virulent strain) with that of H37Ra (avirulent strain) revealed loss and gain of protein binding sites in disordered regions. Comparative analysis of PE24 of H37Rv showed gain of some protein binding sites in disordered regions in PE24 of H37Ra strain (Fig. 10).

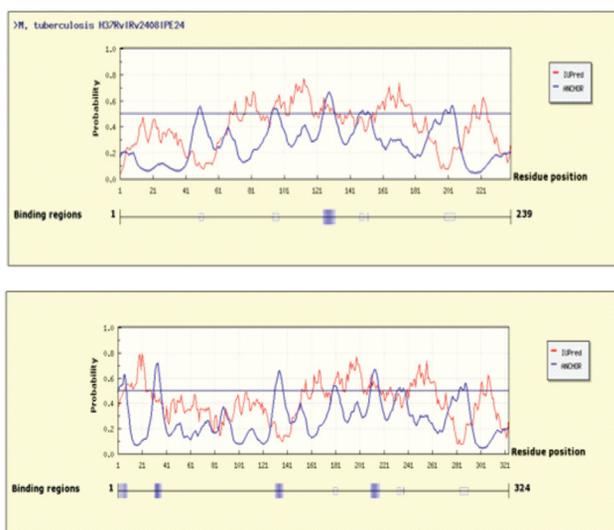


Fig. 10. Comparative ANCHOR analysis of PE24 of H37Rv

CHLAMYDIASIS

Knowledge of the biology of *Chlamydia* is essential in understanding the pathogenesis, diagnosis and treatment of *Chlamydia*-induced reactive arthritis (cReA). At the sight of primary infection (urogenital tract, ocular conjunctiva or respiratory tract mucosa), the bacteria infect monocytes/macrophages and are disseminated via the bloodstream to settle into the joint. Thereafter, the persistent chlamydiae cannot be detected using traditional culture techniques but can be located in synovial membrane and synovial fluid using various methods, including electron microscopy, immunofluorescence and PCR. Morphologically aberrant but viable, metabolically active persistent chlamydiae have been consistently documented in synovial tissue in post-chlamydial ReA. The search for *Chlamydia* or its components at the site of the primary infection or in the joint is the optimal approach to confirm chlamydial aetiology of arthritis. The most specific diagnosis of cReA is made by the detection of the pathogen in the joint itself using PCR and/ or other molecular amplification assays. However, during initial diagnostic testing, the use of immunofluorescence assay has the potential to serve as an easy detection method for *C. trachomatis*. The elementary bodies

(EBs) of *C. trachomatis* were found in the joint fluid of 26% patients with cReA/ uSpA by direct immunofluorescence assay (DFA). Chlamydial heat shock protein 60 (chsp60) which is synthesized in increased amounts and is released into the extracellular milieu during persistence, was also studied. Overall, 23.8% ReA/ uSpA patients were positive for circulatory IgG antibodies against chsp60 protein in the serum. Patients with uSpA showed evidence of higher antibodies to chsp60 (27.2%) as compared to ReA (11.1%) (Fig. 11).

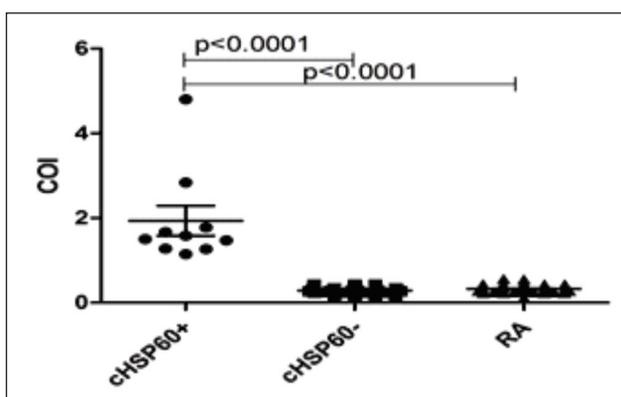


Fig. 11. Cut-off indices (COI) were compared between the ReA/ uSpA chsp60-positive, ReA/ uSpA chsp60-negative and control rheumatoid arthritis (RA) patients. Significant differences were observed between these groups.

ADULT STEM CELL BIOLOGY

The study on the use of SWISS 3T3 cells to develop a culture system for growing cultured epithelial autografts, by adopting a cost-effective strategy of employing mitomycin C (MMC) at a reduced concentration and to characterize the epidermal sheets produced was continued. Mitotic index was estimated in slide flasks in which cultures were initiated with a clonal density of 70 viable 1st passage epidermal keratinocytes per flask containing the best performing MMC treated feeders along with gamma irradiated feeders (γ -Irr) at a density of 15,000 per cm² and the colonies formed after 10 days were labelled with BrdU. The results indicated that it was the stimulation of colony initiation rather than increase in mitosis per colony that turned out to be the advantageous consequences of fine-tuning of MMC treatment by including both concentration and dose per cell as compared to γ -Irr feeders. The importance of dose titration of feeders with mitomycin C is further highlighted through improvement of the otherwise sub-optimal

performance to match with that of γ -Irr feeders in achieving faster growth of keratinocytes.

A study was initiated on framing of a detailed manufacturing protocol for cultured epithelial autografts using Swiss 3T3, but keeping in mind the xenogeneic nature of these culture processes, an investigation has been proposed to identify a cost-effective non-xenogeneic product suitable for resurfacing burn wounds. The present proposal was aimed at achieving an optimal growth stimulatory influence of human dermal fibroblasts as feeders through the earlier modality to produce and characterize the epithelial autograft material. Primarily, cell density gradient experiments were conducted to test if the post-growth arrest cell extinction in human neonatal fibroblasts, while still maintaining low concentrations of mitomycin C, could be regulated by dose per cell towards optimization of their effectiveness on the growth of human keratinocytes. Results showed that the feeder cell extinctions could be determined by dose modulation by still restricting to low concentrations of mitomycin C.

NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY, MUMBAI

To look for polymorphic variations in bilirubin metabolizing genes, hyperbilirubinemia cases (≥ 1.5 mg/dl) with normal haematology, absence of any haemoglobinopathies, normal red cell enzyme levels, liver function tests and who were also negative for hepatitis B surface antigen or anti-hepatitis C antibody were studied along with normal healthy controls. Genotyping of 11 SNPs in the *UGT1A1*, *OATP2*, *HMOX1* and *BLVRA* genes was done in both the cases and controls. Of these, nine SNPs showed polymorphic variations either in the heterozygous or homozygous state for the mutant alleles. A higher proportion of individuals in the patients group had variations in all the SNPs which are polymorphic except one as compared to the control group. The frequency of the mutant alleles was also higher in these cases compared to controls.

Genotyping of five SNPs in *TLR2*, *TLR 4* and *CD14* genes was performed in preterm neonates suspected to have sepsis and controls. Of the five SNPs, three

did not show any allelic variation in both the cases and control groups while the remaining two SNPs, each in *TLR4* and *CD14* genes had a variation (either heterozygous or homozygous of the mutant alleles) and higher proportion of the babies with sepsis showed the presence of these variations.

Newborn screening and molecular characterization for red cell enzyme defects and haemoglobinopathies was done. A centre for screening of hemoglobinopathies and red cell enzymopathies was established at Agartala, Tripura. Of the 241 neonatal samples screened for haemoglobinopathies and red cell enzymopathies (G6PD and PK deficiency), seven (3.0%) were found to have HbE homozygous/HbE thalassaemia, 23(9.5%) had HbE trait and six (2.5%) had HbS trait on HPLC. Four neonates were found to be deficient for G6PD enzyme, of whom two were in combination with HbE trait and one in combination with HbS trait. PK deficiency was also observed in two babies.

A study on newborn screening for sickle cell disease was undertaken to raise a cohort of around 100 newborn babies with sickle cell disease and follow them up regularly to understand the natural history of the disease among tribal populations in south Gujarat and Jabalpur, Madhya Pradesh. A total of 636 newborns mainly from Dhodiya and Kukna tribes from south Gujarat were studied. Of these, 10.5 and 1.4% were found to have sickle cell trait and sickle cell disease, respectively. α gene deletions were observed in 15 of 18 sickle cell traits and one sickle cell disease case. These newly diagnosed sickle homozygous cases along with SCD babies are being followed up and given comprehensive care.

The study on red cell membrane pathology in hereditary spherocytosis was undertaken to see the spectrum of red cell membrane protein defects in cases of hereditary spherocytosis and to characterize the mutations causing these defects. Spectrin deficiency was seen in 4 of 15 cases studied, Ankyrin defect was seen in 6 cases, Band-3 protein deficiency in 4 cases and a combined Spectrin and Ankyrin defect in one case. Molecular characterization of the Ankyrin and Spectrin genes is in progress.

In a study on circulating procoagulant microparticles (MPs) in women with pregnancy loss, on comparison with 70 healthy controls, in 200 women, 42.6, 63.5 and 46% showed significantly elevated annexin V, endothelial (CD62e) and tissue factor (CD142) microparticles (MPs), respectively. In 15 of the 20 women with exaggerated MP levels at the onset of pregnancies, who had history of pregnancy loss and who were on anticoagulant therapy, MP levels decreased considerably as heparin along with ASA therapy progressed (Fig. 12). MPs may serve as important biomarkers for anticoagulation therapy and may provide indications for adjustment of heparin or ASA dosage or other therapeutic options so as to decrease and normalize the MP levels.

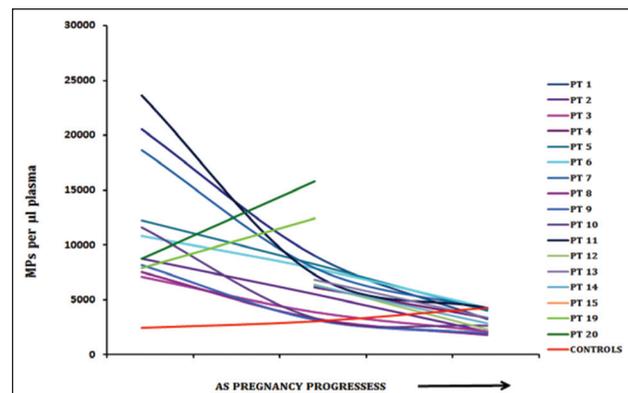


Fig. 12. Circulating microparticles in women with pregnancy loss.

The clinical severity of haemophilia is modulated by coinherited thrombophilia. In a retrospective analysis, 80 patients who were on warfarin for various thrombotic conditions and were also carriers for warfarin sensitive genotypes (VKORC1-1639G>A, CYP2C9*2 or/and CYP2C9*3) were screened for FVL mutation. The data suggested that similar to haemophilia and other bleeding disorders, coinheritance of thrombophilia marker like FVL mutation might have an impact on the amelioration of bleeding manifestations in patients who are carriers of warfarin sensitive alleles.

A countrywide screening of haemophilia patients for inhibitors showed that 6.57% of haemophilia A patients and 0.73% of hemophilia B patients were positive for inhibitors. Highest FVIII inhibitor incidence was observed in Puducherry (11.94%) followed by J & K (9.52%), Tamil Nadu (8.69%) and Maharashtra (8.24%) (Fig. 13).

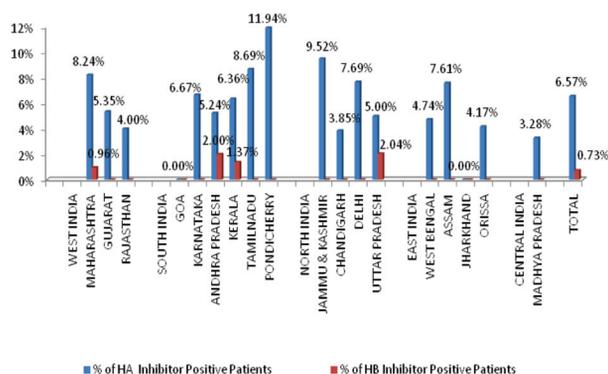


Fig. 13. Inhibitor incidence in haemophilia A and B patients.

The *IL10* promoter polymorphisms ‘GCC/ATA’ and ‘ATA/ATA’ haplotypes showed a significant difference between the two groups. Also, ‘GCC’ haplotypes were significantly higher in the inhibitor positive patients, whereas the ‘non-GCC’ haplotypes were significantly higher in the controls. These were the most significant immune-response related associations detected in the study. The most common HLA alleles in both groups were *DRB1*15* and *DQB1*05*, *DQB1*06*, and the *DRB1*03* allele was significantly higher in these Indian inhibitor negative haemophilia A patients hence could be protective with regard to inhibitor development.

A specific and sensitive APTT-based inhibitor screening assay, using Hemofil-M as the FVIII source instead of NPP was standardized, as an improvement on the conventional assay. It is quick, economical, easy to perform and is now being used for accurate inhibitor diagnoses in haemophilia A patients.

A rapid granule release assay for evaluation of genetic haemophagocytic lymphohistiocytosis (HLH) was developed and being validated. Of the 18 patients with perforin deficiency, molecular characterization was completed in 10 patients. Two nonsense mutations (p.Gln164X and p.Cys176X), both novel, and eight missense mutations including four novel mutations (p.Ala278Asp, p.Asp340Asn, p.Cys395Arg and p.Ala415Thr) and four known missense mutation (p.Gly220Arg, p.Try129Ser, p.Arg177Ser and p.Asp491Asn) were identified in these patients.

In a study on susceptibility to *BCR/ABL* gene mutations *in vitro* and *ex vivo*, the overall incidence

of mutations in the *BCR/ABL* fusion gene was 46%. The median duration of occurrence of mutation was noted to be 41 months for patients with first line imatinib and 85 months for patients pre-treated with non-TKI. Thus, prior treatment with non-TKI drugs reduces the load on the subsequent TKI therapy, possibly increasing its effectiveness and also increasing the median time to mutation occurrence.

Clinical significance of deletions of *BCR/ABL* gene (9q34) and microRNA expression in chronic myeloid leukemia (CML) was studied. The expression analysis showed downregulation of miR-199b and miR-219-2 in the 9q deleted CML patients. However, miR-199b (9q34.11) was significantly downregulated compared to miR-219-2. The follow up study showed that the miR-199b was strongly associated with imatinib resistance. Hence, the deletion on 9q34.1 region (*ABL*) plays an important role in disease pathogenesis. Eventually, miRNAs can provide new therapeutic strategies and can be used as a prognostic indicator.

Quantitative multiplex short fragment assay was established in myelodysplastic syndromes (MDS) to detect copy number changes at 21 gene loci. Chromosome aberrations were detected in 50% cases with MDS. *ASXL1* and *TET2* gene mutations were detected in 20% of MDS cases.

Increased levels of ROS and ≥ 2 fold increase in mtDNA/nDNA ratio has been observed in fanconi anaemia (FA) patients compared to controls, suggesting impairment of mitochondrial function in FA. Twenty six mitochondrial DNA mutations in FA have been found in sub-units of complex I (ND1 to ND6) and complex III of oxidative phosphorylation (OXPHOS), associated with mitochondrial dysfunction.

In a case-control study which involves the identification of various SNPs and haplotypes of the fucosyltransferase 1, 2 and 3 genes and their association with autoimmune disorders such as Sjogren’s syndrome (SS), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), blood and saliva samples were collected from 26 patients (SS = 2; sSS+SLE = 1; sSS+RA = 5; SLE = 15, RA = 3) and 35 normal healthy individuals as the control group. ABO grouping, Lewis typing

and the secretor status were carried out. Detection of autoantibodies such as ANA, dsDNA, Ro/SSA and La/SSB was carried out by IFA /LIA for SS and SLE patients while in case of RA, the RF and anti-CCP autoantibodies were measured by ELISA. PCRs for the *FUT1*, 2 and 3 genes were standardized and the genotyping for patients and controls is in progress.

Extramural Research

ALLERGY

A study was aimed at evaluating the modification in the IgG upon glycation with glucose under physiological conditions at the Jawaharlal Nehru Medical college, Aligarh. Immunogenicity of native and modified IgG was probed by inducing polyclonal antibodies in experimental animals. The induced antibodies have been characterized for their fine antigenic binding specificity towards a variety of glycosylated and non-glycosylated proteins and amino acids to gain insight into the mechanism of autoantibody production in RA. The results suggested structural perturbations (unfolding and fragmentation of protein, loss of β -structure), increased carbonyl content and ketoamine moieties in IgG upon glycation. Rabbits challenged with glycosylated IgG (AGE- IgG) induced high titre of immunogen specific antibodies which showed cross-reaction with glycosylated/non-glycosylated proteins and amino acids in competitive inhibition assay.

Possible involvement of glycosylated IgG in RA was evaluated by analyzing the binding of serum antibodies from RA patients divided into two groups on the basis of onset with respect to age: group I (early onset of disease: 20-32 years) and group II (late onset of disease: 36-54 years). Antibodies present in the serum of group II patients showed preferentially high binding to AGE- IgG as compared to group I patients. No appreciable binding was observed with the antibodies from normal subjects.

Quantitation of AGE- IgG in purified IgG samples of RA patients and healthy subjects was carried out by NBT colorimetric method. Group II serum samples showed a high level of AGE- IgG than group I serum samples, and it was much less in normal subjects. It was observed that the level of AGE- IgG had a good correlation with the disease

activity. Hence, the increased oxidative stress in group II patients could be the reason for high AGE- IgG concentration as compared to group I. The autoantibodies against AGE- IgG may be helpful in monitoring the progress of disease from mild to chronic stage and preventive measures can be exercised in time. Furthermore, level of AGE- IgG in combination with other clinical features of the RA might be a powerful diagnostic tool.

BIOCHEMISTRY

A study was conducted at the Indian Institute of Toxicological Research (IITR) Lucknow, on prevention of lung tumour development by inositol hexa phosphate. Ethyl nitrosourea (ENU) was administered once intraperitoneally at the 17th day of gestation for transplacental lung tumour development. The results showed the chemopreventive effects of inositol hexaphosphate in F1 generation as also its mechanisms of action.

The objective of the study conducted at the All India Institute of Medical Sciences (AIIMS), New Delhi, was to determine serum cytokines levels (IL 6, IL 10 & IL 18 and TNF- α) and their significance in patients with acute coronary syndrome (ACS) with subsequent follow up. The study population included 120 patients [60 patients in Non STEMI (Group-1) and 60 patients in STEMI group (Group-2)] with ACS and 50 controls. Serum cytokine IL 6, IL 10 and IL 18 and TNF- α levels were measured by enzyme linked immunosorbent assay (ELISA).

Serum IL-6, IL-18, TNF- α levels were significantly higher in ACS groups (STEMI and non STEMI) when compared to the control group. In non STEMI, serum level of IL-10 and IL-18 showed significant elevation on 3 days follow up, and for STEMI group serum levels of IL-18 and TNF- α showed significant decline on 3 days follow up. There was no correlation between serum creatinine kinase, creatinine kinase-MB, troponin I and serum cytokine level in both ACS groups. Association between serum cytokine levels and the site of MI (anterior/inferior/ posterolateral) was also found to be insignificant. TNF- α levels of STEMI patients were significantly influenced by drug therapy (analgesics - aspirin, thrombolytics - streptokinase, glycoprotein IIa -IIIb inhibitors such as clopidogrel and inotropics).

A study was carried out at the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, to evaluate the role of HNP-1 as an adjunct to conventional antituberculous chemotherapy in eliminating *Mycobacterium tuberculosis* persisters during experimental tuberculosis. The persisters are hurdle in proper management of the disease. Thus, there is a need to look for alternative options to overcome this challenge. In this regard, HNP-1 could be the alternative option along with conventional therapy.

The study on the development of a simple fluorescent assay for serum lysyl oxidase (LOX) activity was conducted at the Vision Research Foundation, Sankara Nethralaya, Chennai. The objective was to identify modulators of the human LOX and to develop a fluorescent assay for detection of the LOX in the serum. Enzymatically active mature human lysyl oxidase was cloned, overexpressed and purified by Ni-NTA affinity column chromatographic method. The purified enzyme was also used to study the physiochemical property of the enzyme. To identify the modulators of the LOX, the 3D structure of LOX was developed by *in silico* approach. As homology for LOX was only 29 % in the PDB database, the 3D structure of LOX was developed by threading model method and validated.

Coronary artery disease (CAD) affects Indians 5-6 years earlier than in the West, and the disease is diffuse and malignant. Traditional risk factors have failed to explain the high incidence of premature CAD and hence there is an urgent need to identify newer non-traditional risk biomarkers. Since, LDL heterogeneity is an important determinant of lipoprotein composition, size and metabolism, small dense (sd) LDL is viewed as an important CAD risk biomarker. Recently, cystatin-C has also emerged as a potential predictor of impaired cardiovascular events. In this study conducted at Sir Ganga Ram Hospital, New Delhi, two non-traditional CAD risk markers [sdLDL (linear, polyacrylamide electrophoresis) and cystatin-C (latex enhanced immunoturbidimetry)] were evaluated in CAD patients ≤ 45 years of age and compared with age matched healthy controls.

This study demonstrated that in patients in whom the traditional lipid tests failed to detect dyslipidaemia, cystatin-C and sdLDL might help to detect CAD events at an earlier stage.

CELLULAR AND MOLECULAR BIOLOGY

Chronic obstructive pulmonary disease (COPD) is a complex genetic disease that causes considerable morbidity and mortality worldwide. Genetic variability interacting with environmental and ethnic factors is presumed to cause susceptibility to COPD and to tobacco smoke. A study was conducted at Vallabhbai Patel Chest Institute (Delhi University), Delhi, to elucidate the molecular mechanism of development of COPD in smokers in north Indian population. The study included three groups: smokers without co-morbidity with normal PFT; smokers with spirometry proved COPD and without any other co-morbidity; and healthy non-smoker controls. The gene products *i.e.* MMP1, MMP9, MMP12 and ADAM33 proteins were assayed quantitatively by commercially available ELISA kits. Amplification of *MMP1*, *MMP9*, *MMP12* and *ADAM33* genes was performed under different PCR conditions and respective amplicons obtained were subjected to DNA sequencing analysis.

A significant correlation was found between T+1 SNP in *ADAM33* gene and ADAM concentration, *MMP1*, *MMP12* concentration, and the SNP was found to have positive and significant correlation with duration of COPD, number of cigarettes and pack years but not with age or number of years of smoking. Significant negative correlation was also observed between the *ADAM33* T+1 SNP and FEV1/FVC ratio.

Indian childhood cirrhosis (ICC), a disease unique in India, has specific clinical and histopathological characteristics, in which large amount of copper is deposited in the liver. This study was designed to assess the role of autophagy proteins in the pathobiology of this disease. A total of 40 cases of ICC were studied at PGIMER, Chandigarh. Both biopsy and autopsy material was included. Similar number of cases were normal liver tissue with also studied as normal control group. The methods adopted to study the expression of autophagosomal membrane proteins (PI3K, PI3K inhibitor, beclin 1, LC3, LAMP2, p62) to assess the baseline integrity of hepatocytes (HGF, HAI-1, hepatocyte nuclear factor), the apoptotic markers (TNF- α , fas ligand, bax, bac, bcl2), markers of mellyory bodies (cytokeration 8/18, ubiquitin, p-62), proliferative

markers (Ki 67), copper transporters (ctr1, atoz 1, ATP7A, ATP7B) included immunohistochemistry, immunofluorescence, double immunostaining and fluorescent *in-situ* hybridization (FISH) techniques. There were over-expressions of the autophagy proteins, loss of proteins to indicate the baseline hepatocytic integrity, over-expression of the apoptotic markers and mellory denk bodies markers, Ki67 expression was equivocal. The heavy copper deposition observed in ICC liver possibly was associated with abnormal autophagy property of the hepatocytes resulting in abnormal or defective transport of the copper metals.

A study on the role of prostate apoptosis response-4 (Par-4) and its interactive proteins in chemoresistance/sensitivity in gliomas using multicellular spheroids (MCS) as model was conducted at NCCS, Pune. Par-4 was first identified in rat prostate cancer cells undergoing apoptosis when exposed to apoptotic stimuli. The findings revealed that the expression of Par-4 was downregulated in MCS compared to monolayers derived from glioma cells. Microarray analysis of differentially expressed transcripts done using Gene Ontology (GO) revealed that the number of genes most significantly were in the categories of cell adhesion, cell junction and regulation of cell proliferation. Additionally, in MCS, the expression levels of 13 chemoresistant genes were upregulated while Par-4 was downregulated. Tamoxifen (TAM) effectively induced apoptosis in glioma stem cell line- HNGC-2 and was associated with upregulation of Par-4. Secretory Par-4 and GRP-78 were significantly expressed in HNGC-2 cells on exposure to TAM and specific antibodies to these molecules inhibited cell death suggesting that extrinsic Par-4 is important in TAM-induced apoptosis.

A study on a novel arithmetic approach for foolproof production of growth arrest in 3T3 cells suitable for human epidermal culture was conducted at NIOP, New Delhi. The investigation comprised a cost-effective yet efficient processing strategy for SWISS 3T3 feeder cells suitable for human epidermal keratinocyte stem cell proliferation and *in vitro* construction of stratified epithelium. This technique is being prepared for filing a patent and being developed further towards growing cultured epithelial autograft (CEA) for probable exploitation in resurfacing the burn wounds.

Htra2 is a serine protease localized in the intermembrane space of mitochondria. Evidence highlights the importance of mitochondrial form of Htra2 in the progression of Parkinson's disease. However, the biogenesis and precise function of this mitochondrial form of Htra2 in ageing and its role in progression of Parkinson's disease remain to be elucidated. This study conducted at the University of Hyderabad, Hyderabad, was aimed to identify novel substrate of Htra2 in mitochondria and characterize the identified proteins to understand the biogenesis of Htra2 in young and old mouse brain samples. This study identified a novel substrate for Htra2 and further understanding of this protein may yield valuable information of Htra2 biogenesis, ageing and its link to Parkinson's disease.

HAEMATOLOGY

Acute lymphoblastic leukemia (ALL) is a malignant disorder of the lymphoid progenitor cells and the most common type of childhood leukemia. Though 80% of childhood leukemia is curable, relapse occurs in the remaining 20%. A 2D-profile of lymphoblastic proteins was done at Amrita Institute of Medical Sciences, Kerala, and serial analysis of sequential samples of two patients obtained from presentation through remission and relapse were subjected to Western analysis.

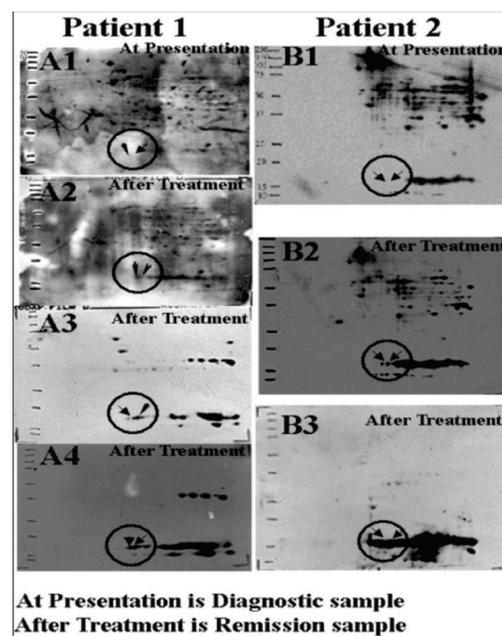


Fig. 14. 2D profiled lymphoblastic proteins from JM-1 cells on blots were probed with biotinylated CSF proteins from CNS of leukemic patients at diagnosis as well as sequential CSF samples from the same patient

This strategy allows identifying changes in reactivity during different stages of the disease to lymphoblastic proteins and can be translated to identify molecules involved in disease status prediction as well as its role in the pathogenesis of the disease. Between the two spots in Fig. 14 (encircled) on analysis, one was found to be eIF5 α , whose role in leukemia has been shown before and the other one was code named as X, a molecule whose expression has been found altered in leukemia. The absence of reactivity of the molecule X at presentation and during relapse (Fig. 15) demonstrates the significance of this molecule in the prognosis of disease as well as its diagnostic potential in clinics.

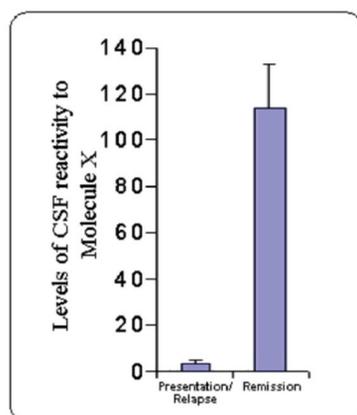


Fig. 15. Significance of X molecule in the prognosis of disease as well as its diagnostic potential in clinics.

Identification of novel molecules that differently react to leukemia CSF is a major finding identified for the first time. The potential of these finding lies in the development of a prognostic marker as well as understanding why the differential reactivity observed during relapse and remission of CNS leukemia is immense. This also opens up potential application of the finding on therapeutical implications.

HUMAN GENETICS

An extensive study conducted at FRIGE, Ahmedabad, on the occurrence of lysosomal storage disorders (LSDs) in the high risk group of children suggests that the frequency of LSDs is 35-45% in children with developmental regression such as neuroregression, visual impairment, hearing loss, skeletal dysplasia, hepatomegaly and/or splenomegaly, failure to thrive, spasticity and presence of cherry red spot. Mutation study

for GM2 gangliosidosis has identified that Tay-Sachs (TSD) and Sandhoff disease (SD) patients have novel mutations in Indian children and enzyme based diagnosis remains the first line of study followed by molecular study. Further analysis has demonstrated that glycolipid storage disorders are the most common disorders followed by mucopolysaccharide disorders. Overall, Gaucher disease is the most common storage disorder observed in Maharashtra followed by GM2 gangliosidosis (Tay-Sachs disease) in Gujarat. Consanguinity played an important role in disease occurrence as nearly 29 % of LSDs were observed among children born to consanguineous parents. Highest occurrence of Batten disease was observed in patients from Belgaum where 72% patients were a result of consanguineous marriages. The study has identified 56 children with GM2 gangliosidosis (41 TTSD and 15 SD). Molecular study of *HEXA* gene has revealed 19 mutations, 13 of which are novel, including 8 missense mutations, four nonsense mutations and one deletion. Previously known missense mutations *viz.* c.508C>T (p.R170W) and c.532C>T (R178C), one 4bp insertion c.1277_1278insTATC (p.Y427Ifs5), 3 splice site mutations c.805+1 G>C, c.459+5 G>A, and another intronic mutation c.672+30 T>G of unknown pathogenic significance were also identified. It has been demonstrated for the first time that c.1385 A>T (p.E462V), c.964 G>T (p.D322Y), c.1277_1278insTATC (p.Y427Ifs5) and c.805+1 G>C mutations are the most common mutation observed in 50% of the children with TSD in India. Among them p.E462V in *Parmar* community of Gujarat and p.D362Y in Muslim patients seem to be the most common one. Half of the patients with Sandhoff disease had 4 novel mutations c.333G>A (p.W111X), c.534_541delAGTTTATC (p.V179RfsX10), c.1563_1573delTATGGATGACG (p.M522LfsX2), and c.1591_1592insA (p.R531KfsX22) and the remaining 50% patients had three known mutations c.1597C>T (p.R533C), c.850C>T (p.R284X) and c.1417 +1G>A. A large study needs to be done in different States of India for identification of common mutations of *HEXA* and *HEXB* genes in TSD and SD patients, respectively; so that information can be utilized for better prognosis, early intervention and care of affected patients.

Sequencing of mutated *COL1A1* and *COL1A2* genes was done in 35 cases of osteogenesis imperfecta (OI) at the Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. These mutations account for only 71% of OI while as per the literature these mutations are seen in 90% cases of OI. The reason is high prevalence of consanguinity in India. Identification of homozygous regions by SNP microarray helped to identify mutations in autosomal recessive genes for OI in 5 cases. These results helped in genetic counselling and can provide prenatal diagnosis to these families for prevention of recurrences. Twenty nine sequences with novel polymorphism of mutations of *COL1A1* and *COL1A2* genes have been submitted in DNA Data Bank of Japan, under the accession numbers AB827380 to AB827409.

Mucopolysaccharidosis IV A (MPS IVA) is a lysosomal storage disease caused by a deficiency of N-acetylgalactosamine-6-sulfatase (GALNS). A study was undertaken at Manipal University, Karnataka, to evaluate the clinical, biochemical and molecular characteristics of Indian patients with Morquio A syndrome and to correlate phenotype with genotype. Thirty six patients with Morquio A syndrome diagnosed by clinical and radiologic features after obtaining informed consent were recruited. The study identified 17 homozygous mutations (in 28 subjects) and 7 different compound heterozygous mutations. There were 19 different mutations and most of the mutations were missense. Sixteen mutations are being described for the first time in *GALNS* gene, of which 13 are missense mutations (p.M1?, p.N32T, p.C79R, p.S82L, p.G188S, p.N204T, p.F216S, p.W230C, p.A291S, p.G317R, p.R386S, p.E450G p.C501S), two splicing mutations (IVS1-1G>C, IVS9-3C>G) and one nonsense mutation (p.Q414X). All the novel mutations were not detected in 50 healthy individuals. The mutations were not present in dbSNP and the 1000 Genomes database.

The most common mutations were p.S287L and p.A291S. The identification of these *GALNS* common mutations has important implications for the design of mutation-detection strategies for the Indian population. This study reveals that Indian patients with Morquio A syndrome have a different spectrum of mutations as compared to others

published in literature. Further studies on common mutations by haplotype analysis will elucidate the origin of common mutations and identify a possible founder effect. Functional studies on splicing mutations will help in understanding the effect on transcription of the gene.

A study on the pattern of gross congenital malformation in children born in Assam Medical College and Hospital, Dibrugarh, Assam, was undertaken. All live and still births born from May 2010 to February 2013 in Assam Medical College, Dibrugarh, were screened for congenital malformations. Equal numbers of controls were also selected among children born after the birth of the malformed baby. Types of malformation, age of the parents, sex of the baby, history of consanguinity, ethnicity, antenatal history of drug exposure, socio-economic status and educational status of the mother were noted. A total of 17052 live birth and 1140 still births were screened, and 206 (187 live births and 19 still birth) malformed babies were detected. Musculoskeletal system was the most commonly involved, followed by CNS with birth prevalence rate of 17 and 16.5 per 10,000, respectively. Cleft lip and cleft palate were the third most common malformations with overall birth prevalence rate of 14.8/10,000. CTEV was the most common individual malformation (12.6/10,000). Low birth weight, male sex of the newborn, increased age of the mother and father, and lower educational status of mother were strongly associated with malformation in newborn. No association was observed with the antenatal registration, folic acid supplementation, ethnicity and religion of mother

Beta amyloid plaques (A beta 40/ A beta 42) have been seen associated with neurodegeneration and these plaques have been implicated in Alzheimer's disease. Similar plaques were observed in the brains of Down syndrome (Trisomy 21) patients. A search led to the identification of the gene for soluble form of beta-amyloid precursor protein (A.P.P) at 21q21. High levels of APP have been identified in Alzheimer's disease, Down syndrome and cases of Fragile X syndrome. Interestingly, high level of plasma amyloid precursor protein (APP) has also been reported in severely affected children with autism. Parameters investigated in a

study conducted at Vasavi Medical and Research Centre, Hyderabad, were DNA damage and repair in lymphocytes of patients with neurodegeneration as compared to healthy controls, plasma levels of APP, amyloid β -40 and amyloid β -42 products and mutational spectrum in the hot spots of *APP* gene.

The results of comet assay showed significant increase in comet tail length, which is an estimate of DNA damage, in all the three disorders (Alzheimer's disease, Down syndrome, and autism) as compared to their controls, indicating instability of the genome in these three neurodegenerative conditions. Amyloid β -peptides have been implicated in the aetiology of Alzheimer's disease. The cleavage of the APP by β -secretase followed by γ -secretase gives rise to the fragment peptides: amyloid β -40 and amyloid β -42. The $A\beta$ 42/40 ratio is thought to be a predictive biomarker for cognitive impairment and eventual Alzheimer's disease. The $A\beta$ 42/40 ratio was found to be higher in subjects with Alzheimer's disease, Down syndrome, and autism as compared to their respective controls. Total APP (tAPP) and secretory APP (sAPP) were found to be higher in patients than in controls. Sequence analysis identified a variation in 4 Alzheimer's patients and 3 autistic patients. Variation or insertion of a single base causes frame shift in the gene resulting in an abnormal truncated protein or non-functional protein (Fig. 16).

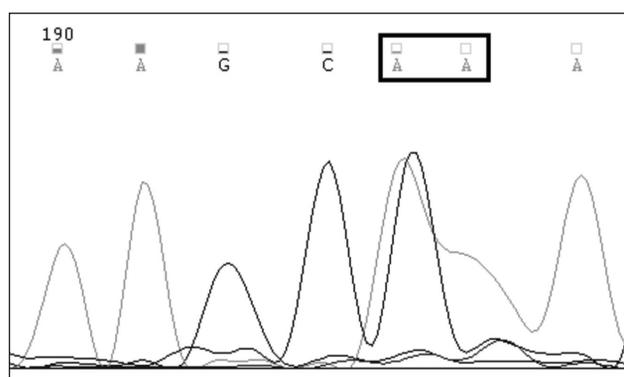


Fig. 16. Showing variation in APP exon 16 flanking region (intron 15) with variation NC_000021.8:g.27270063C>G rs112182125.

Genetic evaluation of patients with mental retardation was done in Udupi and Dakshin Kanada districts of Karnataka State in a study conducted at Manipal University, Karnataka. Screening was carried out for cryptic and subtelomeric rearrangements and imbalances like microdeletions

and microduplications using multiplex ligation-dependant probe amplification (MLPA) analysis and it also revealed the frequency of subtelomeric rearrangements in children with idiopathic intellectual disability.

A total of 385 patients were screened for gross chromosomal abnormalities using conventional cytogenetics and high resolution chromosome banding. One hundred eighty five patients (48%) had gross chromosomal abnormalities which included Down syndrome (47%), Edward syndrome (1 patient), insertional inversion involving chromosome 1 and 2 (1 patient) and heteromorphic variant 15p+ (1 patient), inversion 9qh (1 patient). Centromere banding (C-banding) was done to confirm the 9qh inversion.

BAC FISH probes were prepared from MLSC BAC library and utilized for the confirmation of submicroscopic and subtelomeric rearrangements detected from MLPA analysis (Fig.17).

The purpose of the study was to find out the submicroscopic subtelomere deletions that could be missed by routine conventional cytogenetics. Recent methodologies for assessing the genomic imbalance at submicroscopic subtelomeres concluded MLPA to be a robust technique in a diagnostic set-up, although the use of real-time PCR and microarrays may become more widespread with their availability on an affordable commercial platform.

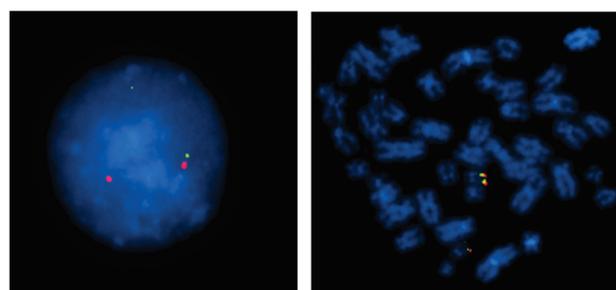


Fig. 17. Interphase and metaphase cell showing fluorescent signals for SHANK3 BAC-FISH probe.

Lysosomal storage disorders (LSDs) are a heterogeneous group of disorders associated with specific lysosomal enzyme deficiency. At present the diagnosis and prenatal diagnosis of these disorders is primarily based on enzyme assay, which has a number of disadvantages. In this study on clinical, biochemical and molecular analysis of treatable

lysosomal storage disorders at the Centre for DNA Fingerprinting and Diagnostics, Hyderabad, the objectives were to identify disease causing mutations in common lysosomal storage disorders and to carry out genotype-phenotype correlation with clinical features and enzyme levels. Some of the LSDs studied were Niemann Pick disease, metachromatic leucodystrophy, sialidosis, Gaucher disease, mucopolysaccharidosis type VI (Maroteaux Lamy syndrome), and mucopolysaccharidosis type II (Hunter syndrome). Techniques used were lysosomal enzyme assay and mutation analysis.

A total of 20 mutations *viz.*, L98R, W450C, P445L, D53N, A237D, W353X, S320R, c.659_660delTA, Y103SfsX9, W450L, c.del1208C, R160Q, c.del496T, H393R, D54N, c.1577 del C, E390K, c.208_215 del CCGCACCT, V376E, and L321P were found in *ARSB* gene in 31 patients of Maroteaux-Lamy syndrome.

A total of 30 mutations were observed in 38 patients of Niemann-Pick disease, of which, 22 were novel. The enzyme levels were severely decreased in the patients with the observed mutations. The study overall showed that 150 patients with these rare disorders had mutations which were novel as compared to mutations reported in other populations. This knowledge will help in better genetic testing for patients with LSDs in India.

Inborn metabolic disorders are common genetic conditions imposing a high burden on the health care infrastructure unless diagnosed and treated. National Task Force study on newborn screening of congenital hypothyroidism and congenital adrenal hyperplasia was completed in over 100,000 newborns. The study identified about 100 affected babies in whom early treatment could be initiated. The study was carried out at 11 centres which included, 5 newborn screening centres, 3 high risk screening centres, one centre each for Quality Assurance, Data Coordination and Central Coordination. The NTF group has prepared a work manual with Standard Operating Procedures related to Newborn Screening In India. A user friendly website (www.icmrmetbionetindia.org) has also been prepared with valuable information regarding ICMR Newborn Screening programme as well as for genetic diseases in the country.

ICMR NTF study explored the feasibility of introducing 'universal newborn screening' in the country and the study has been completed in 2013. Overall, in these five centers total number of births recorded were 104006 and coverage was 72.6% suggesting that even in highly supervised and well staffed settings the coverage could be obtained for three fourth of births eligible. A detailed data analysis was done and a report was prepared.

NANOMEDICINE

An antibody independent low cost serum ferritin sensor was developed at the University of Calcutta, Kolkata, employing citrate coated Super Paramagnetic Iron Oxide Nanoparticles (SPIONs) and 100 mT Static Magnetic Field (SMF). The technique is independent of costly ferritin specific antibody. Up to 1500 ng/ml serum ferritin can be measured by proposed technique. The differential local magnetic field possessed by ferritin can also be probed by the proposed technique (Fig. 18).

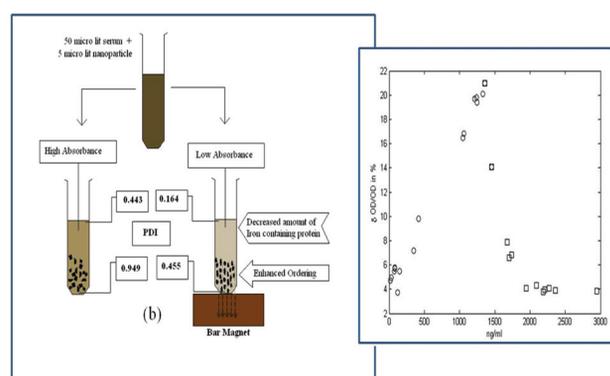


Fig. 18. left panel is the schematic representation of experimental set up and right panel indicates the sensitivity of our proposed technique, where X axis is serum ferritin concentration and Y axis is the % Δ of absorbance at 280 nm.

A study on the development and characterization of functionalized carbon nanotubes for tumour drug targeting and therapy was conducted at the National Institute of Pharmaceutical Education and Research, Mohali. Functionalization density dependent cytotoxicity of oxidized MWCNTs in a murine macrophage, RAW 264.7 cell line was studied. It was demonstrated that MWCNT induced cytotoxicity and apoptosis increased 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. A multifunctional drug-delivery platform based on hyaluronated tethered MWCNTs was developed and successfully explored for tumour-targeted delivery of doxorubicin *in vivo*. Oxidized MWCNTs have been multifunctionalized with folic acid (FA, a well established cancer targeting agent for folate receptor over-expressed cancer cells), methotrexate (MTX, a folate antagonist used in the chemotherapy of certain types of cancer), Alexa-Fluor (AF-488/647, a fluorescent dye used to track the intracellular trafficking of MWCNTs) and Technitium-99m (^{99m}Tc , a radiolabel used to monitor the biodistribution of nanotubes) and successfully explored for tumour-specific drug targeting and therapy.

The ability of small interfering RNA (siRNA) in silencing of specific targeted genes is not only a promising and effective tool to study gene function but it also offers an approach for the treatment of various types of diseases such as cancer. A study on cancer siRNA therapy by tumour selective delivery with ligand targeted nanoparticles conducted at the Aligarh Muslim University, Aligarh, described development of a non-carrier based approach for cellular delivery of encapsulate siRNA. The in-house developed virosome/dendrimer based delivery system was found to possess desirable physio-chemical as well as surface properties. The PI-3K specific siRNA was found to inhibit PI-3K and eventually led to modulation of various pro-apoptotic factors to attenuate cell cycle progression of cancer cells in BALB/c mice.

Non specific expression of suicide gene in normal cells is a serious limitation in the existing suicide gene strategies for cancer therapy. In the study on tissue specific cytotoxicity mediated by nanoparticle-delivered suicidal gene therapy conducted at the Vision Research Foundation, Chennai, and SASTRA University, Tanjore, it was found that there was a mild HSV-*TK* gene expression and cell death in EpCAM negative MIOM1 cell line. This suggested that there was a leaky expression of EpCAM promoter in EpCAM-ve cells also. To control the leaky expression miRNA mediated transcriptional regulation of transgene technique was used.

In conclusion, a novel vector platform combining cellular promoter-based transcriptional targeting

with miRNA regulation for a EpCAM+ve cancer cells suicide gene therapy was developed. This study provides evidence that incorporating miRNA regulation into a transcriptional targeting vector can provide an important layer of control over transgene expression. This dual regulation system (EpCAM and let7) will allow the transgene expression only in EpCAM+ve/let7family downregulated cancer cells. The work offers a two tier level control of the transgene that may lead to a selective transgene expression in the cancer cells without any expression in the normal cells.

In a study on novel target-specific multiplexed nanophytomedicines for cancer therapy conducted at Amrita Centre for Nanosciences & Molecular Medicine, AIMS, Kochi, a novel polymer-protein core/shell nanocarrier based on PLGA-Casein was developed to simultaneously load a combination of an established clinical anticancer drug paclitaxel (Ptx) with a polyphenol catechin (EGCG) in the core and shell, respectively. It was demonstrated that this core/shell nanoconstruct could sequentially release EGCG from casein shell followed by Ptx from PLGA core, which sustained for 7 and 12 days, respectively. Anti-cancer effects of this nanomedicine assessed on metastatic breast cancer MDA-MB-231 cells revealed a synergistic interaction between the drugs. Additionally, the nanomedicine restricted the migration, invasion and adhesion properties of MDA-MB-231 cells and reduced its MMP-9 expression. The enhanced anti-cancer and anti-metastasis effects of Ptx-EGCG nano-combination can be attributed to the sequential release of EGCG followed by Ptx from the shell and core, respectively, which thereby sensitize cancer cells to Ptx treatment in clinical situations of drug resistance to Ptx therapy.

A study for the development of a non-invasive nanoparticulate drug delivery system for the retina was conducted at the IIT-Kanpur, Kanpur. The use of penetration enhancer like matrix metalloproteinase (MMPs) 1, 2 and 14 has been reported to increase trans-scleral permeability and hence increase bioavailability of dextran (70 KD) by 24% in retina. It was hypothesized that drug delivery through the scleral pathway using hydrophobic degradable nanoparticles (less than 200nm) coated with hydrophilic degradable polymers in presence

of permeability enhancer might potentially improve the permeability through sclera and hence the bioavailability of drug to retina.

In developing countries, there has been a resurgence in *Mycobacterium avium* complex (MAC) infections in AIDS patients population, as well as in patients with MAC pulmonary disease. The study conducted at Lifecare Innovations, South Delhi Campus, New Delhi, focused on the potential of lipid glyceryl monostearate (Imwitor) to encapsulate anti- *My. avium* drugs viz. rifabutin (RFB), azithromycin (AZT) and amikacin (AMK). The lipid glyceryl monostearate encapsulated RFB, AZT and AMK efficiently. A single oral dose of the drug- loaded SLNs showed a sustained release of the drugs in plasma for more than 5 days.

In a study on platelet reactivity using nanoscale gold and diamond and development of novel actin polymerization assay conducted at Banaras Hindu University, Varanasi, the effect of nanomaterials (grapheme oxide, GO and nanodiamond ND) on platelet reactivity was investigated. From *in vitro* and *in vivo* studies conducted on human blood as well as mouse models, it was found that both GO and ND induced strong aggregation of blood platelets and elicited extensive thrombo-embolism. With a translational perspective, subsequently a search was done for possible surface-modified isoforms of above nanomaterials with minimal or nil thrombogenicity. The findings suggested that, amine-modified forms of nanodiamond and graphene had no thrombogenic potential and were safe for projected biomedical applications. Thus, the amine-modified forms should replace the commonly available and widely researched oxidized forms of both these nanomaterials while considering their systemic administration for imaging, drug delivery or anti-cancer applications.

The study conducted at University of Madras, Chennai, was aimed to design and synthesise 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.

A study on biosynthesis of silver nanoparticles and their anti-HIV activity was conducted at the Thiruvalluvar University, Fort Campus, Vellore. As per the objectives, attempts were made to synthesize silver nanoparticles with various physicochemical nature using mangroves. Mangroves *Rhizophora lamarckii*, *Bruguiera cylindrica*, *Ceriops decandra* were used to synthesize silver nanoparticles. It was observed that HIV-1 RTase was inhibited by the newly synthesized silver nanoparticles in a dose dependent manner, with an estimated IC₅₀ level of 2, 5, 4, 2 µl/ml by the silver nanoparticles synthesized using the mangroves *Rhizophora apiculata*, *Avicennia marina*, *Excoecaria agallocha*, *Rhizophora lamarckii* against the HIV-1 RTase *in vitro*. Results indicated that the newly synthesized silver nanoparticles had strong inhibitory activity against the HIV-1 RTase.

Drug resistance to first line drug therapy (miltefosine and amphotericin B) against visceral leishmaniasis has been reported. The synthesis of PLGA-PEG nanoparticle conjugated with antibody to CD14 and antileishmanial compounds such as miltefosine, amphotericin B, sitamaquine, vinblastine and sulforaphane has been successful. Characterization of the synthesized nanoparticles by HRTEM confirmed the approximate nano-size of the particle and FT-IR (nanoparticle conjugation). Comparison of IC₅₀ values of miltefosine, amphotericin B and miltefosine conjugated with PLGA-PEG nanoparticle against promastigote and amastigote stages of *Leishmania donovani* at Rajendra Memorial Research Institute of Medical Sciences, Patna, revealed that miltefosine conjugated with PLGA-PEG nanoparticle was more effective in inhibiting the parasite. Hence the efficacy of nano-form of PLGA-PEG conjugated miltefosine or amphotericin B or sitamaquine nanoparticle was higher than that of currently available antileishmanial drugs such as miltefosine and amphotericin B, sitamaquine. Characterization of other synthesized nanoparticles for compounds such as SAG, vinblastine and sitamaquine and drug sensitivity tests has been successful.

In a study on targeted delivery of anti-retroviral drugs using stealth immunoliposomes at SASTRA University, Thanjavur, two types of nano-dimensional carriers have been developed for delivery of anti-retroviral drugs. *i.e.*, chitosan and liposome. These carriers were characterized for their morphology, particle size, colloidal stability, zeta potential, encapsulation efficiency, release kinetics under various conditions, cell uptake and viral inhibition efficiency. Chitosan was found to be effective in encapsulating 72% of the protease inhibitor saquinavir while it was not able to encapsulate the reverse transcriptase inhibitor nevirapine. The uptake of the chitosan nanoparticles loaded with the Alexa fluor 647 was found to be 92% due to positively charged amino group on the chitosan nanoparticles. A significant reduction in the viral load of two sub-types of the virus was observed when treated with the formulated nanoparticles as compared to that of the free drug indicating the potential of this carrier in anti-retroviral therapy. The next category of drug delivery systems, namely, liposomal carriers linked with anti-CD4 was also developed. These carriers were found to be effective in encapsulating both nevirapine and saquinavir. The viral load reduction with the dual drug-loaded carrier seems to be more efficient than the free dual drugs as well as the single drug loaded carrier.

The study on magnetic nanoparticles for effective target drug delivery of carvacrol against liver carcinogenesis conducted at University of Madras, Chennai, showed potential to describe the importance of administration of natural occurring compound Carv-MNPs (Isoflavone) to protect against NDEA induced liver cancer. It is an attempt to evaluate the cytoprotective and chemopreventive effects of Carv-MNPs against NDEA induced HCC in rats and in HepG2 carcinoma cells. Effects of Carv-MNPs on proliferation markers such as PCNA and cyclin D were studied in both NDEA induced HCC animals and in HepG2 carcinoma cells. Analysis of these markers clearly suggested that Carv-MNPs effectively inhibited tumour proliferation. *In silico* analysis of molecular docking interaction of anti-apoptotic protein Bcl-xL and key regulator of Akt signaling pathway PDK-1 active site suggested Carv-MNPs as efficient target inhibiting molecular

drug against Bcl-xL, PDK1 and its downstream components

Nylon 6,12 is an ideal polymeric filler that can be used in dental restorative material because of its properties such as high wear and abrasion resistance, low water absorption, and resistance to alkalis and organic chemicals. Antimicrobial studies were carried out at Mahatma Gandhi University, Kottayam, using *Staphylococcus aureus* and *Candida albicans*. The incremental addition of the Cloisite 30B resulted in significant increase in the antimicrobial activity. The antimicrobial property exhibited by the higher concentrations of nanoclay was comparable to that of the commercially available antibiotic. The nanoclay addition also brought about an increase in the hydrophobicity of the electrospun membranes.

For precise delivery of carvacrol, PLGA (Poly lactic-co-glycolic acid) based superparamagnetic nanoparticles loaded with carvacrol (Carv-MNPs) were synthesized and characterized at the University of Madras, Chennai. Carv-MNPs with a particle size of 223 ± 20.7 nm were synthesized by solvent evaporation method. Fe_3O_4 nanoparticles of 33 ± 3.63 nm encapsulated in the PLGA polymer provides the superparamagnetic property to the Carv-MNPs. The entrapment efficacy of Fe_3O_4 nanoparticles, carvacrol and average cumulative percentage release profile of carvacrol from the Carv-MNPs were investigated. The magnetic nanocarrier enhanced the stability and activity of carvacrol. Release of carvacrol from Carv-MNPs occurred in a controlled manner. The cytotoxicity of Carv-MNPs against human liver cancer cell line (HepG2) was investigated and the 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. Further anti-cancer, anti-metastasis and anti-proliferative efficacy of Carv-MNPs against HepG2 was observed. The result showed that Carv-MNPs had significant anti-tumour activity. Therefore, Carv-MNPs may be considered as an effective anticancer drug delivery system for cancer chemotherapy.

PHARMACOLOGY

This study conducted at the Panjab University, Patiala, was aimed to design oral dosage forms of 5-Fluorouracil (5-FU) using natural, biodegradable

polymers that shall not release the drug in acidic environment of the stomach and to optimize these dosage forms that these shall release the drug after reaching the intestine/colon either due to alkaline pH and/or due to the action of bacterial enzymes on the natural polymers used in the formulation. The result revealed successful derivatization of bael fruit gum (BFG) into carboxymethylated bael fruit gum (CMBFG) and methylated bael fruit gum (MeBFG) and bael fruit pectin (BFP) into methylated bael fruit pectin (MeBFP). MeBFG was found to be suitable for use for direct compression in tablet. Films containing chitosan (CH) and BFG, CMBFG, BFP, MeBFP or MeBFG were prepared and evaluated for their physical properties. The effectiveness of these tablets in treating TNBS induced colitis in rats was observed to be significantly better than that observed after oral administration of Eudragit FS30D or cellulose acetate coated 5-FU tablets. Although the colon to body weight ratio (C/B) in rats administered with 5-FU tablets coated with MG3 film did not return to normal value, the values were insignificantly different as compared to uninduced normal rats. The data revealed superiority of CH:MeBFG (30:70) film over well known polymers like Eudragit FS30D and cellulose acetate for colon release of 5-FU, thereby, resulting in faster treatment of TNBS induced colitis in rats.

A study on formulation, optimization and evaluation of methotrexate (MTX) smart gels for the treatment of rheumatoid arthritis was completed at J.S.S. College of Pharmacy, Sri Shivarathreshwara Nagara, Mysore. Biodegradable injectable *in situ* gels offer versatility in delivering drug at predetermined rate, maintain plasma concentration with a possibility of dose adjustment. The formulations were prepared using pluronic F-127 and varying concentration of pluronic F-68 / carbopol 934. The prepared *in situ* gels were evaluated *in vitro* for FT-IR, sterility, gelation characteristics, content uniformity, viscosity, syringeability and *in vitro* drug release. The results demonstrated that MTX was evenly distributed in all formulations, which were sterile and syringeable through 18 gauge needle. The gels were thermosensitive and thermoresponsive, and were dependent on the type and concentration of co-polymers. The optimized formulation M4 (20% pluronic F-127 and 6% pluronic F-68) exhibited drug release of 99.22% at 120 h. The drug release

was influenced by type and concentration of co-polymers employed. The results showed that the prepared methotrexate *in situ* gels might be a promising drug delivery system for the treatment of RA, which can deliver drugs to specific sites of the body in a controlled manner.

STEM CELL RESEARCH

A study on the characterization of limbal epithelial stem cell (LESC) niche, its regulatory mechanism and justification for 'Niche-Surrogate' therapy was conducted at Calcutta School of Tropical Medicine, Kolkata, to evaluate the alteration or deregulation of LES C and their microenvironment. Diseases related to the corneal blindness have been reported to be caused by limbal stem cell deficiency and are called limbal stem cell deficiency diseases (LSCDD). Like other somatic stem cells, LES C fate is significantly dependent upon the specific micro environmental niche. An attempt was made to weigh up the phenotypic alteration of limbal epithelial cells in UV related corneal diseases like pterygium and also in UV-B exposed experimental mice. In the present experimental set up by using chronic UV-B exposure LESCD mice model which mimic pterygium like condition was successfully developed. Receptor expression profiling of LES C along with cell cycle associated protein levels was evaluated to manifest the disease state. Structural alteration and deregulation of stem cell, its neighbouring niche components were observed by using clinical, morphological, scanning electron microscopy, explants culture study and flowcytometric analysis which demonstrated that the limbal microenvironment played an utmost role for pterygium development. Characterization and understanding of limbal stem-microenvironmental network will open up effective frontiers for the successful management of LSCDD where stem cell transplantation combined with niche modulation could be a novel therapeutic armamentarium in regenerative medicine.

A study on cellular and molecular mechanism of xenoestrogen bisphenol-A (BPA) on the neural stem cell (NSC) proliferation, migration and neurogenesis was conducted at the Indian Institute of Toxicology Research, Lucknow. The effect(s) of BPA on regulatory dynamics of neurogenesis including NSC proliferation, and differentiation

was assessed. Chronic treatment of low dose of BPA (40µg/kg.bw) to pregnant rats from GD6-PND21 was given. It was found that BPA treatment significantly decreased bromodeoxyuridine positive (BrdU⁺) cell proliferation in the dentate gyrus region of the hippocampus and SVZ. BPA significantly increased GFAP⁺ glial cell population in the hippocampus. Number of DCX⁺ and NeuN⁺ neurons was decreased by BPA. Overall, the results showed significant inhibitory effect of BPA on neuronal proliferation, differentiation and Wnt/ β -catenin signaling pathway.

In a study on autologous olfactory ensheathing cell (OEC) transplantation in human spinal cord injury (SCI), 36 individuals were selected to collect olfactory mucosa from nasal cavity. Both qualitative (IHC, ICC & IF) and quantitative (Flow cytometry) analysis of the cell characterization determined presence of OECs in culture conditions. The culture method could achieve 70% purity of OECs. This opens up a clinical possibility to treat patients with SCI with autologous fresh OECs which can be purified from cultures and prepared to be used for neural recovery.

Stress urinary incontinence (SUI) is a widespread health problem that relentlessly impacts quality of life. It has been investigated through several preclinical and clinical trials to establish whether transplantation of patient's own skeletal muscle-derived cells (SkMDCs) can restore the sphincter musculature. It was, therefore, aimed to investigate the efficacy of micro-explant based skeletal muscle stem cell culture and quantification of myoblasts using immuno-cytochemistry, immunofluorescence and flow cytometry assay. Skeletal muscle biopsies were taken from the anterior abdominal wall muscle to obtain cultures from autologous fibroblasts and myoblasts. A total of 70 cultures were performed from the biopsies collected from 37 patients. Immunocytochemistry was carried out after enriching culture with preplating method. The fibroblasts will be injected into the urethral submucosa and the myoblasts will be injected into the rhabdosphincter of patients with SUI. Urodynamic, electromyographic, laboratory parameters, quality-of-life score and a defined incontinence score, have to be evaluated before and after therapy. This study might help design a therapeutic concept for an

effective and least invasive treatment modality to patients with SUI.

The study conducted at CLRD, Deccan Medical College, Hyderabad, on isolation and characterization of neural precursors from human foetal brain reported that CD133 could be used to isolate utmost number of neural precursor cells (NPCs) from different niches of human foetal brain of 18-22wk gestation age. In gestation wk 20 highest percentages of CD133+ve cells were observed as compared to 18-22wk old human foetal SVZ and other regions before and after enrichment. The self-renewal and lineage differentiation potentials of these NPCs were also employed to characterize the CD133+ve and CD133-ve cells. CD133+ve cells developed more number of neurospheres as compared to CD133-ve cells and maintained more capacity to generate secondary neurospheres having more *in vitro* proliferative and self renewal capacity. The study demonstrated that 20 wk gestation aged SVZ served as the most abundant source for the isolation of NPCs. The study provided SVZ as the highest remnant of NPCs as compared to frontal, temporal and occipital lobes to further assess the *in vitro* biology of NPCs. Identification of suitable combination of markers will facilitate further enrichment of NPCs from various sources and provide putative clinical applications of NPCs.

MicroRNAs (miRNAs) are small endogenous non coding RNAs which regulate mRNAs post-transcriptionally. In this study conducted on regulation of mesenchymal stem cells (MSCs) towards osteogenic cell lineage by microRNAs at SRM University, Tamil Nadu, 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 expression of miR-424, miR-106a, miR-148a, let-7i and miR-99a miRNAs was found to be specific in hMSCs; whereas expression of miR-15b, miR-24, miR-130b, miR-30c, and miR-130a miRNAs was found to be specific in differentiated osteoblasts. A bioinformatics approach identified that the MAPK pathway was mostly targeted by hMSCs specific miRNAs; whereas JAK-STAT, p53, focal adhesion, gap junction, ubiquitin mediated proteolysis pathways were targeted by osteoblastic

specific miRNAs. This study identified that miR-15b might act as a positive regulator for osteoblast differentiation and alteration in the expression of miRNAs would be a valuable approach for controlling osteoblast differentiation.

Tissue engineering is an emerging area in the field of treating long bone defects. Biocomposites like chitosan (CHT), polycaprolactone (PCL) and hydroxyapatite (HAP) have osteogenic and biodegradable properties, making them suitable for tissue engineering. A study was conducted at the AIIMS, New Delhi, to explore the chemical, mechanical and biological characteristics of the scaffolds prepared by using these biocomposites along with stem cells. The osteogenic properties were also studied *in vivo* by transplanting them at ectopic site in rats, along with mesenchymal stem cells.

Biocomposites of CHT, HAP and PCL (FDA approved) were chosen for designing of scaffolds by freeze drying method. The results showed that the biocomposites prepared in defined ratio using chitosan, hydroxyapatite and polycaprolactone by freeze drying method was a cost-effective and less laborious method to prepare biodegradable and biocompatible scaffolds that can prove to be a promising tool for tissue engineering in the treatment of long bone defects.

Targeted molecular imaging to detect changes in the structural and functional organization of tissues, at the molecular level is a promising approach for effective and early diagnosis of diseases. Designing of novel and effective imaging probes for targeting biomarkers in live tissues is a major challenge in non-invasive diagnosis of diseases. There is no molecular imaging tool available till date for the detection of liver fibrosis. In a study conducted at CCMB, Hyderabad, a fluorescent probe has been synthesized that comprises collagelin, a specific collagen binding peptide, coupled to fluorescent porphyrin that can effectively detect abnormal deposition of collagen in live tissues by emitting fluorescence in the near infra red (NIR) region. The probe was able to differentiate fibrotic and normal liver tissues.

A study on isolation and characterization of dental pulp mesenchymal stem cells isolated from deciduous and adult humans and their differentiation

into dopaminergic neurons and islet cells was conducted at Manipal Institute of Regenerative Medicine, Bangalore. The findings suggest that cells can be expanded with the best efficiency in KO-DMEM medium supplemented with 10% FBS. In xeno-free (10% human plasma) growth conditions, DPSCs retained their multipotency. Stem cells from human exfoliated deciduous teeth (SHED) showed higher proliferation rate than DPSCs. Influence of hypoxia, high glucose and low serum concentration on growth kinetics and proliferative potential of the human DPSCs and SHED were also detected.

The islet differentiation capacity of both DPSCs and SHED was performed *in vitro*. SHED showed better differentiation potential. The effect of islet like cell clusters (ICCs) derived from SHED was investigated on hyperglycaemia in streptozotocin-induced diabetic mice. ICCs were packed in immuno-isolatory biocompatible macro capsules and then transplanted into streptozotocin (STZ) induced diabetic mice. STZ diabetic mice alone and those transplanted with empty macrocapsules exhibited hyperglycaemia throughout the experiment whereas those transplanted with macro capsules containing ICCs restored normoglycaemia within 3-4 weeks which persisted for more than 60 days. Our results demonstrated for the first time that ICCs derived from SHED could reverse the STZ diabetes in mice without immunosuppression and offer an autologous and non-controversial source of human tissue that could be used for stem cell therapy in diabetes.

This study conducted at Indian Spinal Injury Centre, New Delhi, was designed as a pilot study to establish the safety and feasibility of autologous bone marrow cell transplantation in patients after complete acute spinal cord injury by implantation into the injured spinal cord site with a syringe after exposing the spinal cord or intrathecal injection as compared to a corresponding number of control subjects. Acute injury was chosen, as the potential of regeneration and recovery of the spinal cord may reduce with time. The study was found to be safe on most counts. None of the participants developed either signs or symptoms of meningitis

Primary efficacy variables tested, namely, improvement in the ASIA score did not show

any improvement. This corresponds to natural history of complete SCI. Of the secondary efficacy end points tested, there was a significant improvement in functional examination (SCIM) and ISCIS scores without any significant change in clinical or electrophysiological examination. The improvements in SCIM suggest compensation and exploitation of neuronal plasticity functional training and not because of restoration of function due to cellular regeneration.

A study on use of autologous bone marrow derived stem cell in rehabilitation of patient with dry, age related macular degeneration and retinitis pigmentosa was conducted at AIIMS, New Dehi. The primary objectives was to study the safety of injected intravitreal autologous bone marrow derived stem cells and the secondary objectives was to study the efficacy of the injected intravitreal stem cells in retinal degeneration-retinitis pigmentosa and dry age related macular degeneration. Significant improvement was noted in electrophysiological parameters like multifocal ERG. Improvement in MfERG amplitude was noted in 2-5 degree. Significant improvement was also noted in MfERG implicit time in 5-10 deg.

Intravitreal autologous bone marrow derived stem cell therapy can be a promising and safe treatment for visually disabling retinal degenerative disorders both inherited and acquired.

National Guidelines for Stem Cell Research (2013) have been released on 21st February 2014. (<http://icmr.nic.in/guidelines/NGSCR%202013.pdf>).

BIOMEDICAL ETHICS

At present only clinical trials with new drugs are regulated under the Drugs & Cosmetics Act, 1940 and this law is not applicable to the enormous quantum of biomedical research being conducted in universities, medical colleges, hospitals, and in both public and private sectors. The Biomedical & Health Research Authority is being proposed to be set up for regulating such institutions whose research needs to be monitored to protect the safety, well being and rights of all research participants.

The Draft “Biomedical and Health Research Regulation Bill, was prepared by a small sub-committee and the finalized document was

submitted to the Department of Health Research in November, 2013, from where it has been circulated to the various concerned Ministries/ Departments for comments. The comments were received from 12 Ministries/Departments and were considered by the subcommittee and replies have been prepared and re-submitted to the DHR for consideration. The Bill will be sent to the Department of Legislative Affairs for further processing and approval before submission to the Cabinet by the Department of Health Research.

To understand the newer concerns and issues raised due to recent developments in science as well as to be in line with the regulatory norms, the ICMR in collaboration with WHO-India Country Office jointly organized a 2-day National Consultation Workshop on the subject “Ethical Issues of Biomedical Research on Human Participants”- The present context” on October 28-29, 2013 at New Delhi.

Under a major initiative to strengthen the status and functioning of the ethics committees in India and to assist them in raising their standards to the International level, the ICMR has conducted several training workshops, namely Human Subject Protection Course (HSPC) and Standards Operating Procedures (SOP), Training and Survey of Institutional Ethics Committee at National Institute for Research in Tuberculosis, Chennai, involving participation of several ethics committees from Chennai. The courses were organized by the Council in association with Forum for Ethical Review Committees in Asia and Western Pacific Region (FERCAP) for in-depth training and capacity building of ethics committees.

SHORT TERM STUDENTSHIP

Short term Studentship (STS) is a popular programme among the medical undergraduates pursuing MBBS or BDS in any medical college in India. The programme is open to Indian nationals and from 2014 it has also been extended to NRI/PIO students pursuing their education in India. During 2013-2014 Short Term Studentship programme provided opportunity to a large number of undergraduate students to familiarize themselves with research methodology by undertaking short duration research projects.

macromolecular damages. It was also observed that *G. acerosa* treatment significantly renewed the antioxidant enzymes activities (catalase, superoxide dismutase, glutathione peroxidase glutathione reductase and glutathione-S-transferase). Co-treatment with *G. acerosa* extract significantly reduced apoptosis, mitochondrial membrane potential and EROD level.

A study on stimulatory effect of some mineral compounds on immune response in murine model was completed at Jamia Hamdard, New Delhi Herbo-mineral preparation used in Unani system of medicine such as *Khamira Marwarid* (KM) is prescribed as a tonic for heart and brain, usually as a recuperating agent after disease. The proposed study was planned to evaluate the effect of mineral compounds present in KM for their effect on various immune responses in animal model. Immunomodulatory effect was observed with an increase in serum anti-ovalbumin (OVA) total IgG antibody and IgG titre following oral administration of pearl (P), jade (J) and serpentine (S) to mice. Administration of P and S resulted in elevated levels of IgG2a, IgG2b and IgG3 subtype level while the administration of [1] resulted in elevated levels of IgG I subtype level. Anti-ovalbumin IgE decreased significantly on administration of P, with an increase in anti-ovalbumin IgG but not significantly on administration of [1] and [S]. To assess the effect of oral administration of P, 1 and S on the cell mediated immunity; delayed type hypersensitivity (DTH) response was studied. The level of both Th1 (IL-2/IFN- γ) and Th2 (IL-4/IL-10) cytokines were found to be increased in spleen

cell cultures of mice treated with [P] and [S] but the level of Th1 cytokines were well above than the Th2 cytokines, suggesting the Th1 dominant response stimulated by [P] and [S]. Phagocytic activity was found to be increased significantly in [P], [1] and [S] treated mice.

A study of the effect of leaf extracts of *Clerodendron colebrookianum*, walp(Nefafu) on lipid peroxidation, lipid profile and antioxidant was completed at the Institute of Advanced Study in Science and Technology, Paschim Boragaon, Guwahati . The aim was to find out the antiperoxidative and lipid lowering activity of two different extracts of *Clerodendron colebrookianum* (CC) leaf, viz. crude extract (CE) and hydroalcoholic extract (HE). The study was conducted in two experimental animal models. *i.e.* Triton-WR-1339 (200mg/kg b.wt.) as an intrinsic inducer and by feeding cholesterol (25mg/ kg b.wt.) as an extrinsic inducer. In both the experiments the animals were divided into 5 groups comprising 6 animals in each group. Administration of CE and HE of CC leaf in three different doses to the hyperlipidemic rats caused a significant decrease the plasma lipid levels (quantified using enzymatic kits, Randox). TBARS level reduced significantly both in plasma and tissue in cholesterol fed rats. HE of CC leaf showed interesting results as it reduced significantly total and LDL cholesterol and enhanced the HDL cholesterol in a dose dependent manner. The plant extract also significantly reduced the atherogenic index, a sensitive indicator of cardiovascular diseases. The results observed in the present study suggested that CC leaf could be used to treat hypercholesterolemia also.

REGIONAL MEDICAL RESEARCH CENTRES

To deal with health problems of the regional and marginalised population, ICMR has established a total of 6 Regional Medical Research Centres at Jabalpur (MP), Port Blair (Andaman Nicobar), Bhubaneswar (Odisha), Jodhpur (Rajasthan), Dibrugarh (Assam) and Belagum in Karnataka. The effort of these institutes is to focus on the regional health problems and find suitable solutions with the help from the respective state governments. The significant outcome of the research activities carried out by these centres during 2013-14 is mentioned below:

REGIONAL MEDICAL RESEARCH CENTRE, BELGAUM

HERBAL AND TRADITIONAL MEDICINE

Anti-diabetic activity of IP-156 in rat model

Herbal based Traditional Medicine has been practiced in several regions of India. Preliminary screening of the aqueous extract of plant IP 156 and subsequently one of its fractions was carried out in Streptozotocin (STZ) induced diabetic rat model. Fraction No. 1 was further purified and one compound was isolated, characterized by spectroscopy, and identified. Different doses of this compound, (#01) were further screened for their anti diabetic activity on STZ induced animal model, which showed remarkable activity (Table 1). Detailed pharmacological activities are ongoing for

preparation of effective and safe herbal formulation for management of diabetes.

Elucidating anti-arthritic potential of selected medicinal plants and their fractions in Wistar rats

Plumbagozeylanica Linn. and *HolopteleaIntegrifolia* Planch. hydroalcoholic extracts were selected for further screening, based on safety and efficacy studies. *P. zeylanica* and *H. Integrifolia* hydroalcoholic extracts were subjected for fractionation using partition chromatography (Liquid: Liquid chromatography) with three organic solvents. The solvents were completely evaporated later to obtain dried powders, which were used for experiments. Yield of the fractions were as follows:

H. integrifolia

- HIHY #1: 30.90 mg/g,
- HIHY #2: 03.36 mg/g,
- HIHY #3: 95.10 mg/g

P. zeylanica

- PZHY#1: 40.60 mg/g,
- PZHY#2: 01.16 mg/g,
- PZHY#3: 94.10 mg/g

In vitro cyclo-oxygenase I and cyclo-oxygenase II assay were carried out using Caymen COX inhibition screening kit. Fractions HIHY#3 and PZHY#3 showed maximum COX II inhibitory effect with mild inhibitory effect on COX I. Acute

Table 1. Anti diabetic activity of extract of plant IP 156 (IP156Ext), fraction (#01) and compound (Comp 01) on Stz Induced diabetic rats

Treatment	0 hr	1 hr	3 hr	5 hr	8 hr
Vehicle	488.33 ± 17.45	479.16 ± 22.84	443.00 ± 33.54	430.50 ± 18.27	456.16 ± 19.09
Glibenclamide	487.33 ± 39.44	473.66 ± 25.63	406.66 ± 35.43	358.16 ± 43.76	311.00 ± 47.01
IP156 Ext(200 mg/kg)	536.66 ± 15.57	513.16 ± 16.65	437.50 ± 16.76	390.16 ± 14.20	356.16 ± 10.78
#01 (100 mg/kg)	531.83 ± 37.12	452.33 ± 31.29	401.00 ± 30.60	375.66 ± 20.70	382.00 ± 24.55
Comp 01 (50 mg/kg)	426.50 ± 15.76	425.83 ± 13.15	387.16 ± 9.19	333.33 ± 4.61	308.33 ± 10.14
Comp 01 (5 mg/kg)	333.66 ± 54.84	322.00 ± 31.74	292.00 ± 39.83	265.66 ± 27.72	237.0 14.52

toxicity studies of the fractions were carried out in Swiss albino mice as per the OECD 423 guidelines to explore the safety and find lethal dose (LD50) before performing efficacy studies. All fractions were administered once and observed for continuous 14 days for signs of toxicity. However all the fractions were found safe up to 2000 mg/kg body weight. Therefore LD50 was considered to be more than 2000 mg/kg body weight.

All the six fractions of the active extracts were subjected to screening of anti-inflammatory effect by employing Carrageenan model of acute inflammation in Wistar rats. All the fractions were administered orally at 100 mg/kg. Paw volume of the animals were measured at various intervals to quantify the response. Among the fractions, PZHY#1, PZHY#2, HIHY#1 and HIHY#2 showed maximum inhibitory effect at 2nd hr post challenge of Carrageenan. The anti-inflammatory effect of fractions PZHY#1 and HIHY #1 were almost comparable to aspirin taken as standard drug. All the six fractions were subjected to Complete Freund's Adjuvant (CFA) induced model of chronic inflammation studies. All the fractions were administered in the dose of 25, 50 and 100 mg/kg in the experimental animals. Paw volume and ankle joint diameter were measured at various intervals to quantify the effect. Among the six fractions, HIHY#1, PZHY#1 and PZHY#2 showed maximum inhibition of paw edema. Effects of the fractions HIHY#1, PZHY#1 and PZHY#2

were found to be superior to aspirin with the same dose. Based on results of efficacy studies, PZHY#1 and HIHY#1 have been selected for the further fractionation to identify the active group of compound(s). Estimation of Cytokines, Cell count and Histopathology are in progress.

Phytochemical and *in vitro* studies in *Achyranthes aspera* L.

Achyranthes aspera (family Amaranthaceae) plant is valued for its Ayurvedic importance and high medicinal value.. The major phyto-constituent from the plant is alkaloids and triterpenoids. Oleanolic acid (OA) is the major triterpenoids determined and studied from the plant. The wide array of activities of the plant is attributed to presence of OA in it. Owing to this importance plant was studied for presence of betulinic acid (BA) from *in vitro* grown cultures of *A. aspera*.

Standard methodologies for tissue culture studies were followed. Callogenic potential of leaf explants was studied using different concentration of auxins (2, 4-D, NAA, IAA, IBA). Observations for callus cultures were recorded after 30 days of culturing on LS fortified with 3.00 mg/L 2, 4 D and 0.50 mg/l BAP. It showed a significant growth in callus growth rate. The rate of proliferation increased with increase in 2, 4-D concentration up to 3.00 mg/L (Table 2). During culture period the callus acquired a whitish brown color Table 2.

Table 2. Callus response (%), morphology, growth and content BA and OA (mg/g) obtained as response to MS medium fortified with different levels of 2, 4-D and BAP in *Achyranthes aspera*

Code	2,4-D	BAP	Weight (FW g/ tube)	Response (%)	Callus color	Growth	Content FWB (mg/g)	
							BA	OA
C-01	0.0	0.0	00.03 ± 0.03*	100	DB	----	ND	ND
C-02	2.0	0.5	01.95 ± 0.83**	80	DB	++--	0.004 ± 0.001	ND
C-03	2.0	1.0	01.10 ± 0.98**	100	DB	+++ -	TR	ND
C-04	2.0	2.0	01.62 ± 1.35*	100	LB	+++ -	0.005 ± 0.002	0.002 ± 0.001
C-05	3.0	0.5	02.08 ± 0.92**	100	W	++++	0.012 ± 0.001	0.003 ± 0.000
C-06	3.0	1.0	01.55 ± 0.99**	100	WB	+++ -	0.007 ± 0.004	ND
C-07	3.0	2.0	01.52 ± 0.69**	80	WB	++--	TR	ND
C-08	4.0	0.5	00.51 ± 0.44*	100	WB	++--	ND	ND
C-09	4.0	1.0	01.01 ± 0.81**	70	WB	++--	0.007 ± 0.001	ND
C-10	4.0	2.0	01.86 ± 0.98**	60	LB	++--	TR	ND

DB: Dark Brown; LB: Light Brown; WB: Whitish Brown; W: White; ++++: < 7 days; +++-: < 14 days; ++-: > 30 days; +-: > 45 days; FWB: Fresh Weight Basis; ND: Not Detected; TR: Trace; (≤ 0.001); Values in table represent mean ± SD of 10 replicates per treatment; Significance levels represented as * P< 0.05 and **P<0.01 compared using Dunnett multiple comparison test using one way ANOVA.

Further, RP-UFLC analysis of callus extracts yielded profiles with a retention time of 11.642 ± 0.06 min and 12.819 ± 0.05 min for standard BA and OA respectively. Five point standard linear calibration curves for standards were obtained. Samples prepared were injected and chromatograms were obtained at similar conditions as that of standards. The result of the study is tabulated in Table 2. Highest BA and OA content were obtained in C-05 cultures. It was a likely case that the highest content of BA and OA from callus cultures determined using RP-UFLC analysis were significantly lower than that determined by others in *in-situ*. Conclusively, we have succeeded in initiating callus from leaf explants of *A. aspera* and determining BA, OA from these cultures. The study further scopes for its *in vitro* enhancement.

In vivo and in vitro production of flavanoids in selected species of Clerodendrum

Clerodendrum inerme (L.) Gaertn and *C. phlomidis* (L.) f. are well documented in traditional system of medicines for several formulations and used as folk medicines by various tribes. It was aimed in the present study to develop standardized protocols for *in vitro* propagation of these plants and for *in vitro* production of active metabolites.

The plants of *Clerodendrum inerme* and *Clerodendrum phlomidis* were collected randomly from two different geographically regions (Northern Western Ghat, Belgaum, Karnataka and Aravali Region, Jhalana Doongri, Rajasthan). Various plant parts *viz.*, leaves, stem and flower of both plants were washed, separated, shade dried and powdered for evaluation of flavonoids content. Leaves, nodal segments and axillary apices were used as explants in both plants for *in vitro* studies.

An efficient and reproducible micro propagation protocol based on multiple shoot induction and callus regeneration has been standardized for selected *Clerodendrum* spp. Direct multiple shoot formation was induced in axillary nodal pieces of *C. inerme* cultured on M S (1962) medium supplemented with BA + Kn ($8.88+2.32\mu\text{M}$) and 2ml/L PPM. The best root induction and survival (96%) was achieved on 1/2 strength MS medium supplemented with IAA+ IBA ($5.71+4.92\mu\text{M}$).

Callus induction in leaf explants was optimized by studying the influence of auxins and cytokinins alone and in combinations. Optimum callus was established from leaf explants of *C. inerme* when M S medium was supplemented with phyto-hormones 2,4-D+NAA+KN ($4.52+10.74+2.32\mu\text{M}$) and 1.5 ml/l PPM. A protocol for rapid *in vitro* propagation of *C. phlomidis* being developed. The best response for multiple shoot induction was obtained in nodal explants on MS medium supplemented with BA ($8.8\mu\text{M}$) + IAA ($2.8\mu\text{M}$) and 2.0 ml/l PPM. Rooting of regenerated shoots was achieved on half strength MS medium supplemented with auxins IBA ($4.9\mu\text{M}$), individually. The enormous amount of calli production was obtained in *C. phlomidis* leaf explants when MS medium was fortified with phyto-hormones 2,4-D+NAA+KN ($2.26+5.37+4.64\mu\text{M}$) and 2ml/l PPM. A reproducible and highly efficient protocol for genetic transformation mediated by *Agrobacterium* has been established for *Clerodendrum* species. Apigenin was extracted and quantified from transformed roots (Fig. 1).

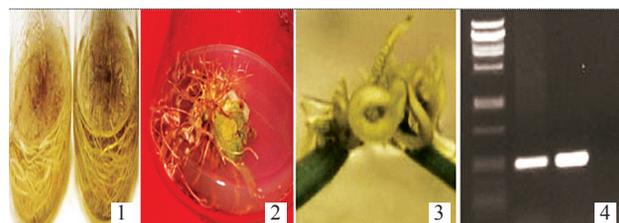


Fig. 1. (1-3). Hairy root culture of *Clerodendrum* species induced by *Agrobacterium rhizogenes* (MTCC532) and (4) PCR amplification of 308 bp fragment of *rol A* gene in hairy roots.

Diseases of Regional Importance and Support to Local Health Authorities/Outbreak of cholera in a backward taluka Bagalkot in North Karnataka

Cholera has been rarely reported from North Karnataka till 2010. However, it appears to be emerging in the recent years with large outbreaks being reported from various areas of Belgaum and Bijapur Districts between 2010 and 2012. RMRC and the state health department jointly investigated an outbreak of cholera in a remote backward area of Bagalkot district in August 2013. A total of 49 persons reported to the health centres from 6th August to 16th August 2013 and attack rate was 4.0 %. All age groups and both sexes were affected. All the isolates were found to be *Vibrio cholerae* O1 Ogawa

El Tor and belonged to a single clone. Interestingly it is the same clone that was circulating in Belgaum and Bijapur districts for the last three four years. Antibiotic sensitivity tests were carried out on these isolates. MIC of chloramphenicol and cotrimoxazole ranged from 32-64 µg/ml and 4/76 µg/ml respectively. MIC of quinolones nalidixic acid and ciprofloxacin ranged between >256 µg/ml and 0.75-1 µg/ml respectively. All the isolates showed reduced susceptibility to ciprofloxacin. MICs for ceftriaxone, (third generation cephalosporin) was 32 µg/ml. All these cephalosporin resistant *Vibrio* strains were confirmed to produce ESBL using the combination disc test. Results indicate that the incriminating strain, common in North Karnataka, appears to have gained resistance to more antibiotics, perhaps due to rampant and indiscriminate use of antibiotics (Fig. 2).



Fig. 2. Cholera-hit Somanakoppa village of Bagalkot, Karnataka

Services to State/District Health Units and Medical Colleges

The Centre has been providing services to the State Surveillance Unit and the District Surveillance Units of the Karnataka Government particularly in identification and confirmation of identities of bacterial pathogens and in investigation of

outbreaks of various epidemics. Similar services have been rendered to the Government Medical Colleges in the region.

RMRC helped the State Administration in identification of an outbreak of Hepatitis in Turmuri village on the outskirts of Belgaum in 2012. It also helped in identification and study of outbreaks of cholera in Harnal and Talikoti in Bijapur District. An upsurge of cases of diphtheria was identified in rural areas of North Karnataka.

REGIONAL MEDICAL RESEARCH CENTRE, BHUBANESWAR

This year the Centre has made all round attempts to address research issues on few most important health problems of this region like vector borne diseases, diarrhoeal disorders, bacterial meningitis, encephalitis, tuberculosis, and non-communicable diseases like under nutrition, diabetes, hypertension and sickle cell disease. Most of the studies were sponsored either by ICMR Task Force or DST, DBT, Gates Foundation, NVBDCP and MoH&FW. Network has been strengthened with the State Health Department, Medical Colleges and Hospitals of the region for referral investigation of sporadic cases and outbreak investigation of viral and other bacterial infections. The centre is also undertaking various HRD programmes for capacity building through PhD and MSc dissertation/training programme, etc.

The field units at Raygada (DHQ Hospital, Raygada) and Kalahandi (DHQ Hospital, Bhawnipatna) have started functioning by way of giving diagnostic services to diarrheal disorders both for treatment and prevention of epidemic, undertaking research activities like strengthening of IMNCI programme to reduce under five child mortality/morbidity and transferring technology to DHQ hospital on use of ELISA and PCR for diagnosis of different infectious diseases.

Proposals have been initiated to establish two new field units at Keonjhar and Kandhamal DHQ hospital as per the request of the state Government and one MRHRU at Tigriria of Cuttack district.

The major research activities of the centre carried out during 2013-14 are highlighted below.

Lymphatic Filariasis

The National Filaria Control Programme (NFEP) targets to eliminate lymphatic filariasis in the country by 2015 through mass administration of single annual dose of DEC (6mg/kg body weight). But one of the major challenges the programme faces is low rate of compliance. To improve compliance the centre has undertaken a study where it is looking for a new advocacy tool by searching the time of onset of lymphatic pathology among the children. Because it has been reported that prevalence of filarial infection in terms of circulating filarial antigen and microfilaria among pediatric age group is more than 30% in different endemic population, who are not currently covered under MDA, but the overt clinical manifestations usually appear in late adolescence and adulthood. It is well known that adult filarial parasites reside in the human lymphatics, where they can elicit an inflammatory response resulting in acute lymphangitis that appear usually in adolescent age group but there is lack of information on pathological changes in the lymphatic system from entry of infection to expression of disease. A lymphoscintigraphy technique has been standardized for the first time on *W.bancrofti* infected children to look for subclinical pathology with help of nuclear medicine experts. Amongst 102 children (50 symptomatic and 52 asymptomatic) enrolled in the study,

around 60% have shown lymphatic pathology. All the enrolled children were given DEC plus Albendazole annually supervised by a physician and were followed for any side reactions. Follow up investigation of children at 6 monthly intervals up to 24 months has indicated reversal of existing pathology in both asymptomatic and symptomatic subjects (Fig. 1). This information has the potential for use as a strong advocacy tool during MDA programme to improve coverage among children, which is very much essential for the LF elimination programme.

In order to strengthen the control strategy the centre has made an attempt to find out whether filarial infection in mothers has any role in increasing susceptibility to infection in the off springs. During this period it has been observed that 34.8% cord bloods collected from 145 CFA positive mothers contains circulating filarial antigen (CFA) indicating transplacental transfer of the filarial antigen. Further filarial specific IgG1, IgG2 and IgG4 responses of cord bloods were found to be positively correlated with CFA of mothers. In contrast IgG3 responses negatively correlated with CFA of mothers. The % of similarity of recognition pattern in the cord blood with maternal blood was high for IgG3 response than IgG4 in all three groups. An increased levels of IL-10 and decreased levels of IFN- γ were observed in cord blood of infected mothers. IFN- γ was positively correlated with IgG3 and negatively correlated with IgG4 level (Fig 2a & b). On the other

POST Rx REVERSIBILITY (Symptomatic)

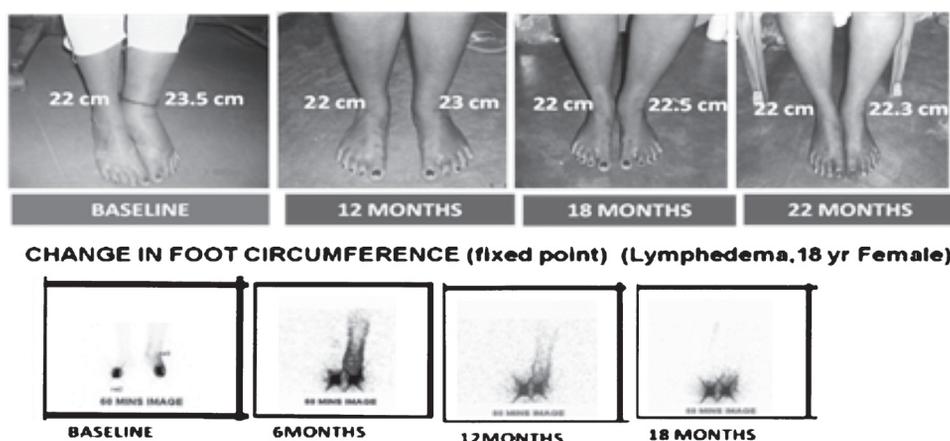


Fig. 1. Post treatment reversibility

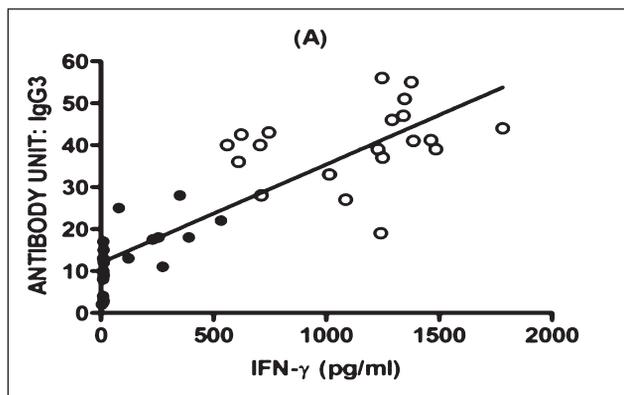


Fig. 2a. Correlation of IFN γ with IgG3 responses in cord bloods of infected and non infected mothers

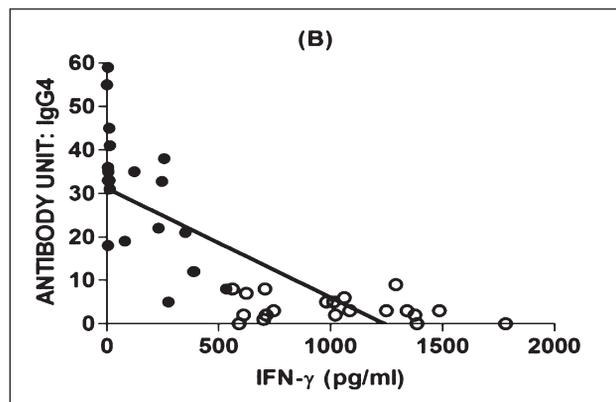


Fig. 2b. Correlation of IL10 in cord blood of in cord bloods of infected and non infected mothers

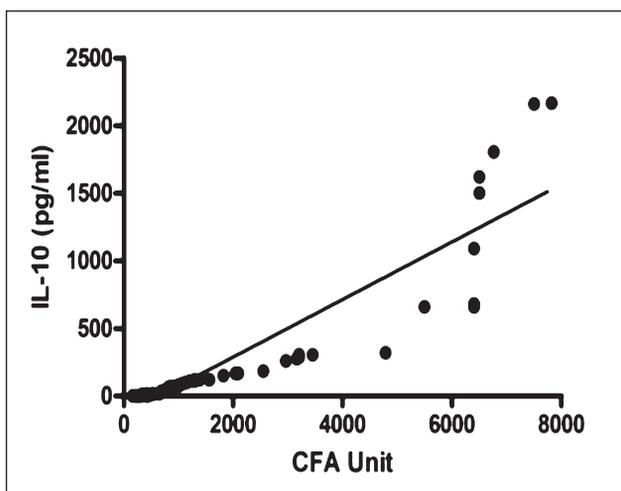


Fig. 3. Correlation of CFA with IL10 responses with IgG4 responses in cord bloods of infected and non infected mothers

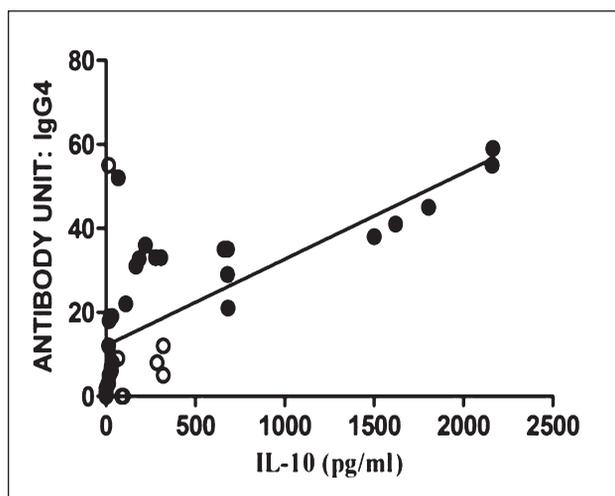


Fig. 4. Correlation of IL10 in cord bloods of infected and non infected mothers.

hand IL-10 was positively correlated with IgG4 and CFA (Figs. 3&4) indicating that cytokines may play a role in modulating the immune responses in cord bloods of sensitized foetus. The findings of the study reveal that *in utero* tolerance or sensitization may influence the filarial-specific immunity to infection in neonates.

In a separate study, a positive correlation between polyreactive antibodies and B-1 cells in filarial-infected human subjects has been observed. Moreover after anti filarial treatment, levels of IgM antibodies to ss-DNA, actin, LPS and filarial antigen increased significantly indicating a role of polyreactive naturally occurring antibodies in filarial infection (Fig. 5).

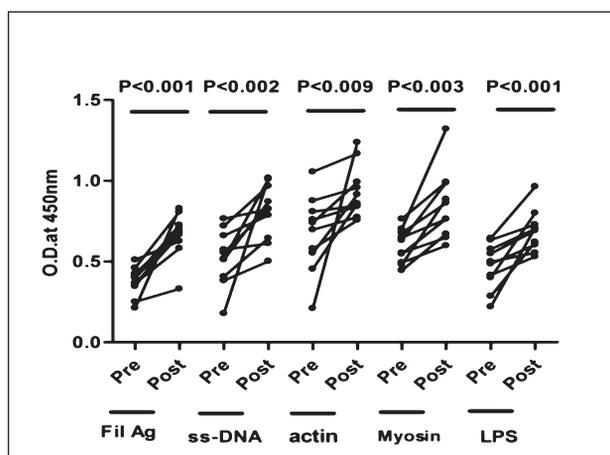


Fig. 5. DEC treatment reconstituted polyreactive Ig M antibodies

As a participating Centre, under National network for genotyping human filarial parasites, allele frequency of different gene loci (β -tubulin, ALT-2, ITS or r-DNA in *W. bancrofti* parasite population of endemic areas of Odisha was studied, which indicated the presence of the single nucleotide polymorphism at 200 position of β -tubulin gene of *W. bancrofti* causing Benzimidazole resistance. These markers can be used for tracking the changes in the genetic heterogeneity of *W. bancrofti* and develop appropriate strategies for the control/elimination of lymphatic filariasis. Similarly L3 stage specific RT-PCR assay for detection of infective stage *W. bancrofti* in vector was evaluated as a part of multi-centric project (Fig. 6). The assay was found to be sensitive and specific and can be useful for monitoring of the national LF elimination programme.

Malaria

Malaria survey conducted using molecular tool in Badampahar CHC (Mayurbhanj Dist) and Ghatgaon CHC (Keonjhar Dist) has revealed *P. malariae* mono infections in 11.6% of cases and mixed infections were 14.2% in Ghatagaon and 6% in Badampahar. Under the current scenario of screen and treat strategy, presence of *P. malariae* poses a difficulty, as the screening is performed by RDT alone. Further in the present study the species specific RDTs were not able to detect neither the mono infection nor the mixed infections of *P. malariae*. Therefore the molecular method can be used as a tool for surveillance to overcome such problems.

In a survey, treatment seeking behaviour, LLIN use and acceptance of IRS by the tribal communities was studied to find out their effect on reduction of malaria. Acceptance was seen to be high (around 90%) and 92% of the households were willing to purchase ITN/LLIN at subsidised cost. However, API was seen to be high (more than 14) in the studied villages of the Phiringia and Gumagarh blocks of Kandhamal district in spite of good LLIN coverage. Hence, other factors need to be addressed.

In a study attempt has been made to identify the sibling species complex and their vectorial capacity in malaria endemic regions of Odisha. Multiplex PCR detected *An. culicifacies* sibling species A, B, C, D and E in the malaria endemic regions of Odisha. *An. culicifacies* E was detected for the first time in Odisha, which was further confirmed by molecular phylogenetics. Highest sporozoite rate and HBF percentage were observed in *An. culicifacies* E in comparison with other sibling species. *An. culicifacies* E collected from Nawarangapur, Nuapara and Keonjhar district showed high HBF percentage and sporozoite rates. *An. culicifacies* B was the most abundant species, followed by *An. culicifacies* C and E. High sporozoite rate and HBF of *An. culicifacies* E indicated that it plays a major role in malaria transmission in Odisha (Fig. 7).

Diarrhoeal Disorders

During 2013-14 the centre carried out an outbreak investigations of severe diarrhoea in tribal dominated areas; Mohana, Laxmipur, Dasmantpur and Kashipur blocks of Gajapati, Koraput and

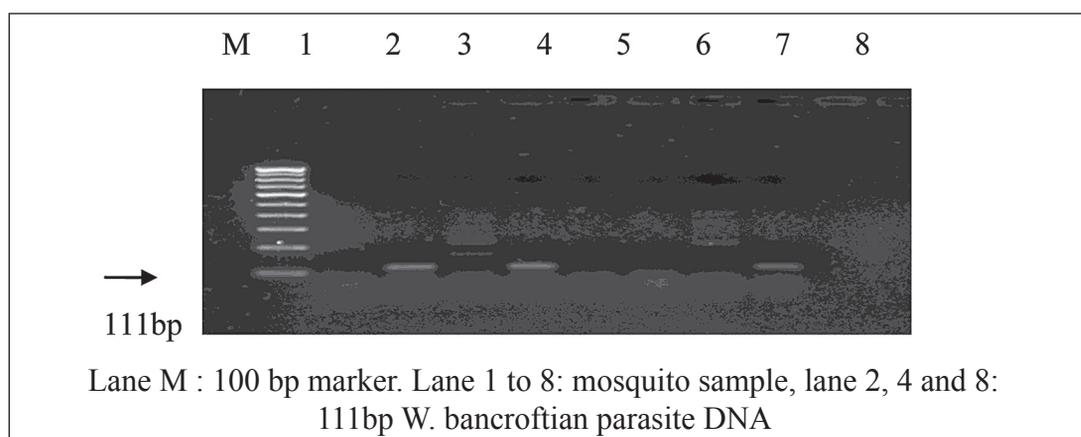


Fig. 6. Detection of *W. bancrofti* L3 by RT-PCR in mosquitoes

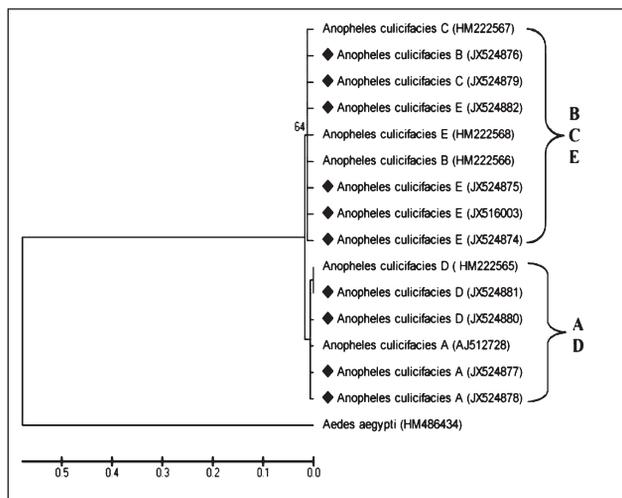


Fig. 7. Phylogenetic tree D3 gene shows that *An. culicifacies* from Odisha were clustered into 2 groups: *An. culicifacies* B, C and E in one group and A and D another group.

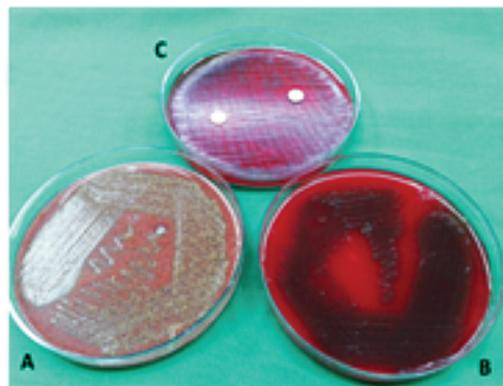
Rayagada districts from October, 2012 to June, 2013. Out of total 107 rectal swabs collected, 81 were culture positive (75.7%) from which 48 (59.2%) were *E. coli*, 16 (19.8%) were *Vibrio cholerae* O1 Ogawa, 11 (13.6%) were *Shigella* spp. and 6 (7.4%) were *Aeromonas* spp, while no *V. cholerae* were isolated from water samples. The early reporting of cholera had helped the health authorities of the district to take adequate control measures which could check the spread of cholera epidemic in this region.

Respiratory Infections

Haemophilus influenzae

The Government of India has planned to introduce a pentavalent vaccine (DPT-Hep.B-Hib) as part of Universal Immunization Programme in a phased manner in selected states of the country. An ongoing surveillance network is critical to facilitate data flow and monitor the changing trends in disease pattern following introduction of potentially lifesaving public health intervention (Pentavalent Vaccine). For this purpose a sentinel surveillance unit has been established at SVPPGIP, Cuttack in 2011-12. During 2013-14 543 CSF and 250 blood samples were investigated from suspected meningitis cases between 1 to 59 months of age. Amongst them 16 samples were found to be latex positive (six for Hib, nine for *S.pneumoniae* and one for group B streptococcus), 3 CSF were culture positive for *Staphylococcus aureus* and 1 was

positive for *Salmonella typhi* and 2 blood samples were culture positive for *Klebsiella pneumoniae* and 4 were positive for *Pseudomonas aeruginosa*. Real time PCR identified 21 cases of *Streptococcus pneumoniae* (fig 6) and 4HiB. Antibiotic sensitivity testing supported case management in the hospital (Fig 8).



A. Growth of *Streptococcus pneumoniae* on chocolate agar plate
 B. Growth of *Streptococcus pneumoniae* on blood agar plate
 C. Plate showing sensitivity of *Streptococcus pneumoniae* to optochin disc

Fig. 8. Figure showing growth of streptococcus pneumoniae isolated from the CSF of a patient suffering from bacterial meningitis.

Tuberculosis

The RNTCP approved Tuberculosis Drug Susceptibility Testing Laboratory has been established at the centre and the lab has been designated as NRL (National Reference Laboratory) during this year. Under surveillance of drug resistance TB in Raygada district, 634 sputum positive tuberculosis cases were subjected for drug susceptibility testing with the four first line drugs, where mono resistance was seen in 35, i.e. to isoniazid in 14 cases & streptomycin in 21 cases and 3 isolates showed resistance to isoniazid and rifampicin (MDR). However MDR was not observed in any of the newly diagnosed TB cases (n=577). The prevalence of MDR TB in Raygada was estimated to be (0- 7%) and the diagnosis of MTB including drug sensitivity results to 634 individuals has been given by the RNTCP for treatment.

Virology Network Laboratory

During this period antibody detection for CMV, EBV, Coxsackie, Westnile and antigen detection for Noro virus has been added and one outbreak

of jaundice, 4 chickenpox, 4 measles, 2 rubella and 1 encephalitis out breaks covering 11 districts have been investigated along with state health department and total 39 different types of viruses have been detected .They were HSV I, HSV II, JE Virus, Dengue, CHIK, Rota, Astro, Adeno(Enteric), Noro G1, Noro G2, Coxackie, Measles, Varicella, Mumps, Rubella, Entero HAV, HEV, HBV, HCV, HDV, HPV, EBV, CMV, Adeno, Influenza A(FluA), FluA(H1N1), Flu B, HMPV A/B, Rhino, Para influenza 1, Para influenza 2, Para influenza 3, Para influenza 4, RSV A/B, Corona viruses(Cor63,Cor229,Cor43, HKU1), Parecho virus, Boca Virus(HBoV) and EV. The enteric virus, Rota, found during this year mostly belongs to G1, G9, G10, and G12 types and P8, P10 types. HAV infection were observed in 6-15 years of age group (42.8%) of patients while, HEV infection was high in adult population (56.8%). Amongst the respiratory infections Adeno Virus was most

common (22.2%) followed by Human Boca, Rhino and Corona Virus. Neurotropic viruses causing acute encephalitis admitted to different hospitals were investigated and clinical manifestations described. HSV, Measles, Dengue and Varicella zooster virus were seen as the major causes of viral AES either as single or co-infection (Fig 9-11).

Maintenance and Isolation of virus

Vero, HEp-2, A 549, RD, C6/36, MDCK, LLC-MK2 viruses are now maintained in the RMRC Virology laboratory. Isolation of HSV is being done from Vero cell line. Inoculation and isolation for viruses like Rota, Dengue, Chik and JE is being attempted. Culture of JEV through intra cerebral inoculation of JEV culture (supplied from KGMU Lucknow) in one day suckling mice has been successfully done for diagnosis and various other research purposes (Fig. 12).



Fig. 9a. Haemagglutination Assay for Influenza A virus .

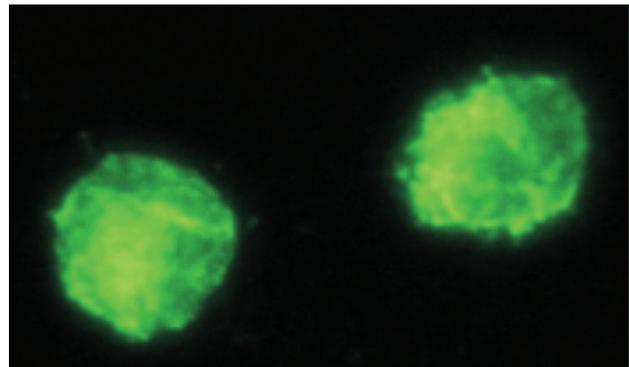


Fig. 9b. Immunofluorescence assay for HSV.

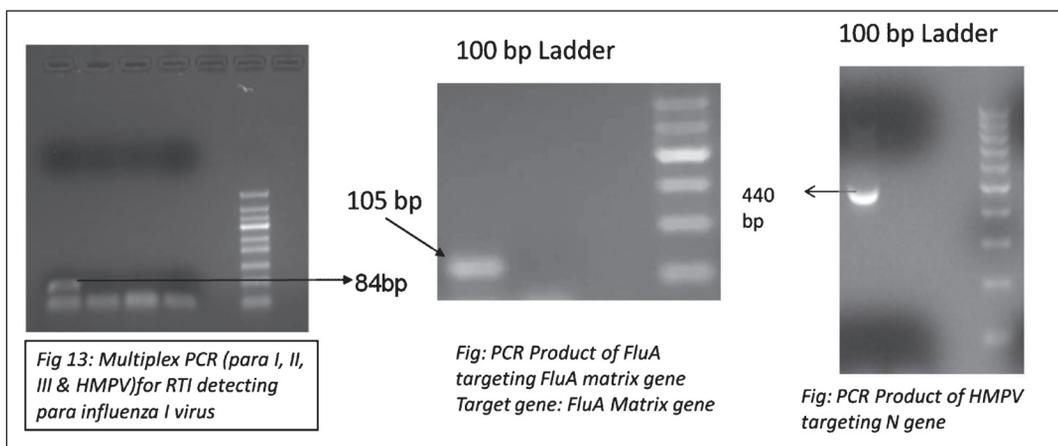


Fig 13: Multiplex PCR (para I, II, III & HMPV)for RTI detecting para influenza I virus

Fig: PCR Product of FluA targeting FluA matrix gene Target gene: FluA Matrix gene

Fig: PCR Product of HMPV targeting N gene

Fig. 10. Detection of ARIs by multiplex PCR.



Fig. 11. A young boy with Varicella Rash.

Dengue & Chikunguniya

The samples from suspected viral diseases (Dengue and Chik virus) were collected from outbreaks or sporadic hospital admissions. Nine districts reported cases of dengue. A total of 1738 number of samples were received; 169 from outbreak investigation and 1569 from different hospitals. Out of the total

samples tested for Dengue IgM 123(18.3%) were positive, where as in 1161 (71.8%) cases NS1 antigen was detected. All four serotypes were detected along with combination of two serotypes (Fig.13). During 2010, serotype II was prevalent where as in 2013 all four serotypes were reported with serotype II as the dominant type. Serotypes I, II and III were predominant in the northern region, where as in southern regions serotype II and IV were common. Three maximum likelihood phylogenetic trees were constructed for 3 serotypes with available reference sequences isolated from different regions of India. A 362 bp product from CprM region were identical in 5 isolates and the sequences had 99% similarity with North Indian strains. All isolates of Serotype I fell into genotype III cluster and grouped with North Indian strains isolated during 2005 and 2010. Sequences of samples positive for Serotype II had clustered with sequences of genotype IV with 99% identity with strains isolated from Kerala and 98% identity with North Indian strains. Sequences for Serotype III are of genotype III and maximum identity (99%) was observed with strains isolated from Delhi during 2003 (Fig. 14).

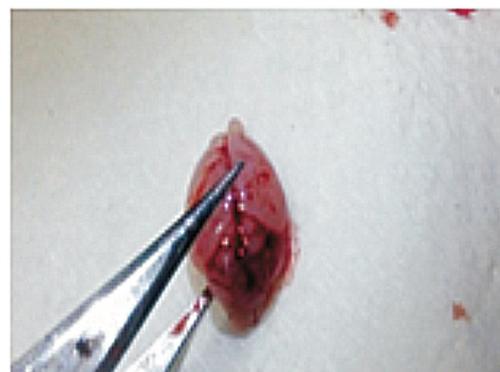


Fig. 12. Inoculation of JE virus in mouse brain and dissection of the brain.

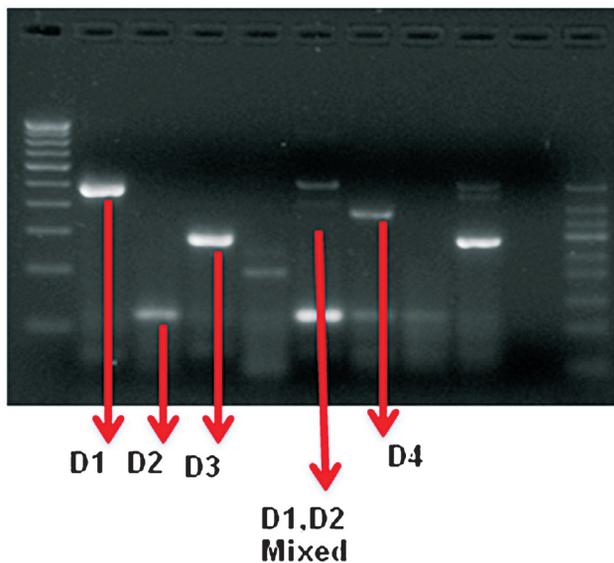


Fig. 13. Dengue Serotypes by PCR.

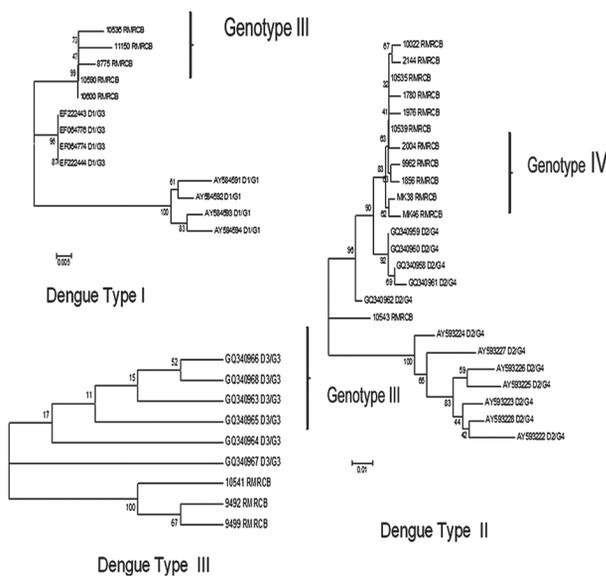


Fig. 14. Phylogenetic tree of dengue serotypes.

However Chik cases are being reported from four districts. Out of total 15 samples received for suspected Chikungunya infection and tested for Chik IgM out of which 4 (23.5%) were found positive for the same.

HPV infection in Women

A total no. 74 of cases with chronic cervicitis or suspected cervical discharge were enrolled during 2013. Cervical swab samples have been collected from the patients attending OPD at AHRCC, Cuttack. Married female aged 21 and above with grades of cervical lesion were included. HPV

detection method was standardized by using MY 11/9 primers which amplify the L1 region. Out of 74 cases, in 14 cases HPV was found to be present. Among 36 subjects of age group between 20-50 yrs, 5 cases were associated with HPV infection. Out of 38 enrolled cases of Age group 50-85yrs, 9 cases are associated with HPV infection (Fig 15).

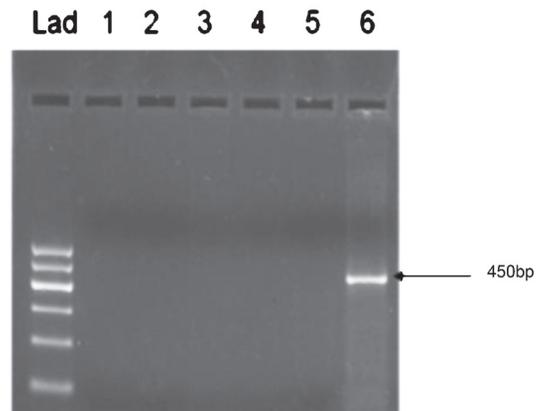


Fig. 15. PCR detection of HPV virus.

Enteric viruses

Total 735 patients with diarrhoeal disorders were investigated for enteric viruses, those were enrolled during 2013. The subjects were mostly children below 5 years who presented with moderate to severe diarrhoea. Rota antigen was detected in 32.68% (n=719) and Adeno in 2.63% (n=223) of cases where as Astro and Noro antigen test was negative for all cases. The most common viral agent was Rota. Specimens were subjected to PCR using type specific primer for genotyping of Rota virus. Majority of G(G1,G3,G9,G10,G12) types and all P(P[4],P[6],P[8], P[11]) types were detected. In all the groups G1and G9 was common among all G types and P8 and P11 were common among all P types (Fig. 16).

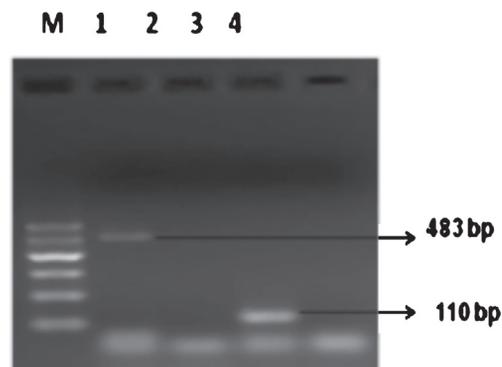


Fig. 16. PCR detection of rotavirus P and G genotype.

Tribal Health

A study was undertaken to evaluate the adolescent reproductive and sexual health programme in Orissa to identify gaps and implement advocacy strategies for effectiveness. Formative research carried out in selecting two blocks (one sector covering 3 sub-centres) in Kalahandi (Dharmagarh and Junagarh) and Rayagada (Jamadehipentha and Gunupur) districts covering 2087 adolescents revealed that very poor knowledge, attitude and behaviour on adolescent reproductive and sexual health issues and quality of care at Adolescent Friendly Health Clinics, accessibility and utilization of health care services. Advocacy strategies implemented for one year in two study sectors quarterly covering adolescent males and females and married adolescents independently conducted peer group education, counselling sessions on adolescent health issues, availability and accessibility of health care services. Orientation training imparted at sector level for stake holders (ANM, Anganwadi Workers, ASHA) about quality of care at community and facility levels. Endline impact assessment revealed that study blocks had considerable improvements (10-45%) in adolescent reproductive (puberty changes, menstrual hygiene, age at marriage, consequences of teenage pregnancy) and sexual (early marriage, contraception, RTI/STI, health programs) and quality of care at facility and service providers levels. The centre has developed logistic IEC materials (pamphlets and flip-charts) in English and Odiya in creating awareness on adolescent health issues and accessibility of quality care and outreach services. This will help to strengthen the programme for effective implementation of quality care and coverage.

An ongoing activity under tribal health research forum is being undertaken to develop a morbidity management strategy for febrile illness through syndromic approach in tribal population of Rayagada district. Baseline morbidity survey was undertaken in 5800 population covering 22 villages from 3 adjacent sub-centres of Jemaidepentha CHC in Rayagada district. Prevalence of febrile illness was 9.5 and 1.8% of the surveyed population during a fortnight period of rainy and winter seasons respectively. Total morbidity including non-febrile

disorders was 21.9 and 6.44% of the population during above seasons. Among the febrile illness, acute respiratory infections (62%), malaria (22%) & diarrhoeal disorders (10%) were common during rainy season, while in winter, URTI was commonest. Both the extremes of ages (<5 yrs & more than 60 yrs) were predominantly affected. Bacterial infections (*S.pneumoniae*, *H.influenzae* & *Staph aureus*) were identified as the cause of ARI in 75% cases followed by viral infections (Corona & Para influenza) in 20% of ARI cases. *P. falciparum* was the dominant (87%) malaria parasite. Diarrhoeal disorders were associated with *E. coli* in 30% & Rotavirus in 10% of cases. Under nutrition in childhood was found as an underlying risk (71.4% in under 5). Average delay in seeking treatment was 2-3 days and their was multiple preferences for seeking treatment but qualititative study findings revealed ASHA/AWW as common source available in village for seeking treatment. The population demography showed a distorted pyramidal structure with low population in higher age group (>50yrs) compared to normal population. To answer the above distortion, cause of death was analyzed by verbal autopsy procedure, covering 52 deaths in past 1 yr. The common causes of death noted were cardiovascular diseases, pulmonary tuberculosis, malaria and liver diseases. Based on preliminary finding of morbidity prevalence including febrile illness its aetiology and treatment seeking behaviour strategy has been developed and implementation initiated, 18 ASHA of 25 villages have been trained on RDT use and syndromic management of febrile illness, 25 community volunteers have been identified and trained on febrile illness identification and warning signal for referral

Health situation of Migratory Population

With respect to the access and responsive of the health system towards migratory population, the centre has found that migrant community usually experience common health problems like common cold & fever, gastritis, diarrhoea, abdominal pain, vomiting, dysentery, asthma and T.B. Around 45 % availed regular health services from government health facilities. The husband or elder people like mother in law / aunt are the decision maker for health issues. Among the pregnant women 80%

opted for institutional delivery and 56% of them had gone for 3 visits of ANC and 90% have received more than 2 doses of injection TT in urban slums of Bhubaneswar MC. The individual component of primary MCH services indicates a good coverage. But when all components combined together, the coverage is very low (around 12.9%) only. Therefore an interventional package combining the approaches of inclusive partnership with government and non-governmental providers, CBOs, community participation and community mobilization for better health service utilisation has been developed and is being implemented. Around 45% migrant population accessing govt health facility and ANC care was around 15% based on these finding strategy has been developed and implemented and evaluation will be carried out shortly

Technology Transfer

Two field units have been established in tribal areas at Rayagada and Kalahandi District Head Quarter hospitals. These units have been equipped to conduct screening and preliminary investigation by serology (ELISA) & PCR on suspected viral diseases. The district health technical staffs were trained on Antigen Antibody detection by ELISA for Dengue, Chikungunya, Measles, Rota virus and Hepatitis infection. This lab is supporting surveillance of endemic and epidemic investigation by the centre in close association with state health unit.

Translational Research

Centre has taken effort to develop two PCR based tools for public health use. One is to monitor the information of vector prevalence, incrimination of vector for malaria transmission, identification of the sibling species of vector and chloroquine (CQ) sensitivity of the parasite ingested by the vector. This technique has been internally validated and the practical aspects of the technique has been demonstrated to the researchers of NIMR and programme personnel of the state NVBDCP in workshop held at Regional Medical Research Centre, Bhubaneswar. The technical manpower has been developed for the programme that will help for rapid monitoring and evaluation of malaria epidemiology.

The other tool developed by the centre is to detect all different serogroups of *V. cholerae* causing cholera in a single PCR test. In-house validation of the technique has been done. Applicability of both the tools is being now field tested. The assay has been validated at NICED, Kolkata and a kit has been developed that can be used for rapid diagnosis of cholera.

REGIONAL MEDICAL RESEARCH CENTRE, NE REGION, DIBRUGARH

The focus of research work of RMRC, Dibrugarh during the year continued to be on cancers, cardiovascular disease & hypertension, diabetes, and haemoglobinopathies among the non-communicable diseases; and vector-borne diseases (including mosquitoes, ticks and mites), viral diseases, bacterial diseases and food borne trematode infections, among the communicable diseases.

EPIDEMIOLOGY AND CLINICAL RESEARCH

Cancer

A population based matched case-control study of Genetic and Molecular Epidemiology of Stomach Cancer in Mizoram of North Eastern Region of India was completed. A total of 105 histologically confirmed stomach cancer cases and 210 matched healthy population controls were recruited. Investigation revealed that the *CYP2E1 RsaI* genotype distributions among Mizo community are 87.3% (C/C), 12.4% (C/A) and 0.3% (A/A). No association between the *CYP2E1 RsaI* polymorphism and overall risk of stomach cancer was observed. Tobacco smokers, smoked dry fish and preserved meat consumers carrying C/C genotype had higher risk of stomach cancer. XRCC3 codon 241 Thr/Met polymorphism had a protective effect against stomach cancer in Mizoram. It was found that NAT2 acetylator status modifies the effects of dietary carcinogens and tobacco smoke on stomach cancer.

In the *Comparative Study of Genetic, Clinical and Epidemiological Factors of Breast Cancer in Rural and Urban Area of India*, 1348 nos. of blood samples (387 breast cancer (BC) cases and 961 controls)

as well as biopsy samples (breast cancer patients) was collected. Betel nut chewing with or without tobacco was found to be an important risk factor of BC in Tripura but not in Mizoram. Smoking habit was found associated with BC in both states. Genetic mutations in BRCA1 gene was found in 12% cases of Mizoram but only 2.44% of cases from Tripura. Carriers of mutations in XRCC1 and XRCC3 and TP53 genes significantly increased the risk of breast cancer subjects from Mizoram and Tripura. Next Generation Sequencing data along with TaqMan allelic discrimination facilitated in identification of a novel mutation in PKHD1 gene which predisposed carriers of the variant allele to increased risk of breast cancer.

Epidemiological investigation of oesophageal cancers were carried under two studies titled (1) *Genome-wide analysis of genetic alternations in patient's oesophageal cancer from North-East India using single nucleotide polymorphism arrays* and (2) *Epigenetic studies in oesophageal cancer in high risk region of Northeast India*. In the genome wide analysis study 135 samples from cases and 140 from controls were collected. Squamous cell carcinoma was found to be mostly predominant (90.7%). Topography inclination is more towards middle-third of oesophagus among both the sexes. Most cases are moderately differentiated (G2) (81%) irrespective of sex. Chewing of tobacco with betel nut-betel leaf (98.4%), smoking (56.1%) and consumption of alcohol (41.5%) is very high among the cases. Under the *Epigenetic study of oesophageal cancers* 65 samples from cases and 68 from controls have been collected. Among the cases 70.8% are males and majority of them belong to the 51-60 yrs age group. Squamous cell carcinoma type was mostly predominant (95.9%) followed by adenocarcinoma (4.1%). Most (79.6%) of the tumours are moderately differentiated, 12.2% are well differentiated and 8.2% poorly differentiated. Tobacco chewing habit is seen among 89.9%, smoking in 71.4% and drinking habits in 36.7% of the cases. 59.2 and 69.4% of the cases consumed smoked food and pickle respectively.

Estimation of cancer disease burden through 12 population based cancer registries in north-east India was continued.

Hypertension & CHD

In view of the high prevalence of hypertension among tea garden workers in Assam, a *community based study to assess the impact of community based dietary salt restriction in the reduction of blood pressure among tea garden workers* is being carried out in two phases. The first phase was a cross sectional study to find out correlation between dietary and blood pressure. It was found out that overall prevalence of hypertension was 50.7% (Male: 52.9%, Female: 47.7%) and consumption of extra salt was high among the hypertensive cases. The second phase was a community based intervention by dietary salt restriction with a target to bring down daily salt consumption of 6 gm/day. It was carried out in two tea gardens: intervention with active IEC activities and control garden with usual care. So far 465 (Male: 236, Female: 229) & (Intervention: 270; Non intervention: 195) participants have been included. It is revealed that participants of intervention vs. non intervention groups are comparable in respect to age, gender and prevalence of hypertension. Range of biochemical parameters in the both groups are within normal limits. In the intervention garden, mean SBP changes from the baseline value of 139.5 ± 26.5 to 135.5 ± 15.2 and DBP changes from 84.3 ± 14.8 to 80.7 ± 10.7 after intensive IEC intervention & follow up after 6- 8 month. The prevalence of hypertension also decreased from the baseline value of 57.6% to 45.6%.

Diabetes

The ICMR *INDIAB study (NE component)*, was completed. In Assam 99 primary sampling units (PSU's) (Urban: 49: Rural 50) involving 3992 households were surveyed in 26 districts with overall response rate of 97.90%. In Assam, the overall type 2 diabetes mellitus (T2DM) prevalence is 5.4% & prediabetes is 11.8% with a higher prevalence in urban compared to rural areas. Overall ratio of known to newly diagnosed T2DM in Assam is 1:0.8 while the ratio in urban & rural areas is 1:0.5 & 1:1 respectively. The prevalence of hypertension, dyslipidemia & metabolic syndrome were also higher in urban compared to rural areas of the state. In terms of glycemic control, in urban areas 37.70% had good control, 45.30% had fair & 17% had poor control & in the rural areas 46.20%

had good, 28.20% had fair & 25.60% had poor control amongst the self reported diabetic subjects. About 64.60% diabetics were managed with oral hypoglycaemic agents (OHA), 19.10% with diet, 9.20% with insulin + OHA & 7.10% on insulin therapy alone. In Mizoram 102 nos of PSU's (52 Rural & 50:Urban) involving 4112 households were included. The prevalence of diabetes & prediabetes was 5.7 & 5.8% respectively with a higher prevalence in urban compared to rural areas. The overall ratio of known diabetes to newly diagnosed diabetes was 1:1, with a ratio of 1:0.9 & 1:1.5 in urban and rural areas respectively. The prevalence of hypertension, dyslipidemia & metabolic syndrome were higher in urban as compared to rural areas. In terms of glycemic control, 23% and 18.90% had poor glycemic control in rural & urban areas respectively.

Mosquitoes and Mosquito Borne Diseases

Malaria

Two collaborative projects are undergoing with Department of Pharmaceuticals Sciences, Dibrugarh University (i) *Synthesis of hybrid 4-Aminoquinoline 1,3,5 – triazine derivatives and evaluation of their antimalarial activity and (ii) Synthesis of hybrid class phenylthiazole 1,3,5 triazine derivatives as novel antifolate for Pf-DHFR-TS inhibition* to screen the newly synthesized hybrid class 4-Aminoquinoline 1,3,5-triazine derivatives and phenylthiazolyl- 1,3,5-triazine derivatives for antimalarial activity. Out of the 20 compounds screened against chloroquine sensitive 3D7 strain 2 compounds showed some appreciable antimalarial activity.

Screening of indigenous medicinal plant varieties for development of potential antimalarial drug/drug intermediates and characterization of potent plant species through DNA barcoding was carried out. A total of 66 crude extracts, belonging to 31 plants have been screened against chloroquine sensitive strain of Pf out of which 7 extracts were detected to contain promising antiplasmodial activity. IC₅₀ values of these 7 extracts were also determined.

In the recently completed study titled *Field bio-efficacy evaluation of DRDO Defender net against mosquitoes*, Defender net, a 100 denier polythelene factory treated LLIN incorporating deltamethrin

55 mg ai/m², developed indigenously by DRDO, Govt of India, was evaluated for bio-efficacy after repeated standard washing. It was revealed that the average deltamethrin content available in Defender net after 5 washings was 13.12 ± 3.97 and after 7 washings was 14.05 ± 0.41 mg ai/m². Defender LLIN was found effective only up to 5 washes in the laboratory bioassays and no significant impact of Defender LLIN on mosquito densities was evident over the plain net in Phase II village hut level bioefficacy trial in Assam.

Under the study titled *Nutritional factors and severity of malaria –a study from North Eastern region of India* a total of 247 blood samples have been collected from suspected cases from different districts of Assam and Arunachal Pradesh of whom 143 cases were found positive. *Plasmodium falciparum* (Pf), *P. vivax* (Pv) and mixed (Pf+Pv) were found in 109, 9 and 25 subjects respectively by slide test while 19, 2 and 24 samples were positive for Pf, Pv and both Pf & Pv respectively by PCR.

Two ongoing studies investigating the therapeutic efficacy of ACT+SP along with molecular parasite diversity and drug resistance 1) *Folate metabolism pathway gene polymorphism among symptomatic and asymptomatic malaria patients from North East India: a possible genetic selection from malaria* and 2) *Molecular Epidemiology of Malaria in India and Qatar with an Emphasis on Parasite Diversity, Drug Resistance and Immune Response* were continued.

Filariasis

Three studies viz. (i) *Multi-centric evaluation of L3 stage specific RT-PCR assay for Wuchereria bancrofti in vectors* (ii) *National network for genotyping of Wuchereria bancrofti from different endemic areas* and (iii) *Lymphatic filariasis endemic mapping in the state of North East India* were carried out. Stage specific (L3) RT-PCR assay was found quite sensitive. Under the genotyping study (project ii) 48.0% of the Mf samples from Dibrugarh had sheath. In the study on microfilaria prevalence and endemicity of lymphatic filariasis a total of 300 selected participants' were screened using ICT filariasis diagnostic kit. Two clinical (elephantiasis) cases were reported from the survey population. In indoor, morning entomological

collections, *Culex quinquefasciatus* was recorded in all the seven locations studied.

Japanese Encephalitis /Acute Encephalitis Syndrome

Under the ICMR task force study to find out the *Aetiology of Acute Encephalitis Syndrome (AES) in India and Establishment of ICMR sample bank of AES cases - a multisite prospective initiative* a total of 563 blood/cerebrospinal fluid samples (or both in some cases) collected from patients with Acute Encephalitis Syndrome (AES) were screened for the presence of Japanese encephalitis (JE) out of which 49.38% were found to be positive. The JE equivocal and negative AES samples were found positive for presence of IgM antibody against Dengue (3/154, 1.95%), Chikungunya (2/89, 2.25%), Leptospirosis (39/151, 25.83%), Scrub typhus (1/34, 2.94%), Rubella (2/82, 2.44%), Measles (3/61, 4.92%), Mumps (4/46, 8.7%), HSV-1 (4/60, 6.67%) and HSV-2 (2/53, 3.77%).

The *Study on Eco-Epidemiological Perspectives of Emerging West Nile Virus in Assam* was continued. 198 clinical serum/cerebrospinal fluid were collected from AES patients from Dibrugarh and Guwahati of which 11.11% (22/198) was positive for WNV. All the positive cases were found to cluster around the months of June to September 2013 and males in the higher age group were predominantly affected. A total of 33.33% convalescent serum of the positive cases showed a 4-fold rise in the antibody titer for WN neutralizing antibodies. Standardization of the end point RT-PCR and two step qPCR was done using WNV strain WNIRGC07 (GeneBank ID: HQ246154).

Visceral Leishmaniasis

An exploratory study in visceral leishmaniasis endemic areas of Assam was done to find out kala-azar endemic areas and vector population responsible for disease transmission. A total of 168 kala-azar cases have been reported till date (old and new) from the different study areas. Active surveillance done by house to house visit in 2013 reported new cases of Kala-azar. During the survey, four PKDL cases have been recorded from the study locations. Entomological collections using CDC light tarp, sticky trap and flash light & aspirator revealed presence of sand fly (*Phlebotomus* spp.)

in the study locations

Paragonimiasis

In the study entitled *Identification of immunodiagnostic antigenic fractions of Paragonimus species by western blotting*, crabs were collected from Assam, Meghalaya and Arunachal Pradesh for detection of metacercariae of lung flukes. 36 out of 675 crabs examined were found infected with metacercariae. Rats fed with *Paragonimus* metacercariae were checked routinely and were sacrificed at different points of time to collect serum and flukes. From the adult worms E/S antigen and egg antigen was prepared. SDS-PAGE of soluble somatic adult worm antigens from *P. westermani* revealed at least 27 bands ranging from 225 to 7 kDa using 4 to 20% gradient gels. The 13 prominent bands were 225, 192, 100, 75, 66, 42, 33, 26.5, 24, 21, 17, 11.5 & 7 kDa. Antigenic fractions below 50 kDa were found to be more prominent.

Bacterial Diseases

To find out the *Genotyping diversity and drug susceptibility and mutation patterns among Mycobacterium tuberculosis complex from Assam, India* 312 sputum samples of smear positive MTB cases were collected from the selected Designated Microscopy Centers from the 5 districts of Upper Assam, out of which 254 patients were newly diagnosed and 58 were previously treated cases. So far 214 samples showed growth on culture. In newly diagnosed cases the prevalence of any drug resistance ranged from 2.6-23.1%, resistance towards rifampicin was about 2.56% and all rifampicin resistant cases were MDR strains. In MTB isolates from previously treated cases, any drug resistance ranged from 18.2-27.3%, resistance towards rifampicin was 18.2% and all the rifampicin resistant cases were also MDR strains. Overall 64% of the isolates were sensitive to all the first line anti-tubercular drugs.

Two studies on neonatal infections i) *study of neonatal meningitis in a tertiary care hospital* ii) *Surveillance of Infection in neonates (0-28 days)* were continued. Out of 224 CSF samples obtained (AMCH, Dibrugarh) 71 were positive by either culture or PCR. In the task force study of *Surveillance of Infection in neonates (0-28 days)* 14 gram negative bacilli and 44 gram positive bacteria were obtained.

The common bacterial isolates obtained were *Acinetobacter* spp, *Enterococcus* spp, *Micrococcus luteus*, *Neisseria meningitides*, *Staphylococcus aureus*, *Klebsiella pneumoniae* spp *pneumonia*, *Escherichia coli*, *Aeromonas salmonicida*, *Leclercia adecarboxylata*, *Serratia marcesens*, *Pantoea agglomerans*. Resistance were observed among commonly used drugs in neonatology: ampicillin (94%), amikacin (56.5%), cefotaxime (64%), ciprofloxacin (64%), gentamicin (59%), meropenem (31%), linezolid (15.3%), vancomycin (27.3%), netilmicin (33.3%), piperacillin (66.7%), piperacillin tazobactam (40%) of the CSF isolates tested. ESBL was detected in (33.3%) of the gram negative isolates (surveillance).

RMRC is investing the *Epidemiology of scrub typhus in selected areas of Assam and Arunachal*. During the reporting period 145 blood samples from suspected cases were collected and screened for scrub typhus (STG) specific IgM and IgG antibodies. Of the 145 samples, 22.06 % (32/145) were found to be IgM positive for *O. tsutsugamushi*. Another 11.03 % (16/145), 0.09% (14/145) and 0.006% (1/145) were IgG positive for Scrub typhus group, Spotted Fever group and Typhus group, respectively.

Viral Diseases

In the ongoing *Phase II multisite monitoring of human influenza viruses* study, 309 nasal/throat samples were collected from 4 PHCs and a referral hospital in Dibrugarh district. About 11% (n=34) samples were found PCR positive for *Influenza B* (n=8), *Influenza A/H3N2* (n=26). 17 influenza viruses (both *Type-B*, *Influenza A/H3N2*) could be isolated.

Under the ICMR Task Force study on *National hospital based rotavirus surveillance network* diarrhoeal stool samples were collected from children ≤ 5 years from AMCH, Dibrugarh and District Hospital, Dimapur. The overall prevalence of rotavirus in Dibrugarh is 38.7% (Rotavirus Ag detection ELISA) with a higher prevalence in infants (52.8%) and in Dimapur was found to be 49.8%. The circulating genotypes of rotavirus detected were most commonly G1P [8], G9P [8], G12P [6], G2P [4] and around 20% P type were untypeable.

Two capacity building project (i) *ICMR Virology network Lab-Grade-I (VDL-1)* and (ii) *Upgradation of the Virology Division to a nodular molecular Virology Lab dedicated to focus on HIV, Hepatitis and Influenza viruses in northeast India* have been initiated. During this period 582 samples were tested for various viral infections. Various outbreak investigations were also carried out in Assam and other Northeastern States. HBV DNA viral load was performed on 60 cases of CHB of which low viral load was seen in 60.3 %, intermediate in 11.6% and high in 28.1%. HBV genotype C (51.3%), genotype A (32.4%) and genotype D (16.2%) were found prevalent. HBV polymerase gene was sequenced and computational docking study was done with phyllanthin and Lamivudine. An in-silico study of 76 natural compounds from *Azadirachta indica* (Neem) against HIV-1 protease showed Naheedine and Nimocinolide as promising drug candidates. HIV partial Gp41 and region gene sequencing showed circulating recombinant form (CRF08-BC) in 4 samples, CRF01-AE in 1 and genotype C in 2 samples.

Two investigations related to hepatitis virus infection (i) *genotyping of hepatitis B virus in north-east India* and (ii) *host innate immunity and hepatitis B persistence* were carried out. Under the *genotyping study* the prevalence of HBV (HBsAg) was 1.3% among blood donors, 1.8% among health care workers, 14.6% among AVH, 40.6% among CAH, 40% among FHF, 16.6% among HCC and 9% among cirrhosis cases. Genotype C was detected in 38.6 %, genotype A was detected in 34.6%, genotype D in 11.5%, mix genotype C/A in 11.5% and genotype C/D mix in 3.8%. *In the host innate immunity and hepatitis B persistence* study a total of 133 subjects have been recruited (control group: 36; study group: 97). Low viral load was seen in 48.8%, intermediate in 17.1% and high viral load in 34.1% of cases. Among the cases 97.9 % (n=95/97) were positive for HBcAb, 29.9% for HBeAg and 61% for HBeAb. In the relative expression study of 26 different genes (innate immunity & six control genes), gene upregulation seen in CHB are IRF3 (3.7 fold), CD40, FOXP3 and GZMB (> 2 fold) while IFNAR2, CD28, FOS and FOXP1 (~ 2 -fold) were down-regulated. TLR1, 2, 4 (> 4 fold) 6 (2-fold), 7, 8, 9 (1.5 fold)

were downregulated while TLR3 and 10 (1.5 fold) were upregulated in CHB compared to controls.

Others Studies

A study has been initiated to *explore and contextualize sexual and injecting risk behaviours among Female Sex Workers (FSW) and sexual interface between FSWs and Injecting Drug Users (IDU) in Churachandpur district of Manipur.*

A task-force 'Biomedical Informatics Centres of ICMR' NE Region Dibrugarh has been initiated this year to promote and support informatics in medical research. Ten compounds of *Brucea mollis* Wall. *Ex kurz* were checked for their antifolate-based antimalarial efficacy of whom 3 (Soulameanone, 9-methoxycanthin-six-one and Inosine) have passed the initial drug likeliness property. To develop National Repository of clinical information/data, high-throughput data, genotype and phenotype" a blue print has been prepared for centralized data storage of studies carried out by scientists of the centre.

The study titled *Socio-cultural features and stigma of leprosy for treatment and control in general health services in India: Cultural Epidemiological Study* was recently completed. 206 individuals were contacted and 145 interviews were completed. A total of 80.8% reported skin patch as the main physical/somatic symptom. Higher proportion of leprosy patients think that others may refuse to visit their home (25.64% vs 22.81%), could not point to a most important perceived cause (2.6% vs. 1.8%), sought first help from private allopathic doctors (9% vs. 1.8%) as compared to non-leprosy patients.

Two surveys on haemoglobinopathy for the Clinical, Anthropometric and Biochemical (CAB) component of i) District Level House Hold Survey -4 (DLHS-4) and ii) Annual Health Survey (AHS) in 16 districts of Arunachal Pradesh and 4 districts of Sikkim were continued.

Outbreak Investigations

The Centre investigated outbreak of acute respiratory tract infection (RTI) with diarrhoea in West Siang Dist. of Arunachal Pradesh. Around 400 subjects were affected of whom most were children with report of 7 deaths. Out of the 37

human respiratory samples collected 8(21.6%) were positive for *Influenza Type A/H3*. The report was communicated immediately to concerned health authority of Arunachal Pradesh.

The Centre investigated diarrhoeal disease outbreak in a tea garden of Dibrugarh district, Assam. Three of the 5 rectal swabs collected showed the growth of *Vibrio cholerae* O1 El tor serotype Ogawa. The isolates were sensitive to *ciprofloxacin*, *tetracycline*, *piperacillin/tazobactam*, ceftriaxone, amikacin and resistant to amoxycylav, cefepime. The investigation report was shared with the state health agencies for taking the control measures.

An outbreak of pyrexia of unknown origin (PUO) in a district of Arunachal Pradesh was also investigated. Twenty nine out of 32 blood samples collected were reactive for IgM antibodies against *Orientia tsutsugamushi* (Scrub typhus). The report was communicated to the concerned health Dept. of Arunachal Pradesh for taking necessary measures.

The centre also investigated an outbreak of acute viral hepatitis in Lakhimpur District of Assam. Acute viral hepatitis A was detected in 18 out of 21 samples. The report was communicated to the health Dept. of Arunachal Pradesh.

REGIONAL MEDICAL RESEARCH CENTRE FOR TRIBALS, JABALPUR

TRIBAL HEALTH RESEARCH FORUM

Tribal health Research Forum (THRF) is a flagship programme of ICMR which was established in 2010 with an aim to address and provide holistic solutions to health issues pertaining to the tribal population. During the period under report two meetings were conducted at VCRC, Puducherry and DMRC, Jodhpur. Recently, 6 new Tribal Health Research Units were established at RMRCT, Jabalpur, RMRC, Bhubaneswar, RMRC, Port Blair, RMRC, Dibrugarh, NIN, Hyderabad, and NIIH, Mumbai. Under the new initiatives following studies were undertaken:

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:

The common protocol was developed by NIIH, Mumbai involving RMRCT, Jabalpur and Valsad Raktadan Kendra, Gujarat.

Developing an innovative tribal health system model to estimate the burden of TB, co-infections and improve the effectiveness of RNTCP in India- A multicentre study

A common protocol was developed by NIRT, Chennai for the proposed/ multicentric study Task Force study on assessing burden of TB (and co-infections) among the tribal population. The primary objective of this study is to develop a tribal health system model with feasible interventions to improve case finding and compliance for TB treatment through a community based approach. This is a multicentre study involving ICMR institutes and Medical Colleges in different regions covering 5 zones (East, West, North, South, and North East).

Tribal Health Research Unit, RMRCT, Jabalpur: Tribal Health Research Unit was established at RMRCT, Jabalpur in the year 2013 and it acts as a nodal unit/ coordinating the Tribal Health Forum's activities across 16 partner ICMR institutes. It has prepared a document on "Overview of tribal population in India" based on Census 2011 data and was published as Special issue of Tribal Health Bulletin, Volume 20, January 2014.

VECTOR BORNE DISEASES

To determine the effectiveness of intensive intervention measures on malaria prevalence in tribal district, Dindori, Madhya Pradesh (MP)

A study was carried out in forest villages of Baiga chak area, district Dindori to evaluate new intervention measures for developing a suitable model for forest malaria control in collaboration with State Vector Borne Disease Control Programme. These interventions are: Two rounds of IRS using synthetic pyrethroid, long lasting insecticide treated nets (LLINs), Rapid diagnostic test (RDT), Artemisinin based combination therapy (ACT) and Intensive Information, Education and Communication (IEC) / Behavior Change Communication (BCC).

Monthly cross sectional fever surveys were carried out door-to-door in study villages. Indoor resting

Anopheles mosquitoes (per man hour), Pyrethrum spray catches (PSC) were carried out once in a month. *An. culicifacies* and *An. fluviatilis* were tested for species specific malaria parasite and their sibling species was identified using polymerase chain reaction (PCR).

Rapid fever surveys revealed a total of 71 malaria positive cases (62 Pf, 8 Pv and 1 mixed infection of Pf and Pv) out of total 2201 fever cases screened in the year 2013-14. The slide positivity rate (SPR) was 3.2 % with 88.7% *P. falciparum*. All indices showed a sharp decline as compared to baseline year particularly > 70% decline in SPR and spleen rate reduced to < 5% during this year. The anopheline fauna of the villages consisted of mainly *An. culicifacies*, *An. subpictus*, *An. fluviatilis* and *An. annularis* in indoor resting collection, total catch and light trap catches. The mean density of *Anopheles* caught per man hour during the year was 10.9. Out of 1102 *An.culicifacies* and 108 *An.fluviatilis* assayed by ELISA for detection of Circumsporozoite protein, none of them were found positive for malaria parasite.

Assessment of the effectiveness of intensive intervention measures on malaria control programme in tribal district, Balaghat, Madhya Pradesh

This study was undertaken in villages of Birsa and Baihar Community Health Centre of district Balaghat for developing a suitable model for malaria control in disturbed area in collaboration with State Vector Borne Disease Control Programme. Malaria control measures in this area included mainly indoor residual spraying (IRS) with a synthetic pyrethroid (alphacypermethrin), insecticide treated bed nets (ITNs), Rapid diagnostic test (RDT) and Artemisinin based combination therapy (ACT).

No reduction was recorded in malaria during the intervention period as the SPR & SFR was 33.8 & 30.2 and 46.5 & 36.7 in 2011 and 2012 respectively. Therefore, a new intervention for mosquito control, ZeroVector® Durable Lining (ZVDL) was installed in Jan 2013 in 6 villages covering 3300 population (provided by Vestergaard Frandsen). This is the Deltamethrin incorporated plastic sheet fixed on the inside wall of the houses and bioassay results revealed that ZVDL showed 100% efficacy upto 11

months. Eight villages with a population of 3500 were kept as control.

Fever surveys carried out during the year 2013-14 revealed that a total of 816 fever cases were screened of which 130 were found positive for malaria (120 Pf and 10 Pv). The SPR was 15.9% with 92.3 % *P. falciparum*. However, in control villages where no ZVDL was installed, malaria prevalence was higher. A total of 293 malaria positive cases (261 Pf, 30 Pv and 2 mixed of Pf and Pv) were found out of 885 screened. The SPR was 33.1% with 89.8 % *P. falciparum*. Likewise the average spleen rate in children was 31% in ZVDL villages while 62% in control villages. During the year, in ZVDL villages a total of 435 *An.culicifacies* and 10 *An.fluviatilis* were tested for vector incrimination of which none were detected as sporozoite positive. However, in control villages 4 *An.culicifacies* were found sporozoite positive (2 Pf and 2 Pv strain) out of 1399 assayed (sporozoite rate, 0.28%).

Clinical and molecular surveillance for monitoring the emerging resistance to antimalarial drugs in *Plasmodium falciparum* in Central India

A study was undertaken for monitoring the clinical and molecular resistance to antimalarial drugs (ACT) in uncomplicated *Pl. falciparum* malaria in Balaghat and Anuppur district of Madhya Pradesh. Therapeutic efficacy test was carried out with ACT orally over a three-day period. Genomic DNA was used to amplify the drug resistance genes (*pfdhfr*, *pfdhps* and *PfATPase6*) using the respective gene specific primers.

Three hundred twenty six patients were screened in Balaghat district, out of which 104 were positive for malaria, 96 *P. falciparum* (of which only 81 enrolled), 7 *P. vivax* and 1 showed mixed infections of *P. falciparum* and *P. vivax*. The therapeutic efficacy outcome was determined for 50 patients as thirty one patients did not complete the study. Over all therapeutic efficacy showed 100% adequate clinical and parasitological response.

One hundred twenty two patients were screened in Anuppur district, out of which 32 tested positive for malaria, 30 *P. falciparum* (of which only 26 enrolled), 1 *P. vivax* and 1 showed mixed infections of *P. falciparum* and *P. vivax*. The therapeutic efficacy outcome was determined for 20 patients

as six patients did not complete the study. Over all therapeutic efficacy showed 95% adequate clinical and parasitological response while one case (5 %) showed late clinical and parasitological failure.

Out of 81 cases, 63 were analyzed for *dhfr* mutations at five codons (16, 51, 59, 108, and 164). Seventy one percent parasite population were harboring the mutation while only 29% with wild type. Majority of the parasite population were having double mutations (65%) with double mutant *pfdhfr* $A_{16}N_{51}R_{59}N_{108}I_{164}$ followed by single mutant *pfdhfr* allele $A_{16}N_{51}C_{59}N_{108}I_{164}$ (5%) and 1% was triple mutant *pfdhfr* allele $A_{16}N_{51}C_{59}S_{108}P_{118}$. Total of 56 isolates were analyzed for *PfATPase6* and 82 % parasite population with wild type allele and only 18 % isolates having single mutation at different codon (336- 450). In Anuppur district, out of 26 cases, 18 were analyzed for *dhfr* mutations at five codons (16, 51, 59, 108, and 164). Eighty nine percent parasite population were harboring the mutation and all are double mutations (*pfdhfr* $A_{16}N_{51}R_{59}N_{108}I_{164}$) while only 11% with wild type.

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

The study was initiated in October 2013 in 2 malarious districts i.e. Bastar and Korea (Koriya) of Chhattisgarh. Two CHCs in the district and 4 villages in each CHC were selected for this study. The overall objective is 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 anopheline fauna of the villages in both the districts consisted of mainly *An. culicifacies*, *An. subpictus*, *An. fluviatilis*, *An. annularis* and *An.vagus* in indoor resting collections. The mean density of *Anopheles* caught per man hour during the year was 13.7 in Bastar district and 16.1 in Korea. Susceptibility of *An. culicifacies* to diagnostic dose of DDT (4%), malathion (5%), deltamethrin (0.05%) and Alphacypermethrin (0.1%) was studied in both the districts. The corrected mortality in Korea was less than 15% to DDT (R), 75% to malathion (R), around 90% to deltamethrin and 100% to Alphacypermethrin in different localities. In Bastar

district, the corrected mortality was <10% to DDT, about 75% to malathion, <90% to deltamethrin and Alphacypermethrin. These results indicate that the species is resistant to DDT and Malathion and tolerant to Deltamethrin and Alphacypermethrin in Bastar.

Species specific breeding site surveys revealed high breeding of anopheline mosquitoes in streams and seepage water in Korea (Koriya) district from which mainly species viz. *An. culicifacies*, *An. subpictus*, *An. fluviatilis*, and *An. jeyporiensis* emerged. More than 22% emergence of *An. culicifacies* and *An. fluviatilis* was recorded. Blood meal test revealed almost 100% positive for bovine blood in Korea (Koriya) while only 2 samples showed human blood positive in Bastar district.

Analysis of *in vivo* transcriptome of *Plasmodium falciparum* from Indian Patients suffering from cerebral malaria and its comparison with that from patients infected with severe malaria (with MOD symptoms)

The study was started in July 2013 to determine the *in vivo* *P. falciparum* gene expression profiling in cerebral and severe (non cerebral) malaria in India. Two study sites have been selected in Chhattisgarh (Maharani Medical College, Jagdalpur and district hospital, Baikunthpur). Patients with symptoms of malaria were screened and patients positive for *P. falciparum* malaria, and fit in the enrolment criteria and consented to participate in the study were enrolled. Blood samples were collected from cerebral, severe and mild malaria from both the hospitals and processed for RNA isolation & Array hybridization at JNCASR.

Evaluation of biomarkers to assess malaria severity due to *P. falciparum*

Under this study all the age groups have been screened at Maharani Hospital and Associated Medical College, Jagdalpur, Chhattisgarh (CG). Overall slide positivity rate (SPR) was 6% and Pf% was 86.1. Case fatality rate in Pf associated malaria was 5.1%.

P. ovale infection was also identified (in 1% cases, out of total 400 microscopic *P. falciparum* samples) which is confirmed by PCR and sequencing for the first time in India. In all the four cases, *P. ovale* infection was mixed with either *P. falciparum*/ *P.*

vivax or both.

Among clinical parameters, coma and respiratory distress were found to be independently associated with risk of mortality in multivariate analysis.

Receiver operating characteristics revealed that cutoff value of IL-1ra ≥ 128.4 pg/ml, was 64.5% sensitive and 61% specific to predict mortality in CM patients at the time of hospital admission (AUC = 0.67). Plasma levels of IL-1ra, sTNFR1 and plasma Ang-2 was significantly declined after 48 hour of admission in patients showed progressive recovery compared to their levels at admission. Combinational biomarkers (IL-1ra, sICAM-1, MIF) showed sensitivity of 71% and specificity of 60% to early identify patients at risk of death at the time of admission into the hospital.

It was also determined that patients whose coma reversed very quickly (6-12 hr) exhibited significantly lower pfHRP-2 (malaria antigen) levels than patients who remain comatose for ≥ 48 hours.

Haemoglobinopathies and malaria

A Tribal Health Research Unit was established in Government Maharani Medical College Hospital, Jagdalpur for screening haemoglobinopathies and malaria particularly for severe and complicated malaria among tribal population. A total of 417 cases were screened for identification of sickle cell trait and diseased patients. Of which 173 were blood smear positive for malaria and 244 were negative and were considered as control. Among the malaria patients, 5.7% had sickle cell trait and one patient had sickle cell disease. Among non malaria patients 17.6% had sickle cell trait and 3.3% had sickle cell disease. Thus frequency of sickle cell gene was less among malaria patients than controls.

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

IEC intervention study to improve KAP related to tuberculosis among Saharia tribe is in progress in

Shivpuri district of Madhya Pradesh. It is being carried out in three phases - Baseline survey (phase I), IEC intervention (phase II) and the endline survey (phase III). The baseline survey includes TB disease & risk factor assessment and KAP in all the selected villages. Intervention in the form of IEC is being carried out in study villages. The endline include assessment of the impact of intervention in terms of KAP, risk factors and TB disease burden after IEC intervention in the area.

The baseline survey has been completed. The findings of the baseline survey show very high prevalence of pulmonary tuberculosis (3003 per 100,000 population) among Saharia tribal community in the study area. The major risk factors for TB *viz.* tobacco use, alcohol consumption, under-nutrition, indoor air pollution are found prevalent amongst them. In addition, the findings highlight their poor knowledge and perception about tuberculosis. Based on these findings, IEC intervention is being implemented in the area in phase II.

Study on pulmonary tuberculosis amongst Saharia tribe in Gwalior district of Madhya Pradesh

On the request from the department of tribal welfare, Govt. of Madhya Pradesh this cross sectional study was carried out amongst Saharia primitive tribe of Gwalior district in Madhya Pradesh. The overall prevalence of PTB was found to be 3294 per 100,000 population. However, the MDR TB situation was not found to be different from other parts of the country (8.2% in re-treatment and 2.3 % in new cases). The overall prevalence of tobacco smoking and alcohol consumption was found to be 23.7% and 15.3% respectively. Fifty percent individuals were found underweight (BMI<18.50). The indoor air pollution, another risk factor for tuberculosis, was also found prevalent among them. Screening was done for diabetes and HIV. A total of 1780 (255 cases and 1525 controls) and 1799 (261 cases and 1538 controls) saharia individuals were tested for HIV and blood glucose levels. Of the 255 TB patients tested for HIV, no case was found to be HIV reactive. One HIV reactive person was however, found among controls. Thirty three of the 261 TB cases (12.6%) had abnormal blood glucose levels while in control group, 128 (8.3%)

had abnormal blood glucose levels. The findings suggest that TB disease remains a major public health problem amongst the Saharia primitive tribal community. HIV however, does not appear to be a problem in this community.

VIROLOGY UNIT

Dengue: This year upsurge in dengue cases was observed in tribal and rural areas. More than 1400 samples collected from dengue suspected cases were tested by the VDL of which almost 30% were found positive. Molecular studies conducted on the samples revealed that dengue virus 3 and dengue virus 2 were in circulation in this part of the country during this year. The major outbreak of dengue virus 2 was documented from tribal district Mandla; this is the first outbreak of dengue in tribals of MP affecting primitive and other tribes. More than 300 dengue cases were confirmed by different diagnostic tests, five dengue positive patients died. The phylogenetic studies on the samples collected from outbreak demonstrated that cosmopolitan strain dengue virus 2 was the aetiology. The timely and accurate diagnosis and advice by the centre helped state health authorities to curb the outbreak.

Influenza and Respiratory Syncytial Virus (RSV): Influenza virus A, B and RSV viruses were detected from the patients suffering with influenza like illness (ILI). Both RSV A and B were detected from the hospitalized patients. RSV subtype A was detected as an important causative agent in ILI in children below age of two years. The centre continues to assist the state health in H1N109pdm diagnosis.

Hepatitis: More than 900 samples from patients suffering with symptoms of hepatitis were subjected to different tests for diagnosing HAV, HBV, HCV and HEV infection. Molecular and phylogenetic studies were conducted on the samples and HAV genotype III A, HBV genotype D, and HEV genotype III were detected from the samples of the patients.

National Hospital Based Rotavirus surveillance Network: RMRCT is a regional laboratory in this study. Standardization of PCR & genotyping PCR has been completed.

COMMUNITY HEALTH

This study aims at assessing prevalence of Hypertension in relation to Urinary excretion of sodium and Serum Creatinine and Blood urea in a tribal district of Madhya Pradesh.

The study was carried out in 33 selected villages and 12 urban wards of Mandla District. In all, 3090 Individuals of different age groups from 1258 households in 33 villages and 12 urban wards were surveyed of which 21.8% male and 18.2% female had high blood pressure and 5% individuals less than 30 years had high blood pressure (Systolic BP more than 160 or diastolic BP more than 90 mmHg). Overall hypertension among urban tribal group was more as compared to never exposed and occasional migration group. The prevalence of hypertension was significantly higher among tobacco smokers (66.1%) as compared to non smokers (13.2%) and among alcoholics (53.5%) as compared to non alcoholics. The intake of salt was directly related to blood pressure. About 8% urine samples were found having high value of urinary sodium, there was positive linear association of urine sodium with blood pressure. The study indicates that hypertension is emerging as a public health problem even among tribals.

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 Baiga Tribe in District Dindori and is planned to be completed in two phases; Phase-I: Implementation of IEC in intervention villages and Phase-II: Impact evaluation survey. IEC intervention are being implemented for creating awareness among women in 12 intervention villages with the support of Block Medical Officer, Health worker, Anganwari, ANM, ASHA, etc. Total 12 village level committees were formulated consisting of ASHA, Anganwari, Dai, one Baiga women and one person from study team in each study village to convey the messages on maternal and child health care among women in their areas. Other activities includes arranging health education camps, pamphlets, slogans on

the walls, banners on common place, Anganwari centre, group discussions etc.

REGIONAL MEDICAL RESEARCH CENTRE, PORT BLAIR

Risk Reduction/Elimination of Infectious Diseases

Diurnally sub periodic form of *Wuchereria bancrofti* is endemic only in a small pocket of five islands in Nancowry group of islands of the Nicobar district. Programme to eliminate lymphatic filariasis (PELF) through mass drug administration (MDA) of a single dose of DEC launched in the islands 2004 in the islands appears to have less than desired impact. 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 population, number of households, number in 2-4 age class has been enumerated and blood smears have been collected for assessing baseline microfilaraemia and antigenemia. Surveys were carried out to assess the type of salt used by the people. A total of 2,154 households were enumerated from all the villages and 6,850 (67.2% coverage) blood slides have been collected. The average consumption of salt per household per month in different islands varied between 2.02 Kg (Katchal) and 2.82 Kg (Chowra). The average consumption of salt per person per month in different islands ranged between 0.481Kg (Katchal) and 0.606 Kg (Teressa). These estimates indicate that fortification of salt with DEC will result in adequate intake of DEC by the population for effective control of LF.

Prevalence of pulmonary tuberculosis in Car Nicobar, its social determinants and hepatitis B co-infection as a determinant of anti-tuberculosis drug induced hepatotoxicity and treatment non-compliance

Between 1986 and 2001, the annual risk of tuberculosis infection (ARTI) and prevalence of smear-positive pulmonary tuberculosis among the Nicobarese of Car Nicobar Island had drastically increased. The revised national tuberculosis control programme (RNTCP) was introduced in Andaman

and Nicobar in 2005 and DOTS+ strategy in 2011. The present study seeks to assess the change in the tuberculosis situation and the social and the determinants of the disease and treatment completion including drug induced hepatotoxicity (DIH). A total of 3,879 people were interviewed for the chest symptoms and 67 (1.72%) chest symptomatics were identified. Sputum samples were collected from 57 (1.47%) chest symptomatics and 12 culture/smear positive cases were identified. The prevalence estimated was 309.3/100,000 population, which was less than half of the prevalence estimated in 2001-02 (735/100,000). A total of 42 pulmonary tuberculosis patients were included in the study of DIH. Till date, follow up investigations are complete for 27 cases and 15 (57.7%) showed raise in either AST/ ALT levels after the initiation of ATT. Four patients were positive for HBsAg, out of whom three had evidence of DIH with ALT levels ranging from 50 IU/L - 350 IU/L. Although the survey is in its preliminary stages and it would not be possible to make an estimate of the prevalence of pulmonary tuberculosis, the preliminary data indicate a probable decrease in the prevalence. The data also shows that DIH is a common occurrence in patients on antituberculosis treatment.

Impact of veterinary public health measures for leptospirosis control in Andaman during the past 15 years

The increased awareness generated after the recognition of the re-emergence leptospirosis in Andaman in late 1980s resulted in independent efforts by human health, veterinary and agricultural the sectors. An initiative was made to assess the present leptospirosis situation in terms of indices of disease occurrence, mortality and burden. The estimated incidence of severe cases of leptospirosis during 2011-12 was 14.6/100,000 and that in 2012-13 was 15.2/100,000. The incidence of severe cases appears to have decreased by 50% during these 15 years. A greater decrease in specific mortality due to leptospirosis was observed during this period (7.47/100,000 during 1996-97, 1.25/100,000 during 2011-12 and 0.82/100,000 during 2012-13). The incidence of all cases of leptospirosis calculated based on PHC based surveillance in Manglutan village was 364 cases per 100,000 for the year 2011-12 and 469 cases per 100,000 for the year 2012-13. The number of cases detected was

much lower than that would be expected from the experience of 1990s. It seems that the incidence has substantially decreased during the past one and half decades. The DALY for the whole UT decreased from 618 in 1996-97 to 117 in 2011-12 and the DALY/1,000 population from 1.91 to 0.31. Similarly the DALY estimated for Manglutan village decreased from 81 in 1998-99 to 0.6 in 2011-12 and DALY/1,000 population from 8.54 to 0.04.

National Hospital Based Rotavirus Surveillance Network

This project involves studies to generate data on the proportion of diarrhoea attributable to rotavirus among the tribal and non-tribal population of Andaman and Nicobar Islands and characterization of prevalent strains of rotavirus to monitor the circulating serotypes. During the period Jan to Dec 2013, a total of 396 possible cases of rotaviral diarrhoea admitted to the wards of the selected hospitals were included in the study and rotaviral aetiology of the diarrhoea was confirmed by rotavirus EIA in 192 (48.5%). Among 337 inpatients from Andaman Islands, 173 (51.3%) were attributable to rotaviral infection, among the 59 inpatients recruited from Nicobar district, 19 (32.2%) were attributable to rotavirus. In this study the most frequent G/P genotype combinations detected were G1P[8], G2P[4] G12P[8] and G9P[4] respectively. Identification of genotype G12 strain with P[8] combination represents the first report of strain belonging to this genotype in Andaman & Nicobar islands.

Antibiotic resistance among enteric pathogens causing childhood diarrhoea and emergence of New Delhi Metallo- β Lactamase-1 possessing multi drug resistant *Escherichia coli*

Existences of New Delhi metallo- β -lactamase 1 (NDM-1) producing *Proteus mirabilis* have been reported in these Islands from cases with urinary tract infection. The present report describes the drug resistance pattern among enteric pathogens, the molecular mechanism of drug resistance and the emergence of carbapenem resistance in bacteria causing childhood diarrhoea in these islands. A total of 677 samples were obtained from paediatric diarrhoea patients during the reporting period of

which 112 samples yielded growth of pathogenic *E. coli* and 15 of *Shigellae* (7 *S. flexneri* and 8 *S. sonnei*). All *E. coli* strains were resistant to first generation cephalosporin, cephalothin, and second generation, cefuroxime and carbenicillin. More than 50% of these isolates were resistant to all drugs other than Imipenem, tetracycline, chloramphenicol and co-trimoxazole. All the *Shigella* isolates were also resistant to carbenicillin and cephalothin. Nalidixic acid resistance was 86% and resistance to other quinolones were in the range of 33.3% to 60%. All quinolone resistant strains had double mutations on *gyrA* gene of QRDR and a mutation on *parC*. A total of 36 *E. coli* isolates harboured PMQR determinants. Two isolates of *E. coli* that were imipenem resistant harboured the NDM-1 gene.

Hospital based surveillance of diseases with suspected viral aetiology

A total of 348 samples were tested for CHIKV and 58 (16.6 %) were positive. A total of 1,020 blood samples from patient with dengue like illness were tested for anti-dengue IgM antibodies and 109 (10.6%) tested positive. Ten (0.88%) out of the 1,129 throat swabs were positive for Influenza A virus. Among the children with respiratory tract infection (>1,200) seven (0.52%) were positive for Human Meta-pneumovirus (HMPV), 24 (1.8 %) for respiratory syncytial virus and 81 (6.38%) were positive for adenovirus. Among the 564 samples from children with diarrhoea, 53 (9.39%) were positive for enteroviruses. The surveillance detected infection with 24 different viral agents. Sixty-three viral sequences were analyzed for characterization and the genotypes and serotypes of various viruses were detected.

Characterization of enteroviruses revealed the existence of enterovirus subtypes such as Human enterovirus 71, Human coxsackievirus A16, Human coxsackievirus A6, Human coxsackievirus A21, Human echovirus 30, Human echovirus 21 and Coxsackievirus B6. A phylogenetic analysis done on hMPV from patients with influenza-like illness (ILI) showed that the strains belonged to subtype 2 of genotype A, closely related to the reference sequence FJ168779 (HMPV A2). Adenovirus identified from respiratory specimens were found to have close genetic relation with serotype 1 (JF

712981) of species C. Further analysis to identify any polymorphism is in progress.

Outbreak of Group A Rotaviral diarrhoea in Diglipur, North Andaman

An outbreak of Rotaviral diarrhoea was suspected in Diglipur area, North Andaman in December 2013. On the request of the Director of Health Services, a team from the Centre was deputed to Diglipur to carry out investigation of the suspected outbreak.

In December 2013, a total of 359 cases of diarrhoea reported to Radha Nagar PHC, which was in excess of the average monthly number of cases thus confirming the outbreak. A total of 28 stool samples were collected from patients with diarrhoea and 21 (75%) stool were positive for Group A Rotavirus by ELISA. The overall attack rate was 6.7% in Radha Nagar PHC areas. The attack rate was highest in the age class 0-6 years (49.6%) followed by age class 7-10 years (20.6%). Attack rates in all age classes above 10 years were 1% or less. Almost every child in the age group of 0-10 years in Radha Nagar village suffered diarrhoea during the outbreak. Water samples from all 24 sampling points were tested for coliforms and all were found to be heavily contaminated with coliforms and not suitable for human consumption. Genotyping and sequence analysis of the rotavirus ELISA positives revealed that all the samples belonged to the genotype G1P[8], the same genotype predominantly seen in sporadic cases in Andaman in the past one year and widely prevalent in mainland India.

Investigation of outbreak of Hand Foot and Mouth Disease in South Andaman

In 2013 during the period from July to December, clinically suspected HFMD cases are being reported in increasing frequency and therefore an outbreak was suspected. The outbreak was investigated to understand the epidemiology of the outbreak and to identify and characterize the etiologic agent. Special studies were also conducted to understand the levels of various cytokines in patients infected with etiologic agent causing HFMD. A total of 221 clinically suspected cases of HFMD were reported in 2013. Specimens for laboratory investigation were obtained from 112 cases and 56 samples were found to be positive for RNA of enterovirus causing HFMD. The phylogenetic analysis for

the identification of etiological agents revealed that 6 subjects were infected with Enterovirus 71 and remaining 7 subjects were infected with Coxsackievirus A-16.

Dengue outbreak among wharf employees at Port Blair and emergence of serotype 3

From mid July 2013, an increase in number of febrile cases with retro orbital pain was observed among the Haddo Wharf staff of Port Blair. Blood samples were collected from the suspected patients and tested for the presence of anti-DENV, anti-chikungunya virus (CHIKV) IgM antibodies by IgM capture ELISA. RNA was extracted from all the serum samples and RT-PCR analysis was carried out for the presence of DENV and CHIKV RNA. PCR product was purified and subjected to DNA sequencing analysis. A total of 23 patients were included in the study and among them three (13.0%) were found to be positive for DENV specific IgM antibodies. Among 23, 12 (52.1%) samples were positive for RT-PCR analysis amplifying a fragment of 511 bp. The nucleotide sequencing of the 511 bp amplicons confirmed that the virus sequence was homologous with DENV-3. The observations from the present study indicate circulation of third serotype of dengue virus in the urban Port Blair, thus confirming the circulation of three serotypes (DENV1, DENV2 and DENV3) within the urban agglomeration of Port Blair.

Health and nutritional status of Onge tribe of Little Andaman

A health and nutritional study was conducted among the Onge tribe of Dugong Creek in Little Andaman in December 2013. The present population of Onges consists of 112 individuals, 54 females and 58 males with an overall sex ratio of 931 females per 1,000 males. Out of the 22 preschool children of the present Onge population, 19 (86.4%) had stunting and among them 17 (77.3%) were severely stunted. All the 22 Onge preschool children had severe wasting. Among the 37 children aged above 5 years, 7 (18.9%) had under-nutrition and 16 (43.2%) had over-weight or obesity. Hb estimation was done on 67 Onges and three (4.5%) had Hb concentration below 11 gm/dl indicating anaemia. Blood pressure was recorded in 39 Onge adults aged > 24 years and 7 (17.9%) had a BP \geq 14/90

mm of Hg. Blood glucose was measured in 51, of which six (11.8%) had diabetes and another 17 (33.3%) had prediabetes. Blood cholesterol levels above 200 mg/dL was observed in 5 (9.8%) of the 51 Onges tested, but none had levels above 250 mg/dl. Eight (16%) Onges had HDL levels below 35 mg/dl. The other health problems identified include a high spleen rate among children aged 2-9 years (8/28, 28.6%), a probably high prevalence of tuberculosis (2 of the 61 Onges aged 15 years and above, prevalence of 32.8/1,000) and congenital heart disease (3/112, 2.7%). The nutritional status of older Onge children appears to have improved since the last survey, but the high prevalence of under-nutrition among the preschool children is persisting and needs to be addressed. A recommendation to this effect has been communicated to the Dept. of Tribal Welfare.

Generation of traditional knowledge from tribal population on usage of plants and seaweeds for their medicinal values

A study was conducted to collect and compile the tribal communities' knowledge of the use of medicinal plants for treating diseases, collect, preserve and propagate these medicinal plants. A total of 174 folk healers or traditional knowledge practitioners (TKPs), 149 belonging to the Nicobarese community and 25 belonging to the Karen community were contacted, interviewed and their knowledge on the use of medicinal plants were documented. All the TKPs together were treating 66 different ailments. The largest number of plant species used was for fever (87 species) followed by body ache (71 species). All the TKPs together used 250 species of plants belonging to 196 genus and 92 plant families. Four plant species are used in six of the eight areas surveyed and another eight are used in five of the eight areas. The use of 11 species of plants is unique to Car Nicobar. Community biodiversity registers of Nicobarese and Karen tribes have been prepared. CBDRs containing information related to traditional knowledge practitioners' methods of preparation of herbal medicines, dose and duration of therapy and the diseases being treated and list of plants used have been prepared.

A study was also conducted to test the indigenous medicinal plants used by the tribes of Andaman and Nicobar for healing purposes, seaweeds and marine

fauna for medicinal properties. Extracts of 36 plants (19 from Karen habitat and 17 from Nicobarese habitats) were studied for anti-microbial activity and 21 (13 from Karen and 8 from Nicobarese) were found to show anti-microbial activity. Fifteen sponge extracts and six seaweed extracts were also tested and seven sponge extracts and all the six seaweed extracts were found to show anti-microbial activity. Six plants and five sponges activity against malaria parasite also. Eight plant extracts were found to be active against *Leptospira* and two plant extracts against TB bacillus. One out of the three plant extracts showed anti-inflammatory and antioxidant activity. Seven crude extracts that showed antimicrobial activity and one that showed anti-inflammatory and antioxidant properties were further purified and analysed and 16 bioactive compounds of various molecular weights have been identified. GC-MS analyses were done for seven crude extracts that showed anti-microbial activity and sixteen bioactive compounds of various molecular weight have been identified. Further studies on these compounds are in progress.

Assessment of current nutritional status of pre-school children

The study was initiated to know the prevalence of under nutrition and micronutrient deficiency, food insecurity, hunger and to understand the factors responsible for such conditions. Although severe food insecurity was estimated to be low at 3.0%, much below the prevalence of 7.5% in the poorest states of India (UNDP Survey, 2008), a large proportion of the households (32.5%) had some degree of food insecurity. This prevalence is similar to the estimated prevalence of food insecurity in the poorest States of India. Prevalence of stunting (40%), under-weight (24.8%) and severe wasting (6.5%) were lower than the rates reported by NNMB in 2008 (40%, 45% and 20% respectively). Prevalence of stunting among the Nicobarese (58.5%) was higher than the other populations of the islands as well as the all India prevalence. However, the prevalence of under-weight (14.7%) and severe wasting (3.5%) are much lower. Anaemia prevalence was high at 70.5%, here again the prevalence among Nicobarese was low (19.4%). The mean dietary intakes of most of the food groups

were lower than RDA. However, the Nicobarese had a better intake. The most pronounced nutrient intake deficiencies were for riboflavin, folic acid and iron. Although the univariate analysis identified a large number of factors as associated with under-nutrition, multiple logistic model showed that only large family and lower socio-economic status evidenced by BPL status and, the surrogate indicator lack of electrification, were the only independent risk factors.

Physico-chemical analysis of soil and surface water in leptospirosis endemic and non-endemic areas

An attempt was made to understand the status of soil nutrients in endemic and non-endemic settings for leptospirosis in the Andaman and Nicobar Islands. The information generated on these lines would enable us to understand the relationship of soil nutrients *vis-a-vis* survival of *Leptospira*. A Total of 48 sampling sites were selected in three districts of Andaman and Nicobar Islands namely North & Middle Andaman, South Andaman and Nicobar. Among the total of 48 samples collected, 35% were from paddy fields, 44% from other crop fields, 13% from forest areas and 8% from inhabited areas. Iron, manganese and copper levels were found to be significantly higher in endemic regions and calcium was found to be significantly higher in non-endemic settings. Ca saturation, Mg saturation and Na saturation were also found to be significantly different between the two settings. Residential area fields of both the settings showed significant difference in sulphur, sodium, manganese, iron, Ca and Na saturation. The study showed that endemic and non-endemic areas, though have identical climatic conditions, have different nutrient profile. Identifying certain nutrients as a responsible factor in leptospirosis endemic region may help in adopting suitable control strategies.

Ecology of leptospirosis – role of biofilms in the transmission dynamics of leptospirosis

The biofilm forming isolate was identified as *Azospirillum brasilense*. Self aggregating strains of *Leptospira* were found to form more readily than other strains. Scanning Electron Microscopy showed that *Leptospira* and *Azospirillum* formed

intertwined networks of attached cells that served as scaffolding for further biofilm development over time. The MBC for the *Leptospira* in biofilm along with *Azospirillum* was significantly higher for antibiotics penicillin, ampicillin and tetracycline. The tolerance of *Leptospira* to UV radiation as well as high temperature was found to be increased in the presence of *Azospirillum*. The mutualism between the two bacteria still remains to be understood further. It is clear that *Leptospira* is benefited from this interaction by acquiring the ability to survive under harsh environmental condition. The information generated is new and it would help to develop effective strategies for the control of leptospirosis.

Bio-ecology of *Aedes aegypti* and *Aedes albopictus* vector of dengue and chikungunya with special reference to its invasion into human habitation

A total of 2422 households were inspected and 747 were found to have water holding containers supporting *Aedes* breeding. A total of 17,314 water holding containers were searched and 1,021 containers were found to support breeding. A total of 4,379 pupae were collected. The Breteau index, was highest in Port Blair tehsil (63.3%), while it was almost similar (35.0% and 33.5%) in Ferrargunj and Little Andaman Tehsils. The container index was 7.3% in Port Blair Tehsil, while in Ferrargunj and Little Andaman tehsils it was 4.6% and 6.9% respectively. The percentage of houses infested with larvae or pupae was highest in Port Blair tehsil (39.5%), followed by Little Andaman (30.5%) and Ferrargunj (26.2%). The pupal index was highest in Port Blair Tehsil (251.6) followed by Little Andaman (198.7) and Ferrargunj Tehsil (132.1). Overall a total of thirty species belonging to thirteen genera were observed. *Ae. aegypti* and *Ae. albopictus* colonized a variety of manmade, artificial and natural breeding places. *Ae. albopictus* was observed to be widely prevalent and dominant mosquito species in the outer. RCC and wooden premises accounted for a larger proportion supporting *Ae. aegypti* and *Ae. albopictus* breeding. The proportion of containers supporting *Ae. aegypti* breeding was more in indoors while containers supporting *Ae. albopictus* breeding is more in outdoors. *Ae. aegypti* breeds in water

holding containers that are used regularly while *Ae. albopictus* breeds in abandoned containers.

Gene expression among the endemic *Leptospiral* strains/isolates recovered from the patients with different clinical manifestation

The study was initiated in an attempt to identify virulence genes associated with the severe complications of leptospirosis by gene expression studies using microarray. Four *Leptospira* strains, on recovered from a mild case and the other three from severe cases were included. Transcripts were retrieved from NCBI database of the isolates used in the study and for four other strains including a saprophytic strain. A total of 11,168 probes were designed for unique sequences of the strains matching NGS consensus sequence and included in microarray.

The number of genes expressed varied between strains and ranged from 3,868 to 4,314 and about 60% of these genes had known or predicted functions. The relative proportions of up-regulated and down-regulated genes ranged from 36.0% to 53.6%. A total of 56 unique genes were predicted to be differentially up-regulated among the strains. Hypothetical proteins, which are found to be highly expressed, may be coding for virulence factors. Highly expressed genes coding for known or hypothetical proteins can be candidates for vaccine development.

Development of DNA Vaccine for *Leptospiral* Infection

An attempt was made to develop a novel consensus-based approach to vaccine development, employing DNA vaccine technology. Leptospiral proteins LipL45 and DnaJ were identified as immunogenic and capable of eliciting long-lasting Immunological memories and were used in the study. Plasmid constructs of optimized sequences of LipL45 and DnaJ were made and administered to BALB/c mice to study the immunological response.

The *in-vitro* expression of these two plasmid constructs (DnaJ and LipL45) were confirmed by plasmid DNA transfection in mammalian cells. The epitope peptides namely HLA –A*01, HLA-A*02, HLA-A*03, HLA-A*11, HLA- A*24,

HLA-A*31, HLA-A*68, HLA-B*44 and HLA-B*8 were identified as dominant T cell epitopes. The T cell mediated immune response (IFN- γ) was found to be significant against the DNA constructs encoding LipL45. The splenocytes of mice which received DNA construct encoding LipL45 showed elevated expression level of IFN- γ (Ct = 14.4), IL-1 β (Ct = 11.4), IL-6 (Ct = 4.9) and IL-12 (Ct = 10.0). The IL-6 response indicates Th2 response in the mice splenocytes. However there is no significant elevation in DnaJ splenocytes of mice that received DnaJ. Anti LipL45 specific IgG antibody in the sera collected from mice immunized with DNA construct encoding LipL45 were significantly higher than in the sera of mice immunized with vector control (pVax1). In conclusion, the DNA construct encoding lipL45 could elicit both cell mediated and humoral immune responses. The animal experiments are scheduled to study the immune response and protective nature of the DNA construct encoding LipL45 using guinea pigs.

Study of the diversity of intestinal microflora of the accessible ethnic communities of Andaman and Nicobar and its association with expression levels of immune related genes, nutritional status and disease prevalence

A cohort of Nicobarese living in the remote villages of Nancowry group of islands was developed for this study. Among them, 26 (43.3%) had never visited any hospital or sub-centre and formed cohort 1. The remaining 34 (56.7%) subjects have undergone medication recently (before 6 months) or in past (C-2). Faecal samples were collected from 60 individuals. DNA was directly extracted from the stool samples. Eight bacterial groups were studied real-time PCR analysis using the primers that were reported earlier. All 60 samples and corresponding standards were amplified and quantified with SYBR Green protocol. The centre identified the lack of *Clostridium coccoides*–*Eubacterium rectale* group in 16 individuals of antibiotic exposed population, and abundance of *Helicobacter*–*Flexispira*–*Wollinella* group in 38 individuals irrespective to their exposure to antibiotics. The mean copy number of 6 bacterial groups among the samples differs in both cohorts.

The usual trend of *Firmicutes/Bacteroidetes* ratio with age was not observed in this population.

Characterization of recombinant proteinases of *Leptospira* and identification of their role in invasion

The project involves the cloning of the proteinase gene, expression, purification and characterization of the expressed recombinant proteinases. Available protease genes identified by searching the whole genome of *Leptospira interrogans* serovar Copenhageni str. Fiocruz L1-130 were selected. Primers were designed to amplify the complete ORF of the proteinases with the restriction sites integrated. The proteinase gene was amplified by PCR performed on genomic DNA isolated from *Leptospira*. The amplicons were gel eluted and digested with respective restriction enzymes and digested fragments were ligated to the expression vector. A total of 20 proteinase genes were selected and all were found to be expressed in *Leptospira*. Two of the 20 proteinase *viz.* metalloprotease (1.2Kb) and microbial collagenase (2.6Kb) were cloned in the vector pXcmkn12. Expression of these proteinase genes, metalloprotease (1.2Kb) and microbial collagenase (2.6Kb) was confirmed. The project is progressing.

Estimation of prevalence of genital tuberculosis among the women

A total of 9,609 women from rural areas were interviewed and subjected to primary screening and among them 628 (6.5%) women were eligible for further screening for FGTB. Till now 173 (1.8% of the surveyed women) have been clinically examined and 129 (1.3% of surveyed women) were found to be eligible for endometrial sampling. Among them, endometrial specimens have been obtained 100 eligible women and processed. One specimen (1%) was culture positive for *M. tuberculosis*. Applying the coverage proportions, the identified case of FGTB represents about 1,500 women in the eligible age group and the prevalence of FGTB would be 66.7/100,000.

National Vector Borne Disease Control Programme sentinel surveillance hospital-dengue and chikungunya surveillance

The Centre is one of sentinel surveillance sites for the surveillance of vector-borne diseases

under the National Vector Borne Disease Control Programme (NVBDCP). Surveillance of dengue and chikungunya is carried out routinely. During the reporting year (2013), a total of 407 suspected cases were screened for dengue infection and 35 (8.6%) were found to be positive in DEN IgM ELISA. The trend showed that dengue transmission occurs round the year with a peak in May. A total of 23 suspected cases of chikungunya were screened and four (17.4%) were positive in CHIKV IgM ELISA. Chikungunya activity is at very low level now. However, sporadic cases still continue to occur.

Intermediate Reference Laboratory for solid culture and drug sensitivity of *M. tuberculosis* - Surveillance of MDR tuberculosis

The Centre has been accredited as intermediate reference laboratory for *M. tuberculosis* culture and DST. When DOTS+ strategy was introduced here in 2011, the Centre became official partner in it with the specific role of providing laboratory support for culture and DST. The results of the surveillance of MDR tuberculosis done in 2013 are presented here. A total of 73 MDR tuberculosis suspect patients were tested and 42 yielded cultures of *M. tuberculosis*. DST was done on 34 isolates and 26 of them were found to be multi-drug resistant strains. Eleven of the MDR TB cases were referred again for follow-up culture and DST and among them, 7 (63.6%) were culture negative in their last follow-up while the remaining four patients continued to be culture positive.

Diagnostic Support in Leptospirosis to Integrated Disease Surveillance Project

The Centre provides laboratory support for the diagnosis of leptospirosis for the Integrated Disease Control Project (IDSP). The results of the surveillance during 2013 are reported here. The total number of eligible patients who attended the selected hospitals and PHCs during Jan –Dec 2013 was 7,382. The number of confirmed cases detected was 1,011 (13.7%). About 40% of the cases reported to Tushnabad PHC and about 20% to Garacharama PHC. Seasonal trend showed two peaks, one in the month of July and the other in September. Among the 1,014 cases, 994 were mild cases, 16 (1.6%) had evidence of pulmonary involvement and one had renal involvement. Based on MAT titres, the common infecting serovar among the confirmed cases was Icterohaemorrhagiae (89.41%).

National Nutrition Monitoring Bureau-Urban Nutritional Survey

National Nutrition Monitoring Bureau was established in the Centre recently. The current urban nutritional survey includes survey of risk factors of chronic non-communicable diseases also. Out of the 18 municipal wards in urban Port Blair, the survey has been completed in four wards. The overall prevalence of hypertension was 10.3% and that of diabetes was 12.4%. Hypercholesterolaemia (>239 mg/dL) prevalence was 2.9% and that of triglyceride levels was 9%. Low HDL levels was seen in 72.4% of the subjects. The urban population of Port Blair has a lower prevalence of hypertension than in other population groups of the territory. Diabetes prevalence was slightly higher and is comparable to all India prevalence. Dyslipidaemia is not highly prevalent except for a high prevalence of low HDL cholesterol level.

Assessment of the on-going MDA programme in Andaman and Nicobar Islands- consumption and compliance during MDA 2013

The programme to eliminate LF was launched in 2004 and is being implemented mass drug administration of DEC and Albendazole. Eight rounds of MDA have been accomplished in these islands. A cross-sectional survey was carried for assessing pattern of DEC distribution and compliance. Cluster survey protocol (WHO 2011) was followed. Drug distribution implemented during 16-18 February 2013 was evaluated. A total of 900 households were surveyed in the whole of Andaman Nicobar Islands and 2615 people were sampled. DEC was successfully distributed to 89.4% (2339/2615) of the population (coverage/distribution rate). 92.3% of these complied with the treatment. Non-compliance was highest in South Andaman (n=189), followed by North and Middle Andaman (n=170). It was the least in Nicobar district (n=96). Only 50 (2.3%) individuals had developed side reactions.

Establishment of leptospirosis reference laboratories in Southeast Asian countries as WHO Collaborating Centre-Indonesia and Nepal

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. Recently National Reference Centres were established in Indonesia and Nepal.

DESERT MEDICINE RESEARCH CENTRE, JODHPUR

DENGUE

Mapping of risk of Dengue Hemorrhagic Fever (DHF) through dengue virus typing in *Aedes* mosquitoes of different settings of Rajasthan

Project concerns with isolation of circulating Dengue virus types (DEN-1, 2, 3 & 4) among *Aedes* mosquitoes (*Aedes aegypti* & *Aedes albopictus*) of different districts of Rajasthan and mapping of circulating DEN types. This year, 11 more districts have been covered *i.e.* by now under the project, 23 different districts in the state have been covered for the collection of mosquito immatures. Total number of systematically sampled 14031 houses was screened in these districts of which 5.3% were positive for the breeding of *Aedes* mosquitoes. *Aedes* larvae were collected from containers found positive for breeding which were then brought to the laboratory for virus isolation studies. Of the total 92,831 containers surveyed, 1929 containers were found to be positive. The key containers observed positive for *Aedes* breeding were cement tanks and clay pots.

Development and testing of ICMR-DMRC module of Dengue control for Rajasthan

Project aimed to generate early warning system of activity of dengue virus in all the 32 districts of Rajasthan. During the period under report, a comprehensive survey in the urban areas (district headquarters) of all the 34 districts of Rajasthan was accomplished. During the course of investigations 1,30,525 domestic containers were examined in 33 districts of Rajasthan. Out of containers examined only 2288 (1.75 %) of containers were found positive for breeding of dengue vectors. At individual district level also the maximum breeding observed in Jaipur district was only 6.1% of containers examined. It was evident that only 1.0 % of 1,30, 525 containers examined showed virus

presence. At individual district level, maximum virus positive containers (3.8%) were observed in Jaipur. As earlier, based on the presence of vertically infected mosquitoes at household level in each district town of Rajasthan, warning points of virus activity has been communicated to state health department for controlling dengue vectors at suggested sites.

Comparative study of Gene sequences of virus isolates of influenza A pandemic (H1N1) 2009 viruses

A preliminary pilot trial of whole genome sequencing of the clinical samples and samples amplified in the chicken eggs was accomplished under technical guidance of NIV, Pune. The throat swab sample of a case of Pandemic Influenza A (H1N1) 2009 virus was taken and inoculated into the Allantoise cavity, Chorio allantoise membrane, yolk sac and amniotic sac of 10 days old embryogenated chicken eggs.

MALARIA

Surveillance of pyrethroid resistance in *Anopheles stephensi* strains of Rajasthan and studies on genetic and biochemical mechanisms of pyrethroid resistance in *An. stephensi*

During the period under report, current susceptibility status of *An. stephensi* was determined against temephos larvicide, which revealed that this species is susceptible to this larvicide in all study districts, however, *An. culicifacies* in one village of Jaisalmer district exhibited intermediate resistance, which needs further verification. The results of the study have direct relevance to the national malaria control programme and the collected information is worth for consideration, while conducting indoor residual spray operations.

Synergistic efficacies of some plant extracts with and without some insecticidal formulations against vectors of malaria and dengue in North-Western Rajasthan

Under Synergistic studies on larvae of *Aedes aegypti* and *Culex quinquefasciatus* to the methanol extracts from yellow ripe fruits of *Solanum xanthocarpum* (SX) and red fruits of *Withania somnifera* (WS), this year, synergistic Factor (SF) and Co-toxicity Coefficient (CTC) were determined, which revealed that except the pairs SX:WS (1:2) & SX:WS

(1:3); which showed antagonistic effects for *Cx. quinquefasciatus*, every other combination showed the synergistic effects to both the species.

Use of insecticide treated nets (ITNs) in alternative forms for the protection against malaria transmission in the desert

Insecticide treated bed nets (ITBNs) have been strongly advocated for use to prevent malaria and are considered to be a significant improvement in the strategy to fight malaria. The base line survey was conducted in the six villages for the collection of the entomological and malaria epidemiology data. The surveys were made in the post monsoon period (September 2013) and in winter season (January 2014). Three *Anopheles* spp., *Anopheles stephensi*, *Anopheles culicifacies* and *Anopheles subpictus* were reported from the villages. Highest slide positive rate (SPR) was reported in the village Nathusar. In the winter base line survey no *Anopheles* spp. was collected from any of the six villages.

Impact of irrigation change on the prevalence of malaria in arid and non-arid areas

The study would identify the eco-environmental factors supporting the vector populations as well as malaria prevalence and based on which the key eco-environmental factors will be identified in view to utilize them in future as early warning tool. Two ecologically different districts, Jaisalmer and Banswara, have been considered to carry-out the studies and in both the districts the study areas have been identified.

KAP study of Dengue and Malaria to design appropriate intervention among Tribal of Rajasthan

Study aimed to assess existing status of Knowledge, Attitude & Practice towards Malaria & Dengue among tribal as well as non-tribal population which may help in reducing Malaria & Dengue infestations among them. The study revealed that the tribal people had knowledge that mosquito bites result in malaria infection, but due to their poor economic condition, they could not afford to purchase personal protection measures like, bed

net, Black Hit or coils/ mats, mosquito repellent crème etc. to curb mosquito bites.

Impact of irrigation change on the prevalence of malaria in arid and non-arid parts of Rajasthan

The project aims study the impact of irrigation change on the ecological conditions with respect to vector prevalence and malaria incidences and to determine key factors influencing vector/malaria prevalence and distribution using RS and GIS. Two ecologically different districts, Jaisalmer and Banswara, have been considered and in both the districts the study areas have been identified.

JAPANESE ENCEPHALITIS

Mapping of mosquito breeding habitats and location of vertebrate hosts in North and Southern parts of Rajasthan state prone for emergence of JE virus using space technology (RS & GIS)

Under this study this year, the Geo-coordinates of mosquito breeding habitats, including ponds, trenches, large water bodies (water reservoir) and the area which is likely to remain under paddy cultivation during monsoon season in study districts were recorded. In addition to this, Geo-coordinates of pig sties & roosting places of paddy birds were also recorded using Global Positioning System (GPS) for the purpose of satellite mapping.

TUBERCULOSIS

Standardization of a rapid method for direct drug sensitivity testing of *Mycobacterium tuberculosis* from sputum samples

The project is to standardize, safe, simple, economic, closed method of direct drug sensitivity testing of *M. tuberculosis* from sputum samples in seven days. Sputum samples from 111 patients admitted at KN Chest hospital with suspicion of MDR-TB were collected. Method was followed blindly and results were available on 7th day for 103 samples. Out of the 111 samples, results of LPA were available for 45 samples. LPA showed 25 as sensitive to rifampicin, of which 18 were also detected as sensitive by our method, giving sensitivity of 72.0%. The method, however, needs to be more refined.

Rapid culture and direct drug sensitivity testing of *Mycobacterium tuberculosis* to ionized and rifampicin using liquid culture media

The study aims to standardize and evaluate rapid method for culture and sensitivity of *M. tuberculosis* and to support RNTCP with culture of *M. tuberculosis* from sputum samples. The new method was used for 11 sputum samples which were also examined by RNTCP using LPA. Method was followed blindly and results were available on 7th day. LPA showed 6 as sensitive to rifampicin, of which 5 were also detected as sensitive by our method.

NON-COMMUNICABLE DISEASES

Effectiveness of diet and lifestyle intervention through Information Education Communication (IEC) tools with Angan Wadi Centres (AWCs) of Tribal population of Udaipur District of Rajasthan as the centre of knowledge dissemination for hypertension (including hypercholesterolemia and diabetes) risk reduction – a cluster randomised controlled trial

In above task force study of hypertension, the team of DMRC surveyed Udaipur district of Rajasthan to check the feasibility and accessibility of the Study area on the basis of highest tribal population and approach. Kotra Tehsil, with highest tribal population *i.e.* more than 90% of tribal population was selected as the study area to conduct the study on hypertension among the tribal population here.

Prevalence of diabetes mellitus and impaired glucose tolerance in the Raika and other communities with similar life style in Rajasthan

The objective of the study was to estimate and compare the prevalence of diabetes mellitus and impaired glucose tolerance in Raika community and other communities with similar life style of Rajasthan, and to find the association of diabetes and impaired glucose tolerance with camel milk consumption, if any. A total population of 12020 was covered from 135 villages of Ajmer, Barmer, Jodhpur and Pali district of Rajasthan, of which raika population was 10003 and non-raika population was 2017. The preliminary analysis of data showed that among raika population, 65.6%

of raika subjects ever consumed camel milk whereas only 10.2% of non-raika subjects ever consumed it. Only 0.2% raika and 0.5% non-raika individuals were having history of diabetes at the time of survey. The prevalence of impaired glucose tolerance (IGT) and diabetes mellitus among raika community consuming camel milk was 1.0% and 0.7% respectively while in raika community not consuming camel milk, the prevalence of impaired glucose tolerance (IGT) and diabetes mellitus was 0.5% and 0.4% respectively. Similarly, the prevalence IGT and diabetes mellitus among non raika community consuming camel milk was 1.5% and 2.1% respectively while in non raika community non consuming camel milk, it was 0.4% and 1.0% respectively.

NUTRITION

Nutritional status of elderly rural population and development of appropriate intervention model using existing health system

Project has been initiated in Luni Tehsil of Jodhpur in collaboration with faculty from Dr. S.N. Medical College, Jodhpur, Director, ICDS and CMHO, Jodhpur. The project aims for clinical assessment, anthropometric measurements, assessment of micronutrient deficiencies, *viz* Iron, Zinc, Vitamin A and E, Calcium, Selenium, Chromium and lipid profile and the assessment of the dietary intake of the elderly population.

Nutritional status along with morbidity and mortality of under five children - a follow up study of earlier registered Neonates and Infants up to 5 years

The analysis of the children belonging to one & half years age group (N= 275) revealed that overall 34.6 percent children were under weight and 6.5 percent belong to severe category. Stunting (Height for age) was 63.6%. Main morbidities observed were, ARI (21.5%), fever (16.4%), GIT (15.3 %) and Ear disease (4.4%). Regarding nutritional deficiency signs, discoloration of hair, was observed 57.1% and Marasmus 0.4 %. It was observed that 96.7 percent of children belong to category of 'Breast feeding & Top Milk consumption' up to the age of 18 months. Mortality between one year - one & half year age group was 10.8/1000 whereas Mortality up to one & half year age (0-18 months)

was 53.3/1000.

Estimation of Zinc deficiency, under nutrition and morbidities especially ARI, Diarrhoea and Fever in school children of Rajasthan

This year data was collected from 255 more school children *i.e.* by now from 465 school children of 6 to 11 years belonging to 15 schools of Jodhpur district. Analysis of the serum zinc of 342 school children revealed that 72.5 percent school children were normal and 27.5 percent children were deficient in serum zinc. The deficiency was more in early age group *i.e.* 31.5 to 32.8 percent in 6 to 7 years age group than 11 years age group (15.4%). It was 28.4 percent in boys whereas 26.4 percent in girls. Analysis of 319 school age children according to Hb estimation revealed that only 26.6 percent children were non anemic. 53.9 percent children belong to mild category (10-11.5 g/dl) and 19.1 percent to moderate category (7-10 g/dl) of anemia. Underweight (Weight for age) in school age children observed 24.2 % and stunting 18.2%. Stunting was observed higher in females than males.

National Nutrition Monitoring Bureau Unit

The preliminary analysis of the survey completed in Jodhpur city, indicated that around 12.7 % of adults are diabetic, 14.53 % are hypertensive and 17.4 % are hypercholesterolemic. Survey is now carried out in Jaipur city. Eight wards have been surveyed till March, 2014. The anthropometric data, basic demographic details were collected and biochemical investigations have been done.

OTHERS

Facilitation of establishment of Model Rural Health Research Unit in Rajasthan

For the establishment of MRHRU, the process of allotment of land of 1200 sq.mt free of cost from

Rajasthan Govt. through local Panchayat in the name of D.H.R. is in progress. MOU with CPWD, Jaipur for civil work of MRHRU has been signed. Two rooms in the premises of CHC Bhanpur Kala and two rooms in Panchyat Bhawan adjacent to CHC have been provided by CHC and Panchayat Samiti of Bhanpurkalan to initiate the work. The first meeting of RAC was held on 1st February 2014 at Bhanpur Kalan, Jaipur. Nine studies were presented and discussed in this meeting. Three research projects have been initiated at MRHRU.

Annual Health Survey- Clinical Anthropometric and Biochemical Component (CAB)

Intensive training was provided to field survey teams consisting of health supervisors and health investigators of surveyed agencies in 4 batches with 15 members in each batch. They were familiarized with techniques for measurements of health indicators/parameters that are being measured in CAB survey (like height and length on stature meter and infantometer respectively, weight on digital weighing balance, blood pressure on digital BP monitor, blood glucose on glucometer).

Biomedical Informatics Centre

One of important objective of this project is to identify genetic loci associated with diseases of National interest such as Diabetes, Cancer, Stress, Mental illness *etc.* Data mining was done on various scientific databases such as pubmed, medline, clinical trials (clinicaltrial.gov), published studies and reviews (cochrane-reviews.org) to develop a knowledge stock of risk factor in consideration with DM risk. Longitudinal studies having different treatment groups for dietary factor in consideration were included. Appropriate combination of MeSH (Medical Subject Heading) terms was used to search relevant literature and clinical studies.

SUPPORTING FACILITIES

During the period under report, the National Institute of Epidemiology (NIE), Chennai, and the National Institute of Medical Statistics (NIMS), New Delhi, provided statistical assistance to various ICMR Institutes. Several new agreements and letters of intent were signed with different national and international organizations.

NATIONAL INSTITUTE OF EPIDEMIOLOGY, CHENNAI

National Hospital based Rotavirus Surveillance Network Project

In 2005, ICMR initiated a multi-centric surveillance system to estimate the burden of rotavirus diarrhoea in the country. The phase I of the surveillance was launched on September 19, 2012 in eight Clinical Recruitment Sites (CRS) under CMC, Vellore and one CRS under RMRC, Port Blair. The phase II of the surveillance was launched in September 2013 in four CRS under NIV Pune; four CRS under AIIMS, New Delhi; two CRS under NICED, Kolkata; two CRS under RMRC, Dibrugarh and two under RMRC, Belgaum. The phase III was launched in July, 2014 involving two CRS under RMRIMS, Patna; two

CRS under RMRCT, Jabalpur; one under RMRC, Bhubaneswar and one under NIE, Chennai (Fig. 1).

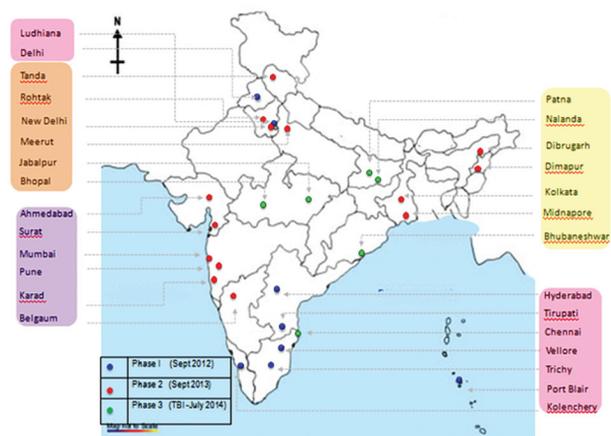


Fig. 1. Rotavirus surveillance sites.

A total of 5574 children were enrolled and 5311 stool samples were collected in the study during September 2012 to March 2014. Of the 5574 enrolled, 47% (2500/5311) of the stool samples were positive for rotavirus. The region-wise rotavirus positivity rates are shown in Fig. 2. *G1P*[8], *G2P*[4], *G9P*[8], *G12P*[6], *G12P*[8] were the major genotypes.

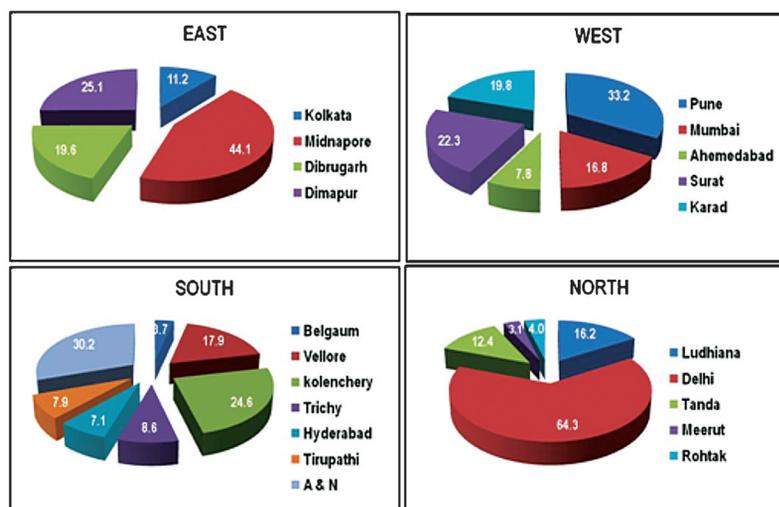


Fig. 2. Distribution of rotavirus positivity by region.

Hospital based surveillance for bacterial meningitis

Following the introduction of pentavalent vaccine in Kerala and Tamil Nadu, the Ministry of Health and Family Welfare decided to initiate hospital based surveillance for bacterial meningitis caused by *H. influenzae*, *S. pneumoniae* and *N. meningitidis* in India to determine trends of bacterial meningitis in children 1 month to 59 months of age at the surveillance sites. NIE is coordinating the activities of the surveillance network.

Since its initiation (March 1, 2012), the project is functional in 10 sentinel sites. The 11th site (New Delhi) has been added in March 2013.

A total of 7024 children were included as per protocol in the Bacterial Meningitis surveillance for a period of two years (March, 2012 to February, 2014) from 11 sentinel sites. Four hundred and six (5.8%) of these cases were confirmed to be due to bacterial meningitis, either by latex agglutination or CSF/blood culture (Table 1).

Table 1: Summary results of the monthly consolidated report sent by sites (March 1, 2012 to February 28, 2014)	
Details	N (%)
Total no. of in-patients in 11 sentinel sites	156154
Total admitted as in-patients with fever	49006
Proportion of suspected bacterial meningitis cases among fever inpatients (n=49006)	7024 (14.3)
Proportion of LP done among the suspected cases (n=7024)	6262 (89.1)
Proportion of probable cases of bacterial meningitis (n=6262)	685 (10.9)
Proportion of confirmed cases of bacterial meningitis (n=7024) (either by Latex, or CSF culture or blood culture)	406 (5.8)
<i>H.influenzae</i> type b (n=7024)	079 (1.13)
<i>S.pneumoniae</i> (n=7024)	313 (4.46)
<i>N.meningitidis</i> (n=7024)	014 (0.20)

WHO-TDR multicentric trial on uniform multi-drug therapy (Uniform MDT) regimen for all types of leprosy patients

There is a need for a simplified treatment regimen that does not require skills to classify disease

and shorten the duration of treatment. This will facilitate sustaining leprosy control activities through primary health care facilities. WHO-TDR supported multicentric trial was aimed at assessing efficacy of six-month multi-drug therapy (MDT) regimen currently recommended for multi-bacillary (MB) patients as uniform MDT for all types of leprosy patients.

This open design trial requiring 2500 newly detected, previously untreated patients each in MB and PB groups, respectively, is being conducted at six sites in India (Tiruvannamalai, Villupuram, Pune, Agra, Gaya and Rohtas) and at two sites in China (Guizhou and Yunnan). In the annual follow up of enrolled patients, clinical improvement (inactive, improved or static) is recorded based on standardized clinical criteria. An individual, who after completion of treatment develops one or more new skin patches consistent with leprosy, without evidence of reactions, is considered to have relapsed.

The study enrolled 3392 patients during 2003-2008. Of these, 38% were MB and 4% had grade 2 disability. Of the 3130 who completed treatment, skin lesions were inactive in 42% of PB (n=798) and in 10% of MB (n=122) patients (P<0.001). At the end of five years of follow up, lesions were inactive in 91% in PB patients and 79% in the MB group (P<0.001). Totally 1165 adverse events were reported and 48% were reported from MB group. In the MB group, 17% migrations and 8% deaths were reported. In the PB group, migrations were 29% and deaths 4.3%. In the MB group, 11 and 12% developed new lesions and neuritis, respectively and 17% had type I and 4% had type II reactions. In the PB group, the adverse events reported were 3.3% new lesions, 5.3% neuritis, 6.8% type I reaction, and 0.5% type II reaction. Six patients (MB=4, PB=2) had clinically confirmed relapse that occurred between the first and third year of follow up. The relapse rate among MB patients was 0.069 per 100 PY and among PB patients was 0.021 per 100 PY. In summary, the interim findings suggested that only a few relapses have occurred and the regimen was found to be effective, acceptable and operationally convenient. In conclusion, the U-MDT regimen was successful in preventing relapse to less than the pre-defined targeted relapse rates of 5% in 5

years in clinical trial set up. However, application of U-MDT in the programme requires several practical considerations.

Socio-cultural features and stigma of leprosy for treatment & control in general health services in India: Cultural epidemiological study

Although leprosy treatment is offered through general health services, there is a need to determine whether and how social and cultural features of leprosy affect access and the quality of clinical services and leprosy control that are required for effective control with integrated services. To address this issue, the NIE is co-ordinating a multicentric task force study, being conducted in five sites in India (Fig. 3).



Fig. 3. Participating sites of multicentric task force study.

The study collected information from patients, health staff (both health services and private sector) and community (leaders and family members). The study gathered information on help-seeking and treatment experience, illness explanatory models, stigma and disease awareness from patients of leprosy and a comparative group of non-leprosy patients (tuberculosis, malaria, and skin diseases other than leprosy/STD) through in-depth interviews using Explanatory Model Interview Catalogue (EMIC) instrument. The planned sample size was 100 patients in these groups. The family and community leaders were interviewed using topic guides on aspects of support, stigma features and community participation. Both government and private health providers were interviewed on

integrated health services, care and treatment-specific issues.

All the participating centers have completed data collection and the data analysis is in progress. The analysis of the data from NIE site (Tiruvannamalai district) indicated that overall anticipated stigma was more prominent among leprosy than non-leprosy patients. Public health facilities were the first help sought by most of them. Awareness about the causes of the illness needed more attention for leprosy and tuberculosis patients.

Mapping and size estimation of Hijras and other transgender populations in States of India

Hijras and other transgenders (TGs, male-to-female, MTF) have been recognized by NACO as important core populations for whom appropriate HIV intervention programmes need to be developed. Mapping of sites (frequented by *hijras* / where they reside) is needed for identification of project sites for HIV prevention/intervention. NIE conducted a study in 17 Indian States to map the areas in which *hijras* and other MTF transgender people reside and/or where they can be potentially reached, and to estimate the population size of *hijras* and other MTF transgender people.

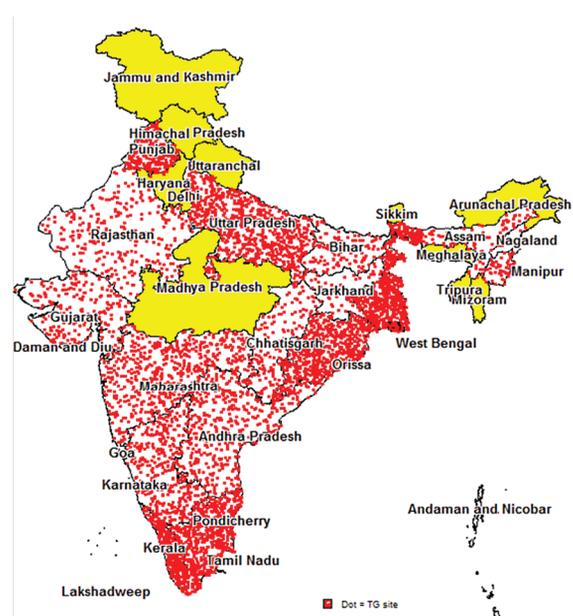


Fig. 4. Map of India depicting the 17 States with TG sites

A total of 5821 TG sites were mapped in the 17 study States, with 1664 (28.6%) in rural areas and 4157 (71.4%) in urban areas. Top five States reporting the

highest number of sites were Uttar Pradesh (825), West Bengal (752), Odisha (696), Tamil Nadu (649) and Maharashtra (586), comprising about 60% of all sites (Fig. 4). The total TG population (point estimate) across the 17 States was 62137, with the range of 53280-74297, having 21% in rural areas and the rest 79% in urban.

Comprehensive approach to condom promotion yields results among long distance truckers who are clients of female sex workers in India: The case of Avahan interventions

As clients of female sex workers (FSW) truckers are vulnerable to HIV and enhance the risk of transmission to their sex partners including spouse. The Avahan interventions in India are aimed to mitigate such risks. The data from 2009 cross-sectional survey for Integrated Behavioral and Biological Assessment (IBBA) were analysed. A composite variable, ‘consistent condom use’, was created using six questions (‘every time’ use with regular FSW AND ‘every time’ use with occasional FSW AND used condom at last sexual intercourse with regular FSW AND used condom at last sexual intercourse with occasional FSW AND ‘every time’ use during anal sex with FSWs AND not even one occasion when condom was not used during anal or vaginal sex with a regular or occasional FSW). Propensity score matching was used to estimate the

impact of various Avahan interventions components on consistent condom use with FSWs by calculating the average treatment effect on the treated (ATT).

Of the 2085 truckers, the data from the highest risk group of 505 (24%) who visited female sex workers in the last year were analysed. Prevalence of consistent condom use by truckers was 75% (379/505) with FSWs, 30% (44/145) with non-paid and non-marital female partners, 19% (14/73) with male partners and 56% (283/505) with all non-marital partners. In the last year, 43% (216) of truckers were contacted by a peer educator, 38% (191) participated in entertainment event, 35% (178) visited the Avahan-branded “Khushi” clinic, 30% (153) received counselling, 24% (121) received condoms from peer educators, and 11% (57) attended training or meeting. Consistent condom use with FSWs was highest among truckers who attended trainings/meetings at 90%, 17.5% higher compared to the matched controls who did not attend (P<0.05). Consistent condom use was also significantly higher for truckers who received counselling (ATT=14%, P<0.01), were contacted by peer educator (ATT=13%, P=0.001), participated in an entertainment event (ATT=12%, P<0.001), visited “Khushi” clinic (ATT=11%, P<0.005) or received condoms (ATT=10%, P<0.02, Table 2).

Table 2. Observed effect of Avahan interventions on consistent condom use with female sex workers among long distance truckers who solicit sex workers, IBBA-2, India (N=505).

Intervention component (% exposed)	Consistent condom use (%)		Average treatment effect for treated (ATT %)	P-value
	Treated	Matched untreated		
Contacted by peer educator (42.6)	81.8	68.7	13.1	0.001
Attended entertainment event (37.7)	81.6	69.4	12.2	0.001
Visited “Khushi” clinic (35.1)	82.6	72.0	10.6	0.005
Received counseling (30.2)	85.4	71.1	14.3	<0.01
Received condoms (23.9)	83.3	73.3	10.0	0.02
Attend trainings/meetings (11.2)	89.6	72.1	17.5	0.03

Coverage and effectiveness of Japanese encephalitis (JE) vaccine, Gorakhpur, Uttar Pradesh

In the Gorakhpur division of Uttar Pradesh, Japanese encephalitis (JE) vaccine was introduced in the routine immunization programme in 2011, as a single dose strategy for children aged between 16-24 months, administered along with DPT/OPV booster. A study was conducted to estimate the coverage and effectiveness of this vaccine. A total of 3200 children from four districts of Gorakhpur division were surveyed. The coverage of JE vaccine in the division was 51% and ranged between 36% in Kushinagar to 66% in Gorakhpur district (Fig. 5). The coverage was not different between gender or caste groups.

To estimate the vaccine effectiveness, 33 cases and 66 controls were included in the case-control study. About 76% of JE cases and 48% of controls were aged >36 months. The cases and controls were similar with respect to gender and caste groups; 18% of cases and 53% controls had a history of JE vaccination. On univariate analysis, the JE cases as compared to healthy controls, were more likely to be older (OR: 3.32, 95% CI: 1.31-8.42), unvaccinated (OR associated with vaccination: 0.2 0.07-0.54) and did not have the vaccination card (OR: 6.1, 95% CI: 2.22-16.77). The age adjusted odds ratio associated with JE vaccination was 0.16 (95% CI: 0.05-0.47). The effectiveness of the vaccine was 84% (95% CI: 53-95). The findings of this study indicated that the coverage of JE vaccine in the Gorakhpur division was low with only half of the

eligible children in Gorakhpur division receiving one dose of the vaccine. The effectiveness of one-dose JE vaccination strategy with SA-14-14-2 strain was high and was comparable with studies conducted in Nepal and China.

Evaluation of hypertension management in the non-communicable disease programme in Chennai, Theni and Villupuram districts : Tamil Nadu Health Systems Project

The government of Tamil Nadu has been implementing community-based and clinic-based interventions for non-communicable diseases in 32 districts of Tamil Nadu since 2012 through the World Bank funded Tamil Nadu Health Systems Project (TNHSP). Interventions include community based awareness to self help groups, awareness through mass media and clinic based screening and treatment for hypertension, diabetes, cancer cervix and cancer breast. Opportunistic screening is being offered in all Primary Health Centers (PHCs) and hospitals for hypertension, diabetes, cancer cervix and cancer breast for those visiting these facilities for any ailment or accompanying the patient. NIE has been working in collaboration with TNHSP, Government of Tamil Nadu, for concurrent evaluation of this programme.

One of the components of the evaluation is the patient survey pre- and post- intervention to assess the change in the treatment practices and self-management among patients with hypertension. The key objectives were to estimate the blood pressure control, level of adherence and self-management

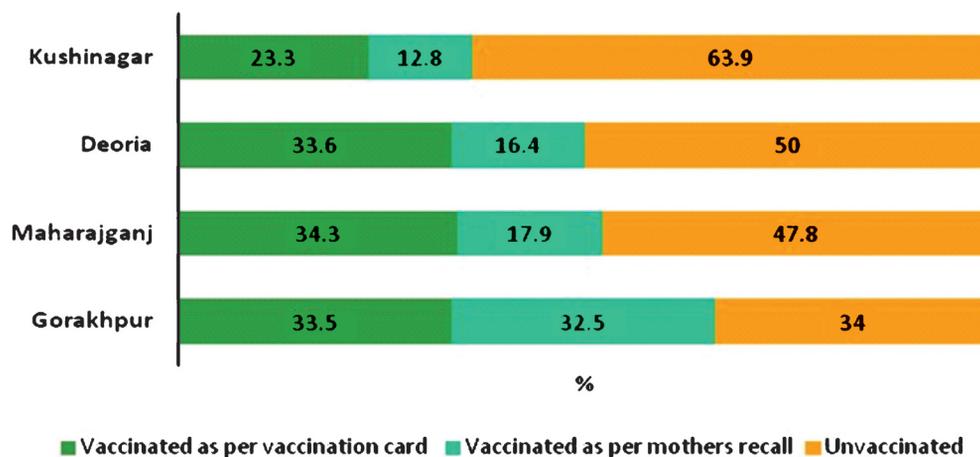


Fig. 5. JE vaccine coverage, Gorakhpur division, 2013.

practices among patients with hypertension in the primary health centers/ government hospitals in Theni and Villupuram districts and the urban PHC in Chennai.

A pre (January to May, 2012) and post-intervention (January to May, 2014) cross-sectional survey of the patients visiting the selected primary health centers (PHC) and all the government hospitals in Villupuram and Theni districts, was conducted, and 18 urban PHC in Chennai where the interventions were initiated were surveyed. The patients in each health facility were recruited consecutively starting from the first hypertension patient reporting on the day of the survey. The sample size for PHCs was 180 from 15 PHCs with a cluster size of 12 patients. A semi-structured, interviewer administered questionnaire was used to collect data. At baseline, 180 patients in Villupuram and Theni PHCs and 216 in Chennai urban PHC were surveyed. During follow up, 185 patients at Villupuram and Theni PHC and 200 in Chennai urban PHC were surveyed. In the hospitals, 339 patients were surveyed at baseline and 350 at follow up in Villupuram and 224

at baseline and 220 at follow up in Theni district. Demographic characteristics of the population were similar at baseline and follow up in all the settings. There was a decline in the atenolol prescriptions in all the settings except urban PHC in Chennai. There was an increase in the amlodipine prescriptions in the primary health centers of Villupuram and Theni. There was an increase in the proportion of patients with co-morbid diabetes taking treatment for hypertension in the primary health centers in Villupuram and Theni probably due to increased detection of diabetes in the screening programme. Remarkable increase was observed in the proportion of patients who were counseled for dietary changes and physical activity by the health staff in all the settings. There was an increase in the awareness regarding target organ complications of hypertension, however, the overall proportion of the patients who had awareness was low. There was an increase in the proportion of patients who achieved blood pressure control in all the settings. Improvement was higher in the PHCs of Villupuram (38 vs. 47%) and Theni (29 vs. 43%) (Table 3).

Table 3. Summary of findings in Primary health centers Villupuram, Theni and Chennai, 2013-14

	Villupuram		Theni		Chennai	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
	N=180	N=185	N=180	N=186	N=216	N=200
Demographic characteristics (%)						
Age >60 years	63	66	70	72	49	60
Females	63	67	57	59	79	71
Never attended school	56	57	40	61	34	41
Labourer (skilled/unskilled/ agricultural)	35	39	38	39	16	10
Drugs prescribed for hypertension (%)						
Amlodipine	28	41	52	55	44	40
Atenolol	70	53	67	44	43	57
Enalapril	15	27	21	25	24	44
Co-morbidities (%)						
Diabetes	26	34	28	36	58	44
Heart disease	5	9	11	1	7	11
Asthma/Respiratory diseases	8	10	5	3	6	13
Lifestyle modifications (%)						
Reduced fried snacks	47	34	53	44	45	40
Reduced salt intake	59	64	41	79	40	47
Started walking regularly	12	8	14	20	22	24
Counselling on lifestyle modification by government medical staff (%)						
Diet	54	84	62	83	57	70
Physical activity	7	34	9	67	13	56
Anti-hypertensive drug adherence for previous 30 days (%)	36	42	47	70	67	63
Awareness of consequences of high BP (%)						
Kidney problem	8	15	7	20	2	20
Eye problem	4	26	11	20	3	22
Health system issues (%)						
Time to reach hospital >30 min	27	29	21	13	4	8
Getting medicines from hospital pharmacy	86	88	95	96	96	76
Advised by staff regarding prescribed drug schedule	86	99	88	96	99	98
Anti-hypertensive drugs issued for 30 days (%)	0	93	0	59	0	10
Achieved BP control (Systolic <140mmHg, Diastolic <90 mmHg) (%)	38	47	29	43	25	30

NATIONAL INSTITUTE OF MEDICAL STATISTICS, NEW DELHI

COMPLETED STUDIES

Extent of integration of Indian system of medicine & homoeopathy (AYUSH) in National Rural Health Mission (NRHM)

The objectives of this study were to measure the extent of main streaming of Indian Systems of Medicine (AYUSH) under NRHM, and to study the impact of NRHM programme in the utilization of AYUSH in the demographically weak districts of U.P. The chief findings of this study were (i) additional doctors from AYUSH have been appointed on contractual basis in all the districts; (ii) the appointment of AYUSH doctors and pharmacists are being renewed timely on completion on their term; (iii) all prescribed medicines of AYUSH are available at different levels in the State; (iv) these medicines are purchased by the district authorities; (v) all AYUSH doctors are prescribing only AYUSH medicines. AYUSH medicines are purchased by the district authorities as per the uniform guidelines of the State government, AYUSH doctors are participating in all National Health Programmes, viz., NRHM, MCH, RCH in the State; and (vi) there is an increase in the allocation of budget under AYUSH in each year of budget.

Multi level modelling to analyze RCH services: Utilization and its correlates

The prime objectives of this study were to assess the inequity in the access and utilization of maternal RCH services (ANC and PNC) and child health care (immunization and treatment seeking behaviour), and to investigate the degree to which the RCH utilization is influenced by the contexts within which the people live and other explanatory variables and to assess the superiority of multi-level modelling approach over the standard logistic regression for the current situation. Comparing with the conventional regression model with the same covariates, it was found that the estimated model was different from the traditional regression model. The results of this study reflected the same thought and the outcome under this structure was influenced not only by the individual level factors,

but also the context in which individuals live. In this analysis the causal pathways to lie at multiple levels simultaneously was anticipated. It is essential to ascertain the contribution of the different sources or levels to the variation in the outcome.

Prevention of HIV/STI among married women in urban India (A five-year NIH funded intervention project)

The specific objective of this study was to develop and evaluate a culturally appropriate, theory-driven, health facility-based intervention utilizing enhanced women's health services and intervention with couples to promote primary prevention of HIV and other sexually transmitted infections (HIV/STI) among married women between 18 to 40 years of age, living in an urban poor community in Mumbai, India. Some of the lessons learned from this project are as follows: (i) The leading problem that women bring to health care providers is white discharge. The syndromic guidelines for treatment of this and related gynaecological symptoms call for antibiotics to address STIs. The RISHTA project found that only one of the first 230 women recruited and presenting symptoms of white discharge had an STI. The results showed that white discharge was more a marker of psychosocial issues rather than biomedical disease. (ii) To address women's psychosocial needs, an individual counselling programme was developed as a complimentary component to medical care. Women who received medical care and individual counselling had significantly more positive outcomes in sexual health, psychological well-being and marital communication than those who received medical care only. These results demonstrated the need to broaden medical care to address the psychosocial issues associated with physical health symptoms. (iii) Involvement of men in women's health and well-being is crucial. Women who received medical care and participated with their husbands in couple's intervention also had significantly more positive outcomes in sexual health, psychological well-being and marital communication than those who received medical care only. Couple's intervention provides a means of engaging men and improving the marital relationship and can be implemented by NGOs and the religious and educational sectors of the community in partnership

with primary care centers. (iv) The positive changes that occur at the individual and family levels must be supported by comparable changes in the cultural and community context. Toward that objective, RISHTA worked the religious sector and the NGOs in the community to promote gender equity and reduce sexual risk. Three annual surveys were conducted and significant improvement was found in men's gender equity attitudes in the intervention as compared to the control community.

A study on the potential gain in life expectancy after elimination of specified causes of death in selected States of India

The findings from this study have implications for practical decision making in setting up health goals, allocating resources, and evaluating health programmes. From an economic point of view, the potential gain in life expectancy for the working population is a better mortality indicator than the number of deaths or its related measures. Hence, an oversimplified approach of allocating research funds based on the number of deaths from different categories of diseases is misleading. The essential question is how to use disease prevention to improve health and reduce health disparities together with policies that aim to reduce socio-economic disparities, reform health care, and improve quality of care.

Maternal health care in rural and urbanized villages of Delhi : A comparative study

This study was conducted to test the hypothesis that utilization of antenatal care, natal (delivery) care and post-natal care among mothers living in urbanized villages was more than that among mothers living in the villages (non-urbanized). A sample of 840 mothers, (420 from each of the two districts of Delhi, South and South West) were interviewed for collecting information on antenatal care, care during delivery and post-natal care and other related aspects. No difference was seen in ANC registration and utilization between the two types of the villages. Further analysis revealed that mother's and husband's education, economic status and religion of the family had significant influence on the utilization of maternal health care services. No significant difference was observed in the number of deliveries conducted in institutions and homes in the two types of the villages. The

utilization of safe delivery care was found to be significantly higher in urban villages than in other villages. In addition to mother's and husband's education, economic status and religion of the family, utilization of safe delivery services was also associated with husband's occupation. Post-natal care was provided to more than 90% of the mothers in both types of the villages. Furthermore, it is important to note that factors like type of family and caste are also associated with post-natal care, besides all those factors that are associated with safe delivery.

National sample survey for assessment of disease burden due to leprosy (Collaborative study with JALMA as an intramural activity)

The objectives of this study was to provide technical and statistical support to NJILOMD, Agra for survey methodology and statistical analysis. As per the inverse sampling plan, survey was carried out in the population until pre-decided new cases were detected. In addition to new case detection, data were collected on the socio-demographic profile of the study population. The socio-demographic profile of the study population *vis-à-vis* the census population of the State was compared. The inverse sampling methodology was simple and could be adopted by the system. Also, repeat survey could be conducted as an active surveillance to detect new cases and thereby treatment to reduce transmission in the population.

A retrospective analysis of a hospital based study of febrile patients in the endemic area of North-East India

This study emphasized the need to strengthen and improve the treatment facilities for malaria in government hospitals and to create more awareness among people regarding early treatment, especially in the rural periphery villages of the endemic areas.

Ongoing Studies

The ongoing studies include the following: (i) The Clinical Trials Registry – India (CTRI) www.ctri.nic.in; (ii) Acceptance level, knowledge, attitude and practice of Indian System of Medicine in North-East; (iii) Baseline Household Malaria Survey in World Bank project States, 2013; (iv) NACO's HIV

Sentinel Surveillance, Modelling, Estimation and Projection; and (iv) Knowledge Network Project on HIV/AIDS.

INTERNATIONAL HEALTH DIVISION

The International Health Division (IHD) in ICMR co-ordinates international collaboration in biomedical research between India and other countries as well as with national & international agencies. Recently, the Department of Health Research (DHR) has signed a Memorandum of Understanding (MoU) with National Institute of Health & Care Excellence (NICE), UK. The regular meetings of Joint Working Group (JWG) or Joint Steering Committee (JSC) with various countries/international institutes/organizations are organized to review, develop and finalize joint collaborative programmes, decide future plans of action and identify priorities for bilateral cooperation. During the year, the JWG/JSC meetings under various MoUs and Joint Statements were held. These included the ICMR – MRC JSC meeting held in London on June 13 to 14, 2013; Indo-EU Group of Senior Officials (GSO) meeting through Video Conference on October 8, 2013; ICMR-INSERM JWG meeting held in New Delhi on October 22, 2013; Working level meeting between officials of ICMR & MRC, UK held in New Delhi on November 11, 2013; Indo-US third JSC meeting on Diabetes Research through video-conference on November 26, 2013; Global Alliance for Chronic Diseases (GACD) Board meeting through teleconference held on December 10, 2013.

The various international workshops/meetings were held under bilateral/multilateral programmes such as; Indo-Norway Workshop on antimicrobial resistance (AMR) was held in Norway in September, 2013; Meeting of Science Counselors of EU States organized by the office of EU Delegation in New Delhi in December, 2013; ICMR- MRC Workshop on Mental Health was held at NIMHANS, Bangalore during February 26 to 28, 2014; ICMR-Academy of Finland workshop on Chronic NCDs at ICMR Headquarters, New Delhi, during March 11-12, 2014.

New Memoranda of Understanding (MoU)

The MoUs signed during this period included, DHR-National Institute for Health and Care

Excellence (NICE)-MoU signed in UK on June 14, 2013 and Addendum two- Global Alliance for Chronic Diseases (GACD) Secretariat Funding and Collaboration Agreement as part of ICMR-GACD MoU signed in New Delhi on March 5, 2014.

Dialogues were initiated and documents were forwarded to Government of India (GOI) for approval of Indo-US Joint Statement on Environmental and Occupational Health; Maternal and Child Health; ICMR-International AIDS Vaccine Initiative, USA and ICMR-University of Sydney/George Institute, Australia and NHMRC, Australia, Drugs for Neglected Diseases Initiative (DNDi) and Bill & Mellinda Gates Foundation (BMGF), USA. Approval of GOI is awaited. Proposed MoUs with Government of Saskatchewan, Canada; German Research Foundation (DFG), Germany; International Consortium on Anti Virals (ICAV), Canada; Indo-US International Centre for Excellence in Research (ICER at NIRT, Chennai), tripartite MoU between DHR, NICE and Ministry of Public Health, Islamic Republic of Afghanistan are under negotiation/consideration. In addition to these meetings, the International Health Division has also represented ICMR in various bilateral/multilateral Joint Committee meetings coordinated by MEA, DST and Ministry of Health & Family Welfare, Government of India for cooperation with various countries such as IBSA, Canada, Germany, Sweden, European Union, South Africa, Australia, Tunisia, Mozambique, UK, Bangladesh, ASEAN, etc. 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 34 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 Government of India through Health Ministry's Screening Committee (HMSC). The projects are peer reviewed by the concerned Technical Divisions at ICMR Headquarters and then placed before the HMSC for consideration and

decision. During the year 2013-14, four meetings of HMSC were organized, wherein 138 projects were considered, and of which, 77 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, eight projects are co-funded by ICMR.

The IHD prepared a document entitled “An overview of International Collaborative Research Projects in Health Research” approved by HMSC Volume II January, 2008 to December, 2012, which was released during the HMSC meeting held on June 6, 2013. The document provides the digest on the international collaborative research projects approved by HMSC during the period with a brief account on Indian PI, Indian intuitions, funding agency, *etc.* The IHD also prepared and submitted the “Report on Outcome of International Collaborative Research Projects approved by HMSC and funded by ICMR during January, 2008 to December, 2012”. This report was presented to the committee members during the HMSC meeting held on September 16, 2013.

International Visitors/Dignitaries

The Division also organized visits by various visitors to ICMR from foreign countries / agencies such as MRC, UK; BMBF, Germany; Institute of Merieux, France; ICAV, Canada; Bill & Mellinda Gates Foundation (BMGF), USA; Messey University, Newzealand; Sheffield, Children’s Hospital, UK; LSHTM, UK; CSIRO, Australia; University of Texas, USA; EMBL, Germany; Mozambique RCN, Norway; Simon Fraiser University, Canada; IVI, South Korea, Academy of Finland (AF), FORTE-Sweden; NCI (NIH), USUH; Harvard Medical School, CDC, USA, *etc.*

The ICMR International Fellowship Programme for Indian biomedical scientists is aimed 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 eleven young Indian scientists during the year 2013-14.

The mission of the virtual Indo-German Science Centre for Infectious Diseases (IG-SCID), established at ICMR is to coordinate joint research in identified areas of infectious diseases and to initiate proactive scientific cooperation with equal participation of Indian and German scientists. The Council has taken up a project entitled, “Managing the Indo-German (ICMR-HGF) Science Centre for Infectious Diseases” and under this programme four collaborative projects were approved, funded and completed.

LIBRARY & INFORMATION SERVICES

The subscription for the e-journals Lancet, Science, Nature, and NEJM for all ICMR Library & Information Centres has been continued in consortia mode. Subscription for full text electronic data base ProQuest Health & Medical Complete including ProQuest Medical Library (covers about 3000+ journals) 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. J-Gate provides access to millions of journal articles available online offered by 11,419 Publishers. It presently has a massive database of journal literature, indexed from 36,709 e-journals with links to full text at publisher sites. J-Gate offers two types of products/services: **J-Gate Portal:** a. Table of Contents (TOC) For 36,709 e-journals. b. Database - A comprehensive searchable database with 36,569,422 articles, with 10,000+ articles added every day. **J-Gate Customized Services:** a. J-Gate Custom Content (JCC)- Local Intranet /Internet solution to libraries, providing e-access for subscribed journals. b. J-Gate Custom Content for Consortia (JCCC) - JCC extended to a homogeneous group of libraries for sharing “subscribed” journal resources.

Subscription to DELNET institutional membership has been renewed. The following databases are available from DELNET which can be accessed through online by the institutional members.

Union Catalogue of Books (1.28 crore bibliographic records)

Union Catalogue of Periodicals (33.9 thousand records)

Database of Periodical articles (9.22 lakh records)

CD-ROM Database (22 thousand records)

Training programme on JCCC@ICMR and J-Gate Plus has been organized on 11.12. 2013 for Delhi based ICMR institutes. Training programme for ProQuest Health & Medical complete was organized at ICMR Hqrs. on 07.03.2014 and regional wise training programmes for J Gate Plus were organized at NIRT, Chennai on 10.03.2014, RMRC, Bhubaneswar on 21.04.2014 and at NIOH, Ahmedabad on 05.06.2014 .

TRANSLATIONAL RESEARCH PROGRAMME OF ICMR

Thirty indigenous affordable technologies for public health use are targeted to be ready by 2014-2015. The following eight indigenous technologies were launched during 2013-2014 by the Hon'able Union Minister of Health & Family Welfare:

- (i) The first indigenously developed Japanese Encephalitis vaccine (JENVAC) under Public Private Partnership was launched on October 4, 2013. The indigenous virus strain was isolated and characterized by the ICMR's National Institute of Virology at Pune and the strains were transferred to Bharat Biotech for further vaccine development;
- (ii) "Thalassemia Detection Kit" was launched on December 17, 2013. This kit was developed by the ICMR's National Institute of Immunohaematology (NIIH), Mumbai;
- (iii) An indigenous device "AV Magnivisualizer" for detection of cervical cancer was launched on December 23, 2013. The device was developed by the ICMR's Institute of Cytology and Preventive Oncology (ICPO), Noida;
- (iv) Two simple and affordable technologies for glucose monitoring devices and testing strips-Suchek and QuickcheQ were launched on January 13, 2014. These two technologies were developed by Nanobios Lab, Indian Institute of Technology, Mumbai and BITS, Hyderabad respectively;
- (v) Three indigenous affordable technologies namely, PCR based Food Pathogen Detection Kit, ELISA kit for Ferritin estimation and Dried Blood Spot (DBS) collection kit for vitamin A analysis, were launched on February 20, 2014. These kits have been developed by the ICMR's National Institute of Nutrition, Hyderabad.

INTELLECTUAL PROPERTY RIGHTS

A total of 18 patents were filed during fiscal year 2013-2014 with the Indian Patent Office. Of these, 11 were from intramural institutes. Maximum patent applications (8) were filed for diagnostics followed by drugs. Six PCT applications were filed during this period with two National phase applications. During this period, three patents (2 from intramural and 1 from extramural institute) have been granted.

Release of ICMR's affordable technologies

Six affordable technologies were released during the year.

Detection kit of Food borne pathogens: Food and water borne diseases are caused by ingestion of contaminated food and/or water with certain bacteria, viruses or parasites and toxins generated by the pathogens. An indigenous method to detect these microorganisms has been developed at National Institute of Nutrition (NIN), Hyderabad with the help of M/s BioServe Biotechnologies (India) Pvt Ltd, Hyderabad in the form of a kit. This PCR based kit is rapid, reliable, and have high specificity and sensitivity, >99% accuracy and is cost-effective against foreign imports. The kit can be used to detect several pathogens ranging from *Salmonella*, *Vibrio cholera*, *Vibrio parahaemolyticus* to *Staphylococcus aureus* and *Listeria* (Fig. 6).

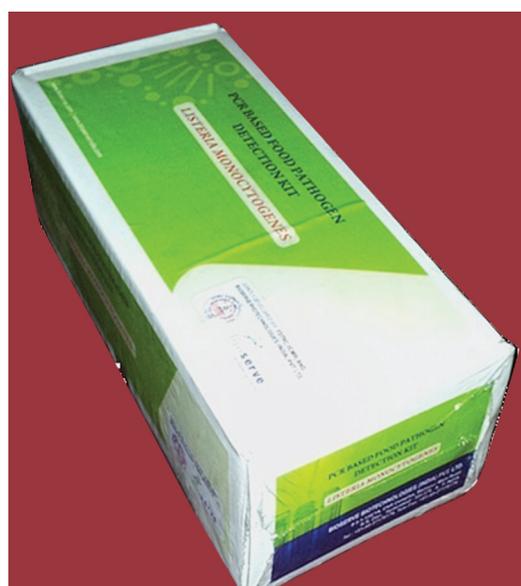


Fig.6. Detection kit of food borne pathogens

A.V. Magnivisualizer for detection of cervical cancer: Magnivisualizer is a low cost, portable, battery operated device used for screening of pre-cancerous and cancerous lesions of cervix and oral cavity. This device is suitable for use in rural areas, where electric supply is erratic. It has been designed and developed by the Institute of Cytology and Preventive Oncology (ICPO), Noida (Fig. 7). This device will provide an opportunity to detect high grade pre-cancerous and early cancerous lesions of cervix and would enable the physicians to treat the lesions in the same sitting without any potential loss to follow up of patients, which is a substantial problem in the rural areas. The technology has been transferred to M/s Smart Scientifics.



Fig.7. A.V. Magnivisualizer for cervical cancer

RDB kit for detection of β -thalassaemia: This diagnostic kit based on PCR and reverse dot blot hybridization is used for the detection of the common β -thalassaemia mutations/syndromes. The kit provides an easy and economical method for detection of six common Indian β -thalassaemia mutations along with two abnormal haemoglobins; HbS and HbE, in a single PCR and hybridization step with accuracy, without using radioactive isotopes and hazardous reagents. It covers about 85 to 90 % of the mutations seen in Indians and can be used for screening thalassaemic babies in the wombs of pregnant ladies. The technology has been transferred to M/s Imgenex India Pvt. Ltd., Bhubaneswar.

Dried Blood Spot (DBS) collection kit for vitamin A analysis: Vitamin A deficiency (VAD) is the leading cause of paediatric blindness and a major determinant of severe infections and mortality among children in the developing world. Measurement of vitamin A in blood (serum retinol levels) can identify

vitamin A deficiency (VAD). Serum vitamin A levels less than 20 $\mu\text{g}/\text{dl}$ is considered as cut-off for defining sub clinical vitamin A deficiency. DBS is a convenient field-friendly method of blood sample collection with reduced risk of infection (Fig.8). It involves collection of small amounts of blood from finger puncture on to a special type of filter paper, which is air dried and transported to the laboratory. Vitamin A is extracted from this and estimated by HPLC analysis. It eliminates the need for venous blood sampling. Vitamin A in dried DBS is stable for seven days at room temperature or at -20°C for 200 days. It has been developed by National Institute of Nutrition, Hyderabad.

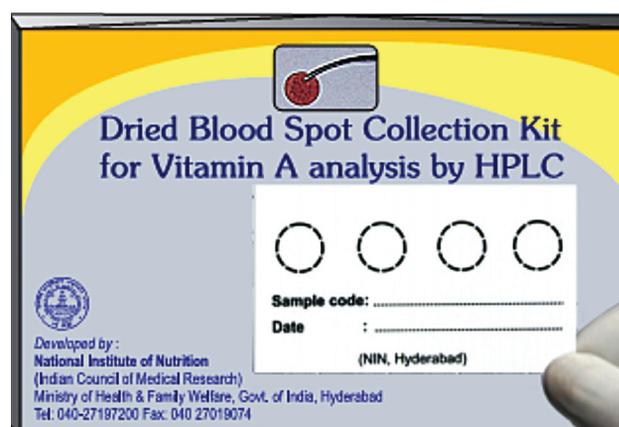
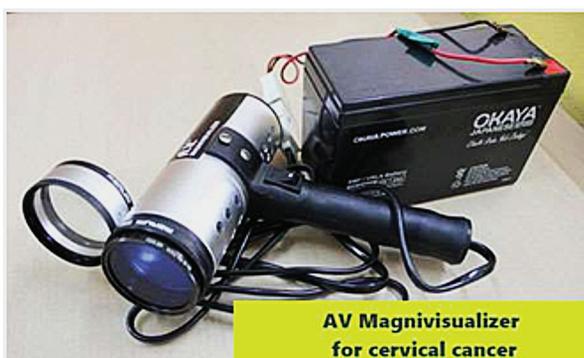


Fig.8. Dried blood spot collection kit for vitamin A analysis

ELISA kit for ferritin: This indigenous ELISA kit is used for assessing serum ferritin and the Caco-2 cell ferritin levels for establishing the measurements of iron bioavailability in the body. The Caco-2 cell line, derived from human colon adenocarcinoma, differentiates and closely resembles mature intestinal absorptive cells. It can be used as a screening tool for testing iron

bioavailability. Ferritin levels in population can be a useful indicator to define the cause and stage of anaemia. This technology has a potential application in iron bioavailability screening in fortified foods and pharmaceuticals, etc. The technology has been developed by National Institute of Nutrition, Hyderabad (Fig.9).



Fig. 9. Blood glucose monitoring device.

Blood glucose measuring device: An indigenous and affordable kit for detection and monitoring of glucose levels in diabetic patients has been developed. The kit will reduce the cost of diabetes management for patients by one-fourth and will make the government’s diabetes-screening programme more cost-effective (Fig.10).



Fig. 10. ELISA kit for ferritin.

Off-shelving of old technology

IPR Unit has off shelved an old technology on *Vijaysar*, an antidiabetic drug generated in 1999 to a company. This technology was an outcome of the joint effort of IIM, Jammu (CSIR) and ICMR. The

technology has been sold after signing a Tripartite Agreement and Assignment deed between the IIM, Jammu and ICMR.

HUMAN RESOURCE DEVELOPMENT

Junior Research Fellowship

During the period under report the ICMR conducted the 13th 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. Approximately 11,000 candidates appeared in the examination conducted at 10 centers (Bhopal, Bhubaneswar, Chandigarh, Chennai, Delhi, Guwahati, Kolkata, Mumbai, Hyderabad and Varanasi).

Financial assistance to MD/MS/DM/MCH thesis in priority areas of biomedical research

Financial assistance is provided to MD/MS/DM/MCH students (50/year) who are in the 2nd year of MD/MS course. The selection committee recommended financial assistance to 107 MD/MS/DM/MCH thesis out of 501 proposals received during 2013-2014. A total of 103 medical colleges/institutes participated in the programme and award to MD/MS given to 47 institutes.

MD, Ph. D. Programme (25 slots per year)

This 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. 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 ongoing at three universities (King George University, Lucknow; NIMHANS, Bangalore & Sri Ramachandra Medical College, Chennai). During 2013-2014 of the 15 allotted slots, 14 were selected. So far 70 candidates have joined the MD/PhD programme. Four candidates have submitted their thesis.

International conference/training/workshops to non ICMR scientist support scheme

Of the 1621 applications received during the year, 354 applicants were supported for international

conference/training/workshops, etc. An analysis of the availed 354 applications showed that 65.8 % (233) applicants were males and 269 (76%) applications were from candidates <35 years of age. A State-Wise analysis indicated that New Delhi was most active with maximum number of applications received, approved and availed (Fig. 11). AIIMS, New Delhi, emerged as the top most institute followed by SGPGIMS, Lucknow; PGIMER, Chandigarh; NIMHANS/ IISc, Bangalore (Fig. 12). Pharmaceutical sciences emerged as the top-most area for which this scheme was availed followed by Ophthalmology, Cancer Research, Neurosciences, Lung Health, Paediatrics etc. (Fig.13).

Post Doctorate Fellowship Programme (50 per yer): To identify and support young Ph.Ds for

conducting research in ICMR Institutes working for priority areas of health research, 63 applications were received and called for personal discussion and 34 were selected (Fig. 14). Programme is ongoing at ICMR Institutes. A total of 188 applications received so far, 73 candidates have joined; 33 PDFs completed the 2 years duration.

ICMR's AWARDS

Process has already been started for processing ICMR awards and Prizes for 216 applications (for the year 2011 & 2012).

Grant-in-aid for organising seminars/symposia/workshops

To update knowledge transfer from laboratory to land at national and international level in the

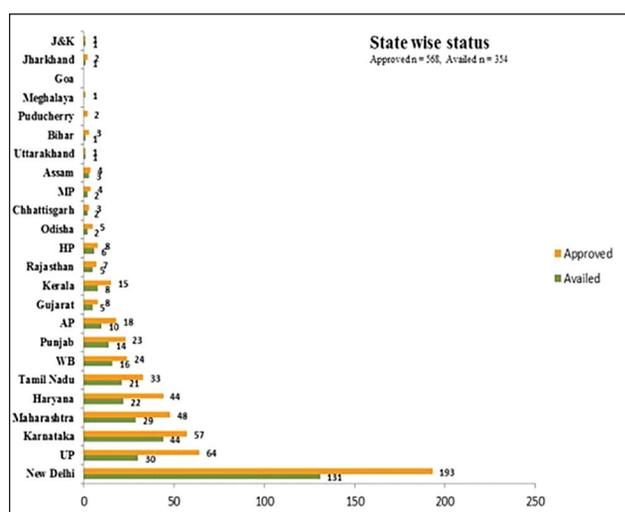


Fig. 11.

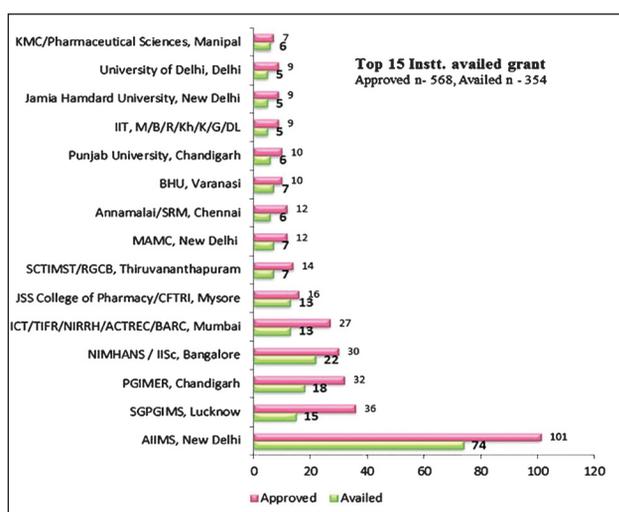


Fig. 12.

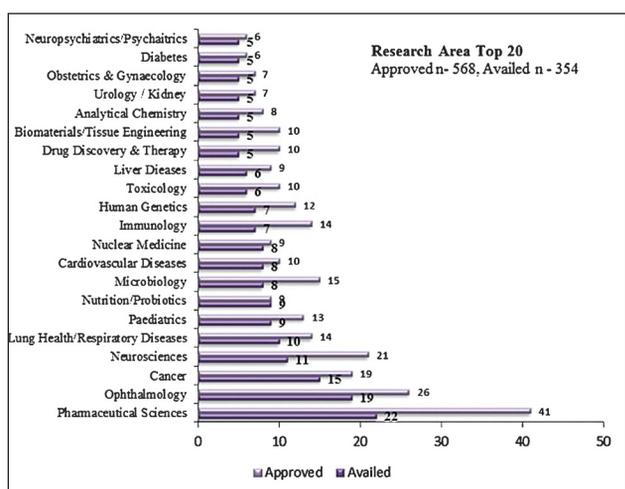


Fig. 13.

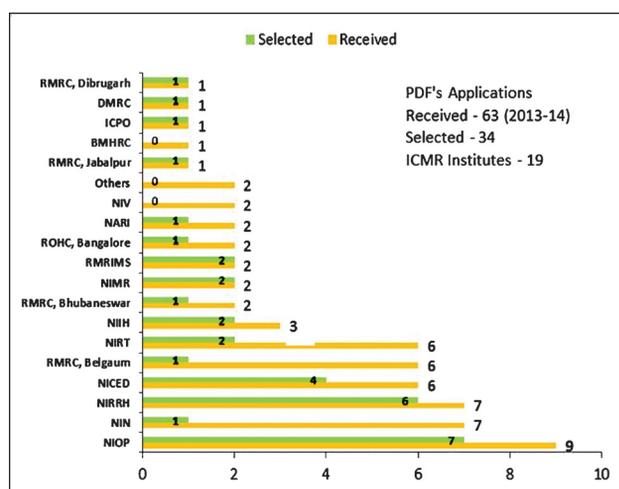


Fig.14.

concerned area, conferences/CME programmes/workshops, *etc.* a scheme is ongoing and of the total 980 applications received, 502 were approved during 2013-2014.

HEALTH SYSTEMS RESEARCH

The Health Systems Research (HSR) division has been working for developing the health systems research in the country and for strengthening the health systems in India by supporting ad-hoc as well as multi-centric Task Force projects for addressing issues related to various aspects of health infrastructure and delivery of health services in the country.

AD-HOC PROJECTS

Adolescent health care

Studies in progress in the States of Andhra Pradesh, Karnataka and Orissa have documented the implementation and impact of government adolescent health programmes. In Andhra Pradesh, the overall prevalence of stunting was 37% and of anaemia was 40% among adolescent girls. A great proportion of adolescent girls (86%) had low serum ferritin levels, of whom only 3% were aware of the government adolescent programmes. In tribal districts of Odisha, the prevalence of stunting was as high as 45% and about 70% of adolescents were suffering from different grades of anaemia. Less than 2% of adolescents were aware of the Adolescent Reproductive and Sexual Health (ARSH) programme. The Odisha study is developing an advocacy based intervention to improve the utilization of government programmes, particularly the ARSH services. Studies from Chandigarh have addressed the impact of mentoring relationship on adolescents. About 70% students thought mentors could help them in overcoming adolescent related problems and about 52% of adolescents felt the need for sex education. This study provided a strong basis for incorporating mentoring at the school/college level. Another study from Pune, assessed the problems of physically-challenged adolescents. Adolescents (25%) and their respective parents (30%) faced stigma due to their disability. At NIMHANS, Bangalore, training manuals on adolescence sexuality, gender discrimination and reproductive health to capacitate social work trainees have been developed. A study

from Karnataka has also qualitatively focused on adolescent health issues pertaining to males, which are neglected in many studies.

Gender perspectives of health

A study from Rajasthan focused on developing competency framework for mainstreaming gender-responsive health services. In a study from Maharashtra, the outcome was the TB-specific quality of care framework with gender perspective for the use in RNTCP. In Jammu & Kashmir State, health system's perspectives of gender in the context of conflict were studied. It revealed that conflict aggravated and intensified the women's problems in Jammu & Kashmir during the last two decades.

Health care delivery and access

Health insurance being an important issue to achieve universal health care, HSR Division has promoted studies on health insurance. Currently two studies are in progress, one in Andhra Pradesh and the other in Karnataka State. These studies are aimed at collecting information of awareness and prevalence of health insurance and other related issues pertaining to the acceptance of health insurance by the community. About 60% of tribal population in Andhra Pradesh was not aware of the concept of insurance. Among those who were aware of it, around 88% were aware of life insurance and only 18% were aware of health insurance. About 70% of tribal households were keen to take health insurance for themselves and their families. In the rural community of Karnataka, about 72% of people were aware of health insurance. Mostly they got this information and availed the health insurance through women self help groups. Of those not having health insurance, only 30% had shown their willingness to pay. Studies have also been devoted to understanding issues related to health care access, particularly among vulnerable population groups. A study from Kerala evaluated maternal and child health care issues among scheduled tribe population with a focus on health infrastructure and manpower deployment.

Other issues

HSR Division has funded a study to develop patient friendly, IT enabled tools for selection of

desired health care services. A study by a medical college in Surat, Gujarat, did critical evaluation of the negative studies published in various Indian biomedical journals. It was found that of the total (7566) original articles published during 2000-2011 in Indian medical journals, only about 4% were negative studies. It also provided some guidelines for the publication of negative studies. A study undertaken by a nursing college in Karnataka, was aimed to document the impact of supervisory support training programme on the performance of health workers posted at PHCs.

Multicentric Task Force projects on migrants' health problems and health systems responsiveness

Community-based interventions to improve the health care access among migrants living in 13 cities are ongoing under two Task Force projects. These cities include six metro cities (New Delhi, Kolkata, Mumbai, Hyderabad, Bengaluru and Lucknow) and seven fast growing smaller cities (Aligarh, Bhubaneswar, Imphal, Jaipur, Ludhiana, Nasik and Visakhapatnam). The interventions were planned and implementing after a comprehensive formative research. The interventions were implemented with the principles of advocacy, partnership and community mobilization. The findings of formative research on the access of migrants to government health care facilities and health system's responsiveness were used during advocacy efforts. Policy briefs were prepared and widely used. Partnerships with State/municipal health officials, non-governmental organizations and industries were developed to address the problems related to low health care access. The supply-side issues were addressed by these partners. Community mobilization activities were undertaken at community level to improve the demand for health care. Health services have undertaken the community mobilization activities in collaboration with local non-governmental organizations and community-based welfare and religious organizations. Thus, strategies have been implemented to address both supply and demand-side issues. The preliminary observations of impact evaluation revealed that both health system's responsiveness as well as migrants' access have improved. It was further observed that the coverage

of geographical areas and the quality of services in the existing areas where migrants reside have improved. The utilization of government health care services by the migrants has also increased during the intervention period. A migrant-specific health care delivery model is the expected outcome of this study.

SOCIAL & BEHAVIOURAL RESEARCH UNIT

During the year, 17 projects were completed, four new projects were funded and 14 projects were approved for funding in the priority area of Gender & Health and CVD related behavioural intervention.

Study on determinants of vasectomy acceptance in a block of Thane District of Maharashtra

The main objective of the study was to investigate the determinants of comparatively high acceptance of vasectomy in Jawhar block in Thane district of Maharashtra State. This exploratory study was conducted for 18 months from March 2011 to September 2012 in the study area with a 45% literacy rate and 90% belonging to tribal population namely Warlis, Koknas, Thakars, Mahadeo Kolis, Malhar Kolis, Dhor Kolis, Katkaris, *etc.* Based on systematic random sampling, 30 vasectomy acceptors from each year (2008-2010) were selected from the service records (*i.e.* a total of 90 vasectomised men - retrospective cases). Also 30 vasectomy acceptors from the study year (2011) were included as prospective cases. Similarly, 30 vasectomies non-acceptors males were selected from each year from the service record (total of 90 non-vasectomised men whose wives have accepted tubectomy). Also, 30 vasectomy non-acceptor males were included as prospective cases. All the participants were contacted at their households and semi-structured interviews were conducted. A total of 121 cases of vasectomy acceptors and 124 cases of vasectomy non-acceptors were interviewed. Vasectomy acceptors were remarkably high in the Warli tribes as compared to the other tribes and this association was found to be significant ($P < 0.05$). Education was also significantly associated with the acceptance of vasectomy. Type of family and holding of BPL card did not have any significant association with the acceptance of vasectomy.

Awareness of other methods or previous use of any contraception was not associated with vasectomy acceptance. In majority of cases it was a joint decision of the couple to accept either vasectomy/tubectomy. The awareness of NSV technique coupled with all the information on incentive was significantly more among the vasectomy acceptor group.

Facilitating factors for vasectomy acceptance were (i) men's concern about their wives health, (ii) efficient community mobilization for generations supported by skilled health providers who advocated vasectomy, and (iii) overall good health service facilities in the block. Major barriers for vasectomy acceptance were (i) fear of health complications/failure post surgery (ii) social stigma, and (iii) untrained health providers with biased opinion on vasectomy.

Self-injurious behaviors and psychopathology among adolescents and young adults in Bangalore

Self-injurious behaviours (SIB) are a growing public health issue and a few troubled youth access mental health services. The study explored the occurrence, methods, characteristics and reported reasons for SIB among school, pre-university and undergraduate college students and its socio-demographic and mental health correlates. Overall, 1571 youth completed the Functional Assessment of Self Mutilation questionnaire and measures of psychopathology, the Youth Self-Report (YSR) or the Adult Self-Report (ASR). Results indicated that 40.7% reported SIB in the past year, with a male preponderance and higher rates among youth between 13 to below 18 years. The rate of non-suicidal self-injury was 33.9%, with 16.7% of self-injurers reporting associated suicidal intent. Moderate/severe forms of SIB were reported by 19.4%. Multiple self-injury methods were most often endorsed and 14.8 years was the mean age of onset. Characteristics like duration of forethought, associated levels of pain and concurrent substance use were described. Distinctive patterns of SIB emerged based on gender; the severity of methods used; and the presence or absence of suicidal intent. SIB served both to regulate internal emotional states (automatic reinforcement) and to influence others in the environment (social reinforcement).

Self-injuring youth had significantly higher levels of internalizing problems, externalizing problems and total problems.

Logistic regression analysis indicated that males (OR = 1.7), youth aged 13 to below 18 years, youth with internalizing (OR=3.8), or externalizing problems (OR=2.4) in the borderline/clinical range had a high likelihood of self-injurious behaviour. Additional logistic regression analyses identified that being a female self-injurer (OR=2.4), a self-injurer with internalizing problems in the borderline/clinical range (OR=3.1), the use of greater number of self-injury methods (OR=1.5) and higher pain levels (OR=3.2) all increased the odds of associated suicidal ideation. The study results suggest for awareness building and targeted preventive intervention approaches among vulnerable school and college youths.

Understanding the sexual and reproductive health (SRH) perspectives and needs of female sex workers and factors determining their access to services.

A study was aimed at exploring various dimensions of SRH related to FSWs from their perspectives and needs. It was conducted by Karnataka Health Promotion Trust (KHPT) in co-ordination with *Niramay Arogya Dham* (NGO) and *Kranti Mahila Sangh* (CBO) working with FSW communities in Solapur for more than 6 years. A total of 500 women participated in this study. The methodology consisted of face-to-face interviews, Focus group discussions and clinical examinations to assess reproductive health status of FSWs. The tool was designed in local language by the sex worker community representatives. The findings of this study described some SRH issues from the perspectives of FSW community itself. Along with STIs and HIV, the study brought in more understanding on issues like menstruation, pregnancy, contraception, abortions, condom usage, connections between literacy, economic conditions, age at marriage, age at entry into sex work, etc.

The learning will help the FSWs and their CBOs in relating this study to their own community members and understanding the SRH status of their members. It will help the prevention programme by National AIDS Control Organisation (NACO) as well as

the National Rural Health Mission (NRHM) in understanding health seeking behavior of the FSW community on SRH matters and identify gaps in the service delivery/access.

Relationship of demographic variables, socio-cultural issues and selected psychological constructs with the positive mental health of North Indian adolescents.

The current study was undertaken on 1932 adolescents residing in urban and rural areas of Delhi-NCR and its adjoining areas. The participants were either school dropouts (N=120) or attending school (N=1812) in the age group of 11 to 19 years. The participants answered the questionnaire in either Hindi or English. Various psychometric scales such as Mental Health Continuum Short Form (MHC-SF), Strength and Difficulties Questionnaire (SDQ), Personal Well-being Index for School Children (PWI-SC), Quality of life (WHOQOL-BREF), Brief Multidimensional Students Life Satisfaction Scale (BMSLSS), Flourishing Scale (FS), and Negative Experiences (SPAN), Depression Anxiety Stress Scales 21 (DASS-210) and Adolescent Resilience Scale (ARS) were administered along with a demographic questionnaire. The results broadly indicated that early adolescents had a higher mean scores on all factors of positive mental health, whereas, older adolescents had higher score on difficulties. Male adolescents were higher on negative positive mental constructs whereas females were higher on positive mental health constructs. Rural adolescents had a higher mean score on positive mental health whereas urban adolescents had a higher mean score on difficulties score. Adolescents who attended government school possessed higher difficulties as compared to private school adolescents. Private school adolescents had a higher mean score on positive mental health. Dropouts broadly showed lesser positive mental health as compared to school going adolescents.

Development, testing and validation of a tool to 'Screen' physical, psychological and mental health issues in adolescents

This study was conducted with the objective of developing and validating a screening tool for common issues during adolescence. Data were

collected from Center for Adolescent Health, Lady Hardinge Medical College, New Delhi during July, 2012 to September, 2013. Several existing screening tools were reviewed by the study team to prepare the first version and the Expert Committee prepared Draft-1. This was tested with a large number of adolescents (512) and factor analysis was performed. Focused group discussions with nurses, parents and adolescents were conducted to identify the psychometric properties and language issues. The group found it acceptable, easy to understand and covering all important issues. They advised to have two separate versions for adolescents below 14 years, and 14 years and above. The proceedings of this phase resulted in Draft-2 of the tool. This tool was validated on 372 adolescents in school and attending OPD. All these adolescents completed the questionnaire and were interviewed. The findings (and classification) of the latter were considered gold standard.

The tool had a good internal consistency (0.83) for questions common to both versions. The tool also had good discriminatory properties to identify adolescents having emergency issues requiring medical evaluation with issues not requiring urgent medical evaluation and normal adolescent. This tool can also provide information on risk factors for development of non-communicable diseases (physical activity, sedentary behaviour, dietary intakes, and tobacco use), and about the health center functioning.

A study on gaps in reproductive health services for adolescents in Delhi

This study was conducted to find out the availability and type of ARSH facilities in both Public and Private Sector to address the reproductive health needs of adolescents in Delhi, and to assess the availability of quality (trained manpower, safe and enabling environment, holistic approach) reproductive health care services for the adolescents in public sector in Delhi. A cross-sectional study was conducted for a period of 18 months on semi-structured questionnaires developed for the study with reference to 'WHO Manual for "Quality Assurance: Quality Reproductive Health Services to adolescent Clients'. The questionnaires were pilot tested and were administered by trained field investigators. The data were collected in a cascade

manner from the district headquarters, Facility managers, care providers and support staff posted in the health facilities. Exit interviews were conducted on the adolescents availing these services in the primary, secondary and tertiary health facilities. The results showed that there was a paucity of data on private and non-governmental health facilities providing ARSH services at all the district headquarters. Though policy makers/planners, managers and care providers were aware of the health needs of adolescents there was a further need to address the issues like privacy, confidentiality, community awareness for stigma alleviation, adolescent friendly ambience, participation of adolescents in planning, and evaluation of ARSH services can help in improving the quality of ARSH services in public health facilities.

Behavioural addiction in the community: An exploration

Behavioural addiction is a recurring compulsion by an individual to engage in some specific activity, despite harmful consequences, as deemed by the user himself to his individual health, mental state, or social life. The present study was aimed to explore the pattern of behavioural addiction in an Indian context. The sample consisted of 2755 subjects, 1392 were males and 1363 were female participants within an age group of 18 years and above from an urban locality in East Bangalore. Cross-sectional study adopting a house-to-house survey methodology was used for collection of data. The survey questionnaire incorporated demographic schedule, the Lie-Bet Toll, Facebook intensity Scale, Exercise addiction Inventory, questions for other behavioral addiction, General Health Questionnaire and Modified mental health screening questionnaire.

The results showed that behavioural addictions have been observed with 1.3% for internet (2% males & 0.6% females); shopping (4%) (male-3.2% & female- 4.8%); cell phone (4.1%-5% males & 3.1% female); eating (1.6%); television (2.9%); 3.3% males & 2.4% females); Work (9.7%) (10.5% males & 8.9% females); sex (0.2%; 0.3% male & 0.1% females); exercise (5.85%; 7.5% males & 3.8% females). 1.2% of the sample had

Gambling addiction. Physical distress was found as a co-morbidity with eating addiction (1.6%); mobile phone addiction (6.8%); and television addiction (5.3%); 18.8% of subjects with eating addiction also had gambling addiction and 13.5% of television addicts had problematic gambling. There was absence of treatment seeking behaviour for behavioural addictions. Focus group discussion revealed presence of unemployment, lack of awareness, cultural differences; easy availability, accessibility, acceptance of the behaviours and easy availability were associated with addictions. The findings have implication for appropriate tool development, developing therapeutic interventions, extending the study to different clinical groups and for policy making.

Assessment of sexual and reproductive health (SRH) needs of HIV infected adolescents in six districts of Karnataka.

This study covered 600 HIV infected adolescents in the age group of 13 to 19 years. The issues ranging from information needs to social support, counselling, treatment, *etc.* were investigated. It was found that HIV infection affected the social life of adolescents as evidenced by the fact that only 50% of them were living with their parents and 31% have dropped out of school mostly due to ill health. Adolescents were found to have poor knowledge related to SRH which is a cause of concern, as 80% of them were on ART and regular follow up. Similarly, disclosure of HIV to adolescents is another major issue. Only 61.24% knew they were on treatment for HIV, though 90% reported that they were on ART. Only 53.3% were fully disclosed regarding their HIV status. It showed that counseling programme was not fully effective in addressing the critical issues related to HIV infected adolescents.

Although the preferred source of information for adolescents was school or health centre, 51% of caregivers brushed aside the queries related to SRH. On the basis of the study results, it is recommended that service providers/ counsellors need to be reoriented with regard to SRH need of adolescents, sexuality needs to be discussed and preventive services need to be strengthened.

Socio-Cultural dimension of sustainability of sensitizing self help groups (SHGS) to reproductive-health via empowerment and engagement (SSSTREE)

The study was aimed to utilize the current model of Self Help Group (SHG) functioning to build capacity to increase awareness and health felt needs to access services for reproductive health and cervical cancer screening among rural women at the community level in a sustainable and cost-effective manner. The study was conducted in five districts spread over three States (Tamil Nadu, Chhattisgarh and Rajasthan). Kolar district was the primary intervention site. The study showed good outcome of improved knowledge in women which is to yield a greater impact of healthier lives and families. In rural areas, women being less educated and with lesser exposure to media and other sources, their knowledge, practice and health-seeking behaviours were low. With SSSTREE intervention, thousands of women were benefitted and hence thousands of families. Through the Health Mela, nearly 1000 women were addressed and through FGDs and

workshops, about 1200 women were imparted with knowledge on all aspects of women's health.

One of the major achievement was that the women learnt and understood more about the health issues like menstrual hygiene, RTI/STI, HIV, sexual hygiene, family planning, pregnancy care, abortion, child care, government schemes and entitlements, cervical cancer and about services available at health care facilities. Most importantly, they were able to shed hesitation to talk about such intimate issues, understand more and empower themselves. Critical issues like awareness on cervical cancer has been now easily conveyed to them, which in turn will keep a check on the incidence and treatment-seeking. The very fact that women are now able to identify symptoms of RTI/STI is a matter of great significance. Also women have shown positive improvements in the health behaviours like menstrual hygiene, sexual hygiene and also they seek better care in terms of ANC, immunization, *etc.* This programme has successfully proved as a holistic approach to address all the major issues of women's health.

PUBLICATION AND INFORMATION

PUBLICATION AND INFORMATION

ICMR continued its multifaceted activities in the field of publication and information for dissemination of biomedical information generated through research to different target groups. The Indian Journals of Medical Research completed 100 years of its uninterrupted publication during the year under report.

PERIODICALS

INDIAN JOURNAL OF MEDICAL RESEARCH

The Indian Journal of Medical Research (IJMR) has successfully completed one hundred years of its existence in July 2013. During the year long centenary year celebrations several new initiatives were taken up. The cover page and the get-up of the Journal was changed and a new section on “Clinical Images” was started. During the Centenary year (July 2012 to July 2013), each issue of the IJMR carried a centenary special review article, and an IJMR Classic article. In addition, 13 most cited

IJMR articles featured during the last 50 years were selected and reproduced again during the centenary year.

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 Letters to the Editor (Correspondence), View Points, Perspectives, Systematic reviews with meta analysis, and Students’ IJMR were also contained to be published. During the period under report, of the 302 articles published under various categories/ sections, 49% were original research articles, followed by review articles and correspondences (Fig.1). The quality of peer review was maintained by involving reviewers both from India as well as foreign countries (Fig.2).

The IJMR was continued to be brought out in two volumes 12 issues every year and has been covered by all global abstracting and indexing services. The journal is available full text free on the net with

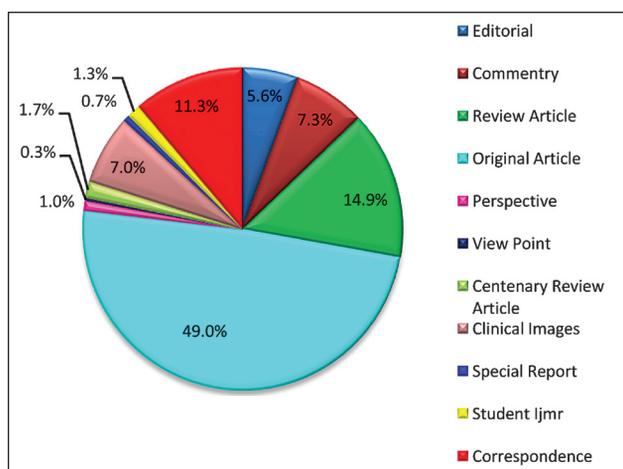


Fig. 1. Article published in IJMR under different categories (2013-14).

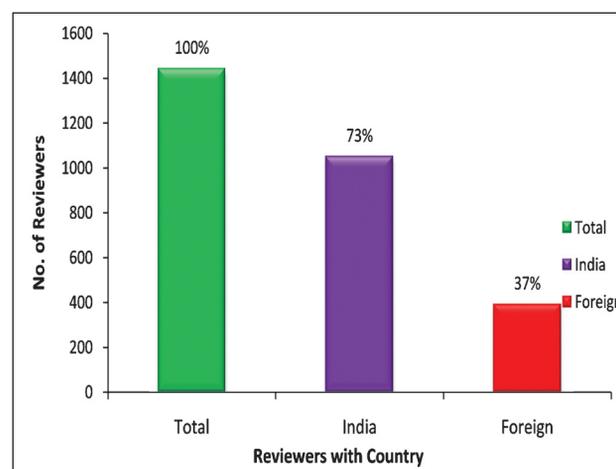


Fig. 2. Number and percentage of Indian and Foreign Reviewers of IJMR (2013-14).

a searchable menu. IJMR archive is available at <http://ijmr.in> with full text of articles available free in pdf format since the inception (July 2013).

A special issue on “Translational Immunology in Health & Disease” was brought out in November 2013 with Dr N. K. Mehra as Guest Editor. A total of 20 review articles contributed by renowned experts in the field and one original research article were published under four Themes in this special issue. A special section on “Nutrition & Food Security” was also brought out in September 2013 with Dr Prema Ramachandran as guest editor. Six review articles contributed by nutrition specialists were published in this Section

The Impact factor of the IJMR for 2013 was 1.661.

ANNUAL REPORT

The Annual Report of the ICMR (both English and Hindi versions) for the financial Year 2012-2013 was brought out in time. The report was made more attractive and informative with improvement in its content, quality and get up. Soft copy of the report was also provided in CDs. The ICMR also published Annual Report of the Department of Health Research. Both these reports can be accessed on ICMR (www.icmr.nic.in) as well as on DHR (www.dhr.gov.in) websites, respectively.

HINDI PUBLICATIONS

ICMR Patrika

The publication of *ICMR Patrika*, a monthly Hindi Bulletin continued to be brought out. The articles on High Blood Pressure, Health Hazards of Tobacco Products, Air Pollution, Rheumatic fever and Rheumatic Heart Disease, Activities of Department of Health Research, Drug Abuse, etc. Which were of general interest to readers were featured in various issues.

NON PERIODICALS

Citations of ICMR Awardees

Both English and Hindi versions Citations of ICMR Awardees were prepared on published for the years 2009 & 2010.

Report of ICMR High Power Committee Other Publications

- A Report of the High Power Committee constituted to review the ongoing research activities of the ICMR was brought out in February 2014.
- Disease specific documents were brought out on 15 disease conditions viz., malaria, filariasis, leishmaniasis, dengue/chikungunya, JE/AES, diarrhoeal disease, cancer, HPV, other viral diseases, nutritional disorders, environmental and occupational health, HIV/AIDS, chlamydia, leprosy and tuberculosis.

MONOGRAPHS ON INDIAN MEDICINAL PLANTS

Review Monographs on Indian Medicinal Plants

The Monograph aims at consolidation of Indian research contributions (published information) in the area of medicinal plants. ICMR published the compiled information in series on Reviews on Indian Medicinal Plants. During the year three Volumes 11-13 (with botanical names F-K) covering monographs on about 1100 medicinal plants species carrying multidisciplinary information were published.

The series 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

Work on other volumes is in progress.

Quality Standards of Indian Medicinal Plants

The publication is a compilation brought out in the form of Quality Standards of important Indian medicinal plants. These publications are useful for developing plant drugs of adequate quality, safety and efficacy of wider acceptance.

The quality standards on 35 medicinal plants were developed, monographs prepared, finalized, technically reviewed and published as Vol. 11 as part of series on Quality Standards on Indian Medicinal Plants. Monographs on another 35 plants are being finalized for 12th volume.

Generation of Phytochemical Markers and Development of a Repository:

The programme aims to develop phytochemical Reference standard markers from select medicinal plants for standardization of drugs for stakeholders engaged in establishing and maintaining quality of medicinal plants and their products.

The work on different volumes in the series is in progress

Medicinal Plants Monographs on Diseases of Public Health Importance

The ICMR has taken up preparation and publication of Monographs on select diseases of public health importance matching with the national health priorities. The monographs incorporate scientific information on diseases (including etiopathogenesis) and plant drugs as given in the ancient texts (Indigenous System of Medicine) and Allopathic System of Medicine on one hand and the multidisciplinary research data generated during various scientific studies on these plant remedies with focus on pharmacological, toxicological, clinical, phytochemical, pharmacognostic aspects, on the other. The Monographs shall also include complete references of the work cited and the photographs of important medicinal plants.

The monograph in the area of Diabetes Mellitus is being finalised.

Safety Review Monographs on Indian Medicinal Plants

An attempt is being made to review all safety related published information on widely used medicinal plants and to bring out monographs on these plants. A format has been devised after consulting experts in this area which would form the basis of these monographs. Monographs on Safety aspects of 40 Important Indian Medicinal plants have been technically reviewed to be included in the first volume in this series

Adhoc Proejct

An Adhoc Project entitled “Herb Drug Interaction Studies of Selected Herbs and Allopathic Drugs in the Treatment of type 2 Diabetes, Rheumatoid Arthritis and Epilepsy” is being conducted at Natural

Remedies, Bangalore and AIIMS, New Delhi deals with the mechanisms of herb–drug interactions which is essential for clinical risk assessment. The project aims to find out the effects of selected herbs which are widely used concomitantly, for the selected conditions, with the conventional drugs for the same conditions. It envisages evaluation of herbal drug interaction in 3 disease conditions requiring chronic medication *viz.*, Diabetes type 2, Epilepsy and Rheumatoid Arthritis with one prototype drug used in each condition and selected herbs used in Traditional system of medicine.

BIOMEDICAL INFORMATION & COMMUNICATION

SCIENTOMETRIC STUDIES

Annual Research Output of ICMR Institutes

The annual document ‘2012 Research Output of ICMR Institutes’ with analysis of publications from all the institutes including Regional Medical Research Centres has been brought out. A total of 633 papers were published by the ICMR institutes during the calendar year 2012. The National Institute for Research in Tuberculosis (NIRT), Chennai topped the tally with 75 papers followed by the National Institute of Nutrition (NIN), Hyderabad (68), National Institute of Cholera and Enteric Diseases (NICED), Kolkata (67), National Institute of Malaria Research (NIMR), New Delhi (52) and National AIDS Research Institute (NARI), Pune (39). Of the 331 journals used for publishing 633 papers, 188 journals had an impact factor (IF) 2012 equal to or greater than 1.000.

The average IF / paper of the Council for the calendar year 2012 was 3.096.

ICMR Awards for Popular Medical Books in Hindi for Biennium 2012-13

Enterier were invited through advertisement in various Hindi and English Newspapers. A total of 9 books were received. Evaluation of these popular Hindi publication has been entrusted to Committee of Experts specially constituted for this purpose.

Hindi Day Lecture

A popular lecture in Hindi was organized at ICMR Hqrs on 29th Oct., 2013. Dr Jitendra Nagpal,

Department of Psychiatry, Moolchand Hospital, New Delhi delivered a lecture on the topic “*Badalte vishwa mein Avasad ki Chunautiyan*”.

Display Advertisement in Media

- ICMR published display advertisement in English, Hindi and Vernacular language across the country on contributions, achievements of ICMR in various field of Biomedical Research coinciding with the
 - Birth Anniversary of Architect of Modern India Pt. Jawahar Lal Nehru (November 14, 2013)
 - National Science Day (February 28, 2014)
- ICMR Published display advertisement in English, Hindi & Vernacular language Newspaper across the country coinciding with launch of affordable indigenous technologies
 - Vaccine for JE (October 4, 2013)
 - Tests for molecular diagnosis of Thalassemia (December 17, 2014)
 - Magnivisualizer for cervical cancer screening (December 23, 2013)
 - Strips and detection system for diabetes (January 13, 2014)
 - New testes for detection of pathogenic bacteria in food & Technologies for vitamin A and Ferritin estimation (February 20, 2014)
- ICMR published an advertorial feature entitled “Putting Health First” on scientific contributions and achievements of ICMR and DHR in the February 2014 issue (Vol.2, Issue 1) of inflight magazine of Air India Shubhyatra.

Dissemination of Biomedical Information

- ICMR put up an exhibition in the Health Pavilion designed by Ministry of Health & Family Welfare, Govt. of India during India International Trade Fair (IITF), 2013 held at Pragati Maidan from 14-27th November, 2013 and displayed informative posters for general public in the area of maternal & child health, tuberculosis, nutrition and general information on ICMR.
- ICMR participated in a Mega Science exhibition “Pride of India” organized as part

of 101st session of Indian Science Congress held in Jammu during February 3-7, 2014 and displayed scientific achievements in the form of attractive posters on various diseases conditions, new innovations and technologies. Video films on the activities and achievements of ICMR and its institutes were also featured in Hindi and English. Large number of Students of the local schools/colleges, scientists, delegates and officials visited the exhibition.

- A public outreach session was also organized at Jammu University on 5th February, 2014 to create awareness among masses. Dr Dipika Mohanty, Former Director, NIIH, Mumbai, Dr Ajit Gorakshakhar, Scientist E, NIIH, Mumbai and Dr Aditya Parashari, Scientist B, ICPO, Noida delivered talk on Genetic Disorders, Haemoglobinopathies and Cancer screening respectively. The programme was attended by large no. of delegates
- ICMR also participated in an information campaign entitled “11th Infra-Educa 2013” held at Jammu from 28-29th June, 2013 and Srinagar from 3-4th July, 2013 and displayed informative posters on various activities of the ICMR. Video films on the significant achievements of ICMR were also shown. Chief Minister of J&K Hon’ble Shri Omar Abdulla visited the ICMR Pavilion at Srinagar.
- ICMR participated in Science Exhibition ‘5th Vision Rajasthan 2014’ at Chomu, Jaipur during 17-19th February, 2014 and displayed its significant achievements through attractive posters and video shows.
- ICMR participated in 17th National Exhibition on the theme “India Advancing towards a World Power” organized by Central Calcutta Science and Culture Organization for youth at Rama Krishana Mission, Belur Math, Howrah from 21-25th September, 2013.
- ICMR participated in an S&T Exhibition held at Panjim, Goa during September 28-30, 2013 and showcased its technologies and significant scientific achievements. ICMR’s National Institute of Malaria Research, Field Station, Goa organized the Health Camp and live demonstration for the control of mosquitoes

and mosquito borne diseases. Large number of local students visited the ICMR stall.

Book Fairs

ICMR participated in World Book Fair held in New Delhi during 4-10th February, 2014 and put up the display and sale of ICMR publications. ICMR stall was visited by large number of people and books on nutrition were in great demand.



ICMR Participation in World Book Fair, held at New Delhi from 15-23rd February, 2014.



ICMR participated in '11th Infra-Educa 2013' held at Srinagar from 3-4th July, 2013.



ICMR participated in Indian Science Congress held at Jammu from 3-7th February, 2014.



ICMR Participation in IITF, held at New Delhi from 14-27th November, 2014.

BIO-INFORMATICS

Task-Force on Biomedical Informatics

A total of 20 Biomedical Informatics Centres (BIC) established in the 2nd phase of Task Force on Biomedical Informatics have initiated 35 collaborative projects on various communicable and non communicable diseases including common diseases such as Cancer, Tuberculosis, HIV, Sickle Cell Anemia, Malaria, Typhoid, Diabetes and rare diseases such as Progressive Familial Interhepatic Cholestasis, Corneal Dystrophy, Hypertrophic Cardiomyopathy. Approximately 15 projects have potential for translation (Drug Development and Diagnostic/Prognostic Biomarkers). The Centres have published 22 research papers and conducted 7 training programs for the benefit of medical professionals.

ICMR Website

Website is used to disseminate information and announcements related to ICMR and its institutes globally. Advertisements, Office Circulars, IJMR monthly issue, STS and other related matter of ICMR Headquarters and its institutes are displayed through the ICMR Website which is being updated with the database of Publications, Projects, Scientists profile, Seminars/Symposia, Circulars etc. Average page view of ICMR Website for the year 2013-14 is 16483. International visits are 37.35% and Visits from India are 61.46% (As per Web Analytical Software designed by NIC)

e-Recruitment Software

Software is designed and maintained by BIC to receive online applications from the candidates

applying for the permanent posts of ICMR Headquarters. Applications are being received through this system from the year 2012-13.

Extramural activities

BIC is processing extramural projects and fellowships in the area of Bioinformatics and Medical Informatics. BIC is managing 33 adhoc projects and 42 fellowships. All projects are processed within three months of receiving as BIC is holding PRC every three months. The extramural funding managed by BIC has produced 58 publications in peer-reviewed journals and one patent entitled '1,3-diacetyl biphenyl analogs, their derivatives and salts thereof as anticancer agents' during 2013-14. Majority of the projects are in the area of structural biology, however, recently BIC has taken steps to strengthen medical informatics and other areas of Bioinformatics.

Time Bound Reports and other Services to the Council:

Reports have been produced from extramural information system for answering Parliament questions and other time bound queries from the Ministry, SAG of Divisions *etc.* The BIC worked as Nodal Point for all ICMR institutes and the Headquarters for ePublishing of tenders on Central Public Procurement Portal of Government of India. BIC continues to provide services to the council like compilation of GSLIS data for Admin II, email correspondence by divisions to directors of ICMR *etc.*

Management of Servers and Network

The Internet, intranet and videoconferencing facilities are provided by BIC to ICMR Headquarters and ICMR 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) and Pay slips are managed by BIC.

ICMR Computational Genomics Centre

BIC has developed proposal for establishing ICMR Computational Genomics Centre, which will be a centralized facility, intended to provide infrastructure and expertise in using Genomics

tools and techniques for medical research. Having a centralized infrastructure reduces redundancy of hardware, software and the expertise and allow development of skills and expertise to an extent that is difficult to achieve in individual laboratories/institutes

The intended users of the Centre will be research groups from ICMR laboratories. The Centre will also provide support and services to medical researchers from Universities, Hospitals and Medical Colleges where infrastructure, expertise or awareness restricts the application of high-throughput Genomics in medical research for high impact publications and translation into products.

The proposal has been recommended by Expert Group, Scientific Advisory Group (SAG) and Biomedical Research Board (BMRB). It is in final stages of clearance from the Department of Health Research.

NATIONAL DATABASES OF INDIAN MEDICAL JOURNALS

National Databases of Indian Medicals Journals is a project funded by ICMR (Publication and Information Division). The project produces important web based resources targeted towards Bio-medical researchers and practitioners from select Indian Medical Journals and generates awareness about these resources among the target community. It produces and updates – IndMED and MedIND. While IndMED indexes over 110 Indian medical and allied biomedical journals, MedIND hosts those Indian journals which are either indexed in PubMed (from US National Medical Library) or indexed in IndMED. Journals covered in IndMED are selected by an expert committee called Journal Selection Committee. For MedIND, the Indexed journals are given an option to host their full text content on MedIND.

Activities Undertaken in the year (April 2013 - March 2014):

Look, feel and description of MedIND and IndMED website has been changed. Indexing coverage has been made available. Help for searching also has been included.

Two Journal selection committee meetings were held on 30th August 2013 and on 25th March 2014.

More than 12,000 new articles had been indexed in IndMED.

MedIND had Full Text of Sixty-Six Medical Journals.

Following Training cum User Awareness events were organised aiming training of target users:

- I. Training - cum - User Awareness programme - 23rd May, 2013, Srinagar, Jammu & Kashmir.
- II. Training cum User Awareness Programme on 29th July 2013 at MM Institute of Medical Sciences, Mullana, Haryana.
- III. Workshop on “BIOMEDICAL INFORMATICS” - 19th October, 2013, Maninagar, Ahmadabad
- IV. Talk on NIC’s contribution in Open Access to Indian Biomedical Literature (IndMED/ MedIND) at National Conference Opening up by Closing the Circle: Strengthening Open Access in India being organised by UNESCO, JNU and CMECA at JNU convention centre on October 21, 2013.
- V. Training - cum - User Awareness programme - 24th October, 2013, PGIMS, Rohtak, Haryana
- VI. Talk delivered on “IndMED” - 16th November, 2013, AIIMS, New Delhi
- VI. Show casing of MedIND in International Conference on Asia – Pacific Digital Libraries, Bangalore, 9-11 December 2013.
- VIII. Training cum User Awareness Programme on 19th Feb, 2014 at NIC HQ, New Delhi.

WEB-BASED EXTRAMURAL PROJECT MANAGEMENT SYSTEM

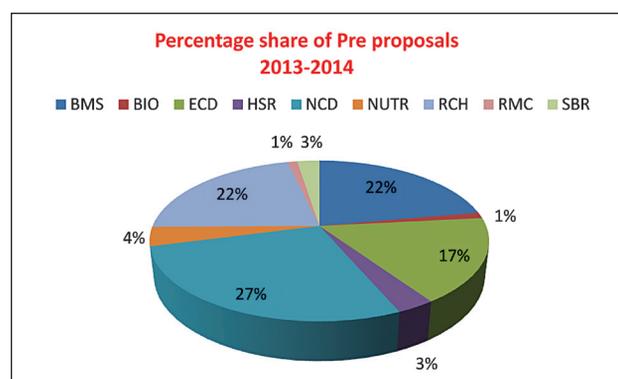
The web-based Extra Mural Project Management System developed by ICMR is now fully functional. The pre-proposals received within a month or on deadline are scrutinized and evaluated by the Committee within a fixed time period and decision is communicated to investigators for both selected

and non-selected proposals by next month. The Principal investigators (PIs) of the selected pre-proposal are asked to expand their pre-proposal into detailed project in the format of the ICMR *ad-hoc* projects within a period of not more than 6 months Online as well hard copies along with a CD with soft copy.

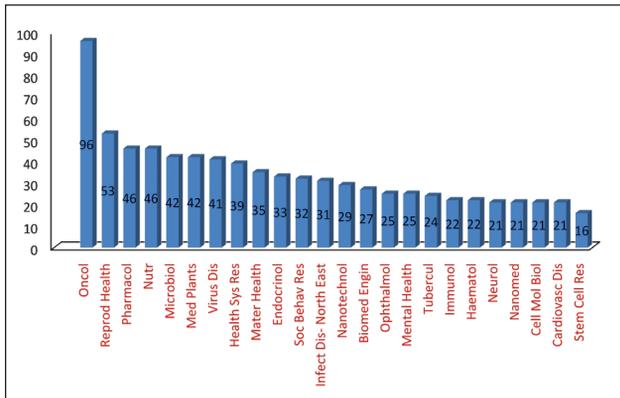
During the year, a new module has been designed and it’s development is in process for PRG recommended budget and ‘Finance’ including generation of Bills by PO’s, modules for Accounts section. ‘Data’ formats along with entry form have been approved by the various ‘stake holders’. The functional ease and practicality has been established by the “Online Processing Unit” of ICMR. All the ‘Forms’ have been uploaded with the appropriate ‘Data’.

The procedure for enlisting, viewing, uploading of documents required for ‘codal formalities’ have also become part of the system and available to PI’s for their use. The PO’s can also view these documents and take necessary action.

There has been an increase in the number of proposals received by ICMR using this system. In the year April 2013- March 2014 ICMR received 1897 proposals in this period using e-PPS. These proposals were reviewed and of these, 843 pre-proposal were shortlisted for submission of detailed proposals. One of the reasons to this increase can be attributed to the ease of proposal submission process in e-PPS.



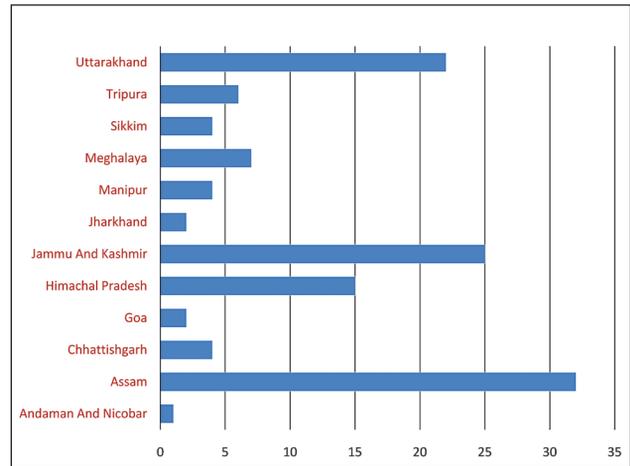
Percent share of pre proposal (Division-wise)



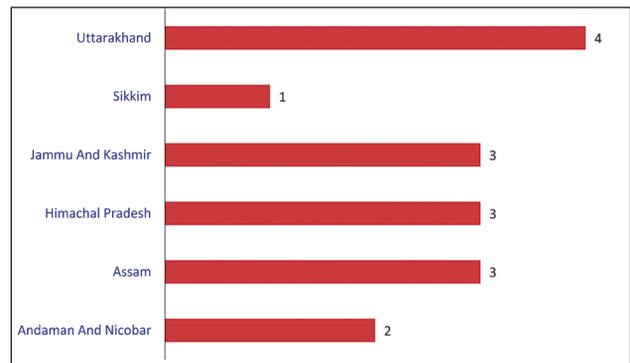
The top most subject discipline was Oncology followed by other important areas of Health Care.

A total of 881 full proposals were received and marked to respective technical divisions for processing, during the year under report.

An initial analysis of the data culled 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 East Khasi Hills, Ri-Bhoi, West Garo Hills, Aizawl, Bhadrak, Dhenkanal, Ganjam, Mayurbhanj, Karaikal, East Sikkim, Dharmapuri, Dindigul, Erode, Midnapore and Nadia.



Pre-Proposal (from Less Privileged Areas) 13-14.



Technically approved full proposals from less privileged area of the country 13-14

ICMR INSTITUTES/CENTRES

1. National JALMA Institute for Leprosy and Other Mycobacterial Diseases
P.B.No.101, Dr. M. Miyazaki Marg
Taj Ganj
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
Acharya Donde Marg
Parel
Mumbai 400012
12. Genetic Research Centre
National Institute for
Research in Reproductive Health
Jehangir Merwanji Street
Parel
Mumbai 400012
13. National Institute for Research in
Reproductive Health
Jehangir Merwanji Street
Parel
Mumbai 400012
14. National Institute of Immunohaematology
13th Floor, New Multistoreyed Building
K.E.M. Hospital Campus
Parel
Mumbai 400012
15. National Institute of Medical Statistics
ICMR Head Quarters Campus
Ansari Nagar
New Delhi 110029
16. Institute of Cytology and Preventive Oncology
I-7, Sector-39, P.O.Box.No.544
Near Government Degree College
Opposite City Centre
NOIDA 201301

17. National Institute of Pathology
Safdarjang Hospital Campus
Post Box No. 4909
New Delhi 110029
18. Rajendra Memorial Research
Institute of Medical Sciences
Agamkuan
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.B. No.11
Pune 411001
23. ICMR Virus Unit (Regional Infectious Disease Laboratory)
GB4, Ist Floor , ID & BG Hospital Campus
57, Dr. S.C. Banerjee Road, Beliaghata
Kolkata 700010
24. National Institute for Research in Environmental Health
Kamla Nehru Hospital Building
Gandhi Medical College Campus
Bhopal 462001
25. National Centre for Disease Informatics and Research
Nirmal Bhawan-ICMR Complex (II Floor)
Poojanhalli Road, Off NH-7
Adjacent to Trumpet Flyover of BIAL
Kannamangla Post
Bangalore 562110

26. Bhopal Memorial Hospital & Research Centre
Raisen Bye Pass Road
Karond
Bhopal 462 038
27. Regional Medical Research Centre
Nehru Nagar
National Highway No. 4
Belgaum 590010
28. Regional Medical Research Centre
Nandankanan Road
P.O. Chandrasekharapur
Bhubaneswar 751023
29. Regional Medical Research Centre
N.E.Region, East-Chowkidinghee
Post Box No. 105
Dibrugarh 786001
30. Regional Medical Research Centre for Tribals
Medical College Campus
Nagpur Road
P.O.Garha
Jabalpur 482003
31. Desert Medicine Research Centre
P.O.Box No. 122
New Pali Road
Jodhpur 342005
32. Regional Medical Research Centre
Post Bag No.13
Dollygunj
Port Blair 744101

ICMR CENTRES FOR ADVANCED RESEARCH

1. Identification and Functional Characterization of Individual Micromnas Specifically Associated With Myocardial Hyperglycaemic Condition In Cardiomyocytes, Postgraduate Institute of Medical Education And Research, Chandigarh.
2. Advanced Centre For Newborn Health Research, All India Institute of Medical Sciences, New Delhi.
3. Centre for Evidence Based Child Health Advance Pediatric Centre, 2015, Postgraduate Institute of Medical Education and Research, Chandigarh.
4. Centre for Advanced Research on Environmental Health: Air Pollution, Sri Ramachandra University, Chennai.
5. Centre for Advanced Research in Cancer Genetics and Genomics Advanced Centre for Treatment, Research & Education In Cancer Navi Mumbai
6. Centre for Advanced Research in Pharmacogenomics, Jawaharlal Institute of Postgraduate Medical Education, Puduchery
7. Emerging Areas In Molecular Medicine, Jawaharlal Nehru University, New Delhi.
8. Centre for Molecular Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, LuckNow.
9. Centre of Excellence In Molecular Medicine, All India Institute of, Medical Sciences, New Delhi.
10. Advanced ICMR Clinical Pharmacodynamic Centre for The Evaluation Of The Pharmacodynamic Effects of Drugs Nizam's Institute of Medical Science, New Delhi.

11. Advanced Centre for Research And Training in Evidence Based Health Care,
Christian Medical College and Hospital,
Vellore.
12. Hepatocyte Progenitor Cells Isolation from Various Sources,
Charachterisation Expansion and Transplantation,
Government StAnley Medical College and Hospital,
Chennai.
13. Advanced Centre for Reverse Pharmacology in Traditional Medicine,
Melghat and Kasturaba Health Society,
Sevagram.