

Annual Report 2014-15



Indian Council of Medical Research
New Delhi

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OVERVIEW

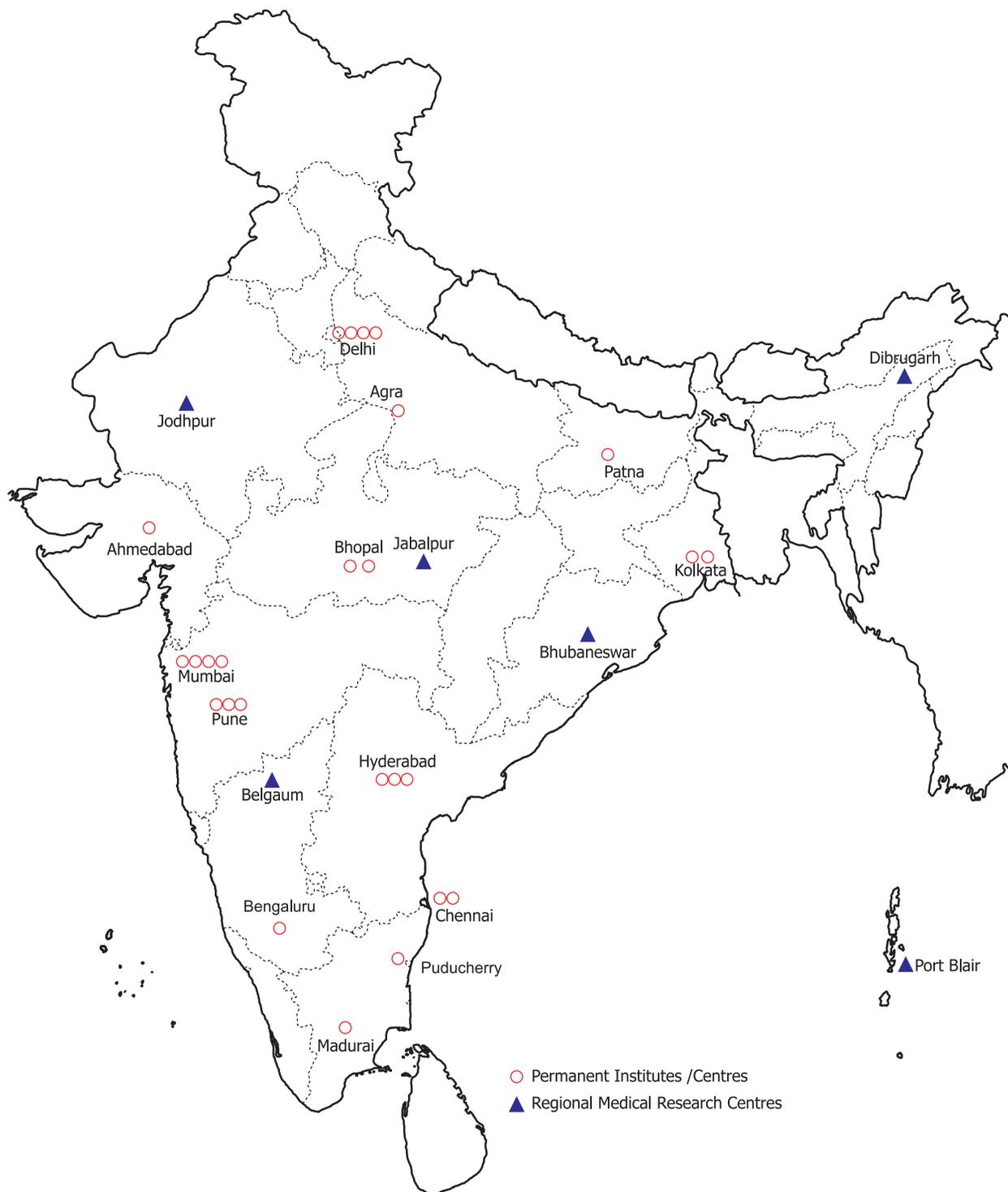
The Indian Council of Medical Research (ICMR), New Delhi, working under the Department of Health Research (DHR), Ministry of Health and Family Welfare, Government of India is the Apex body in India for the formulation, coordination, promotion and conduct of biomedical research. In the context of changing public health scene, the balancing of research efforts between different competing as well as emerging fields, especially when resources are severely limited, is a challenging problem encountered in the management of medical research particularly in the Country like India. In addition to tackling the prevailing health problems being faced by our population, in recent years the research has been intensified progressively on emerging health problems. Strengthening of research capabilities as well as definite and robust research strategy are recognised as vital tools for conduct of successful research and the ICMR has strived hard to adopt new strategies from time to time as per need of the situation. For the fulfilment of its goals, the strategies adopted by ICMR includes careful selection of thrust areas as per the national needs, with further refinement in the light of past experiences, undertaking studies to enlarge the scientific basis of preventive medicine and health promotion, development of simplified, cost effective and mass applicable testes which are specific and accurate for early and definitive recognition of diseases/health problems, establishment of close and effective linkages with social and behavioural scientists, active pursuance of problem oriented and problem solving research.

The ICMR discharges its primary function of promoting biomedical research through intramural as well as extramural research. Apart from 32 permanent research institutes/Centres, ICMR has several regional centres, research units, field stations spread across the Country. Extramural research is promoted by the ICMR through (i) Setting up Centres for Advanced Research in different research areas; (ii) Task force studies and national multicentric collaborative projects and (iii) Open ended adhoc research projects generated spontaneously by active scientists in biomedical institutions/universities, nongovernmental organizations, *etc* from different parts of the Country. Salient research activities carried out under the aegis of ICMR during 2014-15 are highlighted in the following pages.

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 has been developed and validated by the Vector Control Research Centre, (VCRC) Puducherry. The two-stage cluster sampling (30 clusters x 10 pools) protocol involving villages and households can be used for the collection of gravid females by using traps for monitoring vector infection and infectivity. The VCRC has standardized the procedures for preparation of insecticide impregnated papers to establish a National facility for supplying insecticide impregnated papers (IIPs) conforming to WHO standard for monitoring insecticide resistance/susceptibility in malaria vectors to different insecticides used in the National vector-borne disease control programme.

Based on the studies at Rajendra Memorial Research Institute for Medical Sciences, Patna. Single dose ambisome and combination therapy (miltefosine plus paramomycin) has been now introduced in National treatment guidelines for Kala-Azar.

The year 2014 was characterized by a huge dengue outbreak in the country. The National Institute of Virology, Pune tested altogether -7000 samples for dengue virus specific IgM out of which 59.2% were positive in Pune city and 22.3% were found positive in rural Maharashtra. Serotyping revealed that DENV-4 which normally occurs sporadically in India was the dominant serotype in July-August but later it was co-



ICMR INSTITUTIONAL NETWORK

dominant with DENV-2 in Pune city. In the rest of Maharashtra, DENV-2 dominated followed by DENV-3 and DENV-1. The re-emergence of DENV-4 is a cause for concern as it is often associated with severity in secondary infections.

A community based study in West Bengal by the National Institute of Cholera and Enteric Diseases (NICED), Kolkata has identified association of 'adult water management practices at point of use in rural household' with childhood diarrhoea. The incidence rate of rotavirus diarrhoea in this study has been estimated to be highest in children in the age group > 6 to ≤ 18 month (24/100 child-year). As part of the national rotavirus surveillance network, NICED, Kolkata has identified a diverse range of genotypes including G1P[8], GIP[6], G2P[4], G2P[6], G9P[4], G9P[8], G12P[6], and G12P[8] from the eastern part of the country. G1P[8] was the predominant type identified.

Composition of bioactive phytochemicals in commonly consumed Indian foods including regional and tribal foods were evaluated at the National Institute of Nutrition, Hyderabad. It was found that there is a considerable evidence for the role of antioxidant constituents of fruits and vegetables in the maintenance of health and disease prevention. Studies showed that phytochemicals, some of them not necessarily antioxidants, are also bioactive for preventing many chronic degenerative diseases.

Prenatal diagnosis for single gene disorders using molecular techniques have been expanded to include genetic disorders with increased morbidity/mortality such as ichthyosis, infantile hylinosis and osteopetrosis. Mutations in the X-linked cyclin-dependent kinase like 5 (CDKL5) gene have been identified in patients with atypical Rett syndrome, X-linked infantile spasms sharing common features of generally early onset seizures and mental retardation. This is the first report showing the mutation in CDKL5 gene in Indian cases of Rett syndrome.

No wild poliovirus was isolated from Acute Flaccid Paralysis (AFP) cases from AFP surveillance in India, as well as from sewage samples collected in Mumbai, Delhi, Kolkata, Patna, Punjab and Ahmedabad from Environmental surveillance.

A centre for screening and molecular diagnosis of hemoglobinopathies and red cell enzymopathies was established at Agartala Medical College where newborn screening was also undertaken for HbE disorders, Hbs disorders, G6PD deficiency and pyruvate kinase deficiency. This will help us to understand the magnitude of the problem of these inherited red cell disorders in Tripura and their contribution to neonatal jaundice and hemolysis. The Centre established in the north eastern region will be helpful for the diagnosis of common genetic disorders which in turn will help in better management of these cases. This centre can also be further developed of prenatal diagnosis facilities for genetic disorders which will ultimately help to prevent the birth of an affected child.

The DNA vaccine, developed at the Institute of Cytology and Preventive Oncology, Noida against high risk HPV16 is being tested for its immunogenicity. Efforts are on to increase its immunogenicity to make it a vaccination success.

The study carried out by the scientists of National Institute of Occupational Health, Ahmedabad observed that low birth weight, less head circumference, neonatal death, less developed genitalia and need to stay at nursery was more frequent with mothers using biomass fuel when compared with other fuel users. Significantly increased risk of 'low birth and need of newborn to stay in neonatal care unit' in the form of calculated odds ratio was observed in biomass fuel users.

The International Health Division of ICMR liases in biomedical research between Indian and other countries, as also with national and international agencies such as Ministry of Science & Technology, Indian and foreign missions and WHO, *etc.* The division has facilitated signing of agreements by the Ministry of Health and Family Welfare (MoHFW) with other countries as well as between ICMR/DHR and international organizations/institutes. During the period under review the MoUs signed included, addendum-three for GACD Secretariat funding at UCL, London signed in June, 2014 under existing ICMR-GACD MoU; MoU between the Research Council of Norway and ICMR on Health Research, signed on October 14, 2014 in

Norway; MoU between ICMR and IAVI on Cooperation in AIDS Vaccine Development signed in October/November, 2014 in New York and ICMR headquarters, New Delhi respectively. MoU between the ICMR and the Russian Foundation for Basic Research on Cooperation in Health Research signed on December 11, 2014 at New Delhi; MoU between ICMR and University of Sydney, Australia for collaboration in Health Research signed in January/March, 2015.

During the year Non-invasive rapid diagnostic tests for kala-azar (Visceral leishmaniasis) using urine as well as oral fluid samples were launched. Besides, the following technologies developed at various ICMR Institutes/Centres are ready for release-

(i) IgG ELISA diagnostic kit for paragonimiasis (lung fluke infection) developed at RMRC, Dibrugarh; (ii) Recombinant IgM ELISA for the diagnosis of Leptospirosis developed at RMRC, Port Blair; (iii) Latex based agglutination technique for the detection of leptospiral antibodies during acute stage of the diseases developed at RMRC, Port Blair; (iv) PCR based diagnostic kit for *Chlamydia trachomatis* developed at NIRRH, Mumbai; (v) Personal Cooling Garment (PCG) system developed at NIOH, Ahmedabad; (vi) Resazurin Reduction Test (RRT) Kit to Assess Sperm Quality in Men developed at NIRRH, Mumbai; (vii) Urine based immunodiagnostic kits for assessment of infertility (for EIG, PdG, LH and FSH) developed at NIRRH, Mumbai; (viii) Recombinant protein-based assay for diagnosis of hepatitis E developed at NIV, Pune; (ix) Diagnostic kit for *C. trachomatis* in reproductive tract infection developed at NIOP, New Delhi are ready for release.

During the year a total of 17 patents were filed, 11 in India – six from intramural research and five from extramural research. One patent was granted during the year. Four patent Cooperation Treaty (PCT) applications have been filed- two from extramural research and two from intramural research. Two patent applications were filed in the United States of America.

ICMR scientists published a total of 887 research papers in various national and international journals during this year. A total of new 429 adhoc projects, 41 new TF projects and 230 new fellowships were sanctioned during the year under report. The ICMR selected a total of 10 candidates for pursuing MD/Ph.D courses through national level examination. Besides, a total of 12 young Ph.D were selected for Post-doctoral fellowship programme of ICMR during 2014-15, for the conduct of research in priority areas of health sciences in ICMR Institutes/Centres.

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COMMUNICABLE DISEASES

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

Intramural Research

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS, CHENNAI

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

Interim findings of the clinical trial using moxifloxacin indicate promise for 4-month regimens for treatment of TB. The findings show that a 5 drug, 4-month regimen is non-inferior to

the standard 6-month regimen for treatment of sputum smear positive Pulmonary TB in adults.

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

The pharmacokinetic study of anti-TB drugs in children provided evidence to the national programme that the existing drug doses were inadequate and that there was a need to increase the doses. Pediatric TB doses have now been increased and daily fixed-drug combinations (FDCs) recommended for use in RNTCP.

Pharmacokinetics of Rifabutin during atazanavir/ritonavir co-administration in HIV-TB patients

The pharmacokinetic study of rifabutin in HIV-infected patients on second-line treatment with TB demonstrated the need to increase the existing 150mg thrice weekly dose of rifabutin during concomitant ritonavir/atazanavir administration. The RNTCP and NACO have increased the dose of rifabutin from 150mg thrice weekly to 300mg thrice weekly during ritonavir/atazanavir co-administration. Instructions have been issued by NACO to all ART centres.

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

NIRT study showed that self help groups can contribute to TB control by spreading awareness, increasing case finding and serving as DOT providers in the community. These findings have been communicated to the State Government and Women's Development Corporation for further action.

Genetic diversity and molecular characterization of drug resistance in *Mycobacterium tuberculosis* isolates from south India by whole genome sequencing

Whole genome sequencing of approximately 200 strains of *Mycobacterium tuberculosis* from this geographic area was undertaken. A lot of scientific information has been generated regarding drug resistance mutations, spoligotypes and strains circulating in this region. Further, WGS was undertaken for 19 highly resistant XDR strains – the data show high rates of drug resistance to multiple second line drugs in these patients and the same is explained in the Figure 1.

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

An implementation research study in 12 ART centres across the country has shown the poor uptake of the WHO symptom screen for ruling out TB among HIV-infected persons. Further, only 30% of patients with at least one symptom were referred for TB diagnosis and of these, ~20% were diagnosed as TB. Overall, the prevalence of TB was 1% and incidence 2.2/100 person-years. In the second phase of the study, isoniazid preventive therapy is being provided to all eligible HIV+ persons and the

cohort is being followed to document any reduction in TB incidence.

Construction of *M. tuberculosis* double knockout strain lacking *pknI* and *dacB2*

Cloning and over-expression of Rv2159c *M. tuberculosis* has been completed. Construction and characterization of *M. tuberculosis* double knockout strain lacking *pknI* and *dacB2* is being done and will be tested in animal models.

Study to determine factors that influence plasma concentrations of first line anti-TB drugs in adult TB patients receiving treatment according to RNTCP guidelines

A population pharmacokinetic study being conducted in collaboration with Chennai Corporation has shown sub-therapeutic levels of Rifampicin in the majority of TB patients studied. Further, BMI and diabetic status also have an influence on drug levels.

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

Psychosocial issues facing MDR patients: This study brought out the psychosocial concerns of MDR patients and the need for intervention by

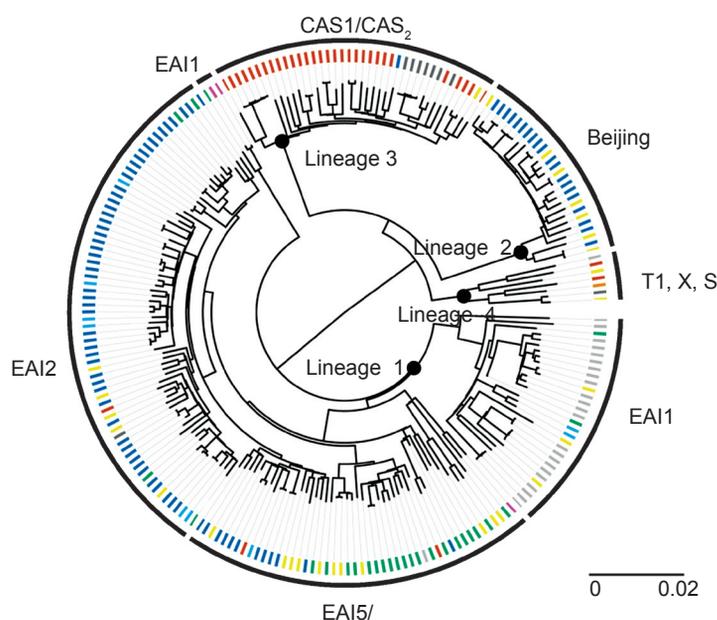


Fig.1. Drug resistance mutations, spoligotypes and strains circulating.

skilled counsellors to ensure treatment compliance (Fig.2).

Role of Chemokines on immunity to TB

This study came out with the following findings:

- (i) Decreased CCL2 expression in -2518GG genotype may be associated with protection against PTB through enhanced Th1 immunity.
- (ii) -403AA genotype of CCL5 and -1447GG genotype of CXCL10 genes are associated with decreased CCL5 and CXCL10 expression respectively which might have a defective role in the recruitment of mononuclear cells to the inflammatory site and granuloma formation and may contribute to progression of TB

Host immune responses in Strongyloidiasis

In order to examine the role of CD4⁺ T cells expressing Th1, Th2 and Th17 cytokines in strongyloidiasis, the Institute compared the frequency of these subsets in infected individuals (INF) to frequencies (F_o) in Ss-uninfected (UN) individuals. INF individuals exhibited a significant decrease in the spontaneous and antigen specific F_o of both mono - and dual functional Th1 cells compared to UN. Similarly, INF individuals also exhibited significantly decreased F_o of mono - and dual - functional Th17 cells upon antigen - stimulation compared to UN. In contrast, both the spontaneous and antigen - induced F_o of mono- and dual - functional Th2 cells were significantly increased in INF compared to UN individuals. In

addition, treatment of Ss infection significantly increased the antigen - specific F_o of Th1 and Th17 cells and decreased the F_o of Th2 cells in INF individuals. Thus, Ss infection is characterized by an IL-10 dependent regulation of mono- and dual-functional Th1, Th2 and Th17 cells, a regulation also reversible by anti-helminthic treatment.

Immunology of helminth-tuberculosis co-infections

In order to define the systemic cytokine levels in helminth-TB coinfection, the Institute measured the circulating plasma levels of Type 1, Type 2, Type 17, other pro-inflammatory and regulatory cytokines in individuals with active TB (ATB) with or without coexistent *Strongyloides stercoralis* (Ss) infection by multiplex ELISA. Similarly, the Institute also measured the same cytokine levels in individuals with LTB with or without concomitant Ss infection in a cross-sectional study. Our data reveal that individuals with ATB or LTB and coexistent Ss infection have significantly lower levels of Type 1 (IFN γ , TNF α and IL-2) and Type 17 (IL-17A and IL-17F) cytokines compared to those without Ss infection. In contrast, those with ATB and LTB with Ss infection have significantly higher levels of the regulatory cytokines (IL-10 and TGF α), and those with LTB and Ss infection also have significantly higher levels of Type 2 cytokines (IL-4, IL-5 and IL-13) as well. Finally, those with LTB (but not ATB) exhibit significantly lower levels of other pro-inflammatory cytokines (IFN γ , IFN α , IL-6, IL-12 and GM-CSF). Data therefore reveal a profound effect of Ss infection on the systemic



Fig. 2. NIRT staff interacting with Self Help Group.

cytokine responses in ATB and LTB and indicate that coincident helminth infections might influence pathogenesis of TB infection and disease (Fig. 3).

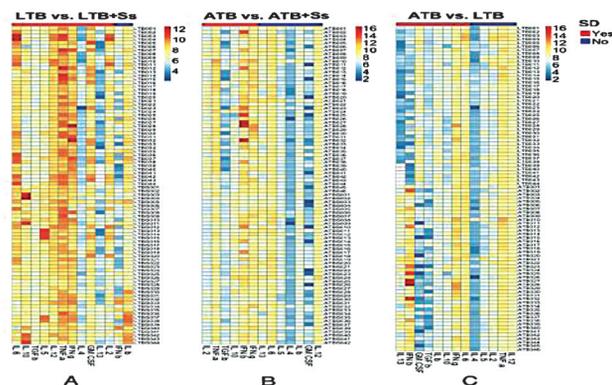


Fig. 3. Heat map showing the expression patterns of various cytokines in the different groups of individuals.

Immunology of diabetes-TB comorbidity

To characterize the influence of diabetes on leucocyte phenotypes in PTB or LTB, the Institute examined the frequency (F_0) of leucocyte subsets in pulmonary TB individuals with (PTB-DM) or without diabetes (PTB); latent TB individuals with (LTB-DM) or without diabetes (LTB) and non-TB infected individuals with (NTB-DM) or without diabetes (NTB). Data reveal that coincident diabetes alters the cellular subset distribution of T cells, B cells, DC and monocytes in both active and

latent TB individuals, thus potentially impacting the pathogenesis of this co-morbid condition.

NATIONAL JALMA INSTITUTE FOR LEPROSY & OTHER MYCOBACTERIAL DISEASES, AGRA

Study on environmental *Mycobacterium leprae* and its transmission link in leprosy

A total of 169 patients were recruited in this study of whom (7.1% were found to be AFB positive and 45.56% were found to be RLEP-PCR positive. In these patients, 54.54% of the soil samples collected from patient dwelling area of 77 RLEP-PCR positive patients were found to be positive while 53.24% drainage water samples from the patient dwelling area were found to be positive for RLEP PCR. Experiments of mRNA for viability of *M. leprae* was done by isolation of total RNA and conversion of total RNA to cDNA and then amplification and sequencing of 16SrRNA gene. The positive amplification of 39 environmental samples showed the viable *M. leprae* present in the soil and water samples from the patient dwelling area of the index case. Short tandem repeat studies showed the same type of strains found in both index case and environmental samples. These findings are of public health importance (Fig 1).

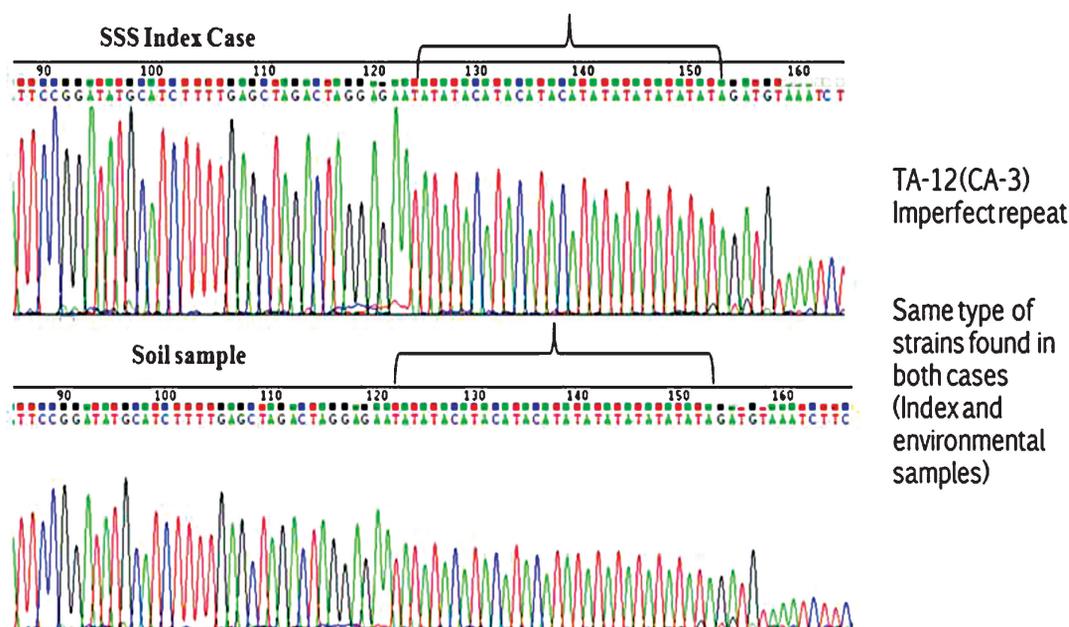


Fig. 1. Comparison of strains of *M. leprae* found in environmental samples with those in SSS samples.

Study on endemicity of leprosy and utilization of health services in a selected area of Uttar Pradesh, Chhattisgarh and Tamil Nadu

The study was conducted in Ghatampur (high endemic) and Banda (Low endemic) of Uttar Pradesh and Mahasamund (High endemic) and Sarguja (Low endemic) of Chhattisgarh. A total of 169 leprosy cases from Ghatampur, 102 cases from Banda, 119 cases from Mahasamund and 26 cases from Sarguja were recruited. In this study, it was found that the molecular technique (*M. leprae* RLEP-PCR) in slit skin smear samples (SSS) helped in definitive early diagnosis of the leprosy and more sensitive than AFB detection in SSS as was simple and could be undertaken in the field conditions. This could be used in the programme for value addition to the early and definitive diagnosis of leprosy. The Phase-II study focusing on the endemicity of leprosy and utilization of health services and role of RLEP-PCR in early detection and treatment among household contacts has been approved.

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

Samples were collected from 54 leprosy patients. AFB was positive in 22.22%, RLEP-PCR was positive in 35.18% and ISSR-PCR was positive in 31.48% of the leprosy patients. In this study, the role of ISSR-PCR markers as a complimentary marker which can assist in case diagnosis of leprosy in combination with RLEP-PCR is being assessed.

Mycobacterial Repository Centre

A total of 5341 mycobacterial isolates from different parts of country are being maintained and are available to support the investigators from different parts of the country. The facility continued to provide the support and references as well as characterized Mycobacterial isolates/DNAs/RNAs/Culture Filtrates/Lysates to different investigators of all over the country. Additional standard as well as clinical strains of mycobacteria will be included in the repository.

Validation of indigenously developed technologies for diagnosis of- (a) pulmonary tuberculosis (b) drug resistant tuberculosis

This multicentric study has been funded by the Department of Biotechnology. During the period, a total of 336 patients were recruited in collected Phase I (n=286) and Phase II (n= 50). The sputum samples were collected and processed for TruNat, MGIT culture, LJ culture, IGS culture and by the GeneXpert test. This multicentric study will result in the development of indigenously developed molecular and rapid culture methods to diagnose drug resistant tuberculosis. NJILOMD is a National Reference Laboratory for first line and second line anti-TB drug resistance testing using solid/liquid cultures as well as by the Line Probe Assay method

Proteomic approach to study drug resistance 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 *E.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 overexpression of seventeen protein spots consistently in all resistant as compared to sensitive isolates. However, five spots matched with already identified proteins and therefore total twelve proteins were found to be upregulated.

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

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

Th 17, gamma delta T cells, T regulatory cells and IgG subclasses in TB patients and their healthy contacts: A follow up study

Higher IFN γ level was noted in culture supernatants of PBMCs of household contacts (HHCs) than patients and similarly higher IFN γ expressing CD4 and CD8 T cells was also observed in HHCs than patients. IL-4 expressing CD4 T cells significantly decreased after 9 months of therapy in patients. The $\gamma\delta$ T cells expressing IFN γ were increased after 6 months of therapy in patients. On the other hand $\gamma\delta$ T cells expressing IL-17 decreased after 6 months of therapy which could relate to the protective response against bacteria. Higher IgG1 and IgG4 were noted against Ag85 complex in patients than HHCs at the time of recruitment. Study of relative frequencies of CD39+CD73+ CD4, CD8 T regulatory cells, Th-17, Th-9, Th-22 cells in leprosy patients with reactions has been initiated.

Understanding the role of CCL 2 and associated gene in leprosy susceptibility and in leprosy reactions

The genes for production of CCL2 chemokine and inducible nitric oxide synthase NOS2 are present in chromosome 17. CCL2 is an important chemokine secreted by monocytes, macrophages which regulates the immune response of an individual through attracting various cells to the site of infection. During this process, it has been reported to regulate other important cytokines such as IL12, IFN gamma, TNF alfa etc. SNPs in *ccl2* and *nos2* gene have been reported to be associated with the susceptibility to TB and Leprosy in various populations.

CC genotype at -362 *ccl2* was found to be the susceptible genotype for leprosy. GG genotype at -2518 *ccl2* and CC genotype at -362 were possibly associated with the risk of suffering from ENL reactions in BL and LL patients. No significant differences in leprosy patients and controls could

be seen in any of the NOS2 snps studied. CC-362 and GG-2518 *ccl2* genotyped patients were having lower level of serum CCL2. The Human Zinc Finger Protein 443, a DNA binding protein was explored to bind to the -2518A/G site and may be responsible for regulation of CCL2 gene expression

Analysis of SNPs in TLRs, TIRAP, MCP1, MiRNAs in tuberculosis and understanding the possible role of these molecules in immunopathogenesis

The frequency of C/C genotype at -362G/C was 12.73% in PTB cases which was significantly higher than that found in healthy controls (7.23%). On haplotype analysis, the A-C haplotype was found to be in higher frequency (0.046) in PTB cases compared to healthy controls (0.004) (P=0.006, OR= 7.23). A-C haplotype was observed to be a risk haplotype for contracting TB. A strong LD was observed between both the sites (D'=0.961, P=0.00) indicating that these two SNPs are in linkage disequilibrium with each other

NATIONAL INSTITUTE OF CHOLERA & ENTERIC DISEASES, KOLKATA

ENTERIC DISEASE INVESTIGATIONS & RESEARCH ON MICROORGANISMS

Childhood diarrhoea in rural West Bengal

NICED conducted a community based study in rural West Bengal in the district of South-24 Parganas. Adult behavior such as 'dipping a pot to draw drinking water from storage vessel in a household' was found associated with diarrhoea in children aged ≤ 2 years in this investigation. The incidence rate of rotavirus diarrhoea in this study was highest in children in the age group > 6 to ≤ 18 month (24/100 child-year). The study also identified bacterial, protozoal, helminthic and viral co-infections in symptomatic rotavirus gastroenteritis in children. Most frequent rotavirus genotype detected in this investigation were G9P[4], G1P[8], G2P[4] and G8P[4]. Information generated through this investigation helped to plan a rotavirus vaccine trial, which is currently in its initial phase of implementation.

Antibiotic decision for treating cases of typhoid

As a part of its research endeavor on typhoid fever, NICED processed blood samples collected from children with clinically suspected typhoid fever attending tertiary care Government hospitals. Routinely the culture reports as well as the antimicrobial susceptibility results of *S. Typhi* isolates (causative agent of typhoid) were sent to the attending physicians, so that appropriate antimicrobials could be initiated for the treatment of the patients. There is notable change in the susceptibility patterns of *S. typhi* over the last few years. Growing resistance to ciprofloxacin, the drug of choice as per WHO recommendation, is a cause of concern leading to many treatment failure cases. NICED recommends judicious use of azithromycin or third generation of cephalosporins, to which most of the isolates (>90%) are currently susceptible (Fig. 1).

Participation in Rotavirus surveillance network

A total number of 914 stool samples from hospitalized diarrhoea patients (<5 years old) from the districts of Medinipur and Kolkata were screened for rotavirus during 2014-2015 as part of country wide surveillance. Stool samples were screened for rotavirus using Rota-ELISA kit detecting the

VP6 antigen. Among 914 stool samples, 487 were detected as rotavirus positive (53%) (Fig. 2). A large variety of genotypes were detected {G1P[8], G1P[6], G2P[4], G2P[6], G9P[4], G9P[8], G12P[6] and G12P[8]} during this study. G1P[8] was the most commonly identified type. Unusual zoonotic strains were detected at low frequency (<2%).

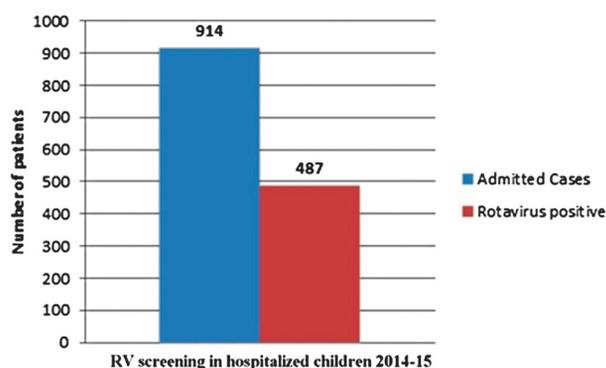


Fig. 2. Surveillance for Group A Rotaviruses in children <5yrs old hospitalized for acute gastroenteritis during 2014-2015. ELISA for VP6 antigen was used for screening.

Intervention among health care providers in urban slum of Kolkata

A multi-component educational intervention was designed to improve the knowledge of non-qualified practitioners of the urban slums of six administrative

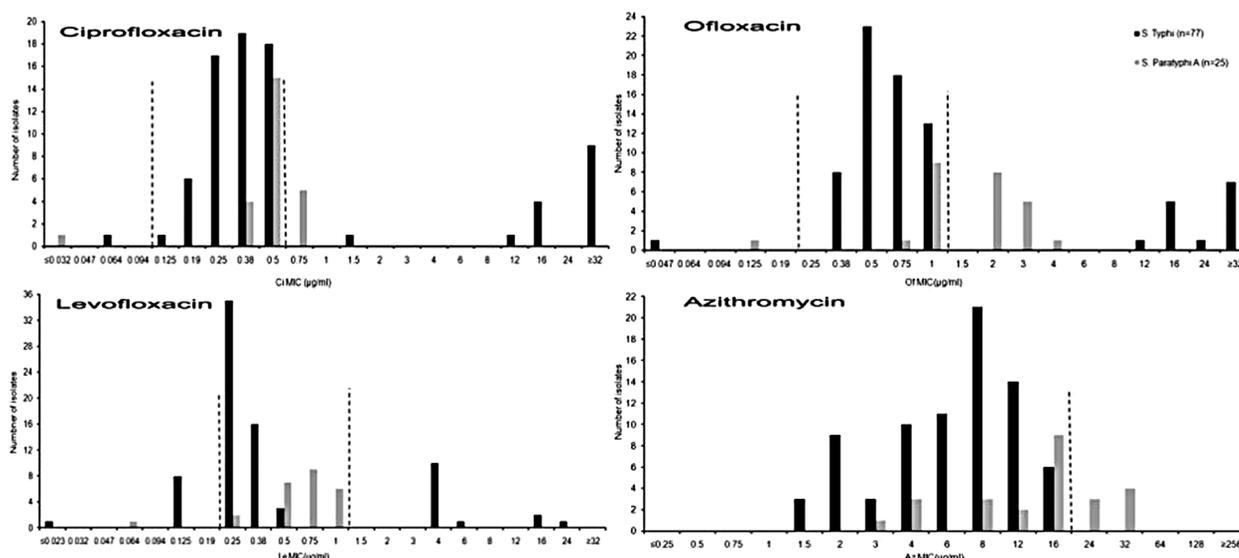


Fig. 1. MIC distribution of fluoroquinolones and azithromycin in all *S. Thphi* and *S. Paratyphi A* Kolkata Isolates during 2009-2013. Interrupted lines denote MIC breakpoints of the antimicrobials for susceptible (left line) and resistant (right line) isolates. Isolates having MICs between two Interrupted lines showed reduced susceptibility. For azithromycin, single interrupted line indicates MIC breakpoint (>16 mg/ml) of resistance.

wards of Kolkata municipal area. Between June and December 2014, 140 such practitioners who were chosen randomly from an exhaustive list of practitioners in the study area and interviewed at baseline, were provided with six modules of educational materials on symptoms and spread of diarrhoeal diseases, cholera (disease as a whole), prevention and management of diarrhoeal diseases along with oral use of rehydration solution. After a gap of 2 months between February and April, 2015, 124 practitioners (16 were lost to follow up due to migration, death or sickness) were interviewed post-intervention. The mean overall [69.25(95% confidence interval: 95%CI=67.66-70.84); paired t-test p value (p) <0.0001] and domain-specific knowledge scores for spread and management of diarrhoea and use of ORS increased significantly

(at $\alpha=0.05$) after intervention compared to that in baseline (Tables 1, 2). Intervention worked better among long-term practitioners having no prior training.

Community based assessment

Stool samples were collected from children aged ≤ 12 years who were residents of semi urban and rural western India. Modified Kato katz method was applied for detection of helminths-ova and polymerase chain reaction (PCR) test was employed to identify diarrhoea causing parasites. The purpose of these tests was to assess the impact of Community Led Total Sanitation (CLTS) campaign undertaken by the State Government of Madhya Pradesh to improve the health and welfare of the

Table 1. Association between changes in domain-specific and overall diarrhoeal-related knowledge level with intervention among the selected non-qualified physicians in urban slums of Kolkata (N=124).

| Domains | Ordinal logistic OR | OR (95%CI) | p value |
|---|---------------------|------------------|---------|
| Symptoms of diarrhoeal diseases | Unadjusted | 0.93 (0.60-1.44) | 0.7426 |
| | Adjusted | 1.29 (0.81-2.07) | 0.2856 |
| Spread of diarrhoeal diseases | Unadjusted | 4.05 (2.53-6.49) | <.0001 |
| | Adjusted | 5.60 (3.36-9.35) | <.0001 |
| Cholera | Unadjusted | 1.49 (0.95-2.33) | 0.0804 |
| | Adjusted | 2.01 (1.25-3.24) | 0.0041 |
| Prevention and control of diarrhoeal diseases | Unadjusted | 0.71 (0.45-1.11) | 0.1313 |
| | Adjusted | 0.89 (0.55-1.45) | 0.6495 |
| Management of diarrhoeal diseases | Unadjusted | 2.15 (1.37-3.39) | 0.0009 |
| | Adjusted | 3.09 (1.86-5.11) | <.0001 |
| Oral rehydration solution | Unadjusted | 1.79 (1.14-2.81) | 0.0111 |
| | Adjusted | 2.28 (1.41-3.67) | 0.0008 |
| Overall knowledge level | Unadjusted | 2.94 (1.88-4.61) | <.0001 |
| | Adjusted | 4.31 (2.63-7.07) | <.0001 |

Table 2. Stratum-specific associations between changes in overall diarrhoeal-related knowledge level with intervention among the selected non-qualified physicians in urban slums of Kolkata (N=124).

| Influence across strata | | OR (95%CI) | p value |
|-------------------------|-------------------------------|------------------|---------|
| Qualification/Training | Alternative Medicine | 2.26(1.28-4.01) | 0.0052 |
| | No such | 4.75(2.24-10.05) | <.0001 |
| Attachment | Having fixed clinic/office | 2.00(1.15-3.46) | 0.0138 |
| | Having no fixed clinic/office | 2.00(0.31-13.03) | 0.4686 |
| | Pharmacist | 8.27(3.33-20.52) | <.0001 |
| Years of practice | ≥ 10 yrs | 3.15(1.85-5.36) | <.0001 |
| | 5-9 yrs | 2.83(1.09-7.34) | 0.0326 |
| | <5yr | 1.46(0.24-8.89) | 0.6809 |

rural poor. Given the average *Ascaris* isolation of 20%, periodic de-worming was recommended for the study region following completion of this assessment research.

New diagnostic approach in relation to antibiotic resistance

Studies conducted at NICED have shown that *Acinetobacter* species are highly antibiotic – resistant, with multiple mechanisms involved. Special attention to these species isolated from in-patient population should be paid. The new combination disc test devised to identify Extended Spectrum Beta-Lactamase (ESBLs) and AmpCs in the presence of metallo-β-lactamases has the potential to be used for routine diagnostic application in laboratories (Fig. 3).

HIV AND OTHER LINKED RESEARCH

Needs assessment of children living with or affected by HIV

An innovative project on assessment of care needs of vulnerable children has been undertaken by NICED in West Bengal. Under this endeavour, a total of 287 children (133 male and 154 female) have been recruited from the district of Paschim Medinipur. The mean age of these children is 11 years (SD±3 years; minimum 6 years and maximum 17 years) and majority of them are Hindu (89%). Of 287 children, 108 (37%) are living with HIV (CLWH) and the rest are affected - defined as one or both parents being HIV sero-reactive. Mothers constituted the largest proportion of interviewees as care givers (248/286; 86%) providing information

on care related issues. About 93% of the care providers (266/287) have reported undergoing HIV test, 84% being HIV sero-reactive. Among CLWH, 63% (68/108) are on anti-retroviral therapy (ART). Under this project, 111 children (93% Hindu) have been recruited from the district of Purbo Medinipur (male:female 62:49). About a third of these children from Purbo Medinipur are living with HIV, of whom 19% are on ART. Mothers in this district also constituted the largest proportion of interviewees as care givers (85%). Children experiencing psychological distress and having other care needs due to HIV related issues have been addressed through the established network of NICED with a Child-Psychiatrist located at Tata Medical Centre, Kolkata (co-investigator of the present project) and the community based organizations named SPARSHA & AASHA, which are the collaborating partners of the current research project (Fig. 4).



Fig. 4. Training and other activities of the ‘Care Needs Assessment’ work; children here are either living with HIV or are affected by HIV/AIDS.

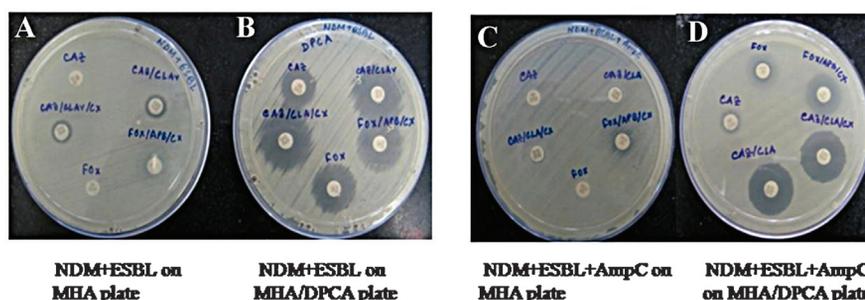


Fig. 3. Failure to detect ESBLs (A) and ESBLs & AmpC (C) in presence of NDM-1 on Muller Hinton agar (MHA) plate by combination disk assay. Addition of dipicolinic acid (DPCA) in the medium resulted in augmentation of zones making ESBLs (B) and ESBLs & AmPC (D) detection possible.

Policy advocacy for rotavirus vaccine in India

In-country knowledge on development of immunization program in India and pros and cons of introduction of rotavirus vaccine in the country was synthesized during the last year by NICED. This synthesis was based on the principles of systematic review and took into account ‘Disease burden’, ‘Host factors’, ‘Macro-social environment’, ‘Agent (rotavirus) related issues’, ‘Immunization program elements in the country’ and ‘Economic factors’. The themes emerging from the cognitive content of the discussion and debate published in various peer-reviewed journals and their critical analysis helped to develop a policy framework based on which the decision to introduce rotavirus vaccine in India could be examined.

Highlighting the need of addressing HCV epidemic among IDUs in India

Another issue of public health importance highlighted during the last year by the scientist of NICED was hepatitis C virus (HCV) infection among injecting drug users (IDUs) in India. This initiative tracked the earliest documented evidence of HCV among IDUs in the country and also its wide geographical spread. The research-review highlighted the importance of recognizing the ability of HCV to spread faster compared to HIV in any unsafe injecting drug use setting. As an outcome, the neglected epidemic of HCV in IDUs in India started drawing attention from policy and program perspectives.

NATIONAL INSTITUTE OF MALARIA RESEARCH, NEW DELHI

Therapeutic efficacy of antimalarials

During the year 2014-15, research in various aspects of malaria and other vector borne diseases was carried out which included bio-ecology of vectors, molecular and proteomic studies on vector-parasite interactions, evaluation of vector control interventions that included efficacy and community acceptance of long lasting insecticidal nets(LLINs) and other interventions and epidemiological studies included molecular studies on population genetic

diversity of malaria parasites, drug resistance and host’s immune responses.

The institute was actively involved in operational research in close collaboration with National Vector Borne Disease Control Programme (NVBDCP). The therapeutic efficacy studies of antimalarials revealed evidence of failure to artesunate+sulfadoxine pyrimethamine (Fig. 1) which led to change in the drug policy to artemether +lumefantrine in the north eastern states. In addition, the institute is also monitoring the therapeutic efficacy of artemisinin along the international borders to detect the possible emergence of resistance to this drug.

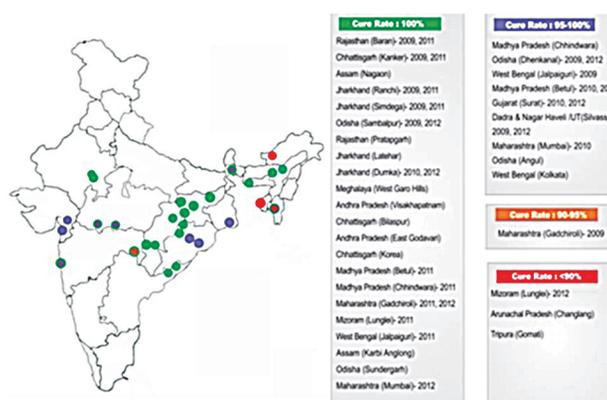


Fig. 1. Therapeutic efficacy of Efficacy to AS+SP.

Clinical development of antimalarials

Clinical trials for safe and effective prevention as well as treatment of malaria in pregnancy were carried out. They showed that the combinations of artesunate+SP as well as artesunate+ mefloquine were safe and effective. They also showed that intermittent screening and treatment can help to identify malaria cases early, and thus prevent complications.

Comprehensive case management of malaria

An operational research project ‘Comprehensive Case Management of Malaria’ is being carried out in collaboration with Government of Odisha to assess the impact of universal access to diagnosis and treatment on malaria incidence in different transmission settings. There has been significant increase in access to diagnosis and treatment in all intervention areas. The proportion of cases being detected and treated within 24 hours of fever has increased significantly (Fig. 2). In the low

transmission settings, improved case management is showing impact by way of reduced malaria.

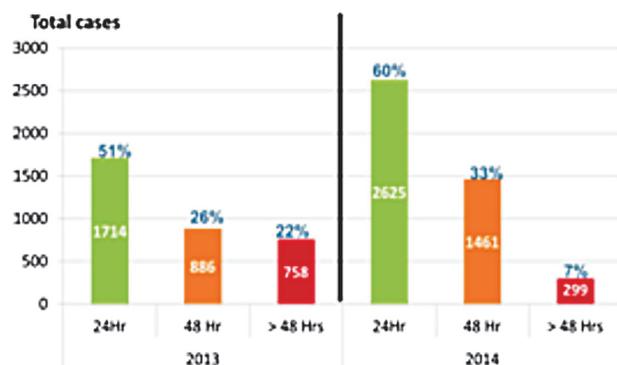


Fig. 2. Time from onset of symptoms to treatment: Athamallik block, Angul.

Mapping of malaria risk in the context of climate change in India

The project aims to generate risk maps of malaria from the viewpoint of malaria prevalence, climatic determinants, anopheline vector's distribution and ecological risk in view of climate change in India. Maps of hotspots of malaria based on temperature and malaria endemicity provide insight that linearly progressing temperature is not likely to increase in high intensity of malaria. The rainfall cut-off resulting in outbreak of malaria in different regions indicated that in states like Assam, 100 mm rainfall in the month of March while in Maharashtra state, more than 200 mm cumulative rainfall in the month of July- September could result into outbreak in the month of December. Work on ecological risk map of malaria using satellite data for all the hotspots of malaria at village level is being undertaken which would serve as baseline map for identifying the ecological features of malaria foci in hotspots and linking future ecological change with malaria endemicity.

Ecological succession of anophelines

During this year, studies were carried out on species succession in North-East where three *Anopheles* species, *An. pseudowillmori*, *An. nigerrimus* and *An. culicifacies* were reported and the prominent vector species *An. culicifacies* was a incriminated as vector. The continued study in G.B. Nagar (U.P.) indicated disappearance of another important vector, *An. fluviatilis* from the study areas that were found earlier in 2012 and this was

reasoned to the absence to thick aquatic vegetation in the main breeding habitat, NTPC canal.

Study of Kdr gene in *Aedes Aegypti*

A study on the insecticide resistance in *Aedes aegypti* showed that F1534C mutation that was responsible for conferring high degree of resistance against DDT and moderate level of resistance against pyrethroids (Fig. 3). The role of a novel mutation T1520I is under investigation.

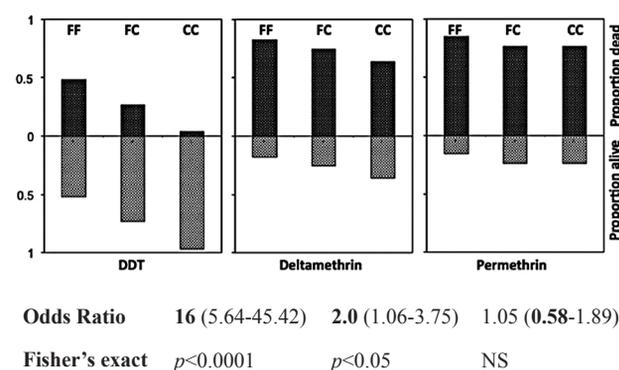


Fig. 3. Association of F1534C (kdr) with insecticide resistance in *Aedes aegypti*.

Evaluation of vector control tools

The Institute conducted field trials on two brands of LLINs in Orissa and Haryana. A new technique for determining insecticide susceptibility using bottle assay was standardized and was found comparable to existing WHO insecticide susceptibility test, and this decreases the reliability on imports. Also a method using common buffers in place of buffers with restricted buffers requiring import of reagents for detecting mosquito blood meal was standardized. In-line with the requirement of new insecticides and tools for insecticide-resistance management, studies with insecticides with novel mode of action were conducted e.g. neonicotinoids and have shown promise. Molecular docking studies to determine alternative insecticides for management of pyrethroid resistance in mosquitoes have yielded encouraging results and validation for feasibility for use of these insecticides on mosquitoes for management of resistance will be initiated.

PARASITE BIOLOGY

Falcipains: potential drug targets

A study is focused to identify the crucial inhibitors which can specifically halt the processing of

falcipains and it has been found that series of non-coded amino acid based inhibitors block the processing of cysteine proteases by inhibiting salt and hydrophobic interactions of the pro and the mature domain. This finding suggests new strategies for the development of anti-malarial agents based on blocking the activation of proteases, and design potential inhibitors based on protease-BC loop interactions.

Study of vir genes in isolates from severe vivax malaria

A study of the virulence (*vir* genes) and transporter of CQR (*Pvcrt-o* genes) has indicated increased expression levels of these genes in severe infections. Increased expression of *Pvcrt-o* genes may be responsible for the changing trends of complicated *P. vivax* malaria (Fig. 4).

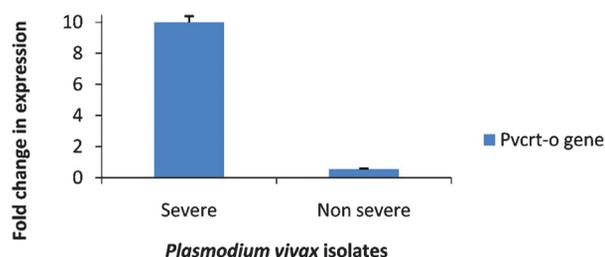


Fig. 4: Expression of *Pvcrt-o* gene in severe and non-severe *P. vivax* isolates normalized with endogenous gene (β -tubulin).

Genetic polymorphism of P. falciparum

Genotyping the *P. falciparum* isolates using *msp1*, *msp2* and *glurp* gene loci was used to assess the multiplicity of infection (MOI) for detecting the number of clones per isolate. The study 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 with two or more alleles present in *msp1*, *msp2* and *glurp* genes (Fig. 5).

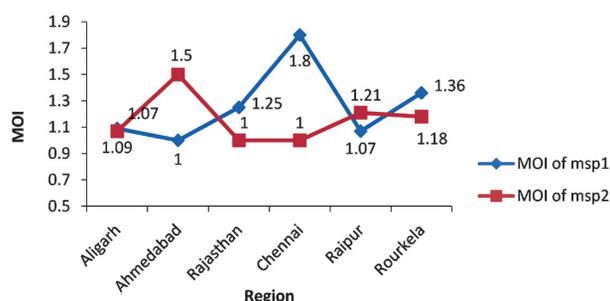


Fig. 5. Comparison of MOI for *msp1* and *msp2* genes in different regions of India.

Role of mesenchymal stem cells in immunity against malaria

Recruitment of MSCs during infection and infusion of these cells into naïve mice was able to confer host resistance against malaria infection.

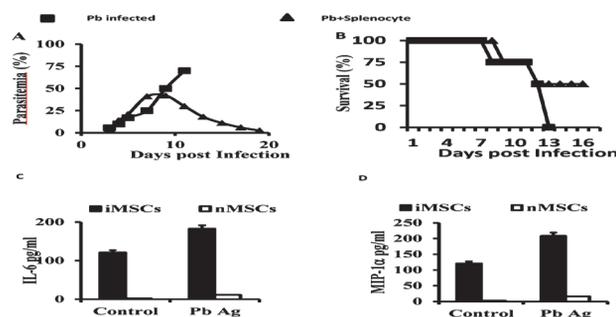


Fig. 6. Immune response in *Plasmodium berghei* infected mice.

MSCs augmented interleukin (IL)-6 productions indicating that MSCs are host-protective and enhance pro-inflammatory cytokine production (Fig. 6).

VECTOR CONTROL RESEARCH CENTRE, PUDUCHERRY

LYMPHATIC FILARIASIS

Morbidity management and disability prevention programme (MMDP) for filarial lymphoedema: Assessment of impact and impediments

Preliminary assessment of the impact of the ongoing morbidity management and disability prevention programme for filarial lymphedema among 260 patients in Villupuram District, Tamil Nadu, showed that the programme has yielded positive results in prevention of disability in the form of reversal of lymphedema to normal condition or remaining in the same grade without further progression. However, grade 1 & 2 cases need additional medical attention to prevent further progression to higher grades.

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

The miniaturization of the electrochemical detector of *W. bancrofti* infection in vectors, developed at VCRC in collaboration with VIT Vellore has been achieved and is under validation. The electrochemical impedance spectroscopy (EIS) signal measurement was found to have reproducibility at 1 pM of the

probe concentration and at 10 pM of the target ss-DNA of the parasite. Based on these optimal conditions, validation of real samples was carried out using the PCR amplicons of filarial parasite *W. bancrofti* (WB) as target DNA and *B. malayi* (BM) & *Cx. quinquefasciatus* mosquito (CQ) as mismatch DNAs for the hybridization analysis. The voltametric experiments showed good differentiation with target DNA against mismatch DNA.

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

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 has been developed and validated. A sample of 210 pools (30 clusters x 7 pools) consisting of minimum 5000 gravid females is sufficient to assess the prevalence of infection in the vectors (0.25%) during post MDA. The consistency of the estimates (vector infection) between surveys immediately after 8 annual round of MDA and two years post-MDA, suggest that the proposed two-stage cluster sampling protocol (village and households) can be used for the collection of *Culex* gravid females by gravid traps for monitoring vector infection and infectivity during post-MDA surveillance. Field validation of the sampling strategies at district level is in progress with further minimization of sample size.

Malaria/Leishmaniasis/Scrub Typhus

Tolerability, efficacy and operational feasibility of Artesunate Combination Therapy (ACT) (Artesunate – Sulphadoxine-Pyrimethamine): as 1st line anti-malarial drug for falciparum malaria control in a tribal area of Odisha state, India

Studies on tolerability, efficacy and operational feasibility of Artesunate combination therapy (ACT) in a tribal area of Odisha suggest that the ACT is well tolerated. The clinical and parasitological response (ACPR) was 90.7%, late parasitological failure (LPF) 8% and early treatment failure (ETF) 1.7%. The major adverse effect was abdominal pain which lasted up to three days after starting the

treatment. However, this symptom was mild as it was easily tolerated by the study patients. There is a need for training of ASHA's at regular intervals and regular replenishment of RDT kits and drugs for early treatment of malaria.

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

Comparative efficacy of indoor residual spraying of DDT WDP 75% and DDT WDP 50% was studied against the malaria vector, *An. fluviatilis* in Odisha. There was no significant difference between the impact of the two formulations on indoor/outdoor daytime resting densities, light trap densities, human blood index, survival rates and vector infection rates. There was also no difference between the two formulations in their ease of application. However, the residual efficacy of sprayed surfaces and post-spray mud-plastered surfaces as assessed by bioassay was higher with DDT 75% arm than with DDT 50%. The quantity of DDT 75% required for a given population is about 30% lesser than that of DDT 50%, which would considerably reduce the cost of transportation, storage space, handling etc.

Entomological and Epidemiological investigations on Leishmaniasis among the Kani forest Tribes in the tribal settlements of Thiruvananthapuram district, Kerala

Indigenous transmission of *Leishmania donovani*, with symptoms of cutaneous leishmaniasis (CL), among the Kani forest tribes of Thiruvananthapuram district, Kerala, was established. Natural infection of *L. donovani* was detected in wild caught *P. argentipes* females through PCR assay. Blood samples from domestic dogs were positive for *L. donovani* DNA indicating their possible role as reservoir host of the parasite. Awareness on the public health importance of CL infection and prevention was created among the Kani tribes through health education. A new sandfly species recorded from the tribal settlements was described and has been assigned a new name *Sergentomyia (Neophlebotomus) monticola sp. nov.* in recognition of the Western Ghats montane rain forests. The DNA barcode sequences of specimens collected from the study area, when analyzed showed that

the overall genetic distance (K2P) between the sequences being only 0.4%, thus belonging to a single taxonomic category.

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

Expertise and facilities for laboratory diagnosis of scrub typhus and entomological research have been developed. Preliminary studies in Puducherry showed higher levels of chigger (*Leptotrombidium deliense*) indices and infection of rodents with *Orientia tsutsugamushi* in areas where human cases of scrub typhus were reported. 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. The Chigger (*L. deliense*) index ranged from 11.0% to 88.5% in the villages surveyed and the overall Chigger index was 26.6%. In all villages surveyed, the Chigger (*L. deliense*) index was well above the critical level of chigger load i.e. 0.69 per rodent indicating that the villages are at high risk of transmission of scrub typhus in Puducherry.

DENGUE/JE

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

Demonstration of vector control and prevention of dengue/chikungunya through partnership and community empowerment in Mandipet and Ramanathapuram PHCs showed that it is feasible to motivate the community through trained school students. Interpersonal communication is more effective than mass communication. Drastic reduction in the number of discarded containers (vector breeding sources) could be achieved in the areas under school based approach in contrast to the comparison areas.

Forecasting JE mosquito vectors abundance through Geo Environmental risk determinants, using Remote Sensing & GIS

Using remote sensing & GIS, a method has been developed to forecast the risk for JE incidence in terms of predicting increase in mosquito vector abundance for areas where conventional paddy

cultivation is in practice. Different approach is required for the areas where there is mixed vegetation.

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

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

Microbial/Chemical Agents for Vector/Parasite Control

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

The citrate salts of B7, B8 and B14, which were found effective in causing mortality of adult worms in gerbils when given intraperitoneally were further tested in *B. malayi* infected *Mastomys coucha* through oral administration of 200 mg/ kg. Animals treated with B7 and B8 were sacrificed after 90 days and the worms alive were recovered indicating that both these compounds are not very effective as macrofilaricidal when given orally to infected animals. Evaluation of B14 is going on *B. malayi* infected multimammate rats.

Development of naphthoquinone analogues as macrofilaricidal agents

In vitro ADME properties of six out of eleven promising macrofilaricidal naphthoquinone (NPQ)

analogues synthesized at VCRC have been studied in comparison with standard drugs. These compounds are found to be stable in the acidic pH of the stomach and have good balance between solubility and permeability for optimal oral absorption and cell membrane permeation. The studies are in progress with remaining five compounds.

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 of an indigenous isolate of *Bacillus amyloliquefaciens* was found to have mosquito larvicidal and pupicidal activity. The metabolite also showed antibacterial effect on clinical isolate of *Staphylococcus aureus*, indicating its potential utility against human pathogenic bacteria.

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 level, of washed polyester nets impregnated with a new LN treatment kit, ICON MAXX, in comparison with polyester nets conventionally treated with the same insecticide, lambda-cyhalothrin (CS 2.5%) at the WHO recommended dosage of 15mg ai/m² was carried out in a *Plasmodium falciparum* endemic area of Odisha State in India. Up to 30 months, the ICON MAXX LN met the WHOPES criteria (80% nets passed by combining the results of cone-bioassays and tunnel tests). However, at 36 months of household use, only 58.8% LNs met the WHOPES criteria. Since, > 20% of the ICON MAXX LNs failed to meet the WHOPES criteria, the study was stopped at 36 months.

FACILITIES

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

Vector susceptibility/resistance monitoring tests are normally conducted in field using WHO tubes and insecticide impregnated papers at the

diagnostic concentrations. Currently, the insecticide impregnated papers are imported from Malaysia. To establish a National facility for supplying insecticide impregnated papers (IIPs) conforming to WHO standard for monitoring insecticide resistance/ susceptibility in malaria vectors to different insecticides used in the National vector control programme, VCRC has standardized the procedures for preparation of insecticide impregnated papers.

Biomedical Informatics Centre of ICMR

One of the units of ICMR's Biomedical informatics centre (BMIC) has been established in VCRC. This unit has initiated a database creation aiming to offer comprehensive data on genomics and proteomics of the arthropod vectors of public health importance in India and finalized an illustrative design vector informatics (VectorInfo) Database homepage. To study the role of *kdr* mutations in voltage gated sodium channel (VGSC) in conferring target mediated resistance to DDT and synthetic pyrethroids in malaria vectors a *De novo* model of Voltage gated sodium channel of *An. gambiae* has been modelled. Based on the complete protein sequence, the *de novo* model of Cuticular collagen of the filarial parasite *W. bancrofti* was constructed and the model has been validated for its stereo chemical quality and used for identifying three epitope regions. Steps are in progress to design specific antibodies against these epitopes.

NATIONAL INSTITUTE OF VIROLOGY, PUNE

Outbreak of Influenza A(H1N1)pdm09 in 2015

A resurgence of influenza with substantial morbidity and mortality was observed in India in 2015. Influenza virus was detected in 831/2476 [812A(H1N1)pdm09, 16 were Type B and 3 were Type A (H3N2)] clinical samples from Pune and other parts of Maharashtra. Thirty two out of 59 clinical samples inoculated in MDCK cell line yielded virus [28 A(H1N1)pdm09, 2 A(H3N2) and 2 Type B Yamagata viruses]. Phylogenetic analysis of 35 *HA* genes (19 in 2014, 16 in 2015) and whole genome analysis of eight A(H1N1)pdm09 isolates was carried out; isolates were similar to A/California/07/2009, 2009-2015 vaccine component and no pathogenic markers were observed.

Immune response gene [non-Major Histo-compatibility Complex (MHC)] polymorphisms in dengue disease pathogenesis

Cytokines play an important role in dengue disease pathogenesis. In order to find out whether single nucleotide polymorphisms (SNPs) in the cytokine genes are associated with dengue disease severity, SNPs in seven cytokine genes were investigated in dengue fever (DF) and dengue haemorrhagic fever (DHF) cases and healthy controls (HC). Interleukin (IL)-8 rs4973 A/T genotype and IL-10 rs1800871 A/G genotype were negatively associated with DHF compared to HCs and DF, respectively. Significantly lower frequencies of the combined genotypes IL-10 A/G-IL-1B A/A, IL-10 A/G-IL8 A/T and IL-10 A/G-IL-17F T/T were observed in DHF cases compared to DF. The results suggest that heterozygous genotypes of IL-8 rs4973 and IL-10 rs1800871 are associated with reduced risk of DHF. Combinations of IL-10 rs1800871 and pro-inflammatory cytokine genotypes influence the risk of DHF.

Study of T regulatory cells in hepatitis E infection

Understanding the crucial role of host factors, in the rapid progression to fulminant hepatitis E and in recovery from hepatitis E is important for the identification of prognostic markers. T regulatory cells (Tregs), Foxp3, IL-10 & TGF- β were identified to be regulating the immune response in self-limiting HEV infection. HEV specific TGF- β 1 was shown to be responsible for enhancement of Tregs in self-limiting HEV infection. Therefore, TGF- β 1 is a possible supplement for boosting Treg response for protection in severe (fulminant) disease.

Detection and characterization of enteroviruses associated with hand-foot and mouth disease (HFMD)

Cases of HFMD were reported from Pune, Maharashtra between October 2013 to November 2014. A total of 158 clinical samples were collected from 70 HFMD cases from five different local hospitals. The study revealed, CVA-16, CVA-6 as the major and other EV types as minor aetiological agents associated in HFMD. Phylogenetic analysis on the basis of full *VP1* gene of CVA-16 strains showed clustering of the Indian

strains with B1c subgenotype which has been reported rarely worldwide.

Assessment of diversity in group A rotavirus

The diversity among rotaviruses remains a challenge to the efficacy of currently available group A rotavirus (GARV) vaccines. Hence, the genomic and antigenic analysis of rotavirus strains circulating in a particular geographic region is essential. Two unusual G9P[4] rotavirus strains one each from 2009 and 2011 were isolated. The *VP7* and *VP4* gene sequences showed that the isolates belonged to sublineage c of the lineage III and P[4]-V, respectively. Multiplex PCR and phylogenetic analysis carried out for 60 rotavirus strains from the years 2011-2013 showed E1-C1 constellation in G1P[8], G9P[8], G12P[8], G12P[6], G2P[6] and G1P[6] and E2-C2 constellation in G2P[4] and G9P[4] strains for *NSP4* and *VP6* genes, respectively.

OUTBREAK INVESTIGATIONS AND SUPPORT

(i) Japanese encephalitis virus (JEV) outbreak, West Bengal: JE outbreak was reported for the first time among adults in northern districts of West Bengal State. Prevention and control measures including consideration of vaccination for adults was suggested following investigations by the central team.

(ii) Kyasanur forest disease (KFD) virus, Kerala: New focus of KFD was detected among the nomadic tribal community inhabiting Western Ghats for the first time in Malappuram district in Kerala by proactive investigation during May 2014. Further, KFD was confirmed in three more districts of Kerala. State Public Health department has thus initiated and has continued enhanced surveillance in the areas along with vaccination of tribals against KFD.

(iii) Crimean Congo haemorrhagic fever (CCHF): During the year, seven CCHF human cases were found positive from Kutch, Anand, Amreli and Aravali districts of Gujarat. Efforts also led to the confirmation of a nosocomial outbreak of CCHF in a private hospital between January-February 2015 in Jodhpur, Rajasthan State. This provided evidence for the disease prevalence in this State that needs to be under surveillance.

(iv) No human transmission noted following avian influenza outbreak, Kerala: Clinical samples from five patients with influenza like illness (ILI)/ severe acute respiratory infections (SARI) symptoms were tested for influenza H5N1 diagnosis during December 2014. Two of these patients were veterinary specialists and the others were symptomatic persons from the affected area. All the five samples were negative for influenza A and B and H5N1 viruses.

(v) Ebola preparedness and the role of apex laboratory: In the scenario of emergence of Ebola virus, a workshop on “Preparedness for quick laboratory diagnosis of Ebola virus” was organized for the 16 medical research/diagnostic laboratories (BSL-3) in India for capacity building in the country. They were also provided with reagents for the purpose of preparedness. NIV, Pune as an apex laboratory provided continuous diagnostic services to the country and also prepared a document, “National guideline to handle Ebola suspected patient and control practices”.

Outbreak investigations

(i) Dengue outbreak: The year 2014 was characterized by a huge outbreak of dengue in Pune. Altogether ~7000 samples were tested for dengue virus specific IgM, of which 59.2% were positive in Pune city and 22.3% were positive in rural Maharashtra. Weekly updates were posted on our website and also sent to the Joint Directorate of Health Services and the National Vector Borne Disease Control Programme (NVBDCP). NIV worked closely with the Pune Municipal Corporation, providing quality control and helped in increasing public awareness. Serotyping revealed that dengue virus (DENV)-4 which normally occurs sporadically in India was the dominant serotype in July-August 2014, but later it was co-dominant with DENV-2 in Pune city. In the rest of Maharashtra, DENV-2 dominated followed by DENV-3 and DENV-1. The re-emergence of DENV-4 is a cause for concern as it is often associated with severity in secondary infections. The introduction of DENV-1 genotype I (Asian) in south India in 2012 associated with one of the largest outbreaks that occurred in Tamil Nadu was also reported. The Kerala outbreak in 2013 was also identified as DENV-1 genotype I.

(ii) Investigation of hepatitis outbreak in Wai, Satara district, Maharashtra: An outbreak of hepatitis E was investigated in Wai town and the adjacent villages in December 2014. Majority of the cases were reported from two places, agricultural produce market for the Wai tehsil, and a company in the industrial area near Wai. Cases were mostly males in the age group of 20-40 yr who probably acquired infection at their workplaces and spread it to the adjoining ten villages where they stayed.

TECHNOLOGY DEVELOPMENT:

- HEV diagnostic ELISA: An ELISA based on the use of recombinant ORF2 proteins for hepatitis E diagnosis, using recombinant open reading frame 2 (ORF2) proteins from hepatitis virus (HEV) expressed in insect cells was developed at NIV. It can be efficiently used to detect infections with type 1 as well as type 4 viruses. The test compared with the widely used commercial kit from Genelabs Diagnostics, Singapore indicated higher sensitivity. The assay requires 2.5 hours and is user friendly. It can be used both as a screening and diagnostic test. It is highly stable at 4°C.
- Kyasanur forest disease virus (KFDV) IgM detection ELISA: The ELISA uses inactivated KFDV antigen and hence can be used at Biosafety Level (BSL) 2 testing. It can be efficiently used to detect IgM antibody against KFDV. The use of avidin-anti KFDV- biotin labeled antibody in the kit indicated higher sensitivity. The differential reactivity among flaviviruses can be differentiated using clinical presentation. It can be used as a diagnostic test. The assay requires 3.5 hours and is user friendly.
- IgG assay for detection of anti Crimean Congo haemorrhagic fever (CCHF) antibodies in sheep and goat: This ELISA uses inactivated CCHF antigen and hence can be used at BSL2 testing. It can be efficiently used to detect IgG antibody against sheep and goat. The test when compared with a CDC kit, was found comparable. The assay requires 2.5 hours and is user friendly. It can be used as a screening test.
- Development of IgG assay for detection of anti-CCHF antibodies in bovine host: This ELISA uses inactivated CCHF antigen and hence

can be used at BSL2 level testing. It can be efficiently used to detect IgG antibody against bovine epitope. The test was compared with a CDC kit and was found comparable. The assay requires 2.5 hours and is user friendly. It can be used as a screening test and is highly stable at 4°C.

- ELISA for detection of anti Chandipura virus (CHPV) IgM in human: The ELISA uses inactivated and concentrated CHPV antigen. Hybridoma cell line producing antibody against CHPV is unique. The use of avidin-anti CHPV biotin labeled antibody in the kit indicated higher sensitivity. It can be used as a diagnostic test.
- Monoclonal antibody based antigen capture ELISA for detection of JEV from mosquito: This test can be used to screen mosquitoes for the presence of JEV antigen. The hybridoma cell line-producing antibody against JEV is unique and the monoclonal antibody is highly specific. The kit uses probing antibody and substrate of the existing MAC ELISA for JE/DEN which are standardized for their stability and supplied all over the country. The ELISA works well with reproducibility and specificity. The assay requires 4hr and is user friendly.
- Realtime RT-PCR, RT-PCR and anti-Ingwavuma human IgG ELISA were also developed.

CENTRE FOR RESEARCH IN MEDICAL ENTOMOLOGY, MADURI

JE virus activity in Cuddalore district, Tamil Nadu

To assess the abundance of JE vectors and its virus activity in the endemic villages of Cuddalore district, Tamil Nadu, the mosquitoes were collected at dusk from Kodikkalam (Sirumangalam PHC), Eraiyur (Sirumangalam PHC) and SS. Puram (Pennadam PHC) during the reported period. The overall vector abundance was found to be increased (PMH 131.98) as compared to last year (PMH 117.38). A total of 14,247 mosquitoes belonging to 21 species and six genera were sampled in 108 man-hours. Among the culicine mosquitoes collected, *Culex tritaeniorhynchus* was the dominant species (PMH 66.08), followed by *Cx. gelidus* (19.25), *Armigeres subalbatus* (1.47) and *Cx. vishnui* (1.40). When dusk

sampling was made, the Dusk Index (DI) of *Cx. vishnui* subgroup was found to peak in October 2014 (166.11), along with that of *Cx. tritaeniorhynchus* (111.68) in November. On comparing the density between *Cx. tritaeniorhynchus* and *Cx. gelidus* it was found to be two and half fold higher than in the preceding year (25.8: 66.08). Among the 124 pools, 194 pools of *Cx. tritaeniorhynchus* (3020 specimens), eight pools of *Cx. vishnui* (123 specimens), 41 pools of *Cx. gelidus* (1521 specimens), two pools of *Cx. quinquefasciatus* (26 specimens), nine pools of *An. subpictus* (135 specimens) and two pools of *An. peditaeniatus* (21 specimens) were subjected to virus assaying. When the vector abundance of *Cx. tritaeniorhynchus* and *Cx. gelidus* was compared with that in last year, the PMH density of the predominant vector species *Cx. tritaeniorhynchus* was found to be increased from 85.14 to 87.17 and *Cx. gelidus* was also found to be increased and became the second most dominant species during this year (PMH 23.33 to 25.39).

To investigate the role of *Anopheles subpictus* along with other JE vectors in JE virus transmission in Tirunelveli district

A study was undertaken to investigate the role of *Anopheles subpictus* along with other Japanese encephalitis vectors in JE virus transmission in Tirunelveli district. A total of 4723 adult mosquitoes in 116 pools were collected, belonging to 13 species from Kuthalaperi and Ariyanayagipuram. *Culex tritaeniorhynchus*, was found to be the predominant species followed by *An. subpictus* in Ariyanayagipuram whereas in Kuthalaperi it was found to be the reverse. The other species found included *Cx. vishnui*, *Cx. gelidus*, *Cx. bitaeniorhynchus*, *Cx. quinquefasciatus*, *Ma. annulifera*, *An. pallidus*, *An. tessellatus*, *Ar. subalbatus*, *An. hyrcans* gp, *Ae. lineatopennis* and *Ae. vittatus*. All 116 pools of mosquitoes collected during the reported period were tested and four pools viz. *Cx. tritaeniorhynchus* (1 pool), *An. subpictus* (2 pools) and *Ma. annulifera* (1), were found positive. *An. subpictus* positive pools were detected in both the study villages.

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

In Gorakhpur district, 15 villages from three blocks (Khorabar, Bhathat and Chargawan) were selected

for entomological studies including dusk collection, outdoor and indoor resting collections to secure samples for vector incrimination and blood-meal identification. In indoor resting collections, a total of 25 different species were collected from human dwellings, cattle sheds and pig sties at selected villages in Khorabar block. The predominant species were *Cx. quinquefasciatus* followed by *Cx. vishnui* group and *An. subpictus*. Similar trend was also noticed in other two blocks (Bhathat and Chargawan). In outdoor resting collections, a total of 20 different species were recorded in outdoor resting collection from Khorabar block, in which, the predominant species was *Cx. tritaeniorhynchus* followed by *Cx. quinquefasciatus*. Similar trend was reported in Bhathat and Chargawan block. During dusk collections, a total of 29 species were collected, namely *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. quinquefasciatus*, *Cx. gelidus*, *Cx. infula*, *Cx. bitaeniorhynchus*, *Cx. epidesmus*, *Cx. whitmorei*, *Cx. fuscocephala*, *Lz. fuscus*, *An. subpictus*, *An. vagus*, *An. philippinensis*, *An. barbirostris*, *An. fluviatilis*, *An. peditaeniatus*, *An. stephensi*, *An. culicifacies*, *Ma. annulifera*, *Ma. indiana*, *Ma. uniformis*, *Ae. aegypti*, *Ae. albopictus*, *Ae. lineatopennis*, *Ae. vittatus*, *Oc. scatophagoides*, *Mi. luzonensis* and *Ar. subalbatus*. Of these species, *C. quinquefasciatus* was the predominant species followed by *Ar. subalbatus* and *Cx. tritaeniorhynchus*. There was an increase in the abundance of mosquito vectors from the month of July and remained upto November 2014 in all the study areas. Mosquitoes were sampled from surrounding areas of pig sties and a total of 22 species, were collected, namely *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. quinquefasciatus*, *Cx. gelidus*, *Cx. fuscocephala*, *Cx. infula*, *Cx. bitaeniorhynchus*, *Cx. epidesmus*, *Cx. whitmorei*, *An. subpictus*, *An. vagus*, *An. philippinensis*, *An. barbirostris*, *An. peditaeniatus*, *An. culicifacies*, *Ma. indiana*, *Ma. annulifera*, *Ma. uniformis*, *Ae. aegypti*, *Ae. albopictus* and *Ar. subalbatus*. Overall, *Cx. quinquefasciatus* was found to be predominant followed by *Cx. tritaeniorhynchus*.

In all types of collections *Cx. vishnui* group was very common with high density whereas *Cx. quinquefasciatus* was found to be predominant throughout the year in all study sites.

JEV detection in mosquitoes

Mosquitoes collected during the reported period from Khorabar, Bhatat and Chargawan blocks of Gorakhpur district were screened for JE virus by antigen capture ELISA. Seventy five pools were found positive by ELISA and further processed for confirmation by RT-PCR / Toxo-IFA. *Cx. quinquefasciatus* was found to be highly (44 out of 75) infected with JEV. Chargawan, Bhathat and Khorabar blocks JEV positive pools were 34, 26, and 15, respectively. The species found positive for JE were *Cx. quinquefasciatus* (44pools), *Cx. tritaeniorhynchus* (9pools), *Cx. vishnui* (3), *Cx. fuscocephala* (2), *Cx. gelidus* (3), *Cx. whitmorei* (1), *Cx. epidesmus* (1), *An. philippinensis* (1), *An. subpictus* (1), *An. subalbatus* (8), *Ae. lineatopennis* (1), *Ma. indiana* (1). The fact that *C. quinquefasciatus* has been repeatedly found infected with the JEV in Gorakhpur during early 2014 is interesting.

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

Long lasting insecticidal nets (LLIN), bed net study is being carried out to evaluate bioefficacy of DRDO Defender Net against the vectors of LF and JE with objectives to assess the efficacy of LLINs on disease vector, bioefficacy relation to number of washes, the persistence of the insecticide on the nets, and its impact on disease prevalence (LF and JE) and social acceptability. Study villages in Tirukoilur such as Thathanur (LLIN), Pardapattu (non-LLIN) and Veeranampattu (no net) were selected. Similarly three villages in Cuddalore district viz., G. Kudikadu village (LLIN), Kollathankurichi (non-LLIN) and Nanthapadi (control) were selected. Both pre-intervention and intervention phases, the entomological evaluation, parasitology and community/social aspects carried out in all study villages.

In Tirukoilur, after distribution of LLIN in the treated village, the density of *Cx. quinquefasciatus* during the intervention phase appeared to decline as compared to pre-intervention phase whereas it showed a high density in the untreated and control villages as compared to treated village. The reduction was 87% (21.21-2.75) in LLIN village, 15% (4.44-

3.77) in non-LLIN village and 7% (6.21-5.79) in control village. During the post-intervention period infectivity and transmission intensity index (TII) were nil in all the study villages. Respondents at different study sites were well aware of the diseases, and the importance of LLIN. The survey conducted during the intervention phase was depicted that the usage of net was 86.7 and 83.3% in the treated village (LLIN) and control village (plain net), respectively.

In Vridhachalam, the density of *Cx. tritaeniorhynchus* during the intervention phase appeared declining in LLIN treated village as compared to pre-intervention phase. The change was from 33.58 to 8.5% in LLIN village, 31.83 to 95.80% in non-LLIN village and 27.55 to 145.89 % in control village.

LYMPHATIC FILARIASIS

Community based strategies to optimize vector control in annual mass drug (MDA) administered rural population to eliminate LF in Villupuram district, Tamil Nadu

A total of six villages were selected as index villages on the basis of >1% microfilaraemia prevalence, in which, about three villages were maintained with vector control and another three villages were without vector control based on the vector breeding habitats, microfilaria (Mf) rate and vector transmission parameters.

During the reported period, entomological monitoring was carried out in all the six study villages in Tirukoilur block. The study villages are Melathazhanur, Padiyendal, Sivanarthangal, Kattupaiyur, Thanaganandal and Kachikuppam. Among the six villages, a total of 594 *Cx. quinquefasciatus* mosquitoes were collected by using indoor resting collection method. During this period, overall per man hour density ranged from 4.29 to 10.43. The highest parity rate was observed in Padiyendal village. No infection and infectivity rate was observed in any of the study villages.

CHIKUNGUNYA

Molecular characterization of Chikungunya viruses (CHIKV) circulating in vectors in Tamil Nadu, special emphasis on the pattern of persistence of CHIKV in nature and the

co-circulation with dengue viruses in vector mosquitoes in Tamil Nadu.

Mosquito samples collected from different breeding habitats such as man-made containers, latex collection cups and pineapple plant axils were found supporting profuse breeding of CHIKV vectors. CHIKV/DENV isolation attempts are presently underway.

DENGUE

Studies on *Aedes aegypti* pupal productivity to correlate transmission of dengue in Coimbatore, Tamil Nadu, India

The past two surveys reported more number of confirmed cases in relation to high pupal production in many high risk areas. Seasonal differences could be observed between the two surveys, however, in both the periods water storage habitats contributed within a ranking of five for pupal productivity, both indoor as well as outdoor due to water storing practices of the community. Strengthening of surveillance based on the pupal productivity of certain specific habitats and information, education and communication (IEC) could be a continuous process.

Development of a recombinant NS1 antigen based diagnostic kit for the early detection of dengue virus infection

Dengue NS1 recombinant bacterial clones were revived and protein expression studies were conducted. Recombinant NS1 antigen expression experiments were optimized and a maximum yield of (152 mg/l) of the protein was achieved. The protein was purified using a metal-chelating chromatographic column (Ni-NTA Fast Start kit, Qiagen) and stored at -20°C after lyophilization for further process. Using this recombinant viral protein, attempts were made for the production of monoclonal antibody in mouse model. A batch of three BALB/c mice of 6-8 weeks age were immunized at a concentration of 50-100 µg/mice. After the final immunization, ELISA was done with the serum collected from the immunized mice and the results were found encouraging against NS1 antigen. Myeloma Sp2/0 cell line was obtained from NCCS, Pune, and the fusion of myeloma and splenocytes was carried out. After seven days, the

plates were screened for the presence of clones and a significant number of clones showed positive reactions. These positive clones were stored in liquid nitrogen for further analysis. Further studies are under progress.

TAXONOMY AND BIODIVERSITY

A survey on the mosquito fauna was carried out in Nilgiris hill ranges, viz., Gudalur and Kotagiri hills of Western Ghats during 2014-2015. A total of 588 mosquito specimens belonging to 26 species, falling under 10 genera and 10 sub-genera were recorded in these two hill ranges. Altitude of the foot hill ranges was between 300 to 1500 metres above mean sea level (MSL). Major emphasis was given to immature collections of mosquitoes and the various breeding habitats viz., slow flowing stream, spring pool, rocky pool, elephant foot print, tree hole, leaf axil and bamboo stump, etc.

Dengue/Chikungunya vector (*Ae. albopictus*) was predominantly recorded in the breeding habitats studied in and around the Tribal area. In pre-DDT era, *An. fluviatilis* was the dominant species for foot hill human malaria in the Western Ghats, but recent surveys have shown that *An. culicifacies* (36.2%) (Rural malaria vector) was more abundant when compared to *An. fluviatilis*. The study was conducted accordingly in different types of tribal populations living in different areas of Nilgiri district. Initially, the study revealed that the entomological aspects of *Ae. albopictus* was dominant in its prevalence in the Kotagiri area (76%).

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

Aedes albopictus was found to be the predominant dengue vector in the Western Ghats region. It could possibly and quickly displace other local mosquitoes since it seeks both animal and human blood posing a risk of easy transfer of pathogens residing in animal hosts to people through bite. The study area was selected based on different types of tribal populations living in Nilgiri district. Baseline details on the entomological aspects revealed that *Ae. albopictus* were dominant in prevalence in the Kotagiri area. While in the pre-DDT era, *An. fluviatilis* was the more dominant species for foot hill human malaria in Western Ghats, recent surveys

indicated that *An. culicifacies* (rural malaria vector) was more abundant than *An. fluviatilis*.

In continuation with our study on mosquito biodiversity in Western Ghats hill ranges an attempt was made to carry out vector mosquito survey in foot hills of different hill ranges of Western Ghats area in Tamil Nadu during the year 2014-2015. Mosquito fauna survey was carried out in four blocks of Nilgiris district (Ooty, Coonoor, Kotagiri & Gudalur) of Western Ghats. Three visits were made during July 14, October 14 and February 2015. A total of 1905 mosquito specimens belonging to 30 species, 21 genera and 19 sub-genera were recorded in these hill ranges during the reported period.

NATIONAL AIDS RESEARCH INSTITUTE, PUNE

RESEARCH ON SEXUALLY TRANSMITTED INFECTIONS (STIS) & HIV INFECTION

Sexually Transmitted Infections

Role of herpes simplex virus (HSV)-2 in HIV-1/HSV-2 co-infection

Epidemiological data suggest a strong correlation between recent HSV-2 infection and higher HIV-1 viral load. In order to investigate this, we studied HIV-1/HSV-2 co-infection in three T-cell lines (CEMccr5, PM-1 and MOLT4/R5) and found that CEMccr5 cell line was best suited for this. Co-infection experiments were also performed in CEMccr5 cells using UV-inactivated HSV-2 virus to investigate whether productive HSV-2 infection is necessary for an increase in HIV-1 replication. Results indicated a 2.47 fold increase ($0.4 \log_{10}$ RNA copies/ml) in the HIV-1 viral load in co-infected CD4 T-cells as compared to those infected with HIV-1 alone. In similar co-infection experiments with HSV-2 virus, there was a significant reduction in HIV-1 viral load [$0.5 \log_{10}$ RNA copies /ml, ($p < 0.05$)], suggesting that productive infection of HSV-2 influences HIV-1 viral load.

Relation between genetic markers of drug resistance and susceptibility profile of *Neisseria gonorrhoeae* isolates

A study has been initiated to identify and analyze mutations in the *tet (M)*, *penA*, *gyrA*, *mtrR*, *porB1*,

parC and *por* genes of *N. gonorrhoeae* isolates and to compare these with their antibiotic resistance profile by bacterial culture (Fig. 1). Thirty three *N. gonorrhoeae* clinical isolates from patients attending sexually transmitted disease (STD) clinics with symptoms of urethral/cervical discharge from different parts of India (25-Delhi, 3-Pune, 5-Hyderabad) were collected in the reporting year. All the isolates showed high level resistance to ciprofloxacin and nalidixic acid; and sensitivity to spectinomycin and azithromycin. However, four (12.1%) isolates showed intermediate susceptibility to ceftriaxone and cefixime. The novel mutations observed from the study done so far are: *penA*-438D, D442N, D442K, N407I, A455E, *ponA*-L425F, *gyrA*-L57A, Y58D, L57P, Y58C, *parC*-M59I, M59N, *mtr*-T31N, L33V, D79N and T86A.

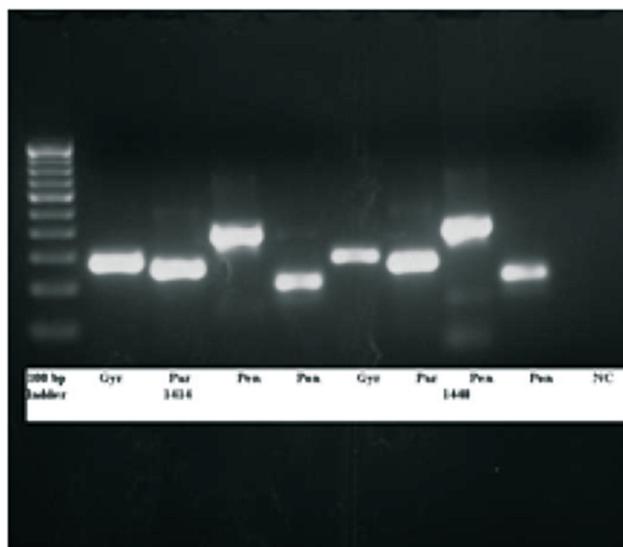


Fig. 1. Agarose gel electrophoresis of PCR products for *gyrA*, *parC*, *penA* and *ponA* genes at 278bp, 255bp, 501bp, 236bp

Improving cervical cancer prevention among HIV-infected women using novel human papilloma virus (HPV) based biomarker assays: An 'Intramural-to-India' study

Given the high prevalence of carcinogenic HPV DNA and higher risk for cervical precancer and cancer among HIV-infected women, there is a need for screening tests that are both sensitive as well as specific. A study evaluated the clinical performance of two novel biomarker assays (i) immunocytostaining by p16^{INK4a}/Ki-67, and (ii) HPV E6/E7 mRNA, for detection of histologically confirmed cervical intraepithelial neoplasia (CIN)

grade 2 and 3 or more severe among HIV-infected women. In all, 1000 participants were enrolled from 3 sites and HPV testing was completed. At the NARI site, 'any HPV genotype' positivity was detected in 162/402 (40.3%) HIV infected women. Carcinogenic 'high-risk' (HR) HPV were present in 117/402 women (29.1%), of whom multiple (*i.e.*, two or more) HR-HPV genotypes were seen in 24/117 (20.5%) cases. HPV genotypes detected in high-grade cervical disease (*i.e.*, CIN 2+) on colposcopy in decreasing order of frequency were HPV16, HPV56, HPV33, HPV35, HPV39, HPV52, HPV58, HPV59 and HPV31. Overall, a trend of increasing HPV positivity (any as well as HR-HPV and HPV16) with increasing disease severity on colposcopy, cytology and histopathology was observed. A high prevalence of high-risk HPV types was found among HIV-infected women. Currently available HPV vaccines do not cover all the HPV types observed in the study participants.

HIV CURE RESEARCH

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

Immune responses play an important role in elimination of latently infected CD4 cells after HIV reactivation. However, upon reactivation, infected cells were shown to secrete interleukin (IL)-10, an immunosuppressive cytokine, which might interfere in elimination of infected cells by inhibiting cytotoxic T lymphocytes (CTLs). The study was conducted to understand pattern of immune response as well as mechanisms responsible for IL-10 secretion, which would have ultimate implications in HIV cure research.

Peripheral blood mononuclear cells (PBMCs) were treated with inhibitors like PD98059 (ERK); tipifarnib (T-bet inhibitor); cyclosporin A (FoxP3 inhibitor) reported to be involved in IL-10 secretion, to determine downstream effect on IL-10 secretion after overnight stimulation by HIV-1 Env. Tipifarnib was found to cause maximum suppression of IL-10 secretion. Although ERK phosphorylation has been reported to be involved in IL-10 secretion by monocytic cells, its blockage resulted in only 1.4 fold reduction in IL-10 secretion. Bioplex assay detected all 9 cytokines; only TNF- α ; IL-10 plus

GM-CSF; and none of the cytokines after treatment with anti-CD3/CD28, bryostatin-1, HIV-1 Env and valproic acid, respectively. Thus the study showed important findings like differential cytokine pattern of anti-latency agents as well as involvement of T- bet pathway in IL-10 secretion by CD4+T cells, which might have therapeutic implications in the HIV infection.

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

The role of miRNAs in the pathogenesis of HIV/AIDS is poorly understood. Published literature suggests that miRNA signatures can be used as biomarkers of HIV disease progression. Downregulation of miRNA29a has been observed among those who have high viral load. We studied miRNA29a levels among HIV-infected individuals in different stages of HIV disease and healthy controls. The findings revealed that miRNA29a levels vary by HIV disease stage indicating that it may be playing a role in HIV latency and reactivation. Study of miRNA146b and 150 is currently ongoing.

RESEARCH ON DRUG RESISTANCE

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

Resistance to azole antifungals is a significant problem in treatment of a common fungal pathogen, *Candida albicans*. An understanding of resistance at molecular level is essential for development of strategies to overcome resistance and for rational design of newer antifungals. We investigated the molecular mechanisms associated with fluconazole resistance in a total of 87 clinical *C. albicans* [fluconazole- susceptible (n=30), -susceptible-dose dependent (n=30) and -resistant (n=27)] isolates. DNA sequencing was used to determine drug target-site alterations (*ERG11*), while real-time PCRs were performed to quantify target and efflux pump genes (*CDR1*, *CDR2* and *MDR1*).

Cross-resistance to fluconazole, ketoconazole and itraconazole was observed in 74.1% isolates. Six amino acid substitutions were identified in *ERG11* gene, including four which have been previously reported (E116D, F145L, E226D, I437V) and two

new ones (P406L, Q474H). An increasing trend of homozygous substitutions was seen from susceptible to resistant isolates. *CDR1* overexpression was seen in 35 (61.4%) isolates including, 14 (46.7%) susceptible-dose dependent and 21 (77.7%) resistant isolates. The *CDR2* gene was exclusively expressed with *CDR1* and their concomitant over-expression was associated with azole cross-resistance. *MDR1* and *ERG11* overexpression did not seem to be associated with resistance.

Our findings highlight the role of drug efflux mediated by ABC transporters in azole resistance and cross-resistance in *C. albicans*. Thus, a combination therapy of azole and an efflux pump inhibitor will be useful in management of severe *C. albicans* infections. There is a need to prioritise research for identifying new efflux inhibitors.

Molecular probing of the *Mycobacterium tuberculosis* isolates, their resistance pattern, and conservation pattern of new drug targets

This project was aimed to understand whether gene targets being considered for development of new anti-tubercular drugs are conserved enough to take the drug development further. Clinical isolates of *M. tuberculosis* with differing resistance profiles from different parts of the country were obtained. Gene sequencing was adapted to assess if the isolates had mutations in genes which are targets of the currently used drugs, as well as to see if the mutations translated into a phenotypic drug resistance. Additionally, four new drug targets were screened for conservation using these well characterized sensitive and resistant isolates.

In all, 116 isolates were characterized and sequenced for proposed drug targets under development, viz., *icl*, *pknB*, *pcaA* and *devS* genes. The regions in these genes if modified could lead to drug resistance, were identified. However, no isolate showed any mutation in these regions or in any other regions of the gene sequenced indicating that these targets remain conserved in *M. tuberculosis* isolates irrespective of their sensitivity to the currently used drugs. Although a large number of mutations are seen in gene targets under drug pressure, genes which are predicted to be the targets for new drug discovery reveal a well conserved pattern.

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

This study was undertaken to examine the role of Gag mutations associated with protease inhibitor(s) resistance in Indian HIV-1 subtype C; to describe the Gag mutations and assess their effect on sensitivity to PIs. The analysis of Gag mutations among the patients failing on protease inhibitor (PI) based ART treatment (n=21) and ART naïve (n=75) HIV-1 subtype C infected individuals were done to find natural variations and association of Gag mutations with the patients failing on PI-based ART treatment. It was found that about one in four patients failing on ART does not show any known PI mutation. A positive association between the PI based ART regimen failure patients and Gag cleavage site substitutions at the p1/p6gag ($\chi^2= 11.20$, $P = 0.001$), NC/p1 ($\chi^2= 11.62$, $P = 0.001$), p17/p24 ($\chi^2= 9.77$, $P = 0.002$) and p6pol/PR ($\chi^2= 3.90$, $P = 0.048$) were observed. A high rate of natural variation at cleavage sites p2/NC, TFP/p6pol and p6pol/PR was observed. The study also highlights the presence of PTAP duplications (TSG101- binding motif in the Gag-p6) of isolates carrying PI resistance mutations. Accumulation of PTAP duplications during therapy may indicate a potential role in antiretroviral drug resistance. The present study also found a novel tetra-peptide insertion in Gag-p6 ALIX-binding motif in the HIV-1 subtype C associated with protease inhibitor failure in Indian patients. The virus with this insertion might increase replication fitness. The clinical importance of the insertion needs to be evaluated in HIV-1 subtype C.

IMPLEMENTATION SCIENCE RESEARCH

Effectiveness of antiretroviral therapy (ART)

A cross-sectional study to assess effectiveness of ART was conducted among 846 eligible consecutive patients who were initiated on ART between 10-14 months in four free ART centres in Pune. The overall frequency of virologic failure at one year was 12.3% (95% CI 10.2-14.7). The ART centre of NARI had the lowest treatment failure of 4.4% among four ART centres. The risk of virological failure was found to be 2.19 (95% CI 1.40-3.44) and 4.39 (95% CI 2.61-7.39) folds among those

who did not live with their partner and those who were not adherent to ART, respectively. Of the 80 patients who failed 17 (21%) did not reveal any drug resistant mutation against nucleoside reverse-transcriptase inhibitors (NRTI) and non-nucleoside reverse-transcriptase inhibitors (NNRTI) indicating that these patients may have been missing recent treatment doses. Of the 80 tested with genotypic drug resistance assays, 18 patients revealed K65R mutation. The high proportion of K65R mutation limits therapeutic options in providing second-line ART and narrows it to zidovudine as a backbone. These findings also indicate that virologic monitoring should be considered in monitoring patients on first-line for early detection of treatment failure without impacting second-line antiretroviral therapeutic options. Mutation pattern indicates that newer antiretrovirals such as rilpivirine and etravirine will not be effective among patients failing first-line antiretroviral therapy. Intensified efforts for counselling among those who do not live with their partners need to be undertaken in the programme.

Loss to follow up

Retrospective data were analysed from 689 patients who were registered as pre-ART patients as their CD4 counts were higher than the cut-off for initiation of ART at NARI ART centre. These patients are expected to undergo CD4 count every 6 months to enable timely initiation of ART. The loss to follow up (LFU) was defined as missing two visits for estimation of CD4 counts. The LFU rate in March 2013 was found to be 11%. Of those who were LFU, 71% were women. Of women who were LFU, 21% were female sex workers. The high rates of LFU among sex workers who belong to the core transmitter group warrants devising appropriate strategies to ensure that they remain linked with the care programme in the ART era where treatment as prevention is assuming the importance in programming.

A pilot study of Early Warning Indicators (EWIs) for HIV drug resistance (HIVDR) monitoring and Quality Care Indicators (QCI) in four programme clinics Pune

In order to strengthen monitoring of the quality of ART services, National AIDS Control Organisation

(NACO) has recommended regular monitoring of quality care indicators in HIV care in addition to EWIs that are deemed most important for monitoring implementation of the ART programme. This study assessed feasibility and usefulness of WHO HIVDR EWI monitoring and QCI in HIV care at four ART programme clinics in Pune city located as an ART programme evaluation tool. All four ART clinics met the WHO EWI target (100%) for ART prescribing practices. The target for 'on time pill pick up' and 'pharmacy stock outs' could be achieved in one clinic and none of the clinics could meet the target for 'retention in care at 12 months'. The overall performance on QCIs fell below the target in three clinics. Efforts are required in improving retention in care, timely pill pick up and ensuring clinic-level drug supply continuity.

Research-cum-intervention (RCI): Community engagement for Japanese encephalitis (JE) and acute encephalitis syndrome (AES) prevention and control

This project is an example of translational research where participatory approaches used for engaging community for biomedical research was replicated at Gorakhpur district in Uttar Pradesh to engage community for JE/AES prevention and control. Experimental design was used wherein the intervention and control arms were selected blocks of Gorakhpur and Deoria districts respectively.

A cluster randomized baseline survey showed non-acceptability of India Mark II hand pumps that were installed by the government for safe drinking water. The major source of drinking water was reported to be shallow hand pumps at homes both in Deoria (88%) and Gorakhpur (85%). Other behavioural aspects pertaining to open defecation, non-use of toilets, and use of shallow hand pumps are important challenges to overcome for prevention and control of JE/AES.

NEW DRUG DISCOVERY

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

Earlier studies identified *Terminalia paniculata* (fruits) and *Polygonum glabrum* (aerial parts) plant extracts and their fractions as potential leads. Two

extracts (acetone and methanol) of each plant part were prepared [NCL 51 (TPA), NCL 52 (TPM), NCL 53 (PGA) and NCL 54 (PGM)], respectively. Based on the bioactivity and natural product library guided fractionation, extracts were further fractionated into fractions [(NCL 51 A to L), (NCL 52 A to M) and (NCL 54 A to K)]. Of the fractions NCL 51(J and K), NCL 52 (K and L) showed promising anti-HIV activity. Anti-HIV activity of 11 compounds of NCL 54 was determined which did not show activity. Additionally, pure compounds of methanolic extract *P. glabrum* (NCL 54), elucidating a new natural product were also tested for anti-mycobacterial, anti-malarial and anti-proliferative activities.

Identification of potential anti-HIV natural product analogs using molecular docking and medicinal chemistry approaches

Analogues of γ -Fagarine, IPSM-179 and IPSM 224 were identified as lead molecules in the previous year. In continuation with this, molecular docking studies of these two leads were carried out which indicated that styrylquinoline nucleus may act through inhibition of integrase activity. Recently, a total of 21 analogues (17 analogues of IPSM-179 & IPSM224 and 4 analogues of tembamide) were tested for inhibition of HIV-1 replication using TZM-bl reporter gene (luciferase) assay. The lead compound NP3551 was further confirmed in primary cells (stimulated PBMCs). The mechanism of action of three leads (IPSM179, IPSM224 and NP3551) was determined using cell based assays for entry (TZM-bl) and fusion inhibition (TZM-bl and HL2/3 cell line). Further confirmation is in progress.

CONTRIBUTION TO THE NATIONAL PROGRAMME

National AIDS control organisation supported – early infant diagnosis (EID) programme

The early infant diagnosis programme has been implemented across India since April 2010 by NACO with the aim of screening HIV exposed infants/children aged between 6 weeks to 18 months for HIV-1 positivity. Screening is done by HIV-1 DNA PCR test on dried blood spot (DBS) samples at 6 weeks, and if HIV antibody is positive

at 6 and 18 months then the HIV status is confirmed by HIV-1 DNA PCR test on whole blood (WB) samples.

In the current reporting year (April 2014-Mar 2015), 827 children were tested for HIV-1 DNA PCR. During the five year period between April 2010 and March 2015, a total of 9006 children were tested at NARI for HIV-1 DNA PCR from Maharashtra excluding Mumbai. The five year average of HIV-1 positive children by DBS testing was found to be 13.4%, while year-wise trend analysis of samples of HIV-1 positive children tested from 2010 to 2015 revealed that there was a decreasing trend of HIV infection from 18.7 to 8.4%.

HIV sentinel surveillance (HSS) among antenatal clinic attendees: Western India region

The 14th round of ANC HIV sentinel surveillance was completed at 191 sentinel sites between 1st January 2015 and 31st March 2015. Three new ANC sentinel sites one each in Palghar, Maharashtra; SDH Sagwara, Rajasthan and Jhabua, Madhya Pradesh and one sub-site in Rajasthan at Dungarpur district, were added this year.

On review of data from consistent sites, there does not seem to be a significant difference in HIV prevalence in these States and UT over the years 2010, 2013 and 2015 although there is some decline in prevalence. However, when we tested for linearity in trends using chi-square test, Mumbai (P=0.026) and Goa (P=0.0003) showed a significant trend for the consecutive years; 2010, 2013 and 2015. (Fig. 2).

Integrated Biological and Behavioural Surveillance (IBBA): Secondary data analysis

Analysis of IBBA data revealed that of the 9667 female sex workers living in 23 districts of four high HIV prevalence States, 15.6% reported anal sex in heterosexual relationships. This finding is important from public health perspective as clinicians treating them should be made aware of anal STIs and their management. It also underscores the importance of prioritizing research on rectal microbicides in addition to vaginal microbicides.

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

The IBBS is being implemented by NACO to generate evidence on HIV prevalence and risk behaviours among female sex workers (FSW), intravenous drug users (IDU), men having sex with men (MSM) and transgenders (TG) and migrants to support planning and prioritization of programme efforts at district, State and national levels. Sampling Frame Development (SFD) for the main community based survey was conducted through NARI in 54 survey domains - 16 FSW, 16 MSM, four each from TG and IDU and 14 for migrants. The SFD field work began in the High Risk Groups (HRG) domains during November and December 2014 and was completed in February 2015. The primary objective of SFD was to list all HRG/ MIG hotspots/ cruising sites and estimate size of HRGs/ MIGs at each of the sites. Hotspots as yet unlisted with State AIDS Control Societies were identified in all States as part of the SFD (Fig. 3).

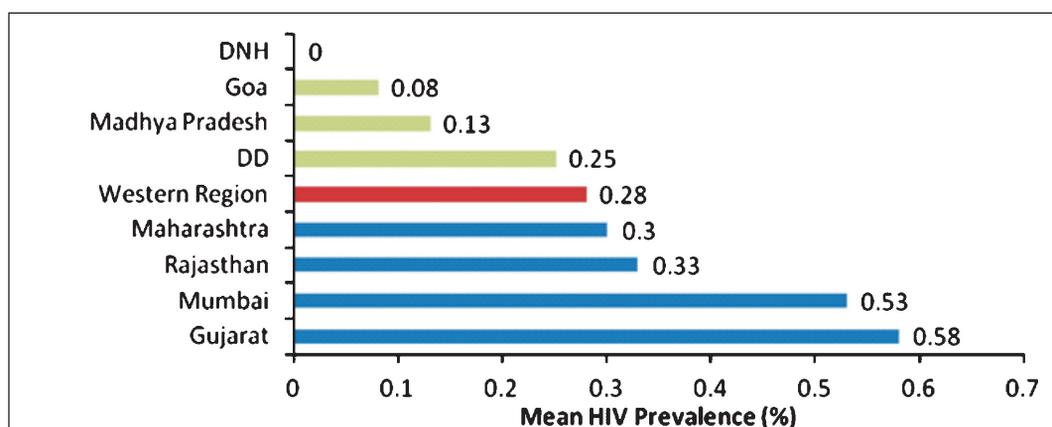


Fig. 2. Mean HIV Prevalence.

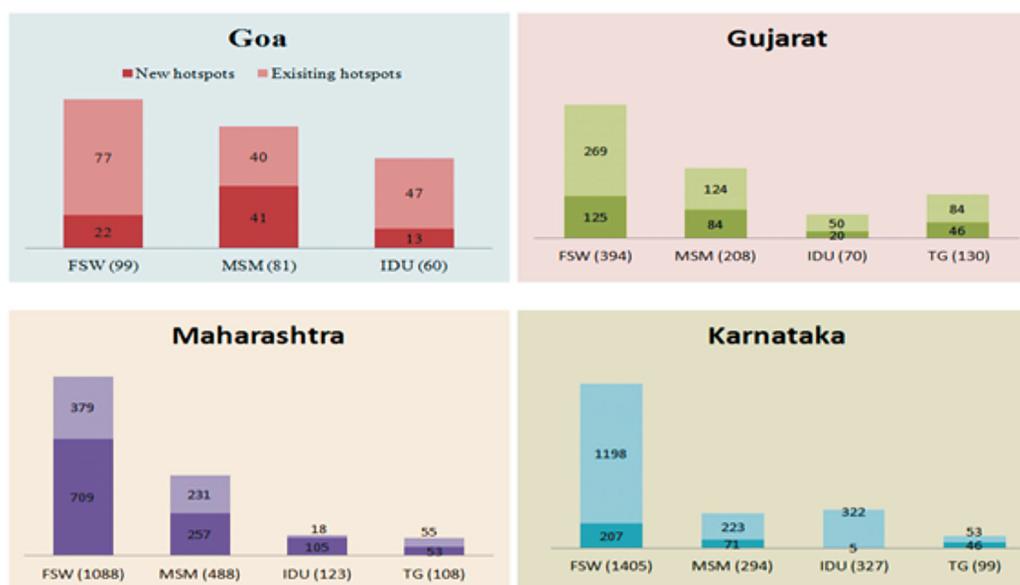


Fig. 3. State-wise distribution of HRG hotspots.

ENTEROVIRUS RESEARCH CENTRE, MUMBAI

Poliomyelitis Surveillance

Enterovirus Research Centre is one of the Global Specialized Laboratories (GSL) of Polio laboratory network in World Health Organization. The Centre is involved in acute flaccid paralysis (AFP) surveillance by virological investigation of faecal specimens from AFP cases reported in Maharashtra, Madhya Pradesh, Chhattisgarh and Goa. Polioviruses isolated from AFP cases showing discordant results in molecular tests are also received for genomic sequencing from other states in India, Bangladesh, Myanmar and Sri Lanka to identify wild, Sabin-like or vaccine-derived polioviruses (VDPV). In 2014, a total of 54780 cases of AFP were reported through AFP surveillance from India, of which 5647 AFP cases were reported from Maharashtra, Madhya Pradesh, Chhattisgarh and Goa and tested for the presence of polioviruses and other enteroviruses. Polioviruses were isolated from 189 (3.34%) AFP cases and non-polio enteroviruses (NPEV) were isolated from 1573 (27.8%) cases. No wild poliovirus or VDPV were isolated from any cases. India continues to be polio-free for the 4th consecutive year.

Apart from the faecal specimens, a total of 4303 polioviruses isolated in the national polio laboratories in the network within and outside India, were also

referred to ERC for intratypic differentiation, genomic sequencing and maintaining virus bank. The Centre sequenced 116 discordant poliovirus isolates from all the network laboratories in 2014. Type 2 VDPVs were identified from three cases; one each from Gujarat, Rajasthan and Delhi, whereas, remaining poliovirus isolates were confirmed as Sabin-like. There was no evidence of circulation of VDPVs in the population.

Environmental surveillance for detection of wild poliovirus

Environmental surveillance was established at ERC, Mumbai in 2001 to provide supplementary information on wild and VDPV circulation in the absence of AFP cases. The centre has been actively involved in monitoring the presence of wild poliovirus in the three high risk blocks in Mumbai. Data collected in the past 13 years have been beneficial in indicating circulation of wild poliovirus /VDPV in the environment. Environmental surveillance was expanded in other parts of India, *i.e.*, Delhi (2010), Patna (2011), Kolkata (2011) and Punjab/ Ahmedabad (2013). In 2014, a total of 156 sewage samples were collected from Mumbai and 77 samples from Patna. Twelve type 2 VDPVs were detected from sewage samples in India out of which four each were isolated from Mumbai and Delhi, two from Patna and one each from Punjab and Kolkata. Along with AFP surveillance, the supplementary surveillance also confirmed the

absence of wild poliovirus transmission in India during 2014.

Comparative evaluation of immunogenicity of bivalent oral polio vaccine (bOPV) with one dose of inactivated polio vaccine (IPV) in standard Expanded Programme on Immunization (EPI) schedule

Polio eradication and Endgame Strategic Plan 2013-2018 includes a global switch from trivalent OPV to bivalent OPV and one dose of IPV for routine immunization against polio. Withdrawal of type 2 OPV is scheduled by April 2016. ERC participated in the clinical trial conducted by the WHO in India for comparative evaluation of immunogenicity of bOPV plus one dose IPV given to infants as per the current EPI schedule. A total of 1960 stool samples were collected and tested for poliovirus excretion; 321 polioviruses were isolated from the stool specimens. Also 3214 serum samples were collected for testing poliovirus neutralizing antibody titres in infants. The study results indicated high sero-conversion rates using the new vaccination strategy.

Studies on poliovirus infections in children with immunodeficiency

Persons/patients with immunodeficiency may harbour poliovirus infection (after natural infection or OPV immunization) for several months or even longer. These patients may suffer from paralytic poliomyelitis because of reversion of Sabin OPV virus attenuation to neurovirulence and will also continue to excrete the virus (immunodeficiency associated vaccine derived polio viruses, iVDPVs). Therefore, persons/patients with immunodeficiency are not only a risk for themselves, but may also become a risk for the community. A study in collaboration with NIIH, Mumbai and Wadia Hospital, Mumbai, is being carried out with the objectives to identify long term poliovirus/enterovirus excretors among children with immunodeficiency. A pilot study was conducted to test the feasibility and operational aspects of the project. The diagnosed PID (Primary Immune-Deficiency) children routinely visiting the PID OPD at Wadia hospital for check-up and intravenous immuno-globulin (IVIG) treatment were enrolled and approached for stool sample collection. A total

of 153 patients visited Wadia hospital from whom 21 (13%) were diagnosed as cases of PID. From 23 stool samples of 13 immunodeficient children tested for polio and other enteroviruses, one child with severe combined immuno-deficiency (SCID) excreted P3 (type 3) VDPV and another child with HLH (haemophagocytic lymphohistiocytosis) excreted P1SL (Type 1 vaccine poliovirus). Monthly stool samples (up to 3 months) collected from the child with SCID were found to be positive for P3 VDPV with 41, 46, 49 nucleotide changes respectively. Screening of PID patients is necessary for identification of poliovirus/enterovirus infection and development of VDPVs in long term excretors in post-polio eradication era. This study will ultimately provide information regarding risk of poliovirus infection in children with immunodeficiency and contribute information for developing polio immunization strategies.

Enterovirus infections in apparently healthy children from AES affected area in Gorakhpur district, Uttar Pradesh

Enteroviruses (EV) are known to cause acute flaccid paralysis (AFP), aseptic meningitis (AM), encephalitis, cardiovascular disease, hand-foot and mouth disease (HFMD), acute haemorrhagic conjunctivitis (AHC) diarrhoea and gastroenteritis. EV surveillance was carried out in healthy children in five selected areas affected by JE/AES in Gorakhpur, UP (Fig. 1) (September 2013 - October 2014) to determine (i) the prevalence of EV infections in healthy children, (ii) circulating EV types and (iii) seasonal distribution of EV types. Stool samples from 6274 healthy children below five years were screened for the presence of EV using virus culture and molecular method of RT-PCR and sequencing. EV isolation was observed among 49.1% (3079/6274) children. A very high prevalence of EV infection (98.8%) was observed throughout the 12 months period with minimum in March 2014 (31.5%) and maximum in July 2014 (71.2%). EV mixed infections (more than one EV) were also observed from 3.7% (114/3042) children. A total of 89 different EV types (out of 108 known types) were isolated. Most prevalent EV types included E7, CVB4, CVB5, CVA20, E3, E33, E30, EV99, EV90, CVA17. Additionally, other EV types (EVA71, E30, E11, E13, CVB5, CVB3, E33), were

also isolated. The study reveals an urgent need for improving environmental sanitation and personal hygienic practices in the region experiencing annual outbreaks of AES with high mortality.



Fig. 1. Map of Gorakhpur district.

Isolation and identification of a new enterovirus serotype EVA121

Enteroviruses pose serious threats to children with a wide range of clinical conditions ranging from mild symptoms to serious conditions. EV serotypes are identified by VP1 gene sequencing of EV. EV serotypes are grouped into four species: HEV-A, -B, -C and -D. EV strains are classified as the same serotype if they share more than 75% nucleotide identity and 85% amino acid identity relative to known EVs and as a different serotype, with less than 75% sequence similarity.

During EV surveillance among healthy children below five years from JE/ AES affected areas in Gorakhpur, UP from September 2013 to October 2014, a total of 6274 stool samples were tested for EV infection. The EV serotypes were identified by VP1 sequencing of EV genome. A new EV was isolated and identified from a 30 month old female child in October 2013 which was designated as EVA121. The new enterovirus EVA121 was accepted by International Committee on Taxonomy of Viruses (ICTV). This is the first report of identification of a new serotype of human enterovirus from India.

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

Enterovirus A71 (EVA71) and Coxsackievirus A16 (CVA16) are the major aetiological agents of HFMD. In India, CVA16 and CVA6 are the

major pathogens of HFMD. Despite genetic similarity, EVA71 leads to severe fatal neurological complications unlike CVA16 and CVA6. Mostly these viruses cause asymptomatic infections with blisters on hand, foot and mouth, and do not cause AFP. We studied the host pathogen interaction of CVA16 and CVA6 isolated at ERC and compared that with EVA71 prototype strain (EVA71 BrCr). Apoptosis caused by HFMD viruses were studied by TUNEL assay, induction of caspases and expression of cytochrome c in virus infected cells by flow cytometry. Expression of IL-6, IL-8, TNF- α , caspases and TLR (toll-like receptor) genes in infected cells were studied by real-time PCR (Fig. 2). HFMD virus infected human neuronal cells showed differential induction of apoptosis to the three different viruses. Percentage of apoptotic cells were found to be comparatively higher in CVA16 infected neuronal cells as compared to CVA6 and EVA71 infection at 24 hpi. CVA16 also induced excess TNF- α as compared to other HFMD viruses in neuronal cells. Further studies are continuing to explore the mechanism of cell tropism and apoptosis in these viruses.

Enterovirus induced changes in cellular gene expression by microarray analysis

Enterovirus 71 (R-13223 IND2001) was isolated at ERC from a case of AFP (Guillain-Barré syndrome) from the State of Haryana reported through the AFP surveillance. The objective of the study was to utilise microarray technique to find out the kinetics and patterns of host gene expression in EV71 R-13223 infected human neuronal cells (SK-N-SH) and to compare with that of un-infected cells. Microarray was performed using Agilent human WG 8 X 60 K array with 60,000 probes at Genotypic Technology, Bengaluru and data were analysed by Gene spring GX version 11 and MS Excel. EV71 infection commonly upregulated 562 genes and downregulated 238 genes at 4 and 8h post infections. The genes upregulated included those required for RIG-I like receptor signaling pathway (10 genes), TLR signaling pathway (11 genes), B-cell receptor signaling pathway (2 genes), antigen-processing and presentation-pathway (9 genes), viral myocarditis (4 genes), apoptosis pathway (4 genes). Most importantly *IFITM*, *IRF7*, *STAT-1*, *NF kb-A*, *HLA-B*, *MDA-5*, *TLR3*, *IL6*, *IL1b*, *TNF*

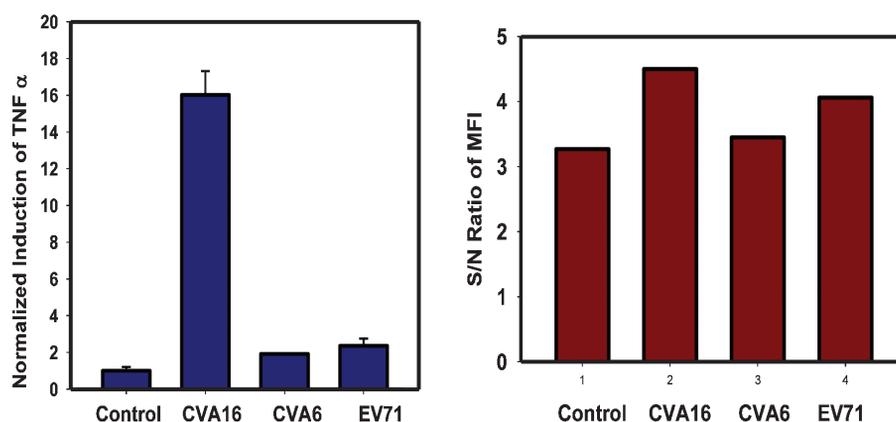


Fig. 2. (A) Induction of TNF- α gene (B) Signal-to-noise ratio of Median Fluorescence Intensity of cytochrome c expression (FITC) in HFMD virus infected human neuronal cell lines (SK-N-SH) 24h post infection.

and *RIG-I* genes were significantly upregulated. The above results were validated by real-time PCR. The study confirmed the involvement of RIG-I like pathway as reported earlier for other EV71 strains. The microarray data are being analysed to explore key proteins/ peptides for further characterization and vaccination studies.

RAJENDRA MEMORIAL RESEARCH INSTITUTE OF MEDICAL SCIENCES, PATNA

EPIDEMIOLOGY

Sentinel surveillance of 2773 kala-azar cases, enrolled at the sentinel sites established in endemic areas of Bihar, revealed rapid diagnostic test (RDT) as the main diagnostic tool (98.8%). Majority of the cases were treated with miltefosine (43.1%), followed by SAG, Amphotericin B and combination therapy. Overall, the final cure rate was about 86% and relapse rate was 8.5%. Pharmacovigilance of miltefosine treated cases did not reveal any major side effect, but 6% of treated case relapsed and one case developed PKDL during 6-months follow up. Further, one year follow up of 1872 kala-azar cases, treated with various drugs at the Institute during 2007-2012, revealed two cases (0.1%) developed PKDL and both had received ambisome for kala-azar treatment. The impact of 2-round training to ASHAs has shown increased referral by 43.48% and thereby its usefulness in active case detection is being assessed.

Diagnostics

A non-invasive method for diagnosis of kala-azar by rK39 test in oral fluid samples was developed with a sensitivity of 99% and specificity of 100%. Third-party re-validation in more samples is underway. Another non-invasive PCR-based diagnostic test for kala-azar using urine samples was found to be 90% sensitive and 95% specific. Molecular detection of *Leishmania* DNA in desquamated buccal swab samples of HIV-co-infected cases (86.3% sensitivity and 98.3% specificity) showed potential for development of a non-invasive diagnostic tool for VL. The immunogenic nature of trypanredoxin and trypanredoxin peroxidase of *L. donovani* observed in VL, asymptomatic and VL co-infected patients, suggests that these proteins may further be explored as diagnostic tool.

Evaluation of blood antigen detection assay by quantitating unique sialoglycoprotein induced on erythrocytes for early diagnosis revealed that antigen-based RBC ELISA had potential to diagnose even asymptomatic infection, whereas antibody-based BSM ELISA was capable for diagnosing VL and PKDL but not asymptomatic infection. The study is in progress to evaluate sensitivity and specificity.

Therapeutics

Efficacy and safety evaluation of single dose ambisome (10 mg/kg body weight) in paediatric VL cases (n=36) revealed initial cure rate of 100%,

final cure rate of 97.2% after 6 months follow up (n=25); one relapsed after initial cure. Apart from rigor, cough, vomiting, elevated SGOT and SGPT (recovered), there was no major adverse event.

In PKDL treatment, three course of amphotericin B in two different doses *i.e.*, 0.5 mg/kg daily and 1 mg/kg on alternate day for 15 injections revealed equal efficacy (final cure 92%) with minimum toxicity; comparatively earlier one was less toxic in terms of laboratory parameters. Further, three course of paromomycin and miltefosine combination showed parasite clearance and disappearance of nodular and papulo-nodular lesions.

Basic Research

The *in vivo* functional characterization of two proteins, argininosuccinate synthase (ASS) and peroxiredoxin (Prx) from *L. donovani*, revealed that both proteins having potential to maintain redox homeostasis in *L. donovani* parasite under exposure to different stress environments (Figs 1, 2).

L-arginine was found to be an essential amino acid for the growth and survival of *L. donovani*. A reconfiguration of metabolic flux from glycolysis towards PPP is crucial for survival of *L. donovani* under oxidative stress and nullifies the deleterious effect of anti-leishmanial drugs. LdUMSBP

regulates amastigotes survival by controlling ETC activity and oxidative phosphorylation.

Fe-S protein activity was found to be upregulated in drug resistant *L. donovani*. About 5-6 fold upregulated Isd11 in sensitive strain, opposite to frataxin, revealed its role in repair and damaged Fe-S cluster in sensitive strain under oxidative stress or ROS generation; suggestive for prospective role of frataxin and Isd11 protein in the biogenesis of Fe-S cluster within mitochondria of *L. donovani*.

Involvement of TryS gene (*LdTryS*), ascorbate peroxidase gene (*LdAPx*) and Silent Information Regulator-2 (*Sir2*) of *L. donovani* was shown in Amp B resistance mechanism.

A DNA vaccine candidate inducing T cell-based immunity in leishmaniasis was developed and its protective nature was proved in a rodent model of infection.

Bioinformatics

Leishmanial Metabolome Database (LMBD) study exhibited that the metabolites' peaks, present in *L. donovani*, differed from miltefosine and Amphotericin B treated *L. donovani* culture. The fragmentation pattern of the protonated (H⁺) of miltefosine was depicted in the ion at m/z 184 in the spectrum of miltefosine, corresponding with

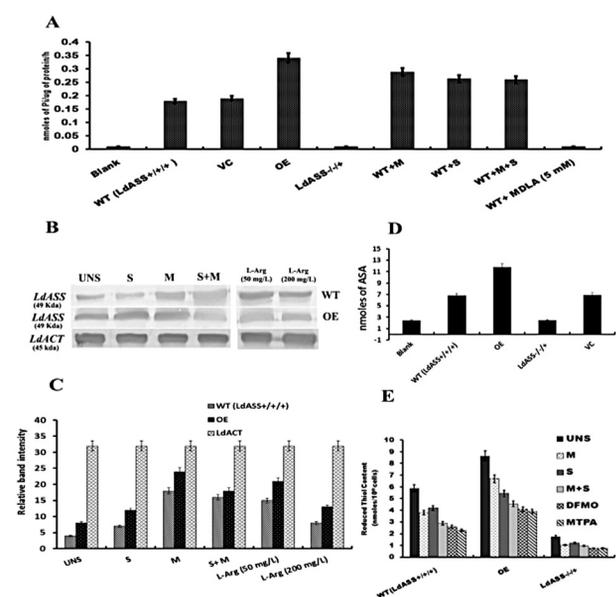


Fig. 1. Western blot analysis and biochemical characterization of mutant *L. donovani* cell line for ASS protein.

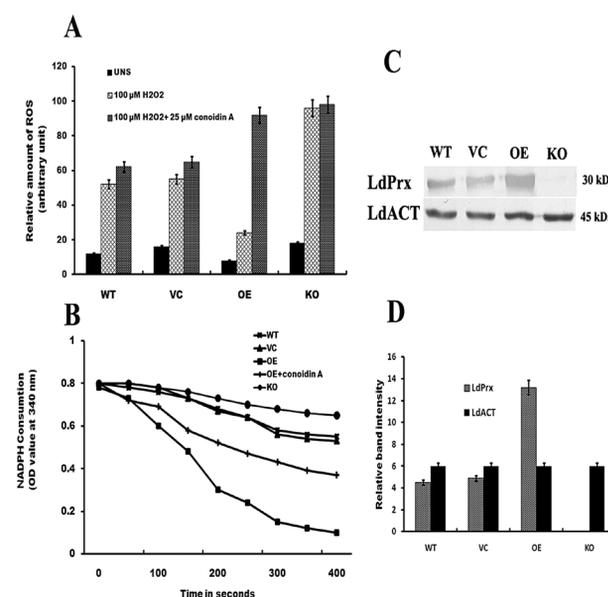


Fig. 2. Western blot analysis and biochemical characterization of mutant *L. donovani* cell line for Prx protein.

the loss of the C16-alkyl chain. Further cleavage of the trimethyl amine from this precursor fragmentation resulted in the product ion at m/z 124.8.

LeishMicrosatDB, a complete curated web-oriented relational database of perfect compounds and clusters of repeats along with their locus coordinates in six sequenced *Leishmania* genome was developed (Fig. 3). It can be accessed as an open source repository at <http://biomedinformri.com/leishmicrosat> for *in silico* identification of polymorphic microsatellite loci and inferring evolutionary relationship between different *Leishmania* strains with a phylogenetic tree.

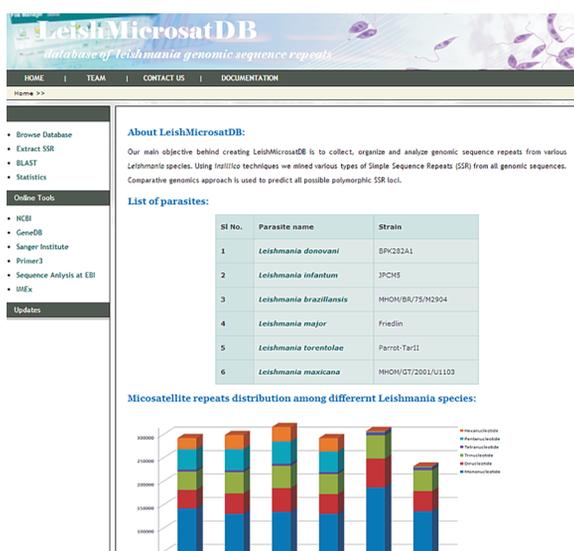


Fig. 3. Leish Microsat DB.

Through whole genome sequencing of *P. argentipes* (vector) by Next-Generation Sequencing (*de novo* sequencing), PE libraries were developed for DDT sensitive and resistant vectors. *In vitro* efficacy of nano-form of PLGA-PEG conjugated miltefosine, Amphotericin B and sitamaquine was found to be higher than the non-nano form.

Nanotechnology based novel drug delivery system against VL showed that efficacy of nano-form of PLGA-PEG conjugated miltefosine or Amphotericin B or sitamaquine nanoparticle is higher than that of the non-nano form.

Operational Research

GIS, geo-statistics and remote sensing based highly significant model was developed to explore breeding site and risk assessment of *Phlebotomus*

argentipes in the kala-azar endemic region of Bihar. Altogether 12 environmental factors including climatic and soil characteristics were identified as significant variables. The adjusted $R^2=72$ indicated nearly 72% of variance of *P. argentipes* abundance, explained by these environmental variables.

Under environmental management of vector control, comparative effectiveness of different interventions: (i) brick cement (ii) brick powder and lime (iii) wire mesh, and (iv) glazed tiles were assessed. Post-intervention assessment revealed a significant reduction in sand fly density in three interventions except wire mesh, which also had minimal acceptance by residents. These interventions can be supplemented to the integrated vector management programme.

Potentiality of Insecticide Quantification Kit (IQK) in quantification of DDT concentration on sprayed surfaces was assessed in sampled households of eight kala-azar endemic districts of Bihar during the 1st round of DDT spray. The IQK analysis on 2280 samples, cross-matched with HPLC test conducted at LSTM, UK, revealed its effectiveness in quantifying the DDT concentration. Further, Disease Database Management System (DDMS) has been taken up in eight kala-azar endemic districts to assess the impact of IRS operation on kala-azar incidence.

Virology

Virlogy laboratory continued its diagnostic support to the State, and also during epidemic/ outbreak of viral diseases such as viral gastroenteritis, AES, influenza, enterovirus, H1N1, VZV, herpes simplex I and II, dengue, chikungunya, EBV, CMV, rubella, measles, swine flu, HAV, HBV, HCV, HEV. A total of 2486 samples were tested (Table I). Apart from rotavirus, other gastroenteritis causing viruses like adenovirus, astrovirus, norovirus were also detected for the first time in Bihar via PCR, which were further analysed via sequencing for serotyping and genotyping. Some uncommon viruses like EBV, HSV, CMV and VZV, rubella and measles were also detected during the year.

Tribal Health Research

(i) *DISHA project*: Followed by training-cum-sensitization meeting, household listing, mapping

and demographic survey conducted in 12 clusters of tribal population of Ranchi district of Jharkhand. Baseline survey of the clusters using pre-tested study tools was initiated. Till date, 846 household questionnaires have been filled up with BP, anthropometry and dietary habits data. Altogether 609 blood samples were collected and sent to the Central laboratory for study relevant investigations. (ii) *Haemoglobinopathies study*: With the objective to estimate prevalence of haemoglobinopathies, viz. thalassaemia, sickle cell anaemia, etc., in tribal population of Bihar, demographic details of 177 tribal households of Jamui district have been captured. Out of 153 blood samples investigated, eight cases (5.2%) had abnormal haemoglobin (β -thalassaemia trait, HbS, HbE) and 73 cases (49.3%) were found to be mild to moderately hypertensive.

Others

In the parasite repository, total 115 Leishmania isolates have been preserved, of which 106 collected from kala-azar patients, five from skin lesions of PKDL patients, one from sand fly gut and three reference isolates. Till date 36 isolates have been characterized by molecular methods for their purity. Apart from this, 1101 serum samples of various categories have also been preserved in serum bank for research purpose.

The Institute provides voluntary counselling and HIV testing through ICTC and the confirmed cases are treated at ART centre (now upgraded to ART plus) as per NACO guidelines. Of the 4241 samples tested, 546 were found HIV positive including 121 HIV-TB co-infection. During the year, 633 HIV positive patients were on pre-ART and 504 on ART.

Table I. Samples tested for viral diseases

| Disease/ Virus | Sample | Tested (N) | Test performed | Positive/ Tested |
|------------------------------|--------------------|-------------|-----------------------|------------------|
| AES (JE) | Serum | 24 | ELISA, PCR | 01/201 |
| | CSF | 201 | | |
| Influenza A & B Swine flu | Throat/ Nasal swab | 15 | Rapid Kit | 01/15 |
| | | 15 | PCR | 00/15 |
| Diarrhoea (Rotavirus) | Stool | 771 | ELISA+Electrophoresis | 52/771 |
| Astrovirus | Stool | 30 | PCR | 03/30 |
| Norovirus | Stool | 100 | PCR | 03/100 |
| Adenovirus | Stool | 40 | PCR | 4/25 |
| Enterovirus | Blood | 18 | PCR | 00/16 |
| | CSF | 18 | | |
| Dengue | Serum | 166 | Ag ELISA + IgM | 31/135 |
| | | | RT-PCR | 04/30 |
| Chikungunya | Serum | 18 | IgM | 00/18 |
| Hepatitis (A,B,C,E) | Serum | 918 | Antigen + Antibody | 99/918 |
| Rubella | Serum | 18 | ELISA IgM | 04/18 |
| CMV(Cytomegalovirus) | Serum | 10 | ELISA IgM | 1/09 |
| HSV-1&2 | Serum | 53 | ELISA IgM | 04/53 |
| VZV | Serum | 42 | ELISA IgM | 09/42 |
| | CSF | 04 | ELISA IgM | 00/04 |
| EBV | Serum | 07 | ELISA IgM | 0/07 |
| Measles | Blood/Serum | 17 | ELISA IgM | 01/17 |
| Total | | 2486 | | 217 |

In the MDR/XDR TB laboratory, out of 500 samples referred from TBD centres, 164 (32.8%) were acid-fast bacilli (AFB) positive and 156 (31.2%) were culture positive. The AFB positivity in samples collected from RMRI OPD and ART centre was 14.7% (96 out of 650) and 25.9% (14 out of 54), respectively.

Extramural Research

The research priorities of the ICMR have always coincided with the health policy and priorities of the country. The research conducted at the ICMR Headquarters in the division of Epidemiology and Communicable Disease (ECD) as well as the institutes working on communicable diseases works closely with the national programme. Apart from the emerging burden of non-communicable diseases, the country continues to face a huge burden of infectious diseases as well, such as vector borne diseases, tuberculosis HIV/AIDS, respiratory infections, *etc.* The management of these infectious diseases is now further complicated by emerging antimicrobial resistance and outbreaks with viral infections in various parts of the country. Efforts were made by the ICMR to help the national programme by directing its research towards nationally relevant problems such that the research outcomes provide the technical advantage in framing the national programmes to control, prevent, eliminate and eradicate the spread of infectious diseases in the country.

VIRAL DISEASES

Network of Viral Diagnostic Laboratories

ICMR's Viral Research and Diagnostic Laboratory Network (VRDLN) established five regional level, six state level and 19 medical college level laboratories in the last year (Fig. 1) apart from the 14 laboratories already funded by ICMR. These laboratories collectively tested more than 60,000 samples and investigated 86 outbreaks. In order to create uniformity and strengthen the quality of data collection in the network, the ICMR established a Resource Centre for VRDL at its apex institute, NIV, Pune, to impart formal training to all categories of staff working in laboratories under the VRDL network. In addition, this centre also conducts QA/QC checks for all laboratories.

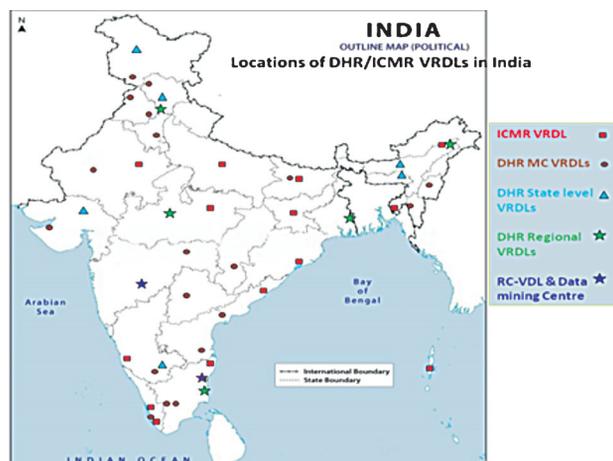


Fig. 1. Locations of DHR/ICMR VRDLs in India.

Wild poliovirus containment in laboratories

The WHO's Global Action Plan for laboratory containment of wild polioviruses, advises that when polio cases are decreasing in countries, the national health authorities are required to alert laboratories regarding the impending eradication of polio, encouraging destruction of all unneeded wild poliovirus materials, and compile a national inventory of all biomedical laboratories choosing to retain such materials. ICMR is leading this effort under the Ministry of Health and Family Welfare (MoHFW). Phase I activities of the National Task Force on Laboratory Containment of Wild Poliovirus were completed, an inventory of 50 biomedical laboratories storing wild polioviruses or potentially infectious materials was prepared and submitted to the National Certification Committee for Polio Eradication (NCCPE). The NCCPE then submitted the report to the Regional Certification Commission. Thereafter, India was declared polio-free on March 27, 2014. Thus WHO South-East Asia region was declared polio-free in 2014, marking a significant leap forward in the global eradication scene, with 80% of the world's population now living in certified polio-free regions.

Acute Encephalitis Syndrome (AES)

A research cum intervention project on AES/JE was launched by DHR/ICMR as part of the multipronged strategy developed for AES/JE in Gorakhpur for prevention, case management and rehabilitation measures. This programme was recommended by a panel of Ministers under the Chairmanship of Health & Family Welfare Minister and actively

involved various Ministries namely, Health and Family Welfare, Social Justice and Empowerment, Rural Development, Urban Development, Drinking Water Supply and Sanitation, Women and Child Development and Animal Husbandry. The project included simultaneous measures for detection of seroprevalence of JE antibodies in pigs, vector control measures using bed nets, indoor residual spraying (IRS) and release of larvivorous fishes in water bodies of five identified blocks of the project. Besides this, studies are being carried out to detect the transmission of enteroviruses in stool samples and correlate these viruses with the disease type; vaccine efficacy studies are also underway to evaluate the compliance and coverage of JE vaccine and identify the reasons for non-compliance in the study by carrying out socio-behavioral studies. Among a total of 3200 children from four districts of Gorakhpur division who were surveyed, the coverage of JE vaccine in Gorakhpur division was low with only half of the eligible children receiving one dose of the vaccine. The coverage of JE vaccine in the division was 51% and ranged between 36% in Kushinagar to 66% in Gorakhpur district. The common reasons cited by the mothers for non vaccination of their children included lack of information about the vaccination, and obstacles for vaccination such as, inconvenience in place/timing of vaccination and busy schedule of mother or her being out of station. ICMR recommended initiation of awareness among mothers about the need to administer two doses of JE vaccine, which were given free of cost in all the public health facilities in the division. The effectiveness of the vaccine was 84% (95% CI: 53-95). Based on the recommendation of ICMR, the Ministry of Health carried out a mass vaccination programme.

Drug Controller General of India (DCGI) approved the Vero cell-derived, purified, inactivated JE vaccine–JENVAC®. It is the first vaccine in the public-private partnership mode between the ICMR and Bharat Biotech International, India, launched in India. The vaccine is under consideration by Ministry of Health to be introduced into the programme.

National Rotavirus Surveillance Network (NRSN)

To build on the success of earlier network established by the ICMR from 2005 to 2009, it was proposed

to extend the surveillance activities at sites located across the country in different geographical zones as recommended by the National Technical Advisory Group on Immunizations (NTAGI) with the goal to have an adequate nationally representative baseline data. The National surveillance is coordinated and funded by ICMR using standardized protocols to determine epidemiologic and virological features of rotavirus and rotavirus disease burden through building on and extending expertise of laboratories that are already carrying out epidemiological studies and by adding new hospitals and laboratories in different geographic regions. The study is being carried out at four major referral laboratories, seven regional centres of ICMR and 23 hospital sites. A double data entry system has been established at the National Institute of Epidemiology (NIE), Chennai for validation of data. The MoHFW has already introduced rotavirus vaccine into the Universal Immunization Programme (UIP) and is planning to roll out in a phase-wise manner across the country soon. The NRSN established across the country will provide information on the impact of the vaccine on the decreasing trend of morbidity and severity of rotavirus diarrhoea cases reported to the hospital over a period as and when the vaccine is rolled out. The network will not only collect more data on rotavirus surveillance but also establish a model surveillance system for other vaccine-preventable diseases. The study revealed that rotavirus diarrhoea peaked during winter months (September–January) (Fig. 2a) across the region with approximately 90% of rotavirus infections occurring in children less than 24 months of age (Fig. 2b) with 40-60% of rotavirus positive cases having moderate to severe diarrhoea (Fig. 2c).

Human Immunodeficiency Virus (HIV):

With a large number of patients undergoing antiretroviral therapy (ART) in our country there is a need to address research questions on ART treatment and also related to toxicity and associated co-morbidities. A study carried out for the first time to assess the effect of ART on viral neutralization potential in HIV-1 infected children at All India Institute of Medical Sciences (AIIMS), New Delhi, revealed that there was an improvement, with time, in viral neutralization potential of plasma antibodies of HIV-1 infected children on ART, indicating a need to initiate ART at early stages of HIV-1 infection in

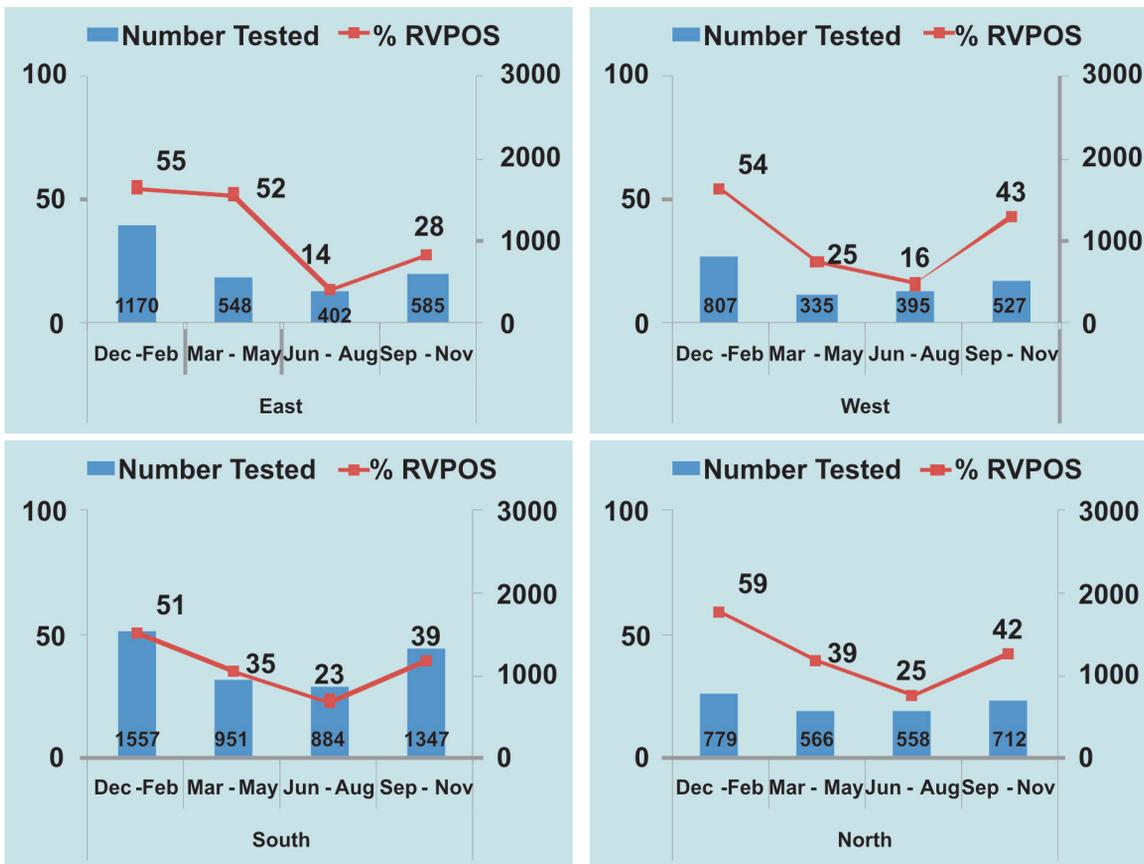


Fig. 2a. Seasonal distribution of Rotavirus Positivity (%) across regions.

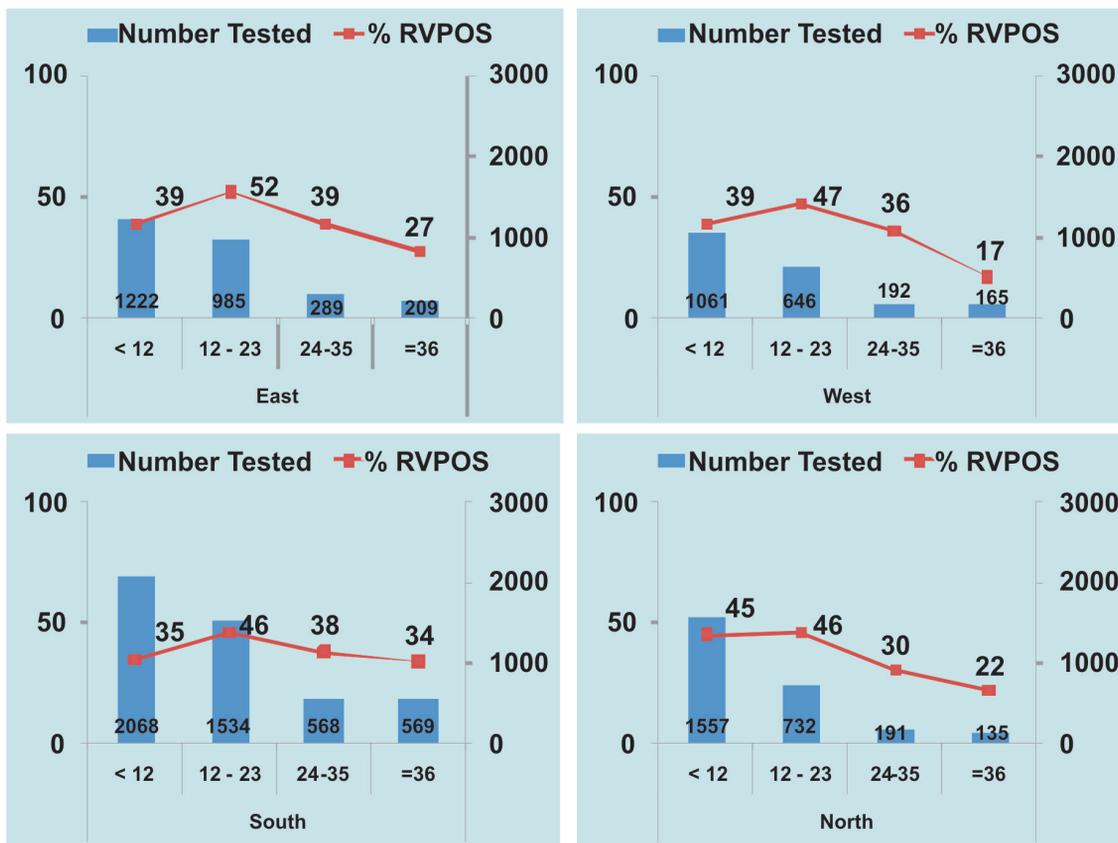


Fig. 2b. Age group wise distribution of Rotavirus Positivity (%) across region (X-axis: age in months).

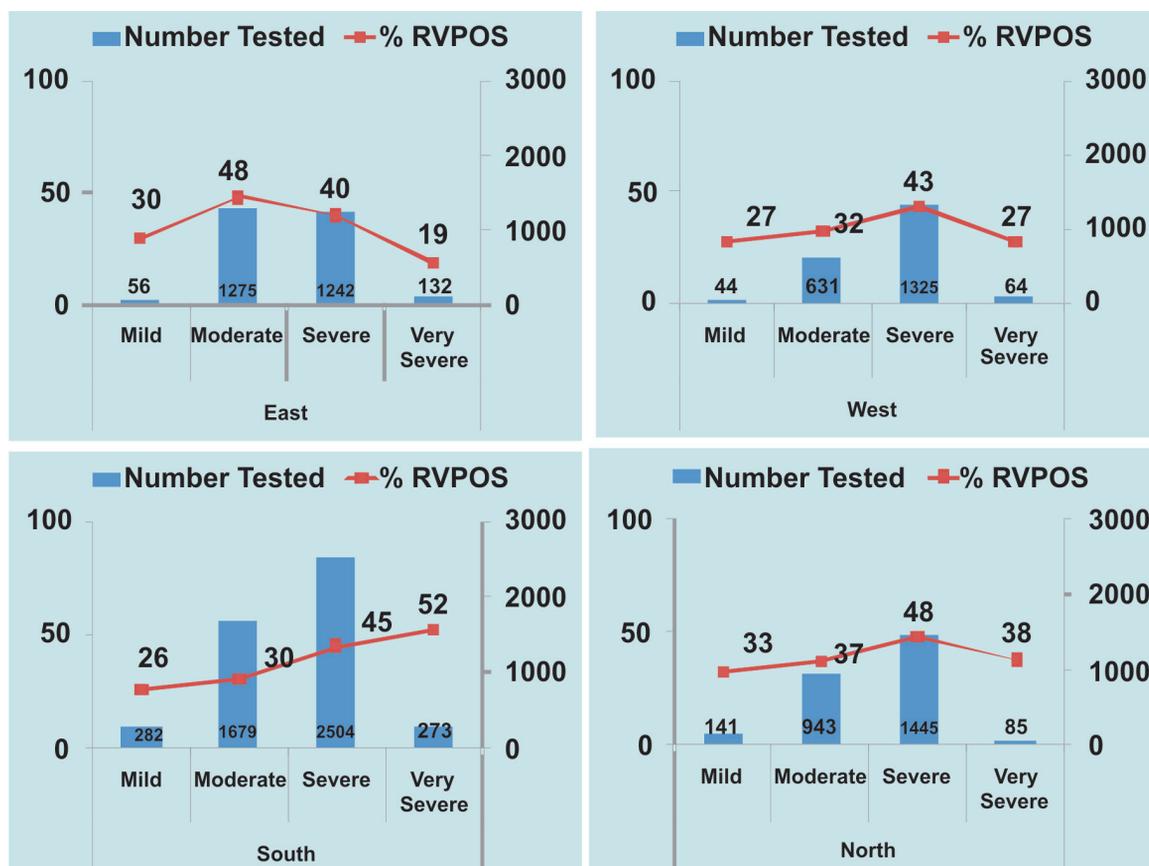


Fig. 2c. Distribution of Rotavirus Positivity (%) in disease severity categories.

children in order to effectively neutralize the virus and control disease progression. A study completed at Y. R. Gaitonde centre for AIDS Research and Education (YRG CARE), Chennai, identified mitochondrial DNA (mtDNA) as a novel biomarker of mitochondrial toxicity induced by nucleoside reverse transcriptase inhibitors (NRTIs) that would predict mitochondrial toxicity at an earlier stage and would facilitate clinical management and careful selection of antiretrovirals (ARVs).

Another study completed at AIIMS, New Delhi, has confirmed that semen is the main carrier of the sexually transmitted virus, including HIV-1. HIV-1 infection resulted in an aberrant production of cytokines and re-activation of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) that further changed the seminal cytokine network. Results showed that suppression of Herpes viruses-induced cytokines might serve as a target for HIV prevention strategy.

ANTIMICROBIAL RESISTANCE

In order to reinforce the surveillance of drug resistance in India, ICMR initiated Anti

Microbial Resistance Surveillance and Research Network (AMRSRN) to enable compilation of data on antimicrobial resistance (AMR) at different levels of health care. So far six nodal centres (NCs) have started functioning and are focusing on: *Enterobacteriaceae*, fungal pathogens, Gram negative nonfermenters, enteric fever, diarrhoeagenic bacteria and Gram positive organisms including methicillin resistant *Staphylococcus aureus* (MRSA) and vanomycin-resistant enterococci (VRE). The standard operating procedures (SOPs) (bacteriology and mycology) have been finalized and are available on ICMR website. Data collection modules have been finalized and the first set of data are being collated. As per the data obtained from the network, *Salmonella* Typhi multidrug resistance (MDR) to ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole showed a downward trend and an increase in resistance to fluoroquinolones and cephalosporins in *S. Typhi* was reported (Fig 3). Of the *Enterobacter* species, *Klebsiella* and *Escherichia coli* caused most of the infections which were 100% sensitive to colistin followed by imipenem and meropenem. Most of the hospital

acquired infections were caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. All isolates of *P. aeruginosa* were susceptible to colistin, followed by imipenem (85%), amikacin (80%), ciprofloxacin (80%), piperacillin-tazobactam (58%) and meropenem (50%) (Fig.4). In *A. baumannii*, maximum susceptibility was for colistin (99%) followed by imipenem (53%) and meropenem (53%). Fluconazole resistance was the highest with *Candida parapsilosis* (11.2%) followed by *C. albicans* (4.5%). (Fig.5). Resistant isolates of 55 *Pseudomonas* and 30 *Acinetobacter species* from CMC, Vellore; nine *Pseudomonas* and nine *Acinetobacter species* from AIIMS, New Delhi and 14 *Pseudomonas* and 30 *Acinetobacter species* from Jawaharlal Institute of Postgraduate Medical Research (JIPMER), Puducherry were completely characterized for their molecular mechanism of resistance (Table 1). Multiple resistances coding gene presence indicates the reason for increased minimum inhibitory concentration (MIC), resulting in requirement of combination therapy with high dose and extended duration and this holds true for both *P. aeruginosa* and *A. baumannii* (Table 1).

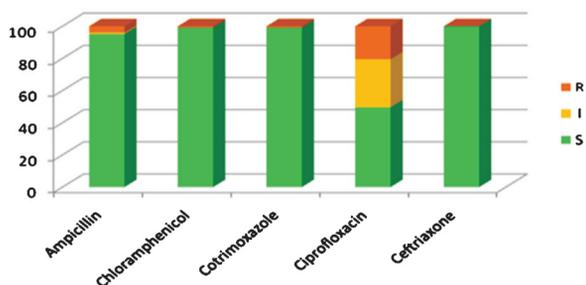


Fig. 3. Susceptibility pattern of *Salmonella Typhi*; (R: resistant; I: intermediate S: susceptible).

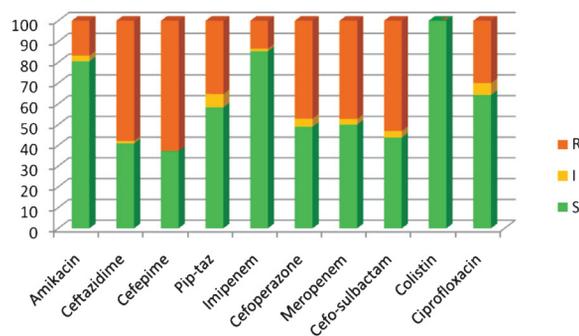


Fig. 4. AMST profile of *Pseudomonas aeruginosa* isolates.

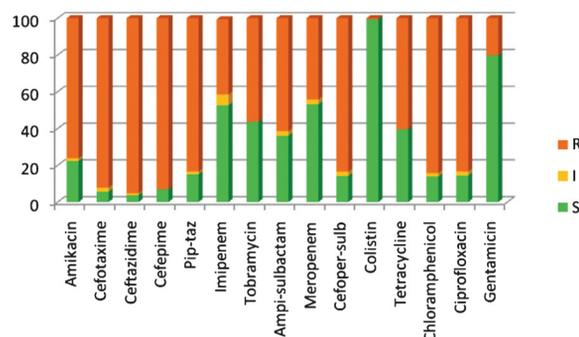


Fig. 5. AMST profile of *Acinetobacter baumannii* isolates.

MYCOBACTERIAL DISEASES

Tuberculosis

A joint venture of ICMR, MoHFW and Department of Biotechnology (DBT) has been initiated to commercialize the indigenous technologies developed by Indian scientists who approach ICMR/DBT so that an affordable diagnostic test/ assay for TB and MDR-TB is available in the country. In view of this a multicentric study has been initiated recently at four participating sites

Table 1. Number of genes identified in ESBL and CRO multiplex PCR reaction.

| Organism | Nodal Centres | No. of Isolates | SPM | IMP | VIM | NDM | OXA-23 | KPC | VEB | PER |
|------------------------------|---------------|-----------------|-----|-----|-----|-----|--------|-----|-----|-----|
| <i>P. aeruginosa</i> | CMC | 55 | 0 | 0 | 15 | 10 | 0 | 0 | 14 | 0 |
| | AIIMS | 9 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| | JIPMER | 14 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0 |
| <i>Acinetobacter species</i> | CMC | 30 | 0 | 0 | 1 | 7 | 51 | 0 | 0 | 11 |
| | AIIMS | 9 | 0 | 0 | 0 | 2 | 9 | 0 | 0 | 3 |
| | JIPMER | 30 | 0 | 0 | 3 | 3 | 23 | 0 | 0 | 14 |

(i.e. National Institute for Research in Tuberculosis (NIRT), Chennai, National JALMA Institute for Leprosy & other Mycobacterial Diseases (NJIL&OMD), Agra, AIIMS, New Delhi and National Institute of Tuberculosis & Respiratory Diseases (NITRD), New Delhi) for validation of the three molecular kits (2 for MDR-TB and 1 for TB diagnosis).

North East

As a part of special initiatives under North-East, and to enhance the research in the communicable diseases in the North East region, research activities in the communicable diseases in the North East are being pursued. The focus of NE projects is primarily to conduct translational research and to cater the problems of North East for the benefit of people and patient welfare.

VECTOR BORNE DISEASES

A Vector Borne Diseases Science Forum has been established at ICMR with the aim to provide a platform to vector biologists, entomologists, epidemiologists, immunologists and other specialists working on vector-borne diseases. During 2014-2015, Regional Medical Research Centre (RMRC), Port Blair in collaboration with Vector control Research Centre (VCRC), Puducherry has initiated on the effectiveness and operational feasibility of mass diethylcarbamazine (DEC)-fortified salt as a supplementary intervention to mass drug administration towards elimination of the lone foci of diurnally sub-periodic *Wuchereria bancrofti* in Andaman & Nicobar Islands. Baseline data collection on microfilaraemia (Mf) prevalence, antigenemia prevalence among children and salt usage pattern of the community has been completed.

Plasmodium liver stages, though clinically silent, yet, biologically are the most active stages preparing the parasites for rapid asexual replication. Each liver stage schizont can generate several thousand merozoites, and each of these merozoites in turn is capable of initiating blood stage infection. Therefore, robust intervention strategies can help eliminate liver stage infection and thereby malaria. A study was undertaken by School of Life Sciences, University of Hyderabad, to unravel the interferon

gamma (IFN- γ) responsive pathway manipulated by the *Plasmodium* liver stages during their attempt to sabotage the host defense mechanisms. It was observed that pretreatment of HepG2 cell line cultures with IFN- γ greatly reduced the growth of exo-erythrocytic forms (EEFs), a phenomenon that was reversed when IFN- γ was given after infection. It could thus be concluded that pretreatment of HepG2 cells with IFN- γ activated defense responses that effectively curbed the growth of EEFs. In order to analyze the global gene expression changes in pre- and post- IFN- γ treated samples, microarrays were performed, that showed dramatic alterations in the gene expressions across control (sporozoite infected), pre-and post-IFN- γ treatment samples.

A study was undertaken by National Institute of Malaria Research (NIMR), New Delhi, to understand the physiological and immunological responses of the mosquito tissues that affect vector competency, i.e., ability to support *Plasmodium* development and transmission. A cluster of sugar fed salivary transcripts encoding plant like proteins (PLTs) was identified, especially photosynthetic molecular machinery components. Their expression validation provided strong evidence on how mosquitoes evolved and adapted for feeding over a plant host. A comparative salivary transcriptomic study, followed by analysis of blood meal impact and molecular responses, confirmed that adult female salivary glands were specialized organs to manage meal specific choices and decisions, through a 'gene switching' mechanism. It could thus be hypothesized that the nature of the mosquito salivary glands may be more complex and carry unique associated microbial flora that may partly facilitate mosquito adaptation to diverse ecologies.

In India, malaria vector control strategy is mainly based on indoor residual spraying (IRS) of insecticides and universal coverage of population with insecticide-treated nets (ITNs) in endemic and difficult to reach areas having API >2 or proportion of *Plasmodium falciparum* >30%. However, one of the major impediments for effective vector control is the development of resistance in vectors to the insecticides. The study is proposed to determine insecticide susceptibility/ resistance status in malaria vectors to the insecticides in use and also to other candidate insecticides that are approved

for use under vector control. Studies under a task force on insecticide resistance monitoring have been planned on malaria and visceral leishmaniasis vectors which will find out susceptibility status of *Anopheles* vectors to dichlorodiphenyl trichloroethane (DDT), malathion and deltamethrin in malaria endemic areas and of *Phlebotomus* species in leishmaniasis endemic areas. These will be carried out in the States of Madhya Pradesh, Uttar Pradesh, Jharkhand, Chattisgarh & North East region.

Among the available vector control options, chemical control remains to be the major method used in the control programmes. National Vector Borne Disease Control Programme (NVBDCP) has been the nodal agency to select and introduce new public health products (PHPs) [which include insecticides/ insecticide formulations/ long lasting insecticide treated nets (LLINs)/ biolarvicides] for vector control under the national programme on the basis of their suitability and adaptability to Indian conditions. Development of a common protocol for uniform evaluation of public health pesticides including biolarvicides has become imperative. Keeping this in view and also to be in line with the WHO Pesticide Evaluation Scheme (WHOPES) guidelines for insecticide evaluation, a common protocol was jointly prepared by the NIMR, New Delhi and the VCRC, Puducherry in 2000, which has been revised/ updated by the Sub-Committee on revision of SOP and common protocol in 2014-2015. Revised Common Protocol for Uniform Evaluation of Public Health Pesticides including biolarvicides for use in Vector Control and SOP for introduction of public health pesticides including biolarvicides in the National Vector Control Programme became available in public domain on NVBDCP, ICMR and National Centre for Disease Control (NCDC) websites. The trials conducted by different institutions at different sites following such common protocols minimized the discrepancies in methodology and thereby their results could be compared more meaningfully to facilitate the NVBDCP to arrive at a decision.

DDT, the most inexpensive and common insecticide used for malaria and kala-azar control is available in two formulations, water dispersible powder (WDP) 50% and WDP 75%. Although, DDT WDP

75% is generally considered as the cost-effective high performing formulation for IRS with long lasting residual properties, more information on the comparative efficacy of the two formulations in Indian conditions is essential to choose DDT 75% for IRS replacing DDT WDP 50%. Hence, the study to assess the comparative efficacy of indoor residual spraying of DDT WDP 75% (one g/m²) with that of DDT WDP 50% (one g/m²) against *Anopheles fluviatilis*, the primary vector of malaria, in the selected endemic areas of Odisha State was undertaken. The study showed that after first round of spraying, there was only a marginal decrease in the parous rate of *An. culicifacies* after spraying in the DDT 50% arm whereas in the 75% arm, the decrease was marked. After two months of spraying, on one time mud plastered surfaces, the mortality was 98.9% with DDT 75% and 49.1% with DDT 50%.

The large scale use of insecticides in disease endemic areas since many years has resulted in the development of resistance among vectors towards certain insecticides, which warrants formulation of resistance management strategies. To formulate and implement resistance management strategies in time, regular monitoring of vector resistance to the insecticides under use is essential. This is done by the zonal entomological teams of State NVBDCP units and other research institutes. To conduct susceptibility tests, the NVBDCP has been obtaining the WHO prescribed insecticide impregnated papers from the WHO recognized institute at Malaysia and distributing to various zonal teams. The NVBDCP often encounters difficulties in the procurement of papers resulting in delay or breakdown in supply system for conducting the susceptibility tests. It is therefore important to have a facility within the country, particularly when the required expertise / skill on this technique is available within country, so that the requirements of the national programme for time to time monitoring of insecticide resistance in vectors to different insecticides are met. At the request of NVBDCP this responsibility was taken up by ICMR. VCRC was identified as the site for establishing such a facility to prepare the insecticide impregnated papers and supply to NVBDCP to monitor the resistance/susceptibility in vectors to insecticides. The papers have been successfully

prepared by VCRC. Validation of the papers is being taken up by NIMR and Institute of Vector Control and Public Health. This will be a national resource for preparation of insecticide impregnated papers.

ICMR through its Vector Borne Disease Science Forum aims to continue its effort towards the elimination of filariasis and leishmaniasis and continues to provide support to the GOI endeavour of malaria pre-elimination. A task force project on insecticide resistance monitoring in malaria and visceral leishmaniasis vectors is planned to be undertaken which will determine the susceptibility status of *Anopheles* vectors to DDT, malathion and deltamethrin in malaria endemic areas and of *Phlebotomus* species in leishmaniasis endemic areas. Various studies on the biology and bionomics of malaria and *Leishmania* vectors will be undertaken. The forum aims to continue supporting projects aimed at developing new molecular diagnostics and treatment strategies for the vector borne diseases. In addition it will also support projects on basic research in relation to vector borne diseases.

Effective surveillance is the corner stone for minimizing the risk of transmission of dengue in the country. In view of conducting an entomological surveillance for the early detection and control of dengue, a study was carried out by ICMR'S National Institute of Malaria Research, New Delhi, in coordination with the Municipal Cooperation of Delhi (MCD), to detect *Aedes* breeding in 20 wards of West Delhi. This study revealed that the key containers such as overhead tanks, water storage tanks, in the residential and office premises as well as curing tanks built at construction sites supported breeding in both transmission and non-transmission seasons.

Studies were also conducted in different parts of the country to find out the transmission of dengue virus by *Aedes aegypti* and *Ae. albopictus*. This revealed that in a single house and in certain cases in individual mosquitoes more than one or two dengue serotypes were found. Further, the vector species and their specific breeding sites were identified, and most importantly, the key containers which contributed to the maximum production of mosquitoes in the areas, were identified. If these

containers were taken care of in pre-monsoon periods they could have reduced the density of mosquitoes and dengue cases in the transmission period. As a result of these interventions, a significant decrease in vector breeding in the key and secondary containers in the transmission period was observed. As most transmission occurs in and around the houses, schools, workplace and other places of day time congregations, ultimate success of the programme therefore depends on community participation.

Tribal Health Research

A study on entomological and epidemiological investigations of leishmaniasis among Kani forest tribes in the tribal Settlements of Thiruvananthapuram district, Kerala, carried out at VCRC, Puducherry, was completed this year. Re-activation of one old cutaneous leishmaniasis (CL) case, after treatment was observed for the first time. A total of 16 species of sandflies were recorded. *Sergentomyia baghdadis* was the predominant species. *P. argentipes* was the second, which was the proven vector of kala-azar in India. Two new species of sandflies were recorded from this area.

Role of a Rapid Diagnostic Kit (RDK) and oral miltefosin to control leishmaniasis in the tribal population of South Bengal was studied by Midnapore Medical College, West Bengal. The results showed the detection of 11 cases of VL and 18 cases of post-kala-azar dermal leishmaniasis (PKDL) by mass screening. Importantly three RDK positive asymptomatic cases developed signs of VL and LD body was detected in their bone marrow during follow up period and was treated promptly. The PKDL cases having no acute crisis and not attending any medical set-up for treatment were also treated. By treatment of the asymptomatic VL and PKDL patients, the chances of disease transmission are reduced.

Two new field units attached with RMRC, Bhubaneswar, in tribal dominated areas of Odisha – Kandhamal and Keonjhar have been opened on request from the State Health Department for improvement of the health indicators of the tribal people of these areas. It will target operational and health systems research activities to increase utilization of the current health programme. Studies

will be undertaken to find out the prevalence of drug resistant TB, RTI and HIV/STDs infections and activities will be done to reduce the under-five child mortality by providing intervention for acute diarrhoea, acute respiratory infections and undernutrition in this district in the first phase. This will help in capacity building through transfer of improved technology and disease control strategies to augment ongoing State Health programmes.

The Tribal Sub-Plan will continue its efforts to support the cause of improving tribal health. It is proposed to initiate ad-hoc studies in already identified specific gap areas on childhood infectious diseases, tuberculosis, STD/ HIV, genetic diseases, diarrhoeal diseases and viral respiratory illnesses. Keeping in view the High Power Committee recommendations it is felt that it would be more pragmatic if research-cum-action kind of studies with interventional component inbuilt in the studies are planned to augment their public health output. Efforts have been made to encourage non-ICMR institutions, especially medical colleges and universities to take up research in priority areas and if required, hand-holding and mentoring also will be carried out to encourage potential investigators.

ZOONOTIC INFECTIONS

Zoonotic infections or zoonoses are infections naturally transmissible between vertebrate animals and humans. These constitute nearly 60% of all known human infections and over 75% of all emerging infectious diseases. A joint ICMR-Indian Council of Agricultural Research (ICAR) panel reviews and supports proposals on zoonotic infections covering both human and animal aspects. These are jointly reviewed by experts on veterinary and human health so as to provide a holistic review of the proposal. These are ICMR funded studies with the technical expertise of veterinary experts. Studies on *Mycobacterium Avium* paratuberculosis, TB in man and animal, food-borne trematodes and cestodes, brucellosis, leptospirosis, rickettsial infections and others have been funded.

A study on clinical, epidemiological, serological and molecular characterization of scrub typhus in South Karnataka by Manipal University has been initiated to evaluate the clinical and epidemiological characteristics of *Orientia tsutsugamushi* in patients

diagnosed with scrub typhus and to determine the validity of various serological (Weil-Felix, ELISA and Immunofluorescence) and molecular methods in diagnosis of scrub typhus. Also this study will narrow down the most sensitive and specific test for diagnosing scrub typhus for better and earlier diagnosis at various stages of the disease. The results showed a comparison of sensitivity and specificity of IgM ELISA, Weil-Felix and PCR *vis-à-vis* immunofluorescence assay (IFA). It was observed that IgM ELISA had the highest sensitivity (85.71%) and specificity (98.67%) as compared to other tests, however, nested PCR had comparable specificity (98.67 %)

Another study on clinical and sero-epidemiology of scrub typhus, spotted fever, Q fever in Puducherry has been supported with the aim to understand the seroprevalence of the above rickettsial infections and for Q fever, ruminants to be screened for antibodies to *Coxiella burnetti*. The results indicated an overall prevalence of 2.46% of coxiellosis in domestic livestock, the seropositivity rate of 14.36% by Weil-Felix was seen in healthy population of Puducherry and surrounding areas, and some cases of *Rickettsia conorii* (a prevalence of 8.84% for spotted fever). Work on mixed infections and confirmation of cases of Q fever in humans is presently underway.

Two studies in Task Force mode have been launched by ICMR at Mahatma Gandhi Institute of Medical Sciences (MGIMS), Wardha and Central India Institute of Medical Science (CIIMS), Nagpur. Under these studies, epidemiology, molecular biology, novel biomarkers and other aspects of tuberculosis, listeriosis, brucellosis, rotavirus infection, rickettsial diseases and leptospirosis will be studied in Wardha and Nagpur districts. Preliminary results show samples positive by IgM ELISA for brucellosis, leptospirosis and scrub typhus.

A study on molecular epidemiology and genetic diversity of *O. tsutsugamushi* from patients with scrub typhus in India was undertaken by CMC, Vellore. The aim of this study was to genotype the 56- kDa cell-surface antigen gene of *O. tsutsugamushi* isolated from patients with scrub typhus from three different geographic locations in India namely Tamil Nadu, Shimla and Shillong. The

study reported the genotypes of *O. tsutsugamushi* circulating in three different geographical locations with endemic scrub typhus. The knowledge adds to the molecular epidemiology which will have significant impact in control of this disease.

Study on important emerging zoonotic diseases of equines used for tourism and pilgrimage was carried out in Jammu and Kashmir with the aim to determine incidence of emerging zoonotic diseases in equines of J & K with respect to glanders, strangles, foal pneumonia, equine influenza and Japanese encephalitis.

Molecular characterization of food-borne trematodes and cestodes prevailing in North East India was done in Shillong with the aim to assess prevalence status of infections, with special reference to North East India by means of molecular epidemiological studies. The survey work resulted in the collection of a total of nine species of trematodes and eight different types of Cestodes parasites. Of these, all of the trematodes species – *Artyfechinostomum sufrartyfex*, *Fasciola species*, *Fasciolopsis buski*, *Paragonimus westermani*, *P. helterotremus*, *Gastrodiscoides hominis*, *Eurytrema pancreaticum* and *Opisthorchis noverca*, and two metacestode forms - hydatid cyst and *Cysticercus cellulosae*, represent important zoonotic potential zoonotic forms.

Rickettsial diseases are considered some of the most covert emerging and re-emerging diseases and are being increasingly recognized in India. Among the major groups of rickettsiosis; commonly reported diseases in India are scrub typhus, murine flea-borne typhus, Indian tick typhus and Q fever. There is a distinct need for physicians and health-care workers at all levels of care in India to be aware of the clinical features, available diagnostic tests and their interpretation, and the therapy of these infections. Therefore, guidelines on diagnoses and management of rickettsial diseases have been developed which are available on websites of DHR and ICMR.

It is envisaged that studies would be developed in the area of joint surveillance, monitoring and management of zoonotic infections of public health importance. Surveillance of food-borne infections, molecular subtyping of clinical isolates to identify cluster of cases of food borne infections needs to be undertaken. Compilation and analysis of antimicrobial susceptibility test (AMST) data of systematic subsets of human clinical pathogens and animal and food isolates against significant antimicrobial agents will be taken up. Antibiotics prescription practices in veterinary practices will also be studied.

REPRODUCTIVE HEALTH

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

Intramural Research

NATIONAL INSTITUTE FOR RESEARCH IN REPRODUCTIVE HEALTH

1. EXPANDING CONTRACEPTIVE CHOICES

1.1 Development of Anti-fertility Vaccines with Sperm Proteins

Synthetic Peptides of Human Seminal Plasma Inhibin (hSPI) and 80kDa Human Sperm Antigen (80kDa HSA) which immunobiologically mimic their respective native proteins have been found to be potential candidates for the development of a male anti-fertility vaccine. The synthetic Peptide1 of 80 kDa was investigated for preclinical safety and was found safe. Sub-acute and chronic toxicity studies are in progress.

1.2 Gender Equity-Focused, Male-Centred Family Planning for Rural India

The study aims to enhance contraceptive knowledge and acceptance of spacing methods among rural young couples through gender equity focused family planning intervention delivered by the Village Health Providers (VHPs).

Following baseline and 9 month follow up surveys in 2013, a total of 46 in-depth interviews of couples from experimental clusters and 22 VHPs have been conducted to assess the quality of services provided through CHARM (Counselling Husbands to Achieve Reproductive Health and Marital Equity) interventions. The data has been cleaned and is in the process of being analyzed. Preliminary results indicate that spousal discussion on either the family size, or on the contraceptive use, together or independently has an effect on the use of any spacing method of contraceptive. The 18 month follow-up survey was initiated during 2014. A total of 891 young married couples from experimental (397) and control (494) villages have been interviewed. All three data sets were cleaned and made readily available for analysis. A small documentary (15 minutes) on CHARM study was prepared with the help of Population Council.

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

The objective of the study was to understand the effectiveness of an intervention to increase contraceptive use among couples where women report unmet need for contraception. The study was conducted in two slum communities namely, Tunga Village and Kajupada in Mumbai. A sample of 1136 currently married women aged 18-39 years having at least one child and reporting unmet need of contraception was selected using systematic random sampling procedure. Baseline data was divided into two groups, namely Group A (women reporting unmet need and domestic violence from husband) and Group B (women reporting unmet need and absence of domestic violence from husband). Group A received counseling sessions on both family planning and marital communication

whereas Group B received information only on family planning. The intervention sessions included individual sessions, couple sessions and group sessions with interval of at least two weeks. A total of 1041 women received family planning counselling. About 137 women received first counselling on marital communication and out of that 99 received couple counselling. To see the impact of the counselling on contraceptive use and domestic violence, endline survey was conducted and the analysis is in progress.

1.4 Acceptability and Feasibility of using Female Condom (FC) in Family Planning Setting

A study conducted on contraceptive and sexual practices among HIV concordant and discordant couples, reported that 85% participants did not know about an alternative method to male condom and 40% expressed the need for female condoms. In NIRRH Family Welfare Clinics it has been observed that some couples avoid using male condom during safe period and also practice withdrawal method and thereby are at risk of unwanted pregnancy. Access to female condom can be an alternative to male condom.

Among 110 women enrolled in the study, 56 women completed the study period. Of these, 54.3% expressed that they were comfortable using female condom and it was not at all noisy during use. 68.6% women were satisfied with sexual pleasure after using a female condom.

71.4% women in the study were satisfied with the method and accepted the method positively. In spite of some limitations in our study, we suggest that female condom should be considered as one of the contraceptive choices in the National Family Welfare Programme.

2. INFERTILITY

2.1 Female Infertility

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

PCOS is a common endocrinopathy in women of reproductive age having both reproductive and metabolic consequences. The association of INSL3 and PON1 genes with PCOS susceptibility

and its related traits were investigated. Insulin-like factor 3 (INSL3), a peptide belonging to the relaxin-insulin family, synthesized by the ovarian theca cells of antral follicles plays an important role in follicular development, its maturation and androgen production. The polymorphisms in the entire exonic region have been screened and a strong association was observed of rs6523 A/G polymorphism in exon 1 with PCOS susceptibility. The other coding region polymorphisms of INSL3 also showed association with PCOS related traits in controls and women with PCOS. PON1, a major anti-atherogenic enzyme, inhibits oxidized LDL formation and facilitates glucose uptake, thereby decreasing cardiovascular disease development and insulin resistance. Its levels and activity are partially regulated by genetic variations. In the reporting year, the association of important promoter and coding region polymorphisms of PON1 with PCOS risk and its related traits was examined. While promoter region and Q192R exonic polymorphisms exhibit similar genotype distribution, the L55M coding region polymorphism was associated with reduced risk of PCOS development in lean women.

2.1.2 Molecular Signature of Human Follicular Fluid in PCOS by Proteomics Approach

Proteomic analysis of human follicular fluid (FF) from PCOS and healthy women indicate defect in cumulus oocyte complex (COC) matrix in PCOS women. Several proteins involved in COC matrix formation and expansion are expressed in granulosa cells. A comparative analysis of the gene expression of several of these COC matrix proteins in granulosa cells from PCOS and controls showed significant downregulation in PCOS. Upstream molecules regulating these genes were studied. Amphiregulin was found to be significantly down regulated in PCOS follicular fluid as well as in granulosa cells. This also confirmed a defect in COC matrix function in PCOS.

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

PCOS is a complex multigenic disorder with characteristic cyst formation in ovary which leads to anovulation and infertility. In PCOS, growth of

ovarian follicles is arrested and this contributes to cyst formation. The present study has been undertaken to delineate the role of apoptotic molecules in cyst formation using a mouse model developed by neonatal estrogenization. In cystic follicles expression of various cell survival and apoptotic molecules were studied. An increase in dead cell population in treated groups was observed by flow cytometry using the Fluorescein Diacetate (FDA) stain. This supported the morphological finding, that with progression of cystogenesis, granulosa cells get dispersed and degraded. This was also confirmed by increased expression of caspase 8 in the treated group in comparison with controls. Ultra-structure study showed apoptotic changes like membrane blebbing, chromatin condensation and vacuolization in the ovary of estradiol treated animals. Studies will be continued to investigate the fertility status of the estrogenized mouse model.

2.1.4 Deciphering the Putative Epigenetic Mechanisms Pertaining to Polycystic Ovary Syndrome

Epigenetic alterations such as DNA methylation and histone modifications have only recently gained recognition as pivotal factors in the onset of complex diseases and their progression. Polycystic ovary syndrome (PCOS) is one such multifaceted disorder that bears a strongly established genetic component but lacks a commonly identified etiopathologic element till date. PCOS being a life-style associated health risk is further influenced by gestational milieu, diet and environmental determinants. Hence, it was decided to investigate the prevalence of epigenetic modifications in PCOS women with special emphasis on the ovarian tissue. The study was initiated by assessing the global LINE-1 DNA methylation levels in women with PCOS in relation to controls. Several CpG site specific differences in DNA of peripheral blood as well as ovarian granulosa cells of these women have been identified. We are currently in the process of validating these epi-marks as a candidate methylation marker for PCOS.

2.1.5 Autoimmune Markers for Diagnosis of Endometriosis

This is a multicentre study for validation of peptide ELISA based on the epitopes of Stomatin like

protein 2 (SLP2), Tropomodulin 3 (TMOD3) and Tropomyosin 3 (TMP3) for non-invasive diagnosis of endometriosis. The study participants recruited during the reporting year in Mumbai, Nagpur, Goa and Kolkata were: Group I (Endometriosis): 121; Group II (Healthy Controls): 222 and Group III (Disease Controls): 20. The total numbers of study participants recruited in this study were: Group I (Endometriosis): 449; Group II (Healthy Controls): 371 and Group III (Disease Controls): 130. The analysis was carried out for 307 women with endometriosis, 104 healthy controls and 71 disease controls. Among endometriosis group, 62% of women were diagnosed as ovarian endometriosis, 23% women as deep infiltrating endometriosis (DIE) and 16% women as peritoneal endometriosis. We observed that around 14% (44 out of 307) of the women with endometriosis showed a family history of endometriosis (mother, sister or both). Around 13% women suffering from endometriosis had history of abortions; of these 73% women were suffering from minimal-mild stage of endometriosis. Mean serum anti-endometrial-autoantibody levels of SLP2a, SLP2b, SLP2c, TMOD3a, TMOD3b, TMOD3c, TMOD3d, TPM3a, TPM3b, TPM3c and TPM3d were significantly elevated in women with endometriosis than in healthy controls as well as disease controls ($p \leq 0.05$). Further experiments are on-going to validate the utility of peptide ELISA for early diagnosis of endometriosis.

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

High Mobility Group Binding 1 (HMGB1) was identified as one of the differentially abundant proteins during the receptive phase in the uterine fluid of humans and rats. Previous studies demonstrated that an excess of extracellular HMGB1 in the uterine cavity leads to pregnancy failure in rats. Studies conducted to elucidate the underlying mechanism revealed activation of nuclear factor kappa b (NF κ B) in the luminal epithelium and increased expression of interleukin 6 (IL-6) in all three compartments of the uterine horn exposed to an excess of HMGB1. Other inflammation associated molecules such as Tumor Necrosis Factor alpha (TNF α) and RAGE (Receptor for Advanced Glycation End products) were investigated for their expression in the horn

administered with HMGB1. TNF α and RAGE expression were found to be deregulated in the horn administered with an excess of HMGB1. Further, implantation sites in the HMGB1 treated horn were not as distinct as seen in the control horn. Collectively, these investigations revealed that an excess of HMGB1 in the uterine fluid is detrimental to pregnancy as it leads to activation of the inflammatory cascade and disruption of endometrial histology.

2.1.7 The Factors of Relevance in Endometrial Adhesiveness to Embryonic Cells

Previous studies on the 2D surfactomes of RL95-2 (displaying higher adhesiveness to JAr, an embryonic cell line) and HEC-1A (less adhesive to JAr cells) identified several cell surface proteins that displayed differential abundance in the two cell lines. HEC-A cell surface had higher abundance of Tubulin beta 2C (TUBB2C), ADAMTS and elongation factor beta whereas RL95-2 cell surface had higher abundance of HSP27, HSPA9, gp96, calreticulin (CRT), Tapasin-ERP57, protein disulfide-isomerase and β actin. During the reporting year, studies were conducted to assess the functional significance of differential abundance of CRT, TUBB2C, and HSPA9. These investigations revealed a pro-adhesive role of the cell surface CRT, HSPA9; and an anti-adhesive role of the cell surface TUBB2C.

In addition to alterations in the intracellular levels, differential localization of proteins may also contribute to a modulation in the cell surface protein repertoire. Considering that the cell surface localization of proteins is mediated by intracellular trafficking machinery, studies were undertaken to investigate the role of Rab11A (a recycling GTPase) in localization of cell adhesion molecules such as integrins and E-cadherin. In the previous year, studies were carried out to generate stable endometrial epithelial (Ishikawa) cell clones that stably over-express the mutant form of Rab11A. In the reporting year, stable cell clones of Rab11A knockdown Ishikawa cells were generated. Together, these stable clones (cells over-expressing mutant Rab11A and cells knocked down for Rab11A expression) exhibited a decrease in the surface localization of integrin α V as compared to the respective empty vector controls. This implied

that Rab11A modulation leads to mis-sorting of integrin α V. Further co-culture of these clones with JAr spheroids (trophoblastic cells), revealed a significant decrease in the attachment of JAr spheroids to endometrial cells. The study highlights the relevance of Rab11A in the trafficking of integrin α V in endometrial epithelial cells.

2.1.8 Role of Homeobox Gene HOXA10 in Endometrial Decidualization

The aim of this study is to decipher functions of HOXA10 in endometrial decidualization. Our earlier studies had shown that HOXA10 is down regulated in the decidua that promotes trophoblast invasion. It is opined that this increase in invasion in response to HOXA10 down-regulation is due to increased expression of gp130 cytokines in the decidua. The expression of IL6 and HOXA10 was found to be reciprocal in the developing mouse decidua with higher expression of HOXA10 at the time of implantation which declined as the gestation progressed. In converse, IL-6 was lowest at time of implantation and its expression increased in the decidua thereafter. The loss of HOXA10 promotes expression of Il-6 which may help in trophoblast invasion.

To investigate the role of HOXA10 in endometriosis the expression on stromal and epithelial cells was knocked down using si/shRNA. In epithelial cells, loss of HOXA10 increased their ability to migrate in wound healing assay. These cells also expressed increased amounts of MMPs. In case of stromal cells, inhibition of HOXA10 expression leads to increased invasion and induction of MMP expression. These results together imply that loss of HOXA10 increases the invasive and migratory potential of endometrial cells which may be a cause of endometriosis.

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

Our earlier studies have shown a cycle-dependent expression of endometrial RAGE and HMGB1 during the estrous cycle in rats. During the reporting year, endometrial RAGE expression was investigated during embryo implantation in rats. Preliminary studies have indicated a modulation in the expression of endometrial RAGE during

embryo implantation in rats. Further, *in vitro* studies showed an increase in the expression of RAGE on stimulation of endometrial epithelial cell line (Ishikawa) with Advanced Glycation End product (AGE). Studies are on-going to investigate the effect of AGE on embryo implantation.

2.1.10 Identification of Novel Biomarkers for Endometriosis

A mouse model has been developed for endometriosis and characterized for its similarity to the human conditions. In this autologous model of endometriosis, the lesion appeared haemorrhagic initially and eventually became large and fluid filled. At later days the lesions were surrounded by extensive adhesions. Histologically, glands and stroma were observed, the glands were healthy and proliferating and later on became dilated and cystic. The glandular epithelium stained positive for cytokeratin, the stroma was vimentin positive. There was extensive glandular proliferation and minimal cell death. In the ectopic tissues, the mRNA expression of steroid receptors mainly estrogen receptors reduced progressively. This mouse model of endometriosis mimicked the classical features of endometriosis in women.

This model was next utilized for biomarker discovery where plasma samples of mice with and without endometriosis were subjected to 2D PAGE and the differential proteins were identified by LC MS/MS. Vitamin D binding protein, serum albumin, fibrinogen β and γ chains, apolipoprotein were found to be differentially present in the two groups. We are presently characterizing these proteins and screening the sera using iTRAQ for more biomarkers.

2.1.11 Development of a Non-human Primate Model for Endometrial Hyperplasia

Studies were undertaken to develop a nonhuman primate model for endometrial hyperplasia, with an objective to elucidate the mechanisms underlying endometrial hyperplasia. We have shown that an unopposed exposure of estrogen in ovariectomized marmosets leads to excessive stromal and glandular epithelial cell proliferation, akin to endometrial hyperplasia. Immuno-histochemical analysis demonstrated dysregulated expressions of PTEN, ER- α , COX-2, PCNA, and pAKT.

These molecules have also been implicated in the development of human endometrial hyperplasia. Endometrial transcriptomes of the estrogen-treated marmosets, exhibiting morphological phenotypes of endometrial hyperplasia, are being investigated to explore whether these animals simulate human endometrial hyperplasia at the molecular level.

2.1.12 Collectins and other Immune Related Proteins in Immunoregulation during Pregnancy Maintenance

The expression of collectins in the first trimester placental and decidual tissues of women undergoing spontaneous abortions with that of women undergoing medical termination of pregnancy was compared. Surfactant Protein-A (SP-A) transcripts were significantly down regulated while Surfactant Protein-D transcripts were significantly up regulated in the spontaneous abortion group. Mannan Binding Lectin (MBL) transcripts were not significantly altered between both groups. Immunohistochemistry showed that SP-D and MBL are localized to cytotrophoblast and syncytiotrophoblast of the first trimester placenta. Decidual stromal cells also showed significant staining for SP-D and MBL. SP-A staining was not significant in these tissues. The results suggest that collectins are differentially expressed at the first trimester fetomaternal tissues during spontaneous abortion and could be relevant in immunoregulation during placental development.

2.2 Male Infertility

2.2.1 Deciphering role of GRP78 in sperm motility

Taking cues from earlier reports of differential phosphorylation of certain proteins in sperm of asthenozoospermic individuals, GRP78 phosphorylation was characterized further to decipher its functional relevance to sperm motility. Phosphorylated GRP78 was about 2 fold less in asthenozoosperm vis-à-vis normozoosperm. Immunofluorescence (IIF) studies indicated surface localization of GRP78 on the equatorial region in case of rat- and in equatorial, midpiece and neck region in case of human spermatozoa. Surface localization of GRP78 was also confirmed by ELISA. GRP78 was observed to be phosphorylated at serine, threonine and tyrosine residues by

immunoprecipitation studies. Nanofluidic Immunoassay coupled with Phosphatase assays revealed three phosphoforms GP4.96, GP4.94 and GP4.85 in mature rat spermatozoa versus two phosphoforms GP4.96 and GP4.94 in testicular spermatozoa. In human spermatozoa, 3 phosphoforms GP5.04, GP4.96 and GP4.94 were observed, of which, GP4.94 and GP5.04 were significantly reduced in asthenozoospermatozoa. Our study thus provides novel information on the spatial distribution of phosphorylated GRP78 during sperm maturation. It may be inferred that GRP78 phosphorylation in spermatozoa undergoes dynamic changes during its maturation. The observation of reduced phosphorylation of GRP78 in asthenozoosperm and identification of the phosphoforms reduced, suggests relevance of GRP78 phosphorylation to sperm function.

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

The aim of the study is 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. Further, the expression of genes associated with sertoli cell and granulosa cell differentiation in the embryonic gonads of XX mice lacking *Lhx2* was profiled. The results revealed that loss of *Lhx2* promoted sertoli cell differentiation and failure of granulosa cell differentiation in the XX gonads. These observations point towards a central role of *Lhx2* in ovarian determination.

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

Immunoregulatory proteins such as Collectins are present in the human testes and may play an important role in regulating the immune response. We have previously shown that expression of collectins is developmentally regulated in mice testis and MBL exhibits stage specific expression during murine spermatogenic cycle. Purified testicular somatic cells were found to bind to recombinant SP-D in a dose dependent manner. Increased transcript levels of immunosuppressive molecules (viz. TGF- β , IL-10 and serpin3n) and increased numbers of F4/80+ and MHC II+ cells

were observed in the testis of SP-D-/- mice than wild type mice.

During the reporting period, we analysed the murine testicular cell types for expression of collectins. Testicular germ cells expressed SP-A, SP-D & MBL-A transcripts and myoid cells expressed MBL-A. Confocal microscopy showed cytoplasmic localization of SP-A, SP-D & MBL-A in germ cells, whereas SP-A also showed nuclear localization in germ cells. MBL-A showed nuclear and weak cytoplasmic localization in myoid cells. Testosterone suppression model using combined treatment with GnRH antagonist, acyline and AR antagonist flutamide was used to assess the hormonal regulation of collectin expression in the testis. Treated mice showed reduced testosterone production and a perturbed spermatogenesis. Importantly, these mice showed significant downregulation of collectin transcript expression in testes suggesting that testosterone positively regulates SP-A, SPD and MBL-A expression. SP-A and SP-D were found to be localized on caudal sperm head and tail, whereas distinct staining for MBL-A was observed on connecting piece and tail of caudal sperm. The presence of collectin expression on sperm could help in the establishment of immune tolerance at the level of male ejaculatory duct and in female reproductive tract.

2.2.4 The Role of DAZ Gene Copies in Infertile Males: Its Impact on Fertilization and Embryo Quality

To determine the genetic basis of male infertility, we focused on the AZFc cluster on the Yq that harbours candidate genes essential for spermatogenesis. Three types of AZFc subdeletions were identified. Among them, the gr/gr and b1/b3 but not b2/b3 subdeletions show a strong association with male infertility. The b2/b4 deletion was not detected in any of the subjects. In men with AZFc subdeletions, loss of two DAZ and one CDY1 gene copy made them highly susceptible to azoospermia and severe oligozoospermia. These subdeletions had no effect on ICSI outcome, albeit there were an increased number of poor quality embryos in AZFc subdeleted group. These results suggest that the AZFc subdeletions are a major risk factor for male infertility and the deletion of both DAZ and

CDY1 gene copies increases its susceptibility to azoospermia or severe oligozoospermia.

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

Congenital bilateral absence of vas deferens (CBAVD) occurs in 2-6% of infertile but otherwise healthy men and is associated with mutations in cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Due to the limited studies in Indian population, there are no guidelines and mutation panel for screening the CBAVD males and female partners, opting ICSI. Therefore, present study has been carried out to identify the spectrum and frequency of *CFTR* gene mutations in Indian males with CBAVD. A total of 64 CBAVD males, their female partners and 50 controls have been recruited in this study. Out of these 64 CBAVD patients, 7 patients were found to have unilateral renal agenesis (CBAVD-URA). Four had left renal agenesis and 3 had right renal agenesis. Two CBAVD-URA males had bilateral absence of seminal vesicles. In all CBAVD-URA patients, only head of the Epididymis was palpable and the rest of the epididymis was absent. The direct DNA sequencing analysis of all 27 exons, exon-intron boundaries and essential promoter of *CFTR* gene in 5 out of 7 CBAVD-URA patients identified IVS8-5T mutation in 4 of the CBAVD-URA males with an allelic frequency of 40%. Nine novel *CFTR* gene variants, four potential regulatory coding *CFTR* gene variant and eight previously reported *CFTR* gene variants were identified in these males. Three potential regulatory *CFTR* gene variants (V470M, T854T, and Q1463Q) were also detected along with IVS8-5T mutation in 3 CBAVD-URA males. The female carrier status was also evaluated. Further studies are on-going to screen the complete *CFTR* gene in Indian CBAVD males to develop mutation panel for Indian population.

2.2.6 Design of Constructs Using Testis Specific Promoters and Generation of Transgenic Infertile Mice

Restricted availability of retinoic acid (RA) in testicular milieu regulates transcriptional activity of

c-kit (KIT, CD117), which concurrently determines the destiny of spermatogonial stem cells towards differentiation. During the reporting year the mode of genomic action of RA has been studied. A novel retinoic acid response element (RARE) positioned at -989 nucleotides upstream of transcription start site (TSS) that provides a binding site for dimeric RA receptor i.e. retinoic acid receptor gamma (RAR γ) and retinoic X receptor (RXR), has been identified. RA treatment influenced *c-kit* promoter activity along with an endogenous *c-kit* expression in C18-4 cells. Promoter deletion assay led to the identification of regions spanning -271 bp and -1011 bp upstream of TSS as minimal promoter and maximal promoter respectively. *In silico* analysis predicted region -1011 to +58 bp comprised of distal enhancer RARE and activators such as PU.1, Sp1 and four *ETS* tandem binding sites at proximal region. The gel retardation and ChIP assays showed binding for RAR γ , GABP α , PU.1 and Sp1 to predicted consensus binding sequences, whereas GABP α occupied two out of four *ETS* binding sites within the *c-kit* promoter region. We propose that in response to RA, an enhanceosome is orchestrated through scaffolding of CBP/p300 molecule between RARE and elements of proximal promoter region and control the germ line expression of *c-kit* gene.

2.2.7 Studies to Elucidate the Molecular Mechanism of Estrogen Action in Spermatogenesis

It is now well evident that estrogen plays a very crucial role in spermatogenesis and male fertility. Estrogen administration or deprivation can have adverse effects on male fertility. Studies are being done to elucidate the molecular mechanism of estrogen action during spermatogenesis using receptor subtype specific agonist and antagonist.

Spermiation, the sperm release process, is imperative to male fertility and reproduction. Morphologically, it is characterized by removal of atypical adherens junctions called ectoplasmic specializations, and formation of transient endocytic devices called tubulobulbar complexes requiring cytoskeleton remodeling and recruitment of proteins needed for endocytosis. Earlier studies in the laboratory demonstrated that estrogen

administration to adult male rats causes spermiation failure due to disruption of tubulobulbar complexes. This was accompanied by a reduction in the intratesticular testosterone levels and an increase in the intratesticular estrogen levels along with deregulation of genes involved in cytoskeleton remodeling (Arpc1b, Evl and Capg) and endocytosis (Picalm, Eea1 and Stx5a). Studies were done to understand the role of estrogen and androgen in regulating these genes independently using seminiferous tubule culture system treated with estrogen, androgen or agonists and antagonists of estrogen receptors. We found that transcripts of Arpc1b, Evl and Picalm are responsive to estrogen while those of Picalm, Eea1 and Stx5a are responsive to androgen. It was observed that the estrogen regulation of Arpc1b and Evl is mediated through estrogen receptor β and that of Picalm occurs through estrogen receptors α and β . Localization of these proteins at or in the vicinity of tubulobulbar complexes suggests a possibility of the involvement of ARPC1B, EVL, PICALM, EEA1 and STX5A in spermiation. Thus, estrogen and androgen regulate specific genes in seminiferous tubules that could play a role in spermiation. Further *in-vivo* studies in rats demonstrated spermiation failure with estrogen receptor beta specific agonist, thereby suggesting that estrogen through its beta receptor may regulate the sperm release.

3. RTI/ STIs/HIV/ MICROBICIDES

3.1 Association of Host Immunogenetic Factors with HIV Transmission.

A comparative study between 30 positive and 62 HIV exposed negative infants born to HIV mothers revealed association of two specific SNPs: IL1R1 (rs2234650) and TNFA (rs1800629) with HIV transmission. CT genotype at IL1R1 was observed in a higher frequency in positive children (76.66% vs. 42.62%, $p=0.002$), while CC genotype frequency was significantly increased in exposed uninfected children (47.54% vs. 16.66%, $p=0.004$). Similarly, the GG genotype of TNFA was significantly high in uninfected children as compared to infected ones (76.66% vs. 46.66%, $p=0.005$), while the GA genotype frequency was high among the infected children (53.33% vs. 21.66%, $p=0.003$). Presence of these SNPs seems to be associated with altered immune response protecting infants from acquiring infection.

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

The effect of SsALF24 on the autophagy response of the host in the presence of LPS was reported last year. Studies were conducted to decipher the role of autophagy during *Candida albicans* infection *in-vitro*. Results demonstrated that *C. albicans* hyphae, the infectious form of the pathogen, enter into VK2/E6E7 cells. Studies are under progress to decipher the exact mechanism by which autophagy responds to *C. albicans* infection and also to assess the effect of SsALF-24 on *C. albicans* hyphae formation.

3.3 Development of a Multistrain Probiotic Lactobacillus Formulation Effective against Reproductive Tract Infections

The objective of our study was to identify the spectrum of vaginal lactobacilli in healthy Indian women, evaluate them for their functional abilities and develop a probiotic for control of infections in reproductive tract. Vaginal swabs from clinically healthy participants were assessed by Nugent scoring and Amsel criteria. The Nugent scoring indicated that 22.3% women, though clinically healthy, were asymptomatic for bacterial infections. Lactobacilli population was found to be heterogeneous in majority of women. *L.iners*, *L.rhamnosus*, *L.reuteri*, *L.gasseri* were the predominant species identified in vaginal samples of healthy women.

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

Studies to evaluate the interaction of HbAHP-25 with gp120 were conducted during the reporting year. It was observed that HbAHP-25 binds to CD4 binding pocket on gp120 with high affinity and blocks interaction of gp120 with CD4. Further, HbAHP-25 exerts its inhibitory activity, irrespective of the tropism of HIV-1. HbAHP-25 showed activity against both CXCR4 and CCR5 tropic viral strains. This is indeed a significant observation considering that majority of the HIV entry inhibitors are either CCR5 antagonists or CXCR4 antagonists. Studies are in progress to assess both *in vitro* and *in vivo* efficacies of HbAHP-25 against clinical isolates of HIV.

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

Immune response is a double-edged sword, as excessive inflammation can cause chronic inflammatory disease. Hence, the innate immune system develops complicated self-regulatory systems, so that this 'sword' does not damage the host. However, mechanisms regulating excessive inflammation that maintain homeostasis at the mucosal surfaces are not known. In the previous year, we demonstrated the identification of micro RNA, LAT7F and its involvement in human vaginal cell immune responses. During the reporting year, Let-7f miRNA expression was validated by qPCR in End1/E6E7 cells. These cells were treated with PRR ligands such as CpG-ODN, poly(I:C)-LL and poly (I:C) for different time periods. The fold change in down-regulation of let-7f miRNA at 1 hr post stimulation was found to be 2 fold with CpG-ODN stimulation as per microarray data. The qPCR results showed 1.98 and 2.5 fold down-regulations of let-7f miRNA at 2 and 24 hrs post stimulation respectively. The fold change up regulation of let-7f miRNA was found to be 1.8 fold at 1 hr post stimulation with poly(I:C)-LL as per microarray data. The qPCR data revealed that a 1.67 and 2.78 fold up regulation of let-7f miRNA at 2 and 24 hrs post stimulation respectively. We infer that CpG-ODN suppress let-7f miRNA (induce tolerance) while poly (I:C)-LL up-regulate let-7f miRNA expression (prevent tolerance).

3.6 Approaches for Controlling Biofilm formation by *Gardnerella vaginalis*

Gardnerella vaginalis is a predominant anaerobe linked with bacterial vaginosis (BV), a genital tract ailment affecting women in the reproductive age. Biofilm formation by *G. vaginalis* is often associated with recurrent BV which does not get resolved with antibiotic treatment. Efforts were continued towards developing agents which could either prevent or limit biofilm formation by *G. vaginalis*. Studies carried out with the rationally designed peptides, GVBAP-7 and GVBAP-11 demonstrated moderate anti-biofilm activity. Suitable sequence modifications will be carried out to enhance the anti-biofilm activity of these peptides. Additionally, peptides reported in literature to

prevent biofilm formation by other organisms are also being explored. Apart from the peptide based approach, the action of certain small molecules such as the antibacterial agent, triclosan and its synthetic derivatives was also tested and these were found to possess potent anti-biofilm activity.

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

Integrin $\alpha_4\beta_7$ has been demonstrated to serve as a receptor for the HIV-1 envelope protein, gp120. However, intracellular events occurring subsequent to binding of HIV-1 gp120 to integrin $\alpha_4\beta_7$ as well as their influence on HIV -1 pathogenesis are not completely understood. Previous work carried out by us and others suggested that the interaction of HIV-1 gp120 with integrin $\alpha_4\beta_7$ on CD4+ T cells leads to activation of a signaling pathway which culminates in the activation of another integrin, LFA-1. During the reporting year, a cell adhesion assay was developed to study the interaction of HIV-1 gp120 simulated CD4+ T cells with different cell adhesion molecules. Results demonstrated enhanced binding to ICAM-1. Blocking with antibodies against integrin $\alpha_4\beta_7$ suggest that both LFA-1 activation and subsequent binding to ICAM-1, two processes important for virological synapse formation, are partially dependent on integrin $\alpha_4\beta_7$. The results provide additional evidence for the involvement of integrin $\alpha_4\beta_7$ mediated signaling in cell-cell transmission of HIV-1. Further studies showed that antibody mediated blocking of integrin $\alpha_4\beta_7$ also results in partial inhibition of HIV replication in $\alpha_4\beta_7$ CD4+ T cells. Studies to elucidate the mechanism by which integrin $\alpha_4\beta_7$ mediated signaling influences HIV-1 replication are ongoing.

3.8 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. Earlier studies on a gel formulation of a recombinant fragment of human surfactant protein D (rhSP-D), 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). It had no adverse effect on the viability of vaginal epithelial cells (VK2) (therapeutic index > 10) 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. rhSP-D significantly inhibited the interaction of both soluble and surface expressed gp120 with CD4. Further, bioinformatics analysis suggested common binding sites on gp120 for SP-D and CD4.

The safety of rhSP-D on vaginal physiology was evaluated by evaluating its effect on activation of NFκB using reporter cell line End/ NFκB (human endocervical cell line), on release of immune mediators from vaginal epithelial cells in presence of lactobacilli and in the rabbit vaginal irritation model. rhSP-D (up to 100µg/ml) did not induce NFκB activation indicating absence of inflammation. The levels of chemokines such as Gro-α, MIP-3α and RANTES did not show any alterations as compared to untreated control. Also, levels of IL-1RA and TGF-β (TGF-β1 and TGF-β2) remained unaltered by rhSP-D. Lower, middle and upper vaginal sections of rabbits subjected to 10 consecutive days for vaginal application of gel formulation comprising rhSP-D, did not show any adverse effects on epithelial tissue morphology or leukocyte infiltration compared to placebo (gel alone). The studies suggest that rhSP-D does not induce inflammation and maintains vaginal homeostasis.

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

The cytokine and hormonal profile of SP-D^{-/-} mice was analyzed during the estrus cycle and early pregnancy. SP-D^{-/-} mice showed higher levels of serum progesterone and pro-inflammatory cytokines and chemokines (IL-1b, TNF-α, IL-10, IL-17, MIP1b etc.) in the uterus during the estrus phase. During the pre-implantation period, SP-D^{-/-} mice showed elevated levels of uterine macrophages (F4/80+) with a skewed M1/M2 phenotype and upregulation of pro-inflammatory cytokines (IL-1β, TNF-α, IL-17, RANTES etc.). Data suggests

dysregulated uterine immune profile of SP-D gene deficient mice during early pregnancy leads to elevated pre-implantation loss suggesting that SP-D plausibly regulates the uterine macrophage phenotype and the cytokine milieu critical for embryo implantation.

3.10 Genotypic Characterization of HIV-1 C Variants in PBMCs and Urogenital cells

Our previous study demonstrated the presence of distinct HIV variants in blood, urogenital cells and secretions of infected individuals. Translated amino acid sequence of C2-V3 region of env gene of HIV1C in PBMCs (n=26 male and 18 females), sperm (n=11), vaginal epithelial (n=14) and cervical cells (n=14) showed the presence of distinct variants in same individuals. Some of the viral variants showed the most predominant conserved region in C2 and V3 region in male and females PBMCs. Identification of these conserved regions in different HIV variants are being further investigated using next generation sequencing (NGS) of HIV variants in different cells and secretions of the same individual. This may be useful in identifying the epitopes responsible for broadly neutralizing antibodies (bnAbs) to HIV isolates and development of peptide based preventive and/or therapeutic vaccine against HIV and response to ART drugs. The genotypic characterization of env gp41 of HIV1 C from circulating plasma viral RNA and proviral DNA from PBMCs using next generation sequencing (NGS), Illumina Miseq demonstrated the presence of multiple variants ranging from single to 28 different variants in the same individual which were further characterized by phylogenetic analysis with HIV-1 M group reference sequences reported globally. The study may also provide additional information regarding the association of these variants with broadly neutralizing antibodies which may be useful for development of vaccine against HIV. The study is being continued using more number of study participants.

HIV variants in PBMCs and urogenital cells of the same individual may have different affinity to host cell receptors/co-receptors. Immune cells may influence sexual transmission of HIV, response to antiretroviral therapy (ART) and progression to disease.

3.11 Next Generation Sequencing Based Characterization of Broadly Neutralizing Antibody Epitopes in HIV Infected Individuals

Under this project, work relating to HIV immunopathogenesis and vaccine development with respect to broadly neutralizing antibodies (bnAbs) has been initiated. These are recognized to be important immune mediators in the control of several viral diseases including HIV. Vaccination with epitopes of these antibodies is an on-going global effort to produce a highly efficacious anti-HIV vaccine. Currently, an exploratory study, undertaking genetic characterization of such linear, non-conformational epitopes present in the membrane proximal external region (MPER) of HIV-1 envelop in HIV-1 infected individuals is ongoing. Both circulating virus (from plasma) as well as proviral sequences from PBMC of primary samples have been analyzed *ex vivo*. Using PCR, RT-PCR and sanger sequencing as well as next generation (Illumina) sequencing (NGS) platforms this study has resulted in the characterization of sequence diversity in epitope sequences present in the Membrane Proximal External Region (MPER) of HIV-1 for bnAbs 2F5, Z13e1, 4E10 and 10E8. The viral load and CD4 counts did not show any correlation with the viral variation. The 2F5 epitope was found to be more conserved along with C terminal region of 10E8 amongst the epitopes analyzed in the study. Haplotype analysis performed for 10E8 epitope revealed 17 sequences that have not been tested for neutralization thus far, out of which 13 sequences are entirely novel. *In silico* docking for predicting the sensitivity of these haplotypes to the 10E8 antibody was performed and 10 out of 17 sequences were found to be potentially resistant. This information will be critical for guiding the composition of vaccines being developed not only for India but also for HIV-1 clade C in general.

3.12 Immune Dysregulation in HIV Pathogenesis

CD4⁺ regulatory T cells (Treg) are known to be associated with T-cell homeostasis and the control of immunopathology associated with persistent pathogens. In HIV/AIDS, they may contribute to viral-persistence through the suppression of specific T-cell responses, whilst simultaneously

limiting damage resulting from continuous immune stimulation. However, investigations conducted till date have focused on the role of circulating Treg with HIV-1 Clade B and HIV2 isolates only. Role of CD4 Treg cell has not been explored in Indian HIV1 C and HIV-1/ HIV-2 dually infected individuals. The present preliminary study evaluated the frequencies of these cells in HIV-1, HIV-2 and HIV-1/HIV-2 (dually) infected individuals using multiparametric flow cytometry.

CD4⁺CD25(IL-2 receptor)⁺ CD127(IL-7 receptor)-FOXP3⁺ Treg cells were identified from Peripheral Blood Mononuclear Cells (PBMC) of 10 healthy, 10 HIV-1 infected and 9 HIV-2 infected individuals on anti-retroviral therapy (ART) by multi-parametric flow cytometry. The frequencies of CD4⁺CD25⁺CD127⁻ T reg cells were found to increase in HIV-1 infected individuals as compared to healthy controls and HIV-2 infected individuals. This suggests the possibility of distinct pathogenesis mechanisms for HIV-1 and HIV-2 infected individuals with respect to IL-2 and IL-7 homeostasis in HIV infection which further need to be evaluated in more individuals.

4. MENOPAUSE AND OSTEOPOROSIS

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

With the increase in life expectancy, osteoporosis has become a formidable public health problem in India and a multidisciplinary approach is needed for its management. Biochemical markers of bone turnover provide valuable insight into the complexities of bone metabolism. Osteocalcin (OC), procollagen peptide of type I collagen (PINP), C terminal cross linked telopeptide (CTX) and pyridinium cross links (PD/DPD) are sensitive markers for assessment of bone health. A project to study multiplexing of these markers in an array was initiated. The key immunoreagents (specific antisera and enzyme label) were generated for the four identified bone markers and used for developing Sandwich ELISAs. These ELISAs were validated for sensitivity, specificity, accuracy. The transformation of these individual ELISAs in an array format is currently on going.

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

Monocytes are precursors of osteoclasts, the bone resorbing cells and thus proteins relevant to osteoclast genesis can be unravelled in monocytes. Through comparative studies employing 4 plex iTRAQ coupled to LC-MS/MS, we previously reported that Heat Shock Protein 27 (HSP27) was distinctly up-regulated in low BMD conditions in premenopausal and postmenopausal categories. We estimated HSP27 (tHSP27) and phosphorylated HSP27 (pHSP 27) in sera from premenopausal and postmenopausal women and observed tHSP27 and pHSP levels were elevated significantly in low BMD conditions in both these categories. However, pHSP 27 exhibited a significant odds ratio (1.647, $p=0.004$) to differentiate between low and normal BMD. A novel inverse relation was observed between serum pHSP 27 and BMD in Indian women.

4.3 Premature Menopause in North East: A Risk Factors Evaluation Study

Premature menopause is known to affect 1% of all women under the age of 40 years and early menopause is reported to affect approximately 5% of women between ages 40 and 45 years. These women are at risk for infertility, psychological distress, cardiovascular events, osteoporosis etc. Ethnicity/genetic factors, smoking/tobacco uses are the important factors determining the age at menopause. Studies have been undertaken to identify the risk factors of premature and early menopause in women from the North East region of India and compare it with their counterpart in Mumbai.

Standardization for estimation of Serum Estradiol, Follicle stimulating hormone, Anti mullerian hormone levels, fasting blood sugar, thyroid stimulating hormone, Prolactin, Luteinizing hormone, DHEAS, leptin, adiponectin by ELISA has been done. Standardization to detect polymorphism in INHA and FSHR gene has also been completed.

5 MATERNAL AND CHILD HEALTH

5.1 A Prospective Observational Study of “Near Miss” Obstetric Events and Maternal Deaths in Tertiary Hospitals of Mumbai

The study was conducted in two tertiary hospitals of Mumbai viz. L.T. Municipal General Hospital (LTMGH) and Sir J.J. group of hospitals (JJH) in which near miss events and maternal deaths were reviewed for a period of one year and interviews of survivors of near misses were also conducted. Data analysis revealed 884 near miss events occurred in 877 women as 7 women were readmitted for these events and 94 maternal deaths were reviewed. Ratio of maternal death to near miss events was 1:9. The study has provided personal, family, community and health system recommendations which could reduce maternal deaths in the state.

5.2 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 the current proposal was to study risk factors associated with preterm birth among women who conceive by Assisted Reproduction Techniques in Mumbai. Objectives of the study were to study 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 (PCOS), related to the artificial reproductive techniques like stage of embryo transfer, cervicovaginal infections and other risk factors) with preterm birth among pregnancies conceived by ART (iii) neonatal outcome in preterm births among women who conceive by ARTs and (iv) To identify risk reduction strategies to prevent preterm birth in pregnancies conceived by ARTs.

409 participants (women being pregnant by use of ART) were contacted for the study. Out of these, 152 participants were enrolled for the study and 113 follow ups could be completed. Out of the 113 pregnancies, there were 12 miscarriages (3

heterotrophic ectopic gestations). The mean age of participants was 33 years. Multiple gestations were observed in 51 participants (45.1%) and singleton pregnancies in 62 participants (54.9%) which were associated with multiembryo transfer. In 17 (15.04) patients pregnancy was complicated by preeclampsia and 12 (11%) developed gestational diabetes which was higher in this cohort. Seventy seven women (73.5%) had preterm deliveries. The incidence of preterm births in this study was observed to be 74.5%. The incidence of caesarean section was very high (97.02%). The commonest indication mentioned for Elective LSCS was IVF pregnancy or precious pregnancy. There were 3 neonatal deaths, two stillbirths and one congenital anomaly involving multiple systems. The two stillbirths were seen in preterm deliveries. Interestingly, 3 cases were recruited who had heterotrophic pregnancy due to multiembryo transfers.

The study reveals very high incidence of preterm birth and caesarean sections among pregnancies conceived by ART. The course of pregnancies and the health of children born after assisted reproductive techniques are two of the most important 'outcome parameters' of the quality of the outcomes of ART. The most appropriate end point after assisted reproduction should also include preterm or term birth as measure of success.

5.3 Lactogenic Properties of Indigenous Medicinal Plants

Breast feeding is the best method for reducing the rate of infant's malnutrition. Many traditional practices have been utilized for increasing milk quantity during lactation. However, there is no scientific validation on these claims of traditional practices established earlier in a scientific way. On considering this aspect, the present investigation is based on finding of galactagogue from herbal remedies of *Cyperus rotundus* (Nagarmotha), *Foeniculum vulgare* (Fennel), *Gmelina arborea* (Shiwan), *Gossypium herbaceum* (Kapashy), *Ipomoea digitata* (Bhui-kohala), *Lepidium sativum* (Aalive), *Pennisetum americanum* (Bajra), *Ricinus communis* (Castor) and *Trigonella foenum-graecum* (Methi) using Holtzman rats. Female rats received oral doses of aqueous extract of these plants during their first lactation. 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 women.

6 ADOLESCENT HEALTH SERVICES

6.1 Strengthening the ARSH Services as per the National Standards of Adolescent Reproductive Sexual Health (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 Government 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.

The study has given inputs to make Adolescent Friendly Health Services functional at grass root level, has been successful in sensitizing the community on adolescent issues through NGO support and involvement of ASHAs and Anganwadi workers. The process of undertaking quality assessment survey to assess the weak areas and develop strategies to address them has helped the ARSH program become functional. A Dissemination meeting was conducted in 2014 and the products and processes have been shared with Government of Maharashtra. Eight adolescent friendly health centres in Karjat block have been included in the Programme Implementation Plan of Government of Maharashtra and will be sustained by the Government.

7. REPRODUCTIVE CANCERS

7.1 Hormonal Regulation of Human Telomerase and its Possible Implications in the Pathogenesis of Prostate Cancer

Telomerase activation allows cancer cells to bypass replicative senescence. Prostate Cancer (PCa) cells, which are initially androgen-dependent and then become androgen-independent, are characterized by high telomerase activity. In the reporting year, studies were undertaken to investigate whether the catalytic unit of telomerase i.e. Human Telomerase

Reverse Transcriptase (hTERT) is regulated by androgen receptor (AR) and whether this occurs in similar fashion in androgen-dependent and androgen-independent PCa cells. Our investigations demonstrated a significant reduction in hTERT protein expression and telomerase activity, following the attenuation of AR expression in LNCaP, an androgen-dependent PCa cell line and paradoxically, also after the forced expression of AR in PC3 and DU145, androgen-independent cell lines (which inherently express low levels of AR). These studies highlighted hTERT expression by AR in a cell-context dependent manner. AR acts as a positive regulator of hTERT expression in androgen-dependent cells and as a negative regulator in androgen-independent PCa cells. These studies also hint at the possibility that the complete neutralization of AR function may not prove beneficial in the management of androgen-independent prostate cancer.

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

Previous results showed the presence of estrogen binding proteins or estrogen receptors on the cell surface of androgen-dependent prostate cancer cells (LNCaP), androgen-independent cells (PC3, DU145), non-tumorigenic prostate epithelial cell line (RWPE1) and also on the cell surface of normal rat prostatic cells. We also demonstrated that the activation of cell surface estrogen receptors leads to phosphorylation of several proteins. MALDI TOF-TOF analysis revealed identity of some of these proteins. Among these were two cytoskeletal proteins such as β actin and cytokeratin 8. Validation studies were conducted to confirm differential phosphorylation of β actin and cytokeratin 8 in LNCaP cells in response to stimulation with E-BSA. Further, flowcytometric analysis revealed higher abundance of cell surface estrogen receptors viz; ER α and ER β in nontumorigenic prostate epithelial cells, compared to tumorigenic prostate cancer cells. Also, investigations revealed that cell surface localization of ERs involves intracellular transport through Endoplasmic Reticulum and Golgi complex. Further studies are underway to elucidate the role of cell surface estrogen receptors in prostate epithelial cells.

7.3 Understanding the Role of CRISP-3 in Prostate Cancer

CRISP-3 is one of the most up regulated genes in prostate cancer. CRISP-3 knockdown experiments using prostate cancer cell line, demonstrated that CRISP-3 affects prostate cancer cell invasion. In order to understand the mechanism of CRISP-3 up regulation in prostate cancer, we cloned CRISP-3 promoter fragments of different sizes in luciferase reporter vector. Luciferase assay identified a region showing strong promoter activity which was active in cell lines expressing (LNCaP) or not expressing (PC3) CRISP-3 endogenously. PC3 cell line showed CRISP-3 expression upon treatment with Trichostatin A indicating that CRISP-3 promoter is deacetylated in PC3 cells. To study the CRISP-3 promoter in the context of chromatin, stable clones using the longest CRISP-3-luciferase construct in PC3 as well as LNCaP cell line were generated. Luciferase assay carried out in stable clones of LNCaP showed ~50 fold higher luciferase activity than the PC3 clones.

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

Cervical cancer in Indian women reveals contrasting association among common sub-family of HLA class I alleles. Study on HLA polymorphism of 75 cervical cancer cases were compared with 175 non cancer controls. Results revealed HLA-B*37, -B*58 were associated significantly with increased risk while HLA-B*40 with decreased risk for cervical cancer. At high resolution analysis after Bonferroni correction, HLA-B*37:01 allele was associated with increased risk whereas HLA-B*40:06 was with decreased risk for cervical cancer. HLA-B*37:01 and -B*40:06 belong to the same super family of HLA-B44. In silico analysis revealed different binding affinities of HLA-B*37:01 and -B*40:06 for the epitopes predicted for E6 and L1 proteins of HPV16. The higher binding affinity of epitopes to B*40:06, as revealed by docking studies, supports the hypothesis that this allele is able to present the antigenic peptides more efficiently than B*37:01 and thereby protect the carriers from the risk of cervical cancer. Thus, there is a clear indication

that HLA plays an important role in development of cervical cancer in HPV infected women. Identification of these factors in high risk HPV infected women may help in reducing the cervical cancer burden in India.

8 STRUCTURAL BIOLOGY AND BIOINFORMATICS

8.1 Functional Significance of Novel Mutations in Follicle Stimulating Hormone Receptor Gene

Interaction between follicle Stimulating hormone (FSH) and its cognate receptor plays crucial role during ovarian folliculogenesis. Naturally occurring mutations in FSH receptor (FSHR) gene have been reported to cause reproductive abnormalities. The two mutations Val⁵¹⁴Ala (novel mutation) and Ala⁵⁷⁵Val in FSHR have been identified in women with OHSS developed during in vitro fertilization (IVF) and primary amenorrhea respectively. The functional characterization of these mutations revealed that the mutation at position 514 increases the cell surface expression and cell signaling at lower dose of FSH stimulation, thereby activating the receptor function. On the other hand, mutation at position 575 was defective in cell surface trafficking and FSH induced signaling, which leads to inactivation of the receptor function. These results suggest that OHSS and primary amenorrhea observed in the two affected woman respectively, could be attributed to the functional characteristics of respective mutant FSHR.

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

Role of specific residues in the extracellular loop2 of follicle stimulating hormone receptor was investigated. Towards this, FSHR mutants L⁵⁰¹F and I⁵⁰⁵V were characterized with respect to their role in FSH induced ERK phosphorylation. Both these mutants showed defect in ERK signaling pathways suggesting that L⁵⁰¹ and I⁵⁰⁵ residues are crucial for FSHR function.

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

Common marmoset (*Callithrix jacchus*), a New World primate monkey, is used as an animal model

in the area of reproductive biology. *Callithrix jacchus* specific FSH and anti-FSH antibodies are required in such studies for superovulation and FSH measuring assays respectively. During reporting period, *Pichia pastoris* expressed recombinant marmoset FSH was characterised for biological activity. Polyclonal antibodies were raised against peptides corresponding to marmoset FSH β -subunit and characterised for marmoset FSH binding.

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

A sequence-based method for identification of antimicrobial peptides (AMPs) using family specific sequence signatures has been developed. Using this method, a large number of AMPs which are absent in popular AMP databases, have been identified.

8.5 Creating a Gene Based Repository for Infertility

Several genetic disorders or genetic polymorphisms/ mutations in genes responsible for reproductive function can lead to infertility in both males and females. Developing a database with information on all these genes and their function along with reported polymorphisms/ mutations will help researchers and clinicians in screening patients with infertility and deducing the cause of infertility. The work on this database is in progress and it currently holds information on sequence, structure, function etc. for 300 genes. Literature mining has been completed for 24 genes.

9 STEM CELL BIOLOGY

9.1 Preclinical Studies with Pancreatic and Tri-Potent Cardiac Progenitors Obtained from In-house Derived Embryonic Stem Cell Lines

Human embryonic stem (hES) cells derived pancreatic progenitors were packed in an immunosolatory device and transplanted in streptozotocin treated mice. The mouse showed detectable levels of human c-peptide after 45 days of transplantation suggesting maturation of the progenitors post-transplantation. This experiment will now be conducted in more number of mice.

Comparison of the polycomb group proteins profile of hES cells derived pancreatic progenitors and adult human pancreas revealed distinct differences, which may ameliorate after transplantation or possibly is the underlying cause to explain that hES cells differentiate into their fetal counterparts and may not be very useful to regenerate adult pancreas.

In another arm to this study, it has been shown that endogenous pluripotent very small embryonic-like stem cells (VSELs) in the adult mouse pancreas can regenerate both islets and acinar cells after partial pancreatectomy. Thus VSELs may be a better candidate to regenerate a diabetic pancreas. Further studies on regeneration of adult diabetic pancreas by manipulating endogenous VSELs will be undertaken.

VSELs in mammalian gonads

Our investigations have demonstrated that VSELs, which survive in chemoablated mouse ovary and testis have the ability to regenerate the gonads. This implied that VSELs can differentiate into gametes, something that has yet not been achieved after 30 years of research on mouse embryonic stem cells and 15 years of research on human embryonic stem cells. The underlying reason is that VSELs are the primordial germ cells (PGCs) derived from the epiblast stage embryo which have a distinct epigenetic status and are pre-programmed to differentiate into gametes. In contrast, embryonic stem (ES) cells are derived from inner cell mass of developing blastocyst and it is difficult to differentiate ES cells *in vitro* into PGCs. Work during the reporting year has shown that VSELs enriched from chemoablated gonads spontaneously differentiate into oocytes and sperm *in vitro*.

10. NATIONAL CENTRE FOR PRECLINICAL REPRODUCTIVE AND GENETIC TOXICOLOGY

10.1 Exposure to Endocrine Disrupter, Bisphenol A (BPA) in Neonatal Rats: Epigenetic Effects on Male Germ Line

BPA induces adverse effects on male fertility which persist well after the exposure. Our earlier study demonstrated, that neonatal exposure to BPA causes

epigenetic changes in ER α and ER β expression by altered DNA methylation and also perturbation in DNA methyltransferases expression in testis during adulthood.

Histone methylation is one of the primary epigenetic modifications of chromatin responsible for regulation of chromatin organization and gene expression. The present study investigated the effect of BPA on histone methylation levels in testis. The site-specific histone methylation is often established and maintained by histone methyltransferases (HMTs). A significant increase in the protein expression of Enhancer of Zeste 2, EZH2 expression in testis was observed upon BPA exposure. Further, a significant down regulation in phospho-AKT levels in BPA treated group as compared to control were observed. The functional significance of increased EZH2 expression was further assessed by examining the expression of H3K27 trimethylation and H3K9 levels. A significant increase in H3K27 trimethylation and H3K9 levels, while decrease in H3K4 trimethylation level was observed in BPA treated group as compared to control, suggesting that neonatal exposure to BPA cause epigenetic changes in testis via aberrant histone methylation.

10.2 Genotoxic and Mutagenic Activity of Bisphenol A, an Endocrine Disruptor and Understanding its Mechanism of Action

A significant increase in post implantation loss was observed in females impregnated by adult male exposed to BPA for 6 consecutive days at NOAEL dose (5.0 mg) or at 10 μ g dose. A significant increase in dominant lethal mutations was observed indicating germ cell mutagenicity of BPA. Further, exposure of BPA resulted in reduction in both daily sperm production and testicular sperm efficiency in male rats. Flow cytometry experiments indicated a significant decrease in the number of 1C cells (round spermatids) and 4C-cells (primary spermatocytes) in the treatment group as compared to the control. This was consistent with the observations demonstrating that estradiol at doses of 20 and 100 μ g/kg for 10 days resulted in a significant decrease in the number of 2C-cells and 4C-cells. Studies are ongoing to elucidate the mechanisms mediating the effects of BPA on male germ cells.

10.3 Effect of Bisphenol-A, an Endocrine Disruptor on Spermatogenesis in Common Marmoset at Cellular and Molecular Level

Bisphenol A (BPA) causes endocrine disruption in laboratory animals and health disorders in humans. To investigate its effects, common marmosets (*Callithrix jacchus*) was chosen, and repeated dose toxicity study was undertaken. Testes were processed for transmission electron microscopy studies. Ultra structural analysis revealed thickening of the basement membrane, cytoplasmic vacuolation in Leydig cells and degeneration of developing spermatid in treatment group as compared to vehicle control. In the treatment group, spermatogonia showed evident characteristics of apoptosis with condensed cytoplasm, vacuolization, swollen mitochondria and margination of chromatin. Considering the similarities between marmoset and humans, the study provides some novel information about BPA induced degenerative changes at the testicular level.

Genetic Research Centre

Genetic Research Centre (GRC) is a permanent centre of ICMR carrying out basic and clinical genetic research with respect to genetic disorders prevalent in India. The aim of the Centre is to reduce mortality and morbidity associated with genetic disorders, by appropriate genetic counseling and prenatal diagnosis. The Centre is a national referral centre for prenatal diagnosis of genetic disorders. Till date, 45 families have benefitted from the benefits of genetic technology. The disorders where prenatal diagnosis was provided include Tay Sachs disease, Sandhoff disease, Robinow syndrome, ichthyosis, dyskeratosis congenita, spondylo-epiphyseal dysplasia, xeroderma pigmentosum, glutaricaciduria type 1 and muscular dystrophy. The Centre 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 basis of lysosomal storage diseases

Lysosomal storage diseases are inborn errors of metabolism due to deficiency of specific enzyme

leading to storage of abnormal substrate in organs such as brain, reticulo-endothelial system, bone, skin, eyes, etc. These disorders can be recognized by clinical symptoms such as neuroregression, coarse facies, and skeletal changes. Biochemical diagnoses are based on specific leucocyte enzyme assay. Till date, 220 patients have been characterized. GM2 gangliosidosis was the commonest group (58 % of patients) followed by mucopolysaccharidoses (10.3 %), Gaucher disease (9.2 %), metachromatic leukodystrophy (7.7 %), Pompe disease (5.4 %), GM1 gangliosidosis (3.9 %) and Niemann Pick disease (3 %). Other rare cases included mucopolipidosis and Fabry disease. Mutation hotspots identified included E462V (HEXA), R284X (HEXB) and L444P (GBA) which were used for rapid screening of respective patients.

Clinical and molecular characterization of genodermatoses

Genetic skin diseases such as the ichthyosis (disorders of keratinization), xeroderma pigmentosum (XP), Griscelli syndrome type 2 (disorder of pigmentation) and epidermolysis bullosa are childhood disorders with significant mortality and morbidity. They are autosomal recessive in inheritance. Clinical affection includes skin pigmentary anomalies such as poikiloderma, ocular and cutaneous photosensitivity, albinism, vesiculobullous or scaly rash and predisposition to hematological and skin cancer. Till date, 169 cases have been characterized and are under follow up. Autosomal recessive ichthyosis and epidermolysis bullosa are the commonest (25.2%), followed by Griscelli syndrome (11.8%), Xeroderma pigmentosum (8.8%), ectodermal dysplasia (6.51 %), infantile hyalinosis (5.4 %) and others. Mutation hotspots identified include exon 3 of XPA gene (neurological type of XP), exon 6 of DKC1 gene (dyskeratosis congenita), exon 10 of LMNA (progeria), exon 13 of ANTXR2 gene (infantile hyalinosis).

Molecular analysis of SCN1A gene in Dravet Syndrome

Dravet Syndrome also known as severe myoclonic epilepsy of infancy (SMEI) is an infantile epileptic

encephalopathy that occurs in the first year of life. Mutations in the voltage-gated sodium channel SCN1A gene encoding the voltage-gated neuronal sodium channel alpha 1 subunit (Nav1.1) is the main genetic cause of Dravets syndrome and are accounted for 70-80% of patients. A study has been undertaken to identify novel and known mutations in SCN1A gene in cases of Indian Dravet Syndrome. A total of 26 mutations in 27 clinically diagnosed patients have been identified; one mutation being common in two patients. Out of these 26 mutations, 17 were found to be missense, one was non-sense, six were found to be frameshift mutations. Two intronic variants have been identified. Out of these, one was found to be present at splice donor or acceptor sites. Extramural Research

CHILD HEALTH

Studies on identification of Bifidobacteria in Breast milk of Indian women by various molecular tools and their bioproducts as probiotics:

Breast-fed infants harbour characteristic intestinal bacteria dominated by *Lactobacilli* and *Bifidobacteria* that contribute to the initial establishment of the microbiota in the newborn. Maturation of the immune system and development of the gut is contributed by the gut microbiota. Present study involves isolation and identification of Bifidobacteria in Human breast milk and comparison of these new bacterial species obtained with the already existing ones.

A total of 100 women, who fed their babies by exclusive breast feeding, participated in the study. The Bifidobacteria are studied for various probiotic properties such as acid resistance, bile salt tolerance, and antibiotic resistance/sensitivity etc. Each isolate of probiotic from human breast milk is original and indigenous in nature. After studying the probiotic properties they can be stocked at the National Collection Centre(s). Since these are of human origin they can easily colonize in human intestine and thus help in the treatment of various gastrointestinal diseases and enhancement of immunity. They will also be useful in incorporating as Food supplements Probiotic curd, Yoghurt and other fermented products.



Fig. Photograph showing positive for F6PPK indicating Bifidobacteria genus confirmation.

Role of molecular neuro-biochemical markers in diagnosis and prediction of outcome of meningitis in children

Meningitis and encephalitis are important cause of childhood morbidity and mortality. There is a pressing need for diagnostic tests for guiding treatment, and for being reliable indicators of brain damage and outcome. We hypothesized that serum and CSF concentrations of neurobiochemical markers will reflect neuronal damage. The objectives for the study included detection of neural tissue proteins: Neuron specific enolase and S-100, acute phase reactant proteins: C reactive protein and selected cytokines: TNF α , IL-6, IL-8, level in serum and CSF of children with acute meningitis (bacterial and aseptic) on the day 1 and 5 of hospitalization and serum level at recovery/discharge. These were correlated with the clinical severity of the disease at presentation to hospital and early and long term neurological outcomes. Two hundred and eighty cases were initially enrolled. However, due to clinical and/or technical reasons, 180 samples were finally available for day 1 as well as day 5 for estimating all the neuro-biochemical markers. There were 117 cases of acute bacterial meningitis and 75 of aseptic meningitis. The mean serum TNF α on day 1 as well as day 5 was higher in patients with aseptic as compared to bacterial meningitis. However, the difference was not statistically significant. The mean serum IL-6 on day 1 as well as on day 5 were significantly higher in aseptic meningitis as compared to bacterial

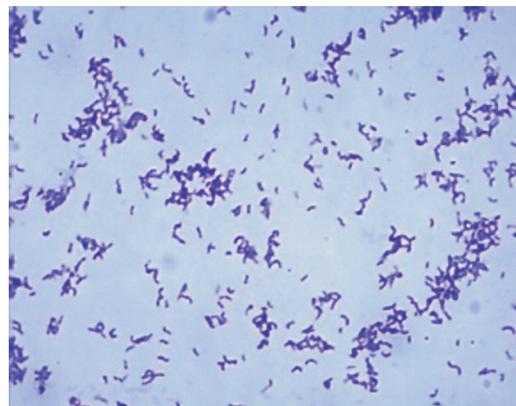


Fig. Photograph showing pure culture of Bifido bacterium isolated from human Breast milk.

(p 0.03). Serum IL-6 was found to be a useful marker to distinguish between bacterial versus aseptic meningitis. Other neurobiochemical markers, including, CSF TNF α , NSE, S-100 and serum IL8 and CRP failed to distinguish between bacterial and aseptic meningitis. Rapid diagnostic test for serum IL-6 should be explored for bedside identification of bacterial versus aseptic meningitis.

Evidenced Based Guidelines are the best external evidence for decision-making about specific health problems. They help in improving the quality of care that patients receive by offering explicit recommendations. The Centre for Advance Research (CAR) on EBCH conducts systematic reviews and educational courses to formulate important evidence informed guidelines which will be helpful to improve child health care programmes in India. Two systematic reviews were published during the year: 1) Distribution of serotypes, vaccine coverage, and antimicrobial susceptibility pattern of *Streptococcus pneumoniae* in children living in SAARC countries: a systematic review. PLoS One. 2014 Sep. 2) Burden of invasive pneumococcal disease in children aged 1 month to 12 years living in South Asia: a systematic review. PLoS One. 2014 May. One review is accepted for publication in World Journal of Pediatrics -Feasibility and usefulness of gentamicin for use at community level: A systematic review based guidance for policy decisions. Two systematic review titles were registered with the Cochrane library in the year 2014. The centre presented 5 posters at the 22nd Cochrane Colloquium held in Hyderabad in September 2014. Based on the published systematic reviews on “pneumococcal burden” and

“distribution of serotypes” the centre was invited to present the findings in the NTAGI sub-committee meeting held on 22/Dec/2014.

An implementation research project **Clusteru** Randomized Trial of a m-Health Intervention *ImTeCHO* to Improve Delivery of Proven MNCH Interventions through Community Based Accredited Social Health Activists (ASHAs) has been initiated in tribal areas of Gujarat *ImTeCHO* is a platform based on mobile phone technology to improve coverage of proven MNCH interventions through improving performance of ASHAs by providing support, supervision and motivation. Primary outcomes of interest are: (1) At least 2 home-based newborn care visits by ASHA within one week of delivery and (2) MACCI (Modified ASHA-centric Composite Coverage Index). After completing baseline evaluation the training of ASHA worker in the job aid is being conducted.

A Community-based Perinatal Death Audit in the Urban Slums of Ludhiana

A cross-sectional study was carried out in 91 urban slums of Ludhiana to investigate the perinatal deaths in the population to identify the causes and quantify their contribution to the overall perinatal mortality and suggest measures. A total 1151 perinatal death was audited. There were 810 (70.6%) still births and 338 (29.4%) early neonatal deaths (END), 77.7% of pregnancy/delivery was associated presence of complications. According to ICD- 10 classification most cases were of Birth Asphyxia (13.5%) and 12.8% were due to Meconium Aspiration, 9.8% due to Antepartum Hemorrhage. 12.9% had low birth weight(< 2.5

KG). The first delay in accessing health care was majorly due to irregular antenatal care (677 cases) followed by home delivery (216 cases). Second delay was due to unspecified transport problems 10 cases. Third delay was due to prolonged labor in hospital 247 cases followed by caesarean section for dead fetus (101 cases). 720 (62.6%) of perinatal deaths had a medical certificate of death. Out 720 medically certified death cases, the certificate was not available in 618, hence the cause of deaths could be ascertained in only 102 of 1151 perinatal deaths. In 67.2% of cases death was not registered. This study provided epidemiological data on perinatal mortality in the under-privileged population under study, and identified risk factors for preventive action. Quarterly meetings at an appropriate slum to discuss perinatal deaths of the area, may help in taking some preventive measures in avoiding a perinatal death in future, by creating awareness. The Municipal Corporation was informed about the perinatal deaths which were not registered.

Neurodevelopmental outcome of LBW infants in a tertiary Health centre (CMC Vellore) (<1250 grams) is being evaluated

A longitudinal study on very low birth weight infants (birth weight < 1.5 kg) born in a tertiary hospital, after they leave the Neonatal ICU is ongoing at CMC Vellore. The neurologic and developmental processes of these infants are being followed up until the corrected gestational age of 18 months to 22 months. Among the 776 infants who were admitted into the NICU, the survival rate in the first phase of the study was 92.26% (89.4%-94.54%, 95% CI). The incidence of poor neurodevelopmental outcome was observed in 11.37% (8.6%-14.9% 95% CI) and incidence of Cerebral palsy in this study in 1.1% (0.39-2.63). None of the antenatal, perinatal or neonatal risk factors were related to the poor neurodevelopmental outcome (GQ <2SD) on the bivariate analysis or multivariate logistic regression model. However post-natal growth restriction (Z-score of weight for age below 2 SD) was significantly associated with poor neurodevelopmental outcome. Perinatal asphyxia, post-natal weight and low maternal education level were associated with poor language abilities on multivariate analysis. Low maternal education, small for gestational status and neonatal hypoglycemia were significantly associated with

remaining underweight at two years of age. There was significant difference in the postnatal growth between small for gestational age (SGA) infant and appropriate for gestational age (AGA) babies but no difference in the survival and neuro-developmental outcomes was observed. Study is ongoing.

Postnatal counselling on exclusive breastfeeding using video

Many mothers are unable to practice exclusive breastfeeding due to several issues like lack of confidence in mother, problems with the infant latching or suckling, breast pain or soreness, perceptions of insufficient milk supply. Video has been found to be an effective tool for health education. Impact of video as an educational tool in promotion of exclusive breastfeeding was assessed in a tertiary hospital in Pondicherry. All primi mothers more than 34 weeks' gestation at the time of delivery without any illness that contraindicate breast feeding were enrolled in the study. An one hour video in Tamil regarding exclusive breastfeeding (advantages and importance of exclusive breastfeeding, correct technique including attachment, common lactation problems, and disadvantages of formula and bottle feeding was prepared and played at least thrice a day including visiting hours. After each session, a dedicated health care worker (Staff Nurse - female) summarized the important points and answered to queries if any. There were total of 43,686 deliveries during the period of October 2012 to march 2014, of them 120685 were counselled. 1126 primi mothers were enrolled and exclusive breast feeding rate at 4 moths was found to be significantly higher in the study group. This simple cost effective strategy of health education could be used for the mass in the hospital.

To study the effect of highly active antiretroviral therapy on B Cell subpopulation in HIV infected children

HIV induced alteration in B cell subpopulations has been well characterized in adult HIV infection. However, a little information is available in the children with HIV infection particularly children less than 5 years old. A prospective cohort case control study was carried out in the Pediatric HIV clinic, Department of Pediatrics, AIIMS. Study subject comprises of 50 HIV-1 infected children and

50 Healthy children. HIV-1 infected children less than 5 years age, Infants (in whom ART is indicated), children in whom antiretroviral therapy is indicated based on clinical criteria or immunological criteria, children in whom antiretroviral therapy is not indicated at time of enrolment in the study were included in the study. HIV-1 infected children older than 5 years already receiving highly active antiretroviral therapy (HAART) and who received a blood transfusion in previous 6 months (B cells' profile may vary) were excluded. The diagnosis of HIV-1 infection in children older than 18 months is confirmed by using immunoassays HIV-1 DNA

PCR as per the guidelines issued by NACO The mean duration of the HAART was 10 ±3months. Laboratory findings of HAART naïve and HAART treated HIV infected children at each follow-up visit are summarized in Table 1; with HAART, there was an improvement in CD4 counts and progressive reduction of viral load Table 2.

Significant alteration in B cell compartment of HIV infected children as compared to healthy children was observed. Following HAART, the median (IQR) frequency of naïve B cells increased significantly at 12 months of HAART

Table 1. Immunological characteristics of study participants.

| Characteristics | HIV infected (0 month)(n=50) | Healthy controls (n=50) |
|---|------------------------------|-------------------------|
| Median (IQR)CD4% | 19(14-26) | 35(32-42) |
| Median (IQR)CD4 count (cells/ μ L) | 1192(599-1595) | 1709(997-2271) |
| Median(IQR) CD19% | 17(13-25) | 18(13-24) |
| Median (IQR)CD19 count (cells/ μ L) | 1067(452- 1773) | 766(525- 1350) |
| Median (IQR)HIV-1 viral load (\log_{10} RNA copies/mL) | 5.4(4.8-6.1) | Not applicable |
| Median (IQR)Serum IgG level (mg/dL) | 3968(n=33)*(2721-4888) | 1104(485-2135) |
| Median (IQR)Serum IgA level (mg/dL) | 141.5(n=30)*(67.6-207.6) | 81(47-142) |
| Median (IQR)Serum IgM level (mg/dL) | 170.6(n=30)*(122.6-274.8) | 151(93-255) |

**The assays will be completed along with the follow up samples.

Table 2. Immunological characteristics of HAART treated and HAART naïve HIV infected children at different time-points.

| Characteristics | Baseline 0 Month (n=30) | | 1 st follow up 6 Months (n=30) | | 2 nd follow up 12 Months (n=30) | |
|--|-------------------------|---------------------------------|---|-------------------|--|-------------------|
| | HAART treated (n=21) | HAART naïve (n=9) | HAART treated (n=21) | HAART naïve (n=9) | HAART treated (n=21) | HAART naïve (n=9) |
| Median (IQR) CD4% | 16 (12-22) | 23* (18-31) <i>p=0.02</i> | 21 (19-29) | 27 (16-38) | 29 (20-33) | 27 (19-29) |
| Median (IQR) CD4 count (cells/ μ L) | 960 (593-1529) | 1341 (634-1788) | 1013 (801-1383) | 1111 (831-1662) | 1348* (1031-1544) <i>p=0.06</i> | 1088 (670-1276) |
| Median (IQR) CD19% | 19 (13-29) | 16 (12-17) | 19 (17-29) | 16 (15-25) | 20 (16-25) | 15 (14-25) |
| Median (IQR) CD19 count (cells/ μ L) | 1047 (441-1751) | 917 (323-1597) | 1134 (803-1722) | 739 (503-1530) | 934 (712-1187) | 743 (368-1411) |
| Median (IQR) HIV-1 viral load (\log_{10} RNA copies/mL) | 5.9 (5.1-6.2) | 5.1* (4.7-5.3) <i>p=0.01</i> | 3.0* (1.9-4.6) <i>p=0.02</i> | 4.9 (4.1-5.0) | 2.7* (1.8-4.1) <i>p=0.001</i> | 4.7 (4.0-5.2) |

($p=0.001$). The study has helped shown that B cell compartment of HIV infected children differs significantly from healthy children. HAART tends to normalize the abnormalities caused by HIV in B cell compartment, although full reconstitution of B cell subpopulation was not observed in this study indicating the fact that small amount of plasma viremia is also able to drive HIV mediated B cell activation and exhaustion.

CAR List

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

Future Plans

To initiate programs in the area of Child Development, Adolescent Health, Fetal origin of adult diseases, addressing child health issues in urban slums, addressing issues related to early neonatal death and still birth, Barriers to implementation of proven interventions & social determinants of Child survival. Interventional studies for improved survival in preterm and LBW infants would be initiated.

It is proposed to initiate multicentre study on etiology of childhood pneumonia in India, short course antibiotic therapy for uncomplicated neonatal sepsis. These projects have been approved technically.

An implementation research project for Improving Access to Qualified Health Care Services for Maternal, Newborn and Childhood Illnesses by Mobile Health Hot Line in rural Uttar Pradesh will be initiated. Project is approved technically.

Another study on morbidity and mortality profile of hospitalised children is proposed.

A proposal for a Centre for advanced research on Paediatric Kidney Diseases at AIIMS, N. Delhi is technically approved is under process for financial concurrence; another centre for advance research on Childhood Emergency Care is under review.

DIABETES

Low cost non invasive Blood glucose monitoring

A cost effective model for noninvasive Photo Glucometer based on the principles of Photo Plethysmography was developed. 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 was found 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. Research work still remains to be carried for Performance evaluation and calibration of Standalone Glucometer for mass screening. Miniaturization of Standalone Glucometer.

An Analysis of the effect of Type II Diabetes Mellitus on Male Fertility rates in Mysore Population

Hormone analysis of 250 Diabetic patients found LH, FSH, testosterone and prolactin abnormality in 41.2%, 30%, 18.6 % , 32.8% of diabetics respectively. The levels of ornithine decarboxylase in semen estimated using ELISHA showed lower levels in Diabetics vs control (1.96 ± 0.77 vs 3.96 ± 0.98). The DNA fragmentation in semen and oxidation stress in serum was more among Diabetics.

Development of blood outgrowth endothelial cell and nanofiber scaffold synergy in wound healing

This study was conducted to explore the utility of rat blood outgrowth endothelial cells (BOECs)

(with and without over expression of endothelial nitric oxide synthase (eNOS)) engrafted onto an appropriate scaffold framework as a therapeutic modality for wound healing. Study achieved preparation and standardization of scaffolds with different architectures, Culture of rBOECs and eNOS-BOECs for inclusion in scaffold, Isolation of Endothelial Progenitor Cells from Sprague-Dawley rats and its differentiation, in to Blood Outgrowth Endothelial Cells (BOECs) in vitro. Tested interaction, viability and functionality assessment of rBOECs and eNOS-BOECs on various scaffolds and standardized BOEC-bandage for in vivo application using the chosen scaffold.

Biomaterials tried so far include chitosan, gelatin, polyurethane and poly lactic acid based composite polymers with and without bioactive coatings. The bioactive materials considered were collagen and fibrin, both known to have functionality enhancement for endothelial cell types. The composite scaffold was synthesized by freeze drying technique as follows.

The limitation with chitosan-fibrin 3D scaffolds was the non-uniformity in distribution of fibrin networks throughout the scaffold.

Photographs of chitosan and chitosan-fibrin composite scaffold.

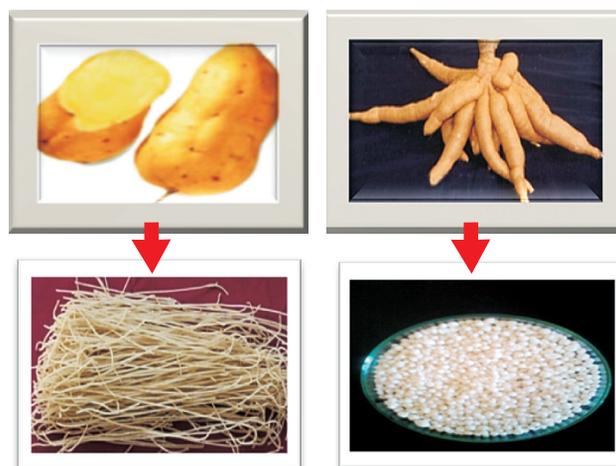


Chitosan-gelatin was prepared by simple co-lyophilisation technique, standardized and characterized by a part of our team. Fibrin was coated on the surface of chitosan and chitosan-

gelatin scaffolds by polymerizing 2% fibrinogen with 50 units/ml thrombin on the surface of the scaffolds. The scaffolds were incubated first in thrombin and then dipped in fibrinogen for 3-5 seconds followed by incubation in room temperature for 30minutes to obtain a fibrin coat. Above figure shows macro porous, fibrous network formation on the surface of the scaffold, indicating fibrin polymerization. 0.05mg/ml of collagen solution prepared in acetic acid was coated on the surface of chitosan and chitosan-gelatin scaffolds and let to dry overnight and the sections were taken for SEM imaging.

Development of low glycaemic noodles from sweet potato and low calorie sago from cassava as anti-diabetic foods

Developed noodles from different sweet potato varieties (white-skinned, cream-fleshed, orange fleshed etc to incorporate sweet potato leaves into noodles (as sweet potato leaves are reported to contain anti-diabetic compounds, tested the noodles for their low glycaemic action under in vitro conditions and in animal models, and developed resistant cassava starch based sago. Study completed its digestibility characteristics and other functional characteristics like dietary fiber, bulk-density, swelling index etc, also tested the low calorie sago for its glycaemic index under in vitro conditions and in animal models. This study showed that RS rich NUTRIOSE was a potential additive to reduce the EGI and produce truly low glycaemic spaghetti.



The study showed that HMT and annealing were excellent techniques to enhance the RS content of

cassava starch. Sago having highest resistant starch (RS) content and low starch digestibility could be made from such starches.

Association of oxidative stress pathway gene polymorphisms with Type 2 diabetes and related complications

Type 2 diabetes (T2DM) is a common disease affecting a huge population in our country and abroad. This non-communicable disease results from both genetic as well as environmental factors. The study explored the role of antioxidant enzymes and association of their gene polymorphisms with T2DM and its related complications in search of newer or modified treatment regimens or prognostic markers, which may emerge based on the polymorphism studies of the various genes of the ROS defense system. The normalized ratio in case of COX2 gene expression indicated that the mRNA levels were up regulated in both cases and controls. The COX2 mRNA expression did not show significant association with T2DM ($P > 0.746$). SOD2 +47C/T 'CC' genotype frequency showed significant association with T2DM cases with the carriage rate of 'C' allele showing an odds ratio of 2.434 indicating risk of T2DM.

The carriage rate of 'C' allele in case of GPx1 +599C/T polymorphism showed 1.362 times higher risk. The expression of eNOS and COX2 contribute to the development and progression of T2DM and related complications. Expression of a number of housekeeping genes was tried and two genes viz. GAPDH and β -actin were selected since they did not show any variability in expression in different tissues as well as cell lines. Both genes showed stability but when cases and controls were compared for their expression, GAPDH was found to be more stable than β -actin ($P > 0.699$ vs > 0.392). COX2 1195T/C PCR products were checked on agarose gel. PCR products were digested by restriction enzyme (HpyCH4III) at 37°C for 2 hours. The digested products were checked on 15 % polyacrylamide gel.

The genotype frequency did not show significant association with T2DM ($p > 0.076$, odd ratio 1.552, CI 0.954-2.522). However, the mutant allele 'C' was significantly higher in T2DM cases ($p < 0.043$)

and individuals with this allele showed 1.765 times higher risk

The assessment of advanced glycation end products (AGE), receptor for AGE (RAGE) and soluble receptor for RAGE (sRAGE), hsCRP in patients of diabetes mellitus with hypertension : Diagnostic & pathophysiological implications

Advanced glycation end products (AGEs) and their receptors are strongly implicated in the development of diabetes complications. When stimulated by AGEs, the receptors for AGEs (RAGEs) induce inflammation and are thought to fuel disease progression. Soluble circulating RAGE (sRAGE) may counteract the detrimental effects of RAGE. The study measured hsCRP, CML, Pentosidine, RAGE, sRAGE levels in stored plasma from the above mentioned groups. The participants, who were aged 40-70 years, had normal kidney function, liver function and had no history of cardiovascular disease.

Low levels of sRAGE were observed in diabetes, hypertension, and diabetes associated hypertension group compared with controls suggesting that low levels are inversely associated with a number of risk factors, including diabetes, hypertension, and diabetes associated hypertension group compared with controls. This fact is further supported by the fact that hsCRP levels in these groups are also significantly high. Increased levels of CML and Pentosidine in diabetes, hypertension and diabetes associated hypertension group compared with controls which clearly shows that the levels of AGE's get raised in these diseased states. Follow up showed that as the disease regresses on giving treatment, the levels of sRAGE started increasing which indicates the improvement in disease. Hence reduced levels of RAGE, hsCRP and AGE's (CML and Pentosidine) correlate with improvement in disease. Study observed an association between lower sRAGE levels and higher RAGE levels in diabetes and diabetes associated hypertension group. Further in the follow up patients on giving treatment sRAGE levels started rising and RAGE levels started declining strengthening the role of sRAGE/RAGE pathway in diabetes and diabetes related hypertension.

Screening Probiotics and Prebiotics for Expression of Glucagon like peptide-1 (GLP-1) A satiety inducing hormones. As prophylactics against diabetes mellitus-2

In this study lactobacillus strains were screened for their ability to enhance secretion of GLP-1 in vitro, to assess the efficacy of prebiotics (inulin and oligofructose) as well as casein hydrolysates for evoking enhanced secretion of GLP-1, to determine the effect of promising strains of lactobacilli / prebiotics / their combination (synbiotics) on co-expression of glucose-dependent insulinotropic peptide (GIP) and peptide YY (PYY) using qRT-PCR also to explore indigenous probiotic strains as natural dietary stimulants for GLP-1 secretion in vitro in human (NCI-H716) and murine (STC-1pGIPNeo) cell line to exploit them as prophylactics against Diabetes Mellitus-2.

The expression and secretion of GLP-1 by probiotic strains both in Live (L) and Heat Killed (HK) forms (postbiotics) appeared to be strain specific. Among the test indigenous probiotic lactobacilli, the probiotic preparations Lf1(L), LrhS3 (L), Lp75 (HK) and LrhS3 (HK) were most stimulative in proglucagon expression. However, in secretion study, HK preparations of Lp91 and Lre120 were most effective in STC-1 cells. Later, the expression and secretion studies with NCI-H716 cells presented better results with forskolin and probiotic preparations indicating the suitability of this human cell model for screening of different agents for GLP-1 secretion and expression. Forskolin, as positive control could induce expression of proglucagon to the significant level by 18.76 fold. The probiotic preparations Lp75 (HK), Lp4 (L) and Lre120 (L) also stimulated proglucagon gene expression to a significant level by 7.72, 6.94 and 4.16 folds respectively. Again heat killed preparations of Lre120 and LrhS3 exhibited maximum secretion of GLP-1 human NCI-H716 cell line. Among casein and whey protein hydrolysates, WP3, CP1, Lh288 (L. helveticus NCDC288 milk fermentate) and Lh292 (L. helveticus NCDC 292 milk fermentate) exhibited the maximum potential to induce proglucagon expression as well as GLP-1 secretion in STC-1 cells. Besides, the prebiotics such as oligofructose, wheat dextrin, maize dextrin and their metabolites (SCFA) after fermentation

with probiotics have also the potential to stimulate the secretion of GLP-1.

In addition, the role of PC3 and FFAR2 in the secretion of GLP-1 has also been established among the promising strains of probiotic lactobacilli. The co-expression GIP, CCK and PYY were also assessed using qRT-PCR with the purified fractions of casein hydrolysates. The peptide CP1 F7 purified from casein hydrolysate upregulated the expression of pro-GIP to a significant level. However, we could not get PYY expression in any of the formulations. The consolidated data indicates that some strains were stimulative in probiotic form, others in postbiotic form and some were effective both in probiotic and postbiotic form. Moreover, the difference in their potential has also been observed at both transcriptional as well as translational level. However, from the outcome of the project, it has been concluded that the dietary strategies using promising probiotic preparations, and milk protein casein as such or their hydrolysates have the prospects to be explored as antidiabetic therapeutics in diabetic patients after carrying out animal studies and human clinical trials (phase II).

A randomized open label comparative study of safety and efficacy of metformin and subcutaneous insulin in Asian Indian Women with Gestational Diabetes

This randomized control trial was to compare the safety and efficacy of Metformin and subcutaneous insulin in Asian Indian women with gestational diabetes mellitus. Two hundred forty five subjects were screened out of which 87 were excluded due to various reasons. One hundred fifty eight were given dietary therapy. Thirty four were controlled on diet till 33 weeks of gestation. Rest 124 were randomized into insulin (n = 60) and Metformin group (n = 64). The glycemic control along with perinatal outcome was compared.

The demographic characteristics were comparable in both groups; mean HbA1C values were 5.27 ± 0.60 and 6.4 ± 1.41 % respectively in group I and II respectively. The average time taken to achieve blood sugar control was 1.6 ± 1.13 weeks and 1.8 ± 1.01 weeks respectively in group I and II which was statistically insignificant. The dose range of Metformin used was 500 mg to 2500 mg with mean 1226.56 ± 397.7 mg. None of the participants

required additional insulin. The dose range for insulin was 2 units to 80 units per day with mean dose 23.7 ± 17.3 units.

The maternal and fetal complications like Pre Eclampsia, urinary tract infection, Vaginitis, Chorioamnionitis, Macrosomia, preterm labour, premature rupture of membrane, intrauterine growth restriction and oligohydramnios were comparable in two groups. The mode of delivery was comparable in two groups. Fetal Outcome: Other complications like respiratory distress syndrome, transient tachypnoea of newborn, hypocalcaemia and hyperbilirubinemia were comparable in both groups. The incidence of SGA, AGA and LGA were 9(14.1%) vs. 2(3.5%), 44 (68.8%) vs. 40 (70.18%) and 11 (17.2%) vs. 15 (26.3%) respectively (P=0.88). Conclusion: The study does not show any significant risk or adverse effects of Metformin on perinatal outcome. Study concluded that Metformin can be substituted as an effective, safe and cheaper alternative to insulin in uncomplicated new onset GDM patients under low resource settings.

Persistent Organic Pollutants – Environmental Risk Factors for Diabetes Mellitus : In a Case-Control Study

Prevalence of type 2 diabetes mellitus in the field practice area of PSGIMS&R was estimated and the relationship between exposure to Persistent Organic Pollutants and Type 2 Diabetes Mellitus was studied. Field survey of sample size of 1750 each for urban and rural field practice areas was conducted in first phase. The estimated sample individuals were drawn from all the villages sub served by the urban and rural centres around PSG. 186 diabetics were recruited for Pos estimation after consent. Results: In all 186 people consented for serum POPs estimation? 112 were non-diabetics, and 75 were diabetics. The comparison of serum levels of OCPs, OPCs, and PCBs among diabetics and non diabetics an unpaired t test revealed that all the values were comparable in both diabetics and non diabetics ($p > .05$). However, for two compounds – Endrin and Endrin ketones showed a significant difference between the two groups. Four organochlorine pesticides and one organophosphate pesticide were strongly associated with diabetes after adjusting for age and body mass index BHC

beta isomer (OR 1.06 95% CI 1 to 1.14, DDD p p (OP 1.06, 95% CI 1.01 to 1.12) DDT o p (OR 1.03, 95% CI.

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 centres in the Country and seven more centres are in process of inclusion under the Trial. Total around 245 subjects have received RISUG injection and all these subjects have been followed for their efficacy and safety. No side effects have been noticed 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 last year but are showing severe oligospermia. The centres have initiated enrolling new subjects and started using the new syringe. 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 has sanctioned the project to up-grade the facility.



Preclinical toxicity studies with recombinant hCG β -LTB vaccine for preventing pregnancy

Human chorionic gonadotropin (hCG) was chosen as a target for immunological intervention, as hCG is not made by any organ of the non-pregnant, non-cancerous woman. It only emerges after fertilization of the egg and plays a crucial role in implantation of the embryo, thereby leading to the onset of

pregnancy. Antibodies inactivating hCG prevent the onset of pregnancy, without any side reactions elsewhere in the body.

The Talwar Research Foundation revived the Vaccine and develop a new vaccine called recombinant hCG β -LTB vaccine. The pre-clinical toxicity studies have been completed both in rodent and marmoset monkey. In both the studies the vaccine was found safe and hence the data of both the studies have been submitted to RCGM for approval. After obtaining approval of RCGM and DCGI the Phase-I clinical trial with recombinant hCG β -LTB vaccine will be initiated.

THE ASSISTED REPRODUCTIVE TECHNOLOGY (REGULATIONS) BILL

The proposed draft ART (Regulation) Bill after the approval of the Ministry of Law and Justices circulated to all the concerned Ministries and Departments of Govt. of India for their comments in 2013 - 2014. The divergent views were received from the concern Ministries and Department of Govt. of India and hence these views were subjected to inter-ministerial consultations. Based on the comments and suggestions emerged from inter-ministerial consultations the draft cabinet note and bill were revised and were again sent to Ministry Law and Justices for consideration. After the formulation of new Government in the Centre the Ministry of Law and Justices advised to again obtain comments from the concerned Ministries/ Department of Govt. of India. Therefore, second time inter-ministerial consultations were held and based on the comments received the draft Cabinet Note and Bill have been revised. The revised draft Cabinet Note and Bill will be sent to the Ministry of Law and Justices for the concurrence of the Hon'ble Minister of Law and Justices after the approval of the Hon'ble Minister of Health and Family Welfare, Govt. of India.

NATIONAL REGISTRY OF ART CLINICS AND BANKS IN INDIA

Currently, 1548 ART Clinics and Banks have been identified. Out of that, around 905 ART Clinics and 169 Banks have confirmed their contact details and remaining are in the process of confirmation. 905 ART clinics have submitted their duly field

performa and out of that 385 ART clinics have been found in compliance with the provisions of the National guidelines and proposed ART (Regulation) Bill therefore these 385 ART clinics have been enrolled under the National Registry of ICMR. An enrolment no. has been given to these 385 ART clinics and their contact details are available on ICMR web site as enrolled ART clinics under the National Registry of ART Clinics and Banks in India of ICMR. The performas of remaining ART clinics are under evaluation. Under the third step, those ART clinics that have been enrolled, will be physically inspected by a team of experts to find out whether the provisions the clinic have mentioned in their performas are available at their clinic. After satisfactory report of the site visit team a unique registration no. will be issue to the ART clinic. The draft National guidelines for accreditation, supervision and regulation of ART banks in India are in the process of finalization and soon will be subjected to the public opinion after the approval of the Director General, ICMR

ENVIRONMENTAL HEALTH

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

This is a multidisciplinary prospective cohort study going on at seven departments of AIIMS, New Delhi and at Jawaharlal Nehru University, New Delhi. After fulfilling the inclusion and exclusion criteria's, 2516 male and female subjects have been enrolled under highly exposed (726), moderate exposed (1255) and control group (535). Out of 2516 enrolled subjects 2122 subjects have been subjected to clinical and laboratory examination and have been followed since last two years. Currently the follow up of the enrolled subjects is going on yearly basis for clinical and laboratory examinations. Simultaneously to complete cohort of the study the enrollment of new subjects is also going on.

MATERNAL HEALTH

Role of PAPP-A, free β -hCG levels, uterine artery doppler and maternal factors in 1st trimester of pregnancy for prediction of preeclampsia

Preeclampsia (PE) complicates approximately 3-7% of pregnancies and is a major cause of maternal

morbidity and mortality. There is need for early identification of this condition as early interventions can improve perinatal outcome. Preeclampsia (PE) complicates approximately 3-7% of pregnancies and is a major cause of maternal morbidity and mortality. There is need for early identification of this condition as early interventions can improve perinatal outcome.

Nearly, 399 (13%) developed hypertension during pregnancy [98 (3.2%) had preeclampsia and 301(9.9%) had gestational hypertension]. Early onset of hypertension (before 34 weeks) was seen in 153 (38.3%) whereas majority 246 (61.7%) had late onset preeclampsia. Other maternal medical disorders such as hypothyroidism, gestational diabetes, and hepatitis were observed in 102 women. Adverse perinatal outcome such as intra uterine growth restriction (IUGR), intra uterine death (IUD), gross congenital anomaly (GCA), and unexplained Still Birth (SB) was seen in 417 (13.5%) women. Lost to follow up rate was 12.3% (431) and 401 women were excluded after

ultrasound examination as they were either more than 13 weeks or 6 days by crown rump length, had missed abortion or had multiple pregnancy. Women with normal maternal and perinatal outcome were taken as controls. Among the maternal factors there was significant difference in age, BMI and mean arterial pressure (MAP) between cases and controls ($p = <0.001$) In the Doppler parameters, the mean pulsatility index (M-PI) and highest systolic/diastolic ratio (H-SD) were significantly higher in cases compared to controls. Biomarker PAPP-A was significantly lower in cases as compared to controls ($p = <0.001$) but free β -hCG (F- β -hCG) was not significantly different (Table 1).

Among the maternal factors there was significant difference in age, BMI and MAP between cases and controls ($p = <0.001$) In the Doppler parameters, the mean PI ($p = 0.016$) and highest SD ratio ($p = 0.007$) were significantly higher in cases compared to controls. Among biomarkers, PAPP-A levels were significantly lower in cases as compared to controls ($p = <0.001$). There was no significant difference

Table 1. Comparison of variables with hypertension of pregnancy and normal outcome.

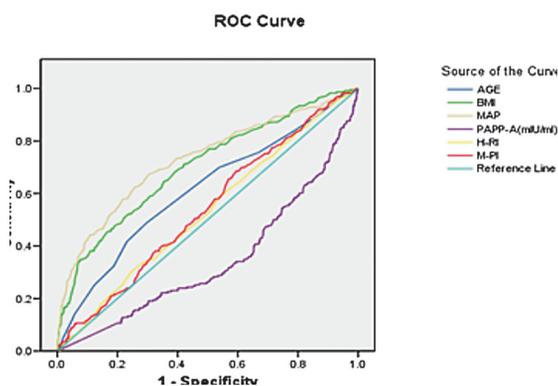
| | Cases with Hypertension | | Controls | | p-value |
|------------------------|-------------------------|--------|----------|--------|---------|
| | Mean | ±SD | Mean | ±SD | |
| Maternal Age | | | | | |
| Age | 25.92 | 4.28 | 24.35 | 3.57 | < 0.001 |
| BMI | 23.91 | 4.76 | 20.76 | 3.39 | < 0.001 |
| MAP | 88.75 | 12.46 | 79.54 | 9.19 | < 0.001 |
| Doppler | | | | | |
| H-PI | 1.65 | 0.48 | 1.59 | 0.49 | 0.053 |
| M-PI | 1.47 | 0.47 | 1.40 | 0.43 | 0.016 |
| H-RI | 0.79 | 0.23 | 0.77 | 0.19 | 0.108 |
| M-RI | 0.73 | 0.19 | 0.71 | 0.14 | 0.066 |
| H-SD | 4.17 | 2.02 | 3.83 | 1.84 | 0.007 |
| M-SD | 4.01 | 1.91 | 4.00 | 1.97 | 0.458 |
| Notch | 104 | 46.02% | 598 | 49.63% | 0.160 |
| Biomarkers | | | | | |
| PAPP-A(mlU/ml) | 4.00 | 2.96 | 5.53 | 3.03 | < 0.001 |
| PAPP-A MoM | 0.82 | 0.60 | 1.13 | 0.62 | < 0.001 |
| F β -hCG (ng/ml) | 48.41 | 32.69 | 49.79 | 35.37 | 0.293 |
| F β - hCG Mom | 1.14 | 0.77 | 1.18 | 0.84 | 0.293 |

found in the free β -hCG levels between cases and controls.

The subjects were subdivided into groups based on maternal and perinatal outcome, different groups were compared with those with normal outcome taken as controls.

| |
|--|
| Normal – group I |
| Hypertension - group II |
| Preeclampsia – group IIA |
| Gestational Hypertension – group II B |
| Early onset hypertension – group II (1) |
| Late onset hypertension – group II(2) |
| Other medical disorders – group III |
| Other adverse perinatal outcome – Group IV |

The comparison of group I and II, group I and IIA, group I and IIB, showed almost similar area under curve (AUC) on receiver operating curve (ROC) for variables such as maternal age, BMI, MAP, PAPP-A and uterine artery Doppler. MAP was the most significant variable as it had highest AUC among the variables across all groups, followed by BMI levels.



When logistic regression was applied, the highest odds ratio for hypertension and gestational hypertension group (group II and II A) was BMI, whereas the highest odds ratio for preeclampsia group (group IIB) was MAP. The sensitivity, specificity, of all variables were higher for early onset hypertension compared to late onset hypertension and controls. Since the early onset hypertension has more adverse effects, needs more intensive care, the high sensitivity and specificity of the markers for early onset can be used for prediction of PE in this group.

Molecular mechanism behind pathogenesis of pre-eclampsia

Hypoxic and angiogenic proteins play important role in the pathophysiology of preeclampsia but the exact molecular mechanism is still elusive. Therefore, a cross sectional study was carried out to find out the role of angiogenic proteins and HIF-1 pathway proteins during early and late gestational ages in the pathophysiology of Pre-eclampsia. Women attending antenatal clinic were screened for preeclampsia using standard criteria at 20-24 weeks gestation and enrolled on confirmation of diagnosis. Controls were matched for age and gestation. Placental samples were collected from cases and controls for analysis of proteins. Immunohistochemistry using specific antibodies was done for studying expression of the key components of angiogenesis as well as HIF-1 signaling pathway proteins in both pre-eclampsia and gestational matched control placental tissues. Western Blotting & Reverse transcriptase PCR was done for revalidation of Immunohistochemistry (IHC) results in representative samples. Quantitative analysis of PLGF, sFlt-1, HIF-1 α using ELISA was done in the serum of patients with Pre-eclampsia. Correlation between clinico-pathological parameters and expression of various angiogenic proteins & HIF-1 pathway proteins was studied.

A total of 100 placental samples of each pre-eclampsia and control groups were collected and serum samples of 320 preeclamptic and 240 normal were retrieved to evaluate the concentration of HIF-1 α , sVEGFR1, PIGF, pVHL, PHD. IHC was performed for 6 proteins. The serum concentration of HIF-1 α and sVEGFR1 was higher in preeclamptic cases than that of controls while PIGF, pVHL and PHD was lower in preeclampsia than controls (Table 2).

Group II - <34 weeks; Group III->34 weeks; Mann Whitney test applied

Immunohistochemistry analysis indicated that HIF-1 α was localized both in nucleus and cytoplasm of syncytiotrophoblast in preeclamptic placental tissue. The immunoreactivity of PIGF, pVHL and PHD revealed a significant downregulation in the

Table 2. Results of ELISA test done in serum of controls (<34 weeks and >34 weeks) and cases.

| Markers | Study Groups | Control | Preeclampsia | P value |
|----------------|--------------|-----------|--------------|-----------|
| HIF-1 α | Group II | 2.86±0.44 | 6.58±0.27 | P=0.0001* |
| | Group III | 1.05±0.33 | 7.86±0.71 | P=0.0001* |
| PIGF | Group II | 4.40±0.94 | 1.72±0.80 | P=0.0001* |
| | Group III | 6.23±0.64 | 1.46±0.74 | P=0.0001* |
| sVEGFR1 | Group II | 2.60±0.69 | 6.69±0.48 | P=0.0001* |
| | Group III | 1.17±0.61 | 8.03±0.64 | P=0.0001* |
| pVHL | Group II | 4.05±0.59 | 1.93±0.76 | P=0.0001* |
| | Group III | 4.67±0.73 | 1.60±0.63 | P=0.0001* |
| PHD | Group II | 5.47±1.06 | 2.23±1.26 | P=0.0001* |
| | Group III | 7.28±1.12 | 1.69±0.78 | P=0.0001* |

cytoplasm of syncytiotrophoblast of preeclamptic placenta. However VEGFR1 and HIF-1 β were upregulated in the syncytiotrophoblast of preeclamptic patients as compared with control ones (Table 3; Figure 1).

Correlation between hypoxic (HIF-1 α , HIF-1 β , pVHL, PHD) and angiogenic proteins (PIGF, VEGFR1) in preeclamptic placental tissues was

done using Pearson’s correlation test (2 tailed). It indicated a negative correlation of HIF-1 α and HIF-1 β with PIGF, pVHL and PHD; and a positive correlation with VEGFR1 which suggests the cumulative effect of these proteins in pathogenesis of preeclampsia (Table 4).

The results indicated that under hypoxic conditions HIF 1 α and β are over expressed due to loss of

Table 3. IHC analysis in normal and preeclamptic placental tissues.

| Protein | Cellular localization | Pre-eclamptic | Control | p-value |
|-----------------|-----------------------|--------------------------|---------|----------|
| HIF-1 α | Nuclear | +++ $\uparrow\uparrow$ | — | p=0.0001 |
| | Cytoplasmic | + | +++ | |
| HIF-1 β | Cytoplasmic | +++ $\uparrow\uparrow$ | + | p=0.0001 |
| PLGF | Cytoplasmic | + $\downarrow\downarrow$ | +++ | p=0.0001 |
| sVEGFR1(sFlt-1) | Cytoplasmic | +++ $\uparrow\uparrow$ | + | p=0.0001 |
| pVHL | Cytoplasmic | + $\downarrow\downarrow$ | +++ | p=0.0001 |
| PHD2 | Cytoplasmic | + $\downarrow\downarrow$ | +++ | p=0.0001 |

(--:no staining; +: mild staining; ++: moderate staining; +++: intense staining; $\downarrow\downarrow$: down regulation; $\uparrow\uparrow$: up regulation)

Table 4. Correlation of hypoxic and angiogenic proteins in placental tissue of preeclamptic patients.

| Protein | No. of cases | HIF-1 α Nuclear | HIF 1 β cytoplasm | PIGF Cytoplasm | VEGFR1 Cytoplasm | pVHL Cytoplasm | PHD Cytoplasm |
|-------------------------|--------------|------------------------|-------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| HIF-1 α Nuclear | 100 | 1 | r= 0.621* p=0.0001 | r=-0.752* p=0.0001 | r=0.619* p=0.0001 | r=-0.467* p=0.0001 | r=-0.342* p=0.0001 |
| HIF 1 β cytoplasm | 100 | | 1 | r=-0.212* p=0.0001 | r=0.309* p=0.0001 | r=-0.298* p=0.0001 | r=-0.462* p=0.0001 |
| PIGF Cytoplasm | 100 | | | 1 | r=-0.581* p=0.0001 | r=0.323* p=0.0001 | r=0.491* p=0.0001 |
| VEGFR1 Cytoplasm | 100 | | | | 1 | r=-0.245* p=0.0001 | r=-0.287* p=0.0001 |
| pVHL Cytoplasm | 70 | | | | | 1 | r=0.632* p=0.0001 |
| PHD Cytoplasm | 70 | | | | | | 1 |

Pearson correlation test (2-tailed); r= correlation coefficient, * Correlation is highly significant at 0.01 level.

ubiquitinating proteins pVHL and PHD resulting in the activation of HIF pathway. HIF proteins interfere with the expression of PIGF and its receptor VEGFR1 probably via increasing the transcription of genes involved in angiogenesis. The altered ratio of PIGF and VEGFR1 leads to abnormal angiogenesis causing endothelial dysfunction. Due to this, there may be incomplete invasion of trophoblast into maternal deciduas that results in constellation of preeclamptic symptoms like high blood pressure, proteinuria etc. (Fig. 2).

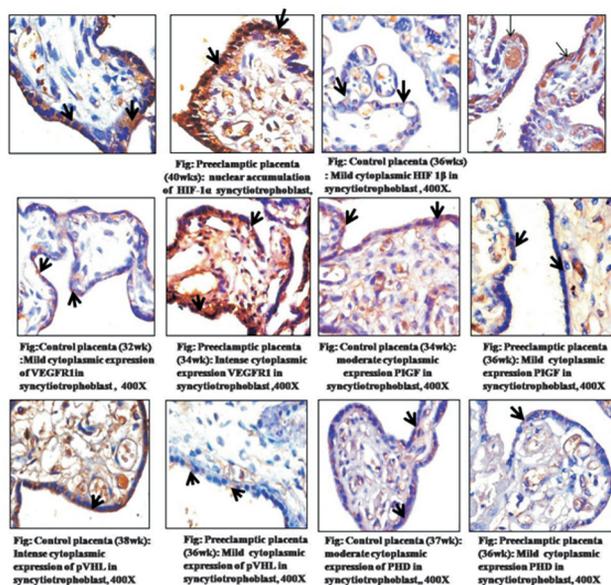


Fig. 1. Depicting the immunohistochemical expression of HIF-1α, HIF-1β, VEGFR1, PIGF, pVHL and PHD.

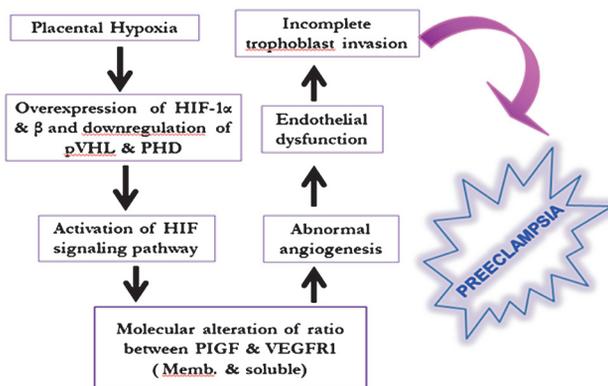


Fig. 2. Hypothesis of development of preeclampsia.

Maternal near miss in a tertiary hospital: causes, associated factors and outcome

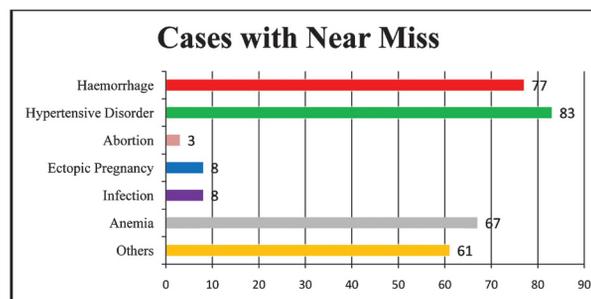
Review of maternal near miss (MNM) cases can provide first-hand information from women who

have survived a life threatening condition. This information can be acted upon to prevent maternal near miss and maternal mortality. A case control study was carried out in a tertiary care referral hospital to study the incidence of MNM and its outcome; the medical causes, associated socio-demographic, obstetric treatment seeking factors and fetal outcome among MNM cases.

Risk factors for MNM and delivery outcome in cases and controls

| | | | |
|---|---|-----------|----------------|
| Risk factors (by multivariate analysis) | <ul style="list-style-type: none"> • Age >30, aOR-2.01(1.02-3.93) • Illiteracy, aOR-2.05(1.11-3.75) • Age at marriage <18, aOR-2.01(1.21-3.32) • Gravida ≥ 4, aOR-2.25(1.21-4.17) • income <5000, aOR-3.79(1.88-7.64) • Residence outside Delhi-NCR, aOR-9.31(4.36-19.90) • Delay in reaching health facility • Incompetent health personnel | | |
| | DELIVERY OUTCOME (%) | MNM n=261 | Controls n=522 |
| Normal delivery | 39 | 97.3 | 29.0 |
| Caesarean Section | 54.8 | 2.7 | 50.2 |
| Live Births | 63.6 | 99.4 | 62.0 |
| Operative Procedure | 35.2 | - | 29.7 |
| Blood Transfusion | 90.4 | - | 72.4 |

Sepsis was seen to be the most common underlying condition in women who died followed by hypertensive disorders, haemorrhage and DIC. Other complications and systemic diseases such as anemia, hepatic causes, fever, ARF, CHF etc was identified as the other major indirect cause of maternal death.



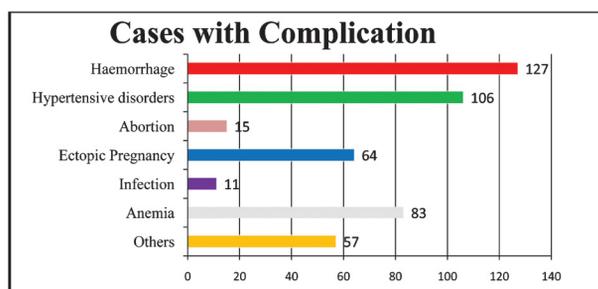


Fig. 3. Risk factors for MNM and delivery outcome in cases and controls.

The three delay model used to identify delays in the management of obstetric complications indicated that the second delay i.e. the delay in reaching the adequate health facility was a major contributor both for development of severe complications cases as well as MNM cases. The most important reasons were that the complaints persisted even after visiting the hospital and incompetence of the health personnel at the first point of contact in managing the patients.

PERINATAL HEALTH

To assess the association between maternal pro-inflammatory cytokine genes and associated polymorphisms with preterm birth: A case-control study

A case- control study was carried out at two hospitals of North India to study the association of preterm birth with genetic, clinical, psychosocial and environmental factors. Mean age of mothers who gave birth to term neonate was 25.67±3.72 years and who gave birth to a preterm neonate was 25.85±4.1 years. Lower Maternal Mid Upper Arm Circumference (MMUAC) and lower post delivery maternal weight was found to be significant in preterm birth (p<0.001). Maternal education was found to be significantly associated with preterm birth (p=0.0457). The study concluded that genetic and psycho-socioeconomic factors have a major impact on preterm birth.

| | OR, (95%CI) | P value |
|------------------------------------|-------------------|---------|
| Pro-inflammatory Cytokines | | |
| IL-1α -889 CT genotype | 1.7, (1.24-1.52) | 0.02 |
| IL-1α (+4845) CT genotype | 1.5, (1.1-1.9), | 0.003 |
| IL-6 (-174) GC genotype | 5.98, (3.3-10.6) | <0.001 |
| IL-6(-174) CC genotype | 2.36,(1.2-4.3) | 0.005 |
| Anti-inflammatory Cytokines | | |
| IL-10(-1082) GA genotype | 0.48 (0.24-0.95) | 0.03 |

Epidemiologic Study Risk Factor Analysis and Impact of Health Care Delivery system on Pre-Term Birth in Gujarat

The study was conducted as mixed methods in four selected districts of Gujarat, with an aim to undertake risk factor analysis and health system response to PTB. Study included follow-up of 2154 ante-natal mothers (with completed 7th month of pregnancy), first follow up was done at 7±1 day of delivery and second after 42±7 days post-delivery. Qualitative methods included Focus Group Discussion in antenatal and postnatal period and In-depth Interviews with other stake holders like Medical Officers, Sub Centre in charge and Block medical officers.

The institutional delivery rate (93%) and enrolment for ANC (100%) were high; however consumption of more than 50 IFA tablet by ante natal mothers was around 55%. Proportion of normal delivery and Caesarean section was 85.5% and 9.76% respectively. Prevalence of PTB in the present study was 9.1%.

Triangulation of quantitative and qualitative data suggests need for modular training to identify risk factors of PTB as a part of ANC and appropriate referral if required. Strengthening of maternal child health tracking system to document PTB is also suggested. Possible policy innovations is to extend IMNCI plus to IMNCI plus “P” i.e. IMNCI plus Pre Term birth care. Verbal death autopsies for Neonatal death and perinatal death should also be initiated on priority.

ADOLESCENT HEALTH

Preparedness for Marriage of Adolescents girls residing in Health and Demographic Surveillance System setting: Vadu HDSS experience

A cross-sectional study was carried out by using mixed methods’ approach complementing qualitative approach with a survey. The objectives were to document the adolescent girl’s perceptions and expectations of marriage, to record their need of knowledge and skills with reference to a healthy sexual and reproductive life and identify innovative interventions for them. Results indicated that the practice of early marriages still exists which is

probably because of low socio economic status (71%) school drop outs (44.2%), No of siblings more (27.9%) [Table-5]. The study findings stated the need to reduce communication gap between parents and their adolescent children. Further research needs to be conducted in identifying barriers in communication and congruent solutions to it. Culturally accepted framework/strategies need to be developed to sensitize parents in the process of preparedness of marriage of their daughters.

Table 5. Reasons for early marriage.

| Reason for early marriage | Frequency (%) (n=172) |
|----------------------------------|-----------------------|
| Socio Economic status | 122(70.9) |
| School Drop out | 76(44.2) |
| No of siblings more | 48(27.9) |
| Prevention of premarital affairs | 32(18) |
| Bad Behavior/character of a girl | 32(18) |
| Social prestige | 19(11) |
| Difficulty in finding groom | 20(11.6) |
| Role of menarche | 9(5.2) |

GYNAECOLOGICAL MORBIDITIES

Role of ATT for positive endometrial aspirate DNA-PCR reproductive outcome in infertile patients in Indian setting

Diagnosis of genital tuberculosis (GTB) is challenging in asymptomatic infertile women and in the absence of direct demonstration of bacteria through microscopy or culture. DNA-PCR (polymerase chain reaction) has revolutionized the diagnosis of paucibacillary GTB, yet it is not clear whether ATT should be administered to infertile women only on the basis of positive endometrial DNA-PCR. A prospective randomized study was carried out to determine the effect of ATT versus no ATT on reproductive outcome of 100 infertile women with patent tubes on laparoscopy and all other tests being negative for genital TB except

endometrial aspirate DNA-PCR. Spontaneous conception using timed intercourse was encouraged post-laparoscopy and repeat endometrial DNA-PCR was performed at 6 months and 12 months in patients who did not conceive and response to treatment was noted in the two groups.

Study details are given in Table 6. The reproductive outcome in the treatment and non-treatment group was not statistically different when ATT is administered on the basis of only positive EA DNA-PCR. Repeat testing for endometrial DNA-PCR at 6 months and 12 months does not provide any additional information.

PREVENTION OF HIV/AIDS/MICROBICIDES

Utility of an additional HIV test near term during pregnancy in strengthening prevention efforts of Mother-to-Child transmission of HIV

A study was carried out to evaluate utility of an additional HIV test near term during pregnancy in strengthening prevention efforts of Mother-to-Child Transmission of HIV and its cost-effectiveness. A total of 9097 pregnant HIV uninfected women were offered a second HIV test near term (34 weeks or beyond) or within 4 weeks of postpartum period. A decision analysis model was used to evaluate cost-effectiveness of a second HIV test in pregnant women near term. The key outcome measures included programme cost with addition of second HIV test in pregnant women and quality-adjusted life years (QALYs) gained. Four new HIV infections were detected in the second test. Thus HIV incidence among pregnant women was 0.12 (95% 0.032 to 0.297) per 100 person women years (PWY). Current strategy of a single HIV test was found to be 8.2 times costlier for less QALYs gained as compared to proposed repeat HIV testing of pregnant women who test negative during the

Table 6.

| | On ATT n=50 (%) | No ATT n=50 (%) | P- value |
|--------------------------------------|--------------------|--------------------|-----------------------|
| Pregnancies | 25 (50%) | 21 (42%) | 0.422 (CI 11%, 0.27%) |
| Term deliveries | 21(84%) | 19 (90.5%) | |
| +ve PCR at 6 months in non-pregnant | 31 (96.9%) | 34 (100%) | 0.299 (CI 2.9%,9.1%) |
| +ve PCR at 12 months in non-pregnant | 23 (92%) | 26 (89.7%) | 0.767(CI 13.0%,17.7%) |

first test. The results warrant consideration at the national level for including a second HIV test of all pregnant women in the national programme. However prior to allocation of resources for a second HIV test in pregnancy, appropriate strategies will have to be planned for improving compliance for prevention of mother-to-child transmission of HIV and reducing loss-to- follow-up of those women detected with HIV.

| | Rural sites | Urban sites |
|---|------------------------------|------------------------------|
| No. of women tested for 1st HIV test | 4803 | 13109 |
| No detected with HIV in first HIV test | 20 | 60 |
| Baseline HIV prevalence (1st HIV test) | 0.42 | 0.46 |
| No of women enrolled for 2nd HIV test | 2229 | 6868 |
| No of women detected with HIV in the 2nd HIV tests | 2 | 2 |
| HIV incidence rate per 1000 Persons Women Years (PWY) | 2.3 (95% CI 0.27 to 8.19) | 0.8 (95% CI 0.09 to 2.82) |
| Overall HIV incidence-1000 PWY | 1.2 (95% CI 0.32-2.97) | |

Knowledge and willingness to use rectal microbicide by the men who have sex with men

Very little information is available about the knowledge and acceptability of new biological prevention tools such as Pre exposure prophylaxis (PrEP) and Rectal Microbicides (RM) among Men who have sex with men (MSM) in whom the HIV risk is high. A Qualitative study using repeated in-depth interviews among 39 consenting MSM was conducted in Pune to explore knowledge and willingness about these tools among MSM community in India. MSM were between 20-55 yrs of age, mostly educated till 10th grade, 12 were highly educated, and 4 MSM were HIV positive, 9 MSM engaged in sex work regularly and 10 occasionally. Few MSM had correct knowledge about HIV. Despite using condoms 23 MSM perceived the risk of HIV infection because of fear of condoms tear/non-use owing to liquor consumption. Majority lacked knowledge about other prevention options while 2 mentioned Post

Exposure Prophylaxis (PEP). PrEP was acceptable as it provided added protection in cases of condom non-use and failure, multiple partners and for a stress free sex life. The study brought out critical need of increasing awareness about new prevention technologies for successful positioning whenever efficacious options become available.

Study to Understand Prevention and Explore Respective Barriers for Women: A multi-stakeholder perspective on vaginal microbicides and other NPTs

A total of 214 in-depth/ key informant interviews and six focus group discussions were conducted in Pune (Maharashtra), Belgaum (Karnataka) and Chennai (Tamil Nadu) using purposive sampling. The highest preference was for vaccine as people felt that it gives lifelong immunity. Mixed response was received for other technologies. The qualitative data was analyzed to understand the process of decision making for using NPTs which can inform researchers, product developers and researchers together. Overarching theme was efficacy and the themes for decision to use NPT come under Product expectation; Socio-cultural issues and Apprehensions.

INSTITUTIONAL STRENGTHENING

National Animal Resource Facility (NARF) for Biomedical Research

The Ministry of Finance has approved the EFC proposal of Rs.338.58 Crores in its meeting held on 13th Aug., 2014. This will be a new institution under the ICMR therefore Ministry of Finance recommended approval of the Cabinet Committee of Economic Affairs (CCEA). Accordingly the Cabinet Note both in Hindi and English was prepared and sent to the Prime Minister Office (PMO), Cabinet Secretariat and also to the Ministry of Finance for consideration. The Ministry of Finance and the Cabinet Secretariat has accepted the Cabinet Note and recommended to put up in the CCEA. The PMO held a meeting with the official of the ICMR and DHR on 8th July, 2015 and accepted the proposal for consideration in CCEA. The proposal, most probably, will be placed in the next meeting of CCEA.

National Centre for Primate Breeding & Research (NCPBR)

Presently, the project is already under implementation as per the revised design. At present the structural work of the Quarantine Building has been completed and installations of various services in the quarantine building are pending because of

lack of funds. Even Corals and Concribes have also being installed. The revised cost estimate of Rs. 290.90 Crores has been evaluated by the Dept. of Expenditure, Planning Commission and CPWD. The CPWD has raised certain comments and these comments have been clarified by the NIRRH, Mumbai and are under evaluation in ICMR.

NUTRITION

The ICMR continues to be instrumental in developing public health activities with many significant contributions in the country in the field of nutrition. It has been made possible by undertaking the laboratory and hospital based research with community based participation. Specifically, the research efforts essentially aimed to achieve optimal nutrition of vulnerable segments of population such as women of reproductive age, children, adolescent girls and elderly by 2020. The salient features of various research activities undertaken during 2014-2015 are given below.

Intramural Research

NATIONAL INSTITUTE OF NUTRITION, HYDERABAD

COMMUNITY STUDIES

- An epidemiological study was carried out at the request of the erstwhile Planning Commission, to assess the effect of consumption of *Khesari dal (Lathyrus sativus)* on human health in the State of Chattisgarh. Mean consumption of *Khesari dal* was only 3.5 g/CU/day, out of 50g of total pulses consumed daily. Only nine suspected old cases of neurolethyrism have been identified in the State.
- A Task Force study of ICMR was carried out in two sites in Hyderabad and Delhi to develop reference curves of body fat (content and distribution) among Indian children aged six to 18 years. The study also aimed to identify the best possible cut-off measures of body fat that predict unacceptable cardio-metabolic risk factors for defining body fatness among Indian children. The study identified determinants of childhood body fatness that are operating at different levels across

five diverse socio-cultural and geographical contexts of India.

- The prevalence of undernutrition especially among under five year children may vary geographically. Therefore, for the development of area specific intervention strategies, divisional/district level mapping of undernutrition was taken up in the State of Haryana. The prevalence of undernutrition (<median -2SD) among <5 year children such as underweight, stunting and wasting was 28, 34 and 11per cent, respectively. However, the magnitude of undernutrition was higher in the divisions of Gurgaon and Hissar compared to Ambala and Rohtak. The results may help in effectively channelizing the limited resources for high-burden area.

BASIC STUDIES

- 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 in the State of Telangana. The 12 month intervention for infants was a home-based four-arm trial with multiple micronutrient powder (MNP), early learning, combination and a placebo. For preschoolers (36-48months) a point-of use cluster-randomized two arm trial (MNP and placebo) was conducted for 8 mo. MNP intervention resulted in significant anaemia reduction in both infants (67 to 50 % in MNP against 66 to 72% in placebo) and preschoolers (46 to 10 % in MNP against 48 to 35 % in placebo). There was a significant improvement in biomarkers of iron in both groups. MNP and early learning improved cognition in infants.

- A study has shown that procyanidin-B2 (PCB2) inhibits formation of advanced glycation endproducts (AGE) in vivo, which in turn ameliorated renal changes in diabetic rats. Feeding 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. Finally PCB2 also prevented proteinuria in diabetic rats (Fig.1).

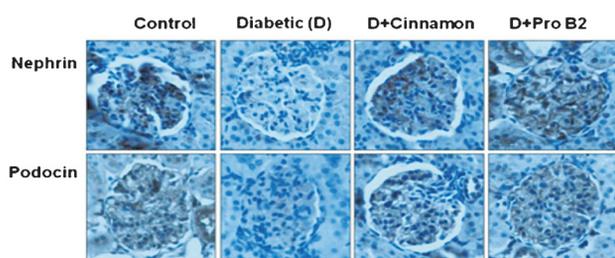


Fig. 1. Immunohistochemical analysis of glomerular expression of nephrin and podocin in control and diabetic rats treated with either cinnamon or PCB2.

- A cross-sectional case-control study that examined plasma vitamin D status in type 2 diabetics with and without retinopathy reported that mean plasma vitamin D levels were significantly lower in T2D patients without and with DR groups compared with the control group. The prevalence of VD deficiency (VDD) was higher in non-diabetic retinopathy (NDR) and DR groups (66 and 63%) compared to age-matched controls (45%), suggesting that VDD was higher in diabetic groups, irrespective of presence or absence of retinopathy.
- Using a range of in vitro protein glycation models, it was reported that ellagic acid (EA), present in many dietary sources prevents advanced glycation endproducts (AGE) formation. EA inhibits AGE formation and activation of receptor for advanced glycation endproducts (RAGE) in retina under hyperglycemic conditions. Further, AGE-RAGE mediated cellular events and subsequent functional abnormalities are modulated by EA which throw light on the potential of therapeutic avenues for treatment of diabetic retinopathy.
- When the response of small heat shock proteins (sHsp) in diabetic rat retina was studied, it was

observed that there was an increased expression of sHsp: α A-crystallin (α AC), α B-crystallin (α BC) and Hsp22 in diabetic retina; there was also increased phosphorylation of α BC under diabetic conditions. Moreover, diabetes activated the p38MAPK signalling cascade by increasing the p-p38 MAPK in retina. These results suggest that specific sHsp are important for neuronal protection in diabetic retinopathy (DR) and could aid in developing therapeutic strategies for DR.

- Using a spontaneous obese rat (WNIN/Ob) neurodegeneration due to obesity was described. Altered ubiquitin-proteasome system (UPS), existence of endoplasmic reticulum (ER) stress, up-regulation of apoptotic markers and apoptosis were found in the cerebral cortex of obese rats. This study highlights the role of altered UPS in neurodegeneration due to obesity (Fig.2).

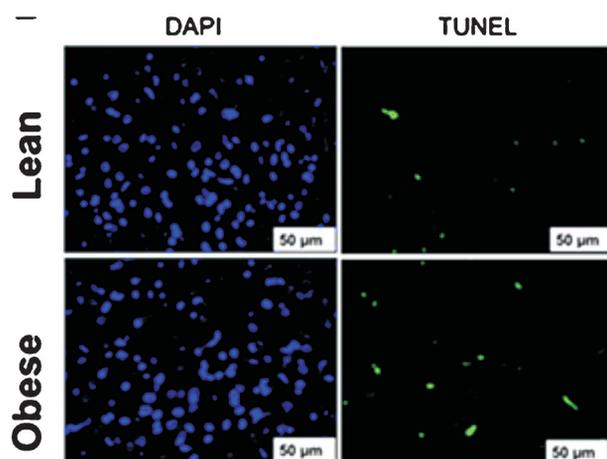


Fig. 2. Apoptosis in cerebral cortex of obesity rat.

- The molecular basis for the effects of growth hormone (GH) on podocyte depletion is not adequately understood. Studies carried out in the Institute have revealed that GH increases expression of transforming growth factor-beta-induced protein (TGFBIP) and also increased secretion of extracellular TGFBIP. Both GH and TGFBIP induced apoptosis and epithelial mesenchymal transition (EMT) of podocytes. Administration of GH to rats induced EMT and apoptosis in the kidney. This indicates that GH-dependent increase in TGFBIP in the podocyte

could be one of the mechanisms responsible for podocyte depletion in DN.

- A study was designed to validate the existing methods (hydro-densitometry, air displacement pletismography (ADP), bio-electrical impedance analysis (BIA) and skinfolds (SKF)) to assess body composition and to develop regression equations using skin fold measurement for accurate appraisal of body composition to suite Indian female population. The study revealed that in preadolescents, there is a good agreement between ADP and BIA when compared to ADP and SKF method. Based on the results, regression equations for 15 different combinations of skinfold thickness were drawn suitable to Indian female population.
- The role of antenatal and perinatal magnesium deficiency in foetal programming for neuro musculoskeletal development in the rat offspring was evaluated. The findings suggested that maternal magnesium restriction induced alteration of promoter DNA methylation in addition to glucocorticoid stress and proinflammatory status could be a mechanism underlying changes in the expression of relevant genes responsible for adiposity and associated stress.
- 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.
- A study that assessed the efficacy of polyphenol-rich dietary ingredients as proteasome inhibitors and their role as anticancer agents. In vitro studies demonstrate that *Murraya koenigii* leaf extract (MLE) is cytotoxic only to cancer cells but not normal cells. Further, the extract inhibited the proteolytic activity of the 26S proteasome only in cancer cells. The study also provided evidence that MLE acts as a proteasome inhibitor and a pro-apoptotic agent in vivo, and highlighted the potential benefits of *M. koenigii* leaves in the prevention and treatment of breast cancer.
- To appreciate and to understand important minimal constituents of plant foods to our health, the composition of bioactive phytochemicals in commonly consumed Indian foods including regional and tribal foods were evaluated. It was found that there is a considerable evidence for the role of antioxidant constituents of fruits and vegetables in the maintenance of health and disease prevention. Studies also showed that phytochemicals, some of them not necessarily antioxidants, are also bioactive for preventing many chronic degenerative diseases. This finding also helped in standardizing the separation methods of many polyphenolic constituents of foods.
- The possible role of changes in neuronal metabolism and neurochemical profile in reduced longevity in WNIN/Ob rats was investigated. The micro-environmental alterations and metabolic changes normally seen in the ageing brain were observed in the young age in obese rat brain. The results further strengthened the inference that changes associated with normal ageing appear to be occurring much earlier in the brain of the WNIN/Ob obese rats and could therefore underlie their accelerated ageing / decreased longevity.
- A study aimed to understand whether oxidative stress has a direct link with the observed immunosuppression in pathophysiological conditions, in simple words whether it has any actual direct effect on the T cell priming responses and the macrophage signaling cascades required for development of subsequent adaptive immune response. Results indicate that inhibition of antigen presentation by Reactive oxygen species (ROS) might be due to modulation of calmodulin-c-rel signaling pathway, rather than weakened co-stimulatory signaling.

CLINICAL, MICROBIOLOGY AND IMMUNOLOGY

- 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.

PATHOLOGY

- Knowledge, attitude and practices (KAP) of medical practitioners regarding food allergy (FA) were studied. A total of 300 medical professionals were contacted and administered a questionnaire. More than 50 per cent knew that FA is a serious problem but did not responded correctly to questions on difference between food allergy and intolerance. Over 53 per cent had a little knowledge about prevalence and published literature on FA, 37.6 per cent rarely referred such patients for further evaluation.
- Effect of severity of maternal anaemia on placental and foetal outcome and molecular basis of fatty acid metabolism in the placenta was studied. Maternal and cord blood as well as placentas of 42 pregnant mothers, with varying grades of anaemia were collected. It was seen that with increasing severity of maternal anemia, there were changes evident in the placental tissue by way of increased expression of markers (VEGF, PLGF, eNoS, etc.) indicating a hypoxic state, and which in the long run may be a precursor for development of hypertension in the offspring.

NUTRITION EDUCATION & COMMUNICATION

- A nutrition education intervention study evaluated the impact of a label information reading kit on usage of food labels among adolescents for promoting healthy food choices. The kit titled 'Read-B4-U-Eat' was developed (Fig.3) and theories of social-cognition and shared-learning were used to develop the module's five components – interactive sessions for guided learning; booklet for self-learning; nine posters for shared learning; animation film (4 min) for edutainment; and notes for teachers

for reiteration. The kit was efficacious nutrition education module to inculcate label reading skills among adolescent consumers.



Fig. 3. READ B4 U EAT Ready-reckoner booklet on food label reading.

- A study assessed nutritional awareness among primary school children using pictorial questionnaires and evaluated the use of interventional approaches with reiterative colorful imagery and demonstration of live samples of foods. The study showed that the intervention improved not only the children's ability to recognize various foods but also to differentiate between healthy and unhealthy options.

FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE, HYDERABAD

- A study was conducted on prevalence of fluorosis in Doda District of Jammu and Kashmir. The fluoride levels were highest in 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.
- In an investigation, fish egg waste has been identified as potential source of protein which can become a value added product. The bioactive Protein hydrolysate has been prepared from underutilized rohu fish using pepsin trypsin and Alcalase enzymes. The compositional analysis of protein hydrolysates revealed that it has good amino acid profile along with essential micronutrients such as ω -3 fatty acids, especially docosahexanoenic acid (DHA).

- Assessment of allergenicity potential of novel proteins expressed in genetically modified (GM) plants under varying conditions of digestion and thermal treatments. The assessment of thermal stability of recombinant barnase (RNase) and barstar (RNase inhibitor) proteins expressed in transgenic *Brassica juncea* was undertaken. The enzyme activities of these proteins did not indicate significant changes upon heat treatment showing high heat stability of these proteins. The potential for food allergy risk from these proteins is considered limited.
- Dose and time dependent effects of *Mucuna pruriens* Linn. ethanolic seed extract on stress related parameters in WNIN/GR-Ob rats was studied. Physical parameters like growth, food intake, feed efficiency ratio, activity was monitored for the experimental period of 45 days. By supplementing *M. pruriens* which contains dopamine and improving environmental conditions the animals exhibited less stress levels.

ACHIEVEMENTS WITH PUBLIC HEALTH IMPORTANCE

PRECLINICAL TOXICOLOGY

- The Centre has conducted studies on acute toxicity study in mice and sub chronic toxicity study in rats, *in vitro* allergenicity test of seed and leaf of transgenic mustard containing Hybrid Dmh-11 and a preclinical bioavailability and safety evaluation of Temozolamide Co-Crystal.
- The Centre has also taken up the investigation on safety evaluation of iron fillings in tea. The report has been submitted to Tea Board of India and it is further evaluated by Experts of the Food Safety and Standards Authority of India (FSSAI) on considering the safety limits of 250 ppm iron filing in tea.
- Pre-clinical safety evaluation of liquid pentavalent vaccine (DTwP+HepB+Hib) for local tolerance. This product is now translated for clinical trial by DCGI.

NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES, HYDERABAD

- Studies on anti obesity properties of *Garcinia* species indicated that the maximum activity against hyperlipidemia was exhibited at high dose level of *G. 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 may explore its mechanism of action so that a good antihyperlipidemic and antiglycating agent could be developed.

- An epidemiological study carried out to assess the effect of consumption of Khesari dal on human health in the State of Chattisgarh, indicated that only nine suspected old cases of neuroleptism were identified in the State.
- The prevalence of undernutrition especially among under five year children may vary geographically. A study identified undernutrition and causative factors in the specific districts and the study results may help in effectively channelizing the limited resources for high-burden area.
- A randomized controlled interventional trial with a 12 month home-based intervention four-arm trial with multiple micronutrient powder (MNP), early learning, combination and a placebo proved that the MNP intervention resulted in significant anaemia reduction in both infants and preschoolers. There was a significant improvement in biomarkers of iron and cognition in infants.
- Composition of bioactive phytochemicals in commonly consumed Indian foods including regional and tribal foods were evaluated. It was found that there is a considerable evidence for the role of antioxidant constituents of fruits and vegetables in the maintenance of health and disease prevention. Studies showed that phytochemicals, some of them not necessarily antioxidants, are also bioactive for preventing many chronic degenerative diseases.
- Knowledge, attitude and practices [KAP] of medical practitioners regarding food allergy (FA) were studied. More than 50 per cent

knew that FA is a serious problem but did not responded correctly to questions on difference between food allergy and intolerance.

- A meta-analysis of randomized, controlled feeding trials of iron fortified foods evaluated haemoglobin (Hb) concentration using meta-regression analysis. This indicated that iron fortified foods could be an effective strategy for reducing iron deficiency anaemia in children.
- A nutrition education intervention study evaluated the impact of a label information reading kit on usage of food labels among adolescents for promoting healthy food choices. The kit titled 'Read-B4-U-Eat' was developed and was efficacious nutrition education module to inculcate label reading skills among adolescent consumers. This could be used to improve label reading skills among adolescents and develop healthy eating habits.
- A study was conducted on prevalence of fluorosis in Doda district of Jammu and Kashmir. The fluoride levels were the highest in Golibagh followed by Malwas village resulting in high intake of fluoride.
- A study revealed that exogenous oxytocin injections do not influence its content in milk and oxytocin administered orally is rapidly digested.
- Assessment of allergenicity potential of novel proteins expressed in genetically modified (GM) plants under varying conditions of digestion and thermal treatments proved that the potential for food allergy risk from these proteins could be limited.

Extramural Research

In the year 2014-2015, the Division of Nutrition is supporting 25 projects, five projects were completed whereas approx. 33 projects are under consideration. The projects received from different parts of the country including the North-East and tribal regions are being supported. The projects range from community based intervention studies on impact of dietary salt restriction in the reduction of blood pressure among tea garden workers of

Assam to supplementation trials and molecular level studies.

The recently completed study on impact of community based dietary salt restriction in the reduction of blood pressure among tea garden workers of Assam reported the overall prevalence of hypertension to be 50.7 per cent (male: 52.9%, female: 47.7%). Also, following intervention and follow up of 6-8 months, mean systolic blood pressure (SBP) reduced from the baseline value of 139.5 ± 26.5 to 135.5 ± 15.2 mmHg ($P=0.018$) and diastolic blood pressure (DBP) reduced from 84.3 ± 14.8 to 80.7 ± 10.7 mmHg ($P=0.03$); whereas no improvement was observed in the control group.

Another study on Iodine status in the State of Uttarakhand revealed that in all the three districts (Nainital, Udham Singh Nagar and Pauri Garhwal), the iodine deficiency existed in the neonates as seen by thyroid stimulating hormone (TSH) levels. The high TSH value amongst neonates indicated the low intake of iodine amongst pregnant women. Further, the study also reported that pregnant women in all the three study districts were iodine deficient as indicated by low median urinary iodine concentration and lower levels of adequately iodized salt.

Some of the other ongoing studies include dietary exposure assessment of polychlorinated biphenyls (PCBs); role of omega-2 fatty acids against chronic lead induced neurotoxicity. *etc.*

During the period under report; a total of 50 proposals are ongoing; six projects have been completed; whereas approx. 10 proposals are under consideration. A number of fellowship proposals covering varied areas of nutrition are currently being supported.

A 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 sanctioned for 54 districts in the country where prevalence of undernutrition and anaemia was

reported to be high as per DLHS-2 survey or where Model Rural Health Research Units have been established by Department of Health Research. The baseline survey has been initiated, which will be followed by intervention component of 18 months with an aim to improve health and nutrition status of vulnerable segment of population.

Another Task Force study entitled “Effectiveness of diet and lifestyle intervention through information education communication (IEC) tools with Anganwadi Centres (AWCs) as the centre of knowledge dissemination for hypertension (including hypercholesterolemia and diabetes) risk reduction – a cluster randomised controlled trial” is being carried out at 10 sites across the country, including seven in tribal belt. The study involves collection of baseline information on knowledge, attitude and practice of people about hypertension, which will be followed by 18 months intervention period involving individual, group and mass activities to educate people for diet and lifestyle modification for reduction in BP. All biochemical analysis (haemoglobin, glucose and lipid profile) of the biological samples collected under the study is being done at NABL accredited laboratory at ‘Centre for Promotion of Nutrition Research and Training... (ICMR)’. A total of 60,000 samples have to be analyzed under the study. Till date, around 15,000 samples have already been collected and analyzed under the project.

Centre for promotion of nutrition research and training with special focus on North-East, tribal and inaccessible population

The laboratory at the Centre carried out the analysis of over 12,000 serum, urine samples, blood spots on filter paper for various nutritional parameters like haemoglobin, glucose, lipid profile, ferritin, folic acid, vitamin B12, vitamin A, vitamin E, *etc.* collected under the Multicentre Intervention Task Force study on hypertension or 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. Currently four Ph.D students are enrolled with the Centre.

NATIONAL NUTRITION MONITORING BUREAU (NNMB)

The NNMB was established by the ICMR in 1972 in the States of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa, West Bengal and Uttar Pradesh. The DG, ICMR and Secretary, DHR, GoI, has sanctioned six more new NNMB Units in the States/UTs of Assam, Andaman & Nicobar Islands, Bihar, Delhi, Rajasthan and Pudhucherry in the year 2012. The house-to-house survey is going on and Final Report of NNMB will be submitted to the ICMR.

MAJOR ACHIEVEMENTS HAVING PUBLIC HEALTH IMPORTANCE

The Task Force on Hypertension is an intervention study being carried out with an aim to reduce the prevalence of hypertension in the country including the tribal population with the help of diet and lifestyle modification. So far, approx. 15,000 whole blood/ serum samples collected from the community have been analyzed for haemoglobin, glucose and lipid profile and advisory have been issued to all those whose biochemical investigations showed high levels for glucose or lipid profile or low levels of haemoglobin. The study has helped in identifying those with impaired glucose tolerance and the population is being counseled for dietary modifications to address the issue. Further, cases of newly diagnosed diabetics and hypertensives have been advised for treatment.

The Task Force on Nutrition sanctioned for 54 districts in the country on the directives of the Planning Commission, has an inbuilt intervention component to improve the health and nutritional status of vulnerable segment of population through use of existing health personnel in the study locale.

The laboratory at CNRT analyzed serum samples collected under WHO trial on vitamin A supplementation among infancy. The work has recently been published in *Lancet*.

ENVIRONMENTAL & OCCUPATIONAL HEALTH

Research in priority areas of occupational and environmental health relevant to national needs for various working groups is actively undertaken by the National Institute of Occupational Health, Ahmedabad, the National Institute for Research in Environmental Health, Bhopal and the Bhopal Memorial Hospital and Research Centre, also in Bhopal. Major highlights of various programmes undertaken by the ICMR in the areas of occupational and environmental health during the year 2014-2015 are given below.

Intramural Research

NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH, AHMEDABAD

Association of adverse neonatal/perinatal outcome with biomass fuel use

This comparative study was initiated to compare adverse neonatal/perinatal outcome with normal subjects in relation to their fuel use characteristics. The study included 609 subjects having mean age of the mothers as 23.1 ± 3.5 years, majority (55.5%) was in the age group of 21-30 years. Sixty five per cent of them had normal body mass index. More than half of the subjects were only primary level educated (54%). About 21 per cent had open kitchen and 34 per cent cooked in living rooms. A total of 44.7 per cent families used biomass fuel (either wood or wood and dung in combination). All the subjects had exposure of at least 10 cooking hour years from the fuel use. The study observed that low birth weight, lesser head circumference, neonatal death, less developed genitalia and need to stay at nursery was more frequent with mothers using biomass fuel when compared with other fuel users. Significantly increased risk of 'low birth weight'

and 'need of newborn to stay in neonatal care unit' 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. Lower education level of mother was also observed to be a near significant contributor of adverse neonatal outcome. The study also assessed the exposure of people to volatile organic compounds (VOCs) and particulate matter while cooking and found that coal and wood as major source for VOCs and particulates followed by kerosene and LPG. This study provided an indication of exposure pattern in respect to different fuels.



Occupational health of workers in ceramic industries

A cross sectional study on 329 male ceramics workers in Gujarat was conducted to determine overall health status among the working populations engaged in ceramics industry. The participants responded to self reported health questionnaire, infrared thermography, blood pressure, heart rate and oral temperature measurements. Thermal microclimate was monitored during the study

period. Results revealed that sleeplessness, muscle cramps and fatigue, excessive feeling of thirst, heavy sweating, elevated body temperature and headache were among the main responses of workers during their daily work schedule. General systemic health complaints were musculoskeletal discomforts (60%), digestive discomforts (39.1%), respiratory discomforts (34.3%), cardiovascular discomforts (18.2%) and visual discomforts (29.5%) (Figure 1). Pain in the lower extremities (~64%) and upper extremities (~26%) was reported by respondents which may be due to awkward posture of work for long hours and manual material handling (Figure 2). Self reported pain perception was mainly reported in lower back (42%) followed by knee (24%) and legs (23%). Job specialization, physical activity, skill acquisition, training and promotion, organizational commitment and task situation were the prime work stressors. The study iterated that long duration of work in ceramic industries is potential health risk to heat stress, musculoskeletal discomforts and work stressors.

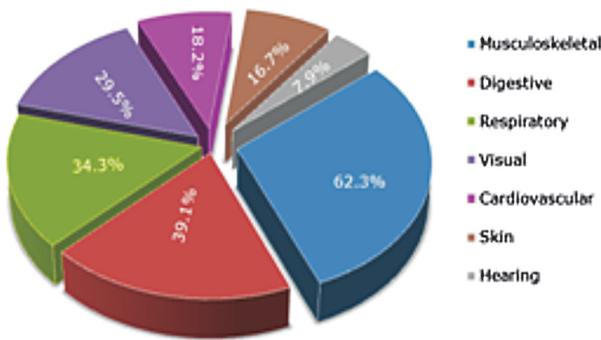


Fig. 1. Health complaints among ceramic.

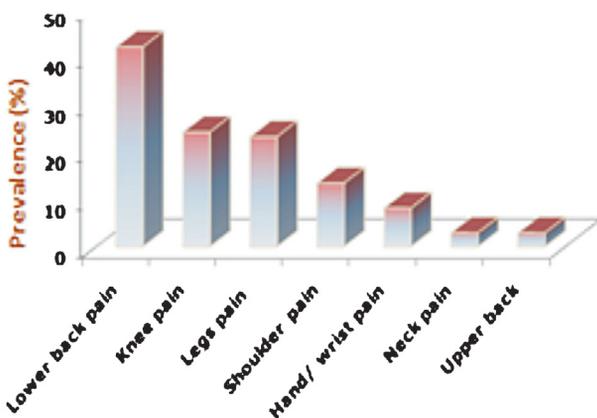


Fig. 2. Musculoskeletal pain and discomforts among ceramic workers.

Sero-prevalence of human brucellosis and work-practices among veterinarians in Gujarat

The study aimed to investigate the human sero-prevalance of brucellosis risk factors associated with human brucellosis in veterarians in brucella affected endemic districts of Gujarat using three different methods (RBPT test, IgM & IgG ELISA) and to assess the possible occupational risk factors associated with human brucellosis. A total of 365 veterinary personals (363 males and 2 females) participated in the study working in private dairy cooperative societies situated in Vadodara and Anand districts of Gujarat and State Government Animal Husbandry Department, Govt. of Gujarat, Gandhinagar. Of the 365 veterinarians, 50 (13.15%) were found positive in RBPT test, a rapid and simple spot agglutination test for primary screening of bovine population but it is also effective with the human blood serum and frequently use for brucella screening widely. RBPT test gave ~ 40-50 per cent accuracy. Another 59 (15.52%) were positive in IgM antibody titre test which gives information about recent infection or acute infection and 74 (19.47%) were positive in IgG antibody test which gives information about possible chronic infections and is significantly diagnosed by IgM and IgG antibodies titre respectively in the human serum having > 90 per cent accuracy of the test results. Sero positive screening for brucellosis cases among high-risk groups recorded a high positive rate among veterinarian, para veterinarian, livestock inspector and semen collector of these districts. Similar results were obtained by previous serological survey conducted among high-risk populations from different parts of India.

Occupational health of workers in iron foundries

This study was conducted in five iron foundries from the State of Gujarat. Heavy workload, sharp edges of raw materials, contact with hot parts, loading and unloading, spilling of molten metal during pouring process into moulds, moving hot iron bars in rolling mill and maintaining work pace with automated moving machines, make the workplace vulnerable to accidents and injuries.

A total of 259 workers from the production process participated in the study. The average age of

workers was 28.8 ± 9.4 years. The mean job tenure of the workers was 5.5 years. Nearly all the workers worked more than eight hours. The mean ambient temperature was 43.4°C , whereas the relative humidity was as low as 22.5 per cent. The WBGT index was 31.4 ± 1.7 . Figure 3 shows the thermal responses of workers exposed to ergonomic-related injury risks, such as lifting heavy items, bending, reaching overhead, pushing and pulling heavy load, working in awkward body postures and performing the same. Lower back pain was reported by 64.8 per cent followed by knee pain (44%) and legs pain (31.5%) .

The general systemic health complaints as reported by the iron foundry workers are shown in Figure 4. Hearing problems was also reported by 13.9 per cent workers. It is suggested to protect the workers health by work-rest schedule, job rotation, adherence to limit working hours, periodic health check up and use of PPE, among others.

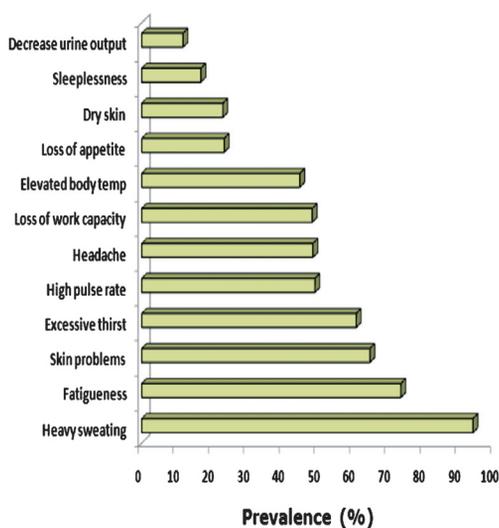


Fig. 3. Response to heat stress disorder among iron workers.

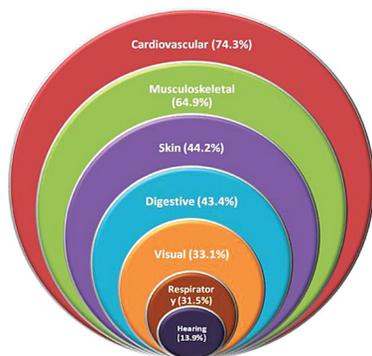


Fig. 4. Health complaints among iron foundry workers.

Musculoskeletal disorders with long-term exposure to lead in workers from lead acid-storage battery

The study was carried out to assess the prevalence of musculoskeletal disorders, blood lead levels and nutritional assessment in 482 workers exposed to lead (Pb) from lead acid storage battery plant. The musculoskeletal disorder (MSD) was assessed by using a Nordic musculoskeletal disorders questionnaire (NMQ). Blood lead levels (BLLs) were determined using atomic absorption spectrophotometer. Highest prevalence of MSD is noticed in knee followed by low back pain, shoulders, neck, ankles, wrists/hands, elbows, upper back and hips/thighs (Figure 5). BLL among lead exposed workers was associated with odds of musculoskeletal morbidities. The Hb%, serum calcium, magnesium and handgrip in both hands were lower in workers with musculoskeletal disorders. The inflammatory marker of highly sensitive C-reactive protein was significantly increased in lead exposed workers having MSD compare to non symptomatic subjects. The workers, having MSD have shown decreased muscle strength in both hands as compared to non symptomatic workers.

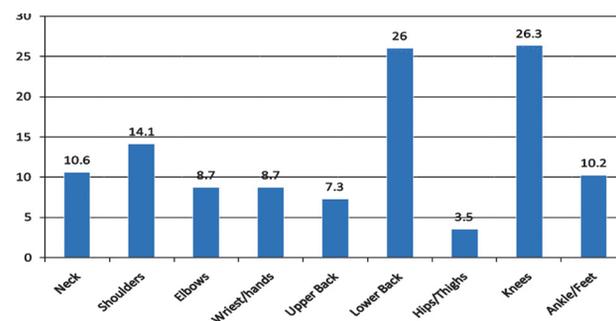


Fig. 5. Prevalence of MSD in lead exposed workers.

Health effects of workers in sewage treatment plants(STPs)

This study aimed to evaluate the respiratory health problems associated with bioaerosols and spirometric measurement of the workers of sewage treatment plants. The study included enumeration of airborne microorganisms, endotoxins, beta, 1-3 D glucan and to assess the seasonal variation of different types of cultivable airborne microorganisms in sewage associated environment in order to correlate with health risk of workers. Three different outdoor plants [180, 106 and 126

MLD (metric liter per day)] of Ahmedabad city adopting different technology were monitored. The 180 MLD capacity plant was aerobic and using ASP (activated sludge process) technology while other two plants were anaerobic and the technology used was upflow anaerobic sludge blanket (UASB). While medical examination was carried out in five different sewage treatment plants.

A total of 107 workers [93(86.9%) males and 14(13.1%) females] were subjected to clinical examination and spirometry in this cross sectional study. The median duration of exposure was 24 months. Seventy one (66.4%) of the workers were directly exposed as they were working on the plant. The spirometry analysis revealed that 88.8 per cent of the subjects were not having any pulmonary impairment while 5.6 and 2.8 per cent had restrictive and obstructive type of pulmonary function impairment, respectively. The morbid conditions among the study showed that nine (8.4%) subjects had skin ailment while other symptoms included cough in one (0.9%), headache in two (1.9%) and joint pains also in two (1.9%) workers. The aerobiological and health monitoring has also been carried out in two seasons *viz.* summer and winter to check the seasonal variation in the number of airborne microorganisms as well as endotoxins and beta 1-3 D glucan concentrations in order to correlate with the health risk of workers. The results suggest that the viable bacteria were recorded higher during winter season compared to summer. This might be due to the moderate temperature during winter season. As all the plants are outdoor, the duration of exposure of the workers was comparatively low. These factors must be considered in order to obtain relevant exposure determinations and establish preventive measures from a health risk perspective.

Chronic health effects caused by repeated exposures have not been established. Common symptoms to non-acute exposure levels include eye irritation, fatigue, headache, and dizziness. The concentrations of CO and CH₄ (methane) were not detected during monitoring in these STPs because of efficient digestion system and the technology involved in collection and storing the CH₄ in the gas collection systems below the UASB reactors which is stored in gas holders. The methane is flared in gas holders.

But the concentrations of hydrogen sulphide (H₂S) have exceeded the permissible exposure limit (PEL) of 10 ppm as per Indian Factories Act 1948 at distribution chambers, UASB reactor, reactor outlets and final outlet channel areas. The workers might be exposed to H₂S at these areas. Regular air monitoring for H₂S and health monitoring among employees is recommended at least at an interval of once a year.

Health study of population on and around coalfield area of Northern Coalfields Limited

This study included coal miners from 10 mines of Northern Coalfields Limited. A total of 719 subjects were recruited in the study: 441 workers (working on the direct mining activities of Northern Coalfields Limited) selected by proportionate stratified random sampling method and 278 residents in the immediate vicinity of the mines.

The demographic characteristics of the mine workers and residents in the vicinity indicated that the age group was 45-54 and 25-34 years in most of the mine workers and the residents group, respectively. Most of the subjects were married and attained middle school level of education in both the groups. Most of the study subjects were non smokers and did not have the alcohol drinking habits. More or less the equal proportion of coal miners and residents had tobacco chewing habits. The occupational characteristics of coal mine workers showed that most of them were working in the coal mines for more than 20 years and the mean duration of exposure was 23.2 ± 8.8 years. Maximum 77(17.5%) miners were dumper operator followed by 70(15.9%) fitters and 60(13.6%) miners working as helpers or general mazdoor.

The common respiratory complaints observed among coal miners were cough in 29(6.6%) workers of which only 17(3.9%) had productive cough. Breathlessness and haemoptysis were reported by only 11(2.5%) and three(0.7%) coal miners, respectively. Similarly in the resident group, only seven (2.5%) reported cough, of which four (1.4%) had productive cough. The other symptoms observed among coal miners were tiredness (3.4%), backache (6.6%) and difficulty in hearing (5.2%). Similarly among residents the other symptoms included tiredness (2.9%), backache (11%),

digestive disturbances (2.2%), sciatica (1.8%) and difficulty in hearing (1.8%).

Profile of psycho social stressors, mental health problems and its electroencephalographic correlates in workers exposed to chronic occupation associated noise

This preliminary study attempted to explore the effect of chronic noise’s exposure on mental health among textile workers and somatic symptoms in the workers associated with the chronic occupational exposure (>2years) to excessive sound ($\geq 80\text{dB}$). A total of 76 male workers were enrolled (aged 48.2 ± 10.3 years) who were working with high noise exposure for a mean duration of 18.26 ± 11.42 years. On General Health Questionnaire (GHQ) eight workers (10.5%) reported positive and 46.1per cent (n=35) workers reported somatic symptoms with predominantly, chest tightness, muscle soreness and hot/cold spells. On Modified Mini International (MINI) 14.5per cent (n=11) workers reported positively with predominant anxiety and depressive symptoms.

The study observed that, in addition to hearing related problems, excessive sound may be associated with various mental stress and psychological problems in the form of anxiety and depressive spectrum of disorders, annoyance, poor concentration, mental fatigue, reduced alertness and disruption of sleep. This preliminary study suggests prevalence of depression and anxiety among workers, exposed with high workplace noise, as also to plan larger studies to examine and understand ill effects of the long standing sound exposure; electrophysiological correlates to understand the occupation associated mental health problems.



Fig. Photo showing the weavers working in the weaving area with multiple weaving machines placed in at a single place with high noise levels.

Effect of indoor air pollution on women and children in India

This study is primarily aimed to better understand the complex linkages between exposure to particulate air pollution and respiratory diseases in India. Specifically, a total of 1265 questionnaires are filled from the low socio income areas of Ahmedabad. They were categorized as LPG and kerosene users on the basis of use of cooking fuel. Medical examination of 504 non-working women and their 806 children (below 10 years) was performed. Of the 504 subjects (non-working women) ,only 483 subjects (non-working women) were co-operated for pulmonary function test (PFT). Of the 483, 457 subjects had normal PFT, whereas 26 subjects had restrictive, obstructive and combined impairments. Cytokines (IL-1, IL-2, IL-4, IL-8, IL-6, IL-10, TNF- α , TNF- β , IFN- γ and RANTES) were analysis in 96 sputum samples (LPG- 52, kerosene- 43 and wood- 1). All were found in normal range. Real time indoor air monitoring for 24 hours of 81 houses (LPG- 37, kerosene- 43 and wood-1) was carried out for the parameters like particulate mass, particle surface area, particle count, carbon dioxide (CO₂), carbon monoxide (CO), temperature and relative humidity. Work for air monitoring and data analysis of processed samples is in progress.



Environment Information System (ENVIS) on occupational and environmental health

Environment Information System (ENVIS) Centre, at NIOH is a thematic Centre of Ministry of Environment, Forests and Climate Change based on the theme of Environment and Occupational Health. It is engaged in collection, collation, storage,

retrieval and dissemination of information related to its theme. It is a resource Centre for those engaged in research, policy making and implementation, students, working class and general population through its website and printed materials.

During the year, this Centre brought out the ENVIS Newsletters “WorkOSH” [ISSN: 2393-8943] as also four newsletters on E-waste management, Agricultural health hazards, Major industrial disasters in India and Environmental health legislations in India. Two Hindi newsletters were also published. Two issues of bibliography viz. “Occupational skin disorders” and “Industrial effluents” were compiled using JabRef software and made accessible through website. Awareness programmes were conducted for workers engaged in construction, ceramic industry and iron foundry.



NATIONAL INSTITUTE FOR RESEARCH IN ENVIRONMENTAL HEALTH (NIREH), BHOPAL

Population based long term epidemiological studies on health effects of Bhopal toxic gas exposure

This study has been continuing since 1985 (1985-1994 by ICMR under BGDRC; 1996-2010 under Centre for Rehabilitation Studies, Government of M.P; 2011 onwards under NIREH) wherein part of the original following cohort of exposed and unexposed persons is being surveyed at six monthly intervals for morbidities and mortalities. During the period under report, 48th (Feb-June, 2014) and 49th (Sept-Dec, 2014) rounds of survey were completed and 50th round was initiated in Jan 2015. During the 48th round of survey, a cohort of 27,181 individuals (21,840 exposed and 5,341 unexposed) from severely exposed (n=7,827), moderately

exposed (n=8,089), mildly exposed (n=5,924) and unexposed/control (n=5,341) areas were followed up. Any morbidity recorded was 22.8 per cent in severely exposed, 17.6 per cent in moderately exposed and 18.2 per cent in mildly exposed areas compared to 9.8 per cent in unexposed areas. Crude death rates were similar in the exposed (4.48/1,000 population) and unexposed cohort (4.49/1,000 population). Respiratory disorders were the primary cause of death recorded in the exposed cohort with nearly three times higher mortality due to respiratory disorders (2.15/1000 population) than in the unexposed (0.74/1000 population) cohort.

During the 49th round of survey a cohort of 29,657 individuals (23,534 exposed and 6,123 unexposed) belonging to severely affected (n=7,851), moderately affected (n=8,515) and mildly affected (7,168) areas and unexposed/control areas (n=6,123) were followed up. The morbidity recorded was 23.6 per cent in severely exposed areas, 16.3 per cent in moderately exposed areas and 17.4 per cent in mildly exposed areas which were higher than 9.5 per cent morbidity recorded in the control areas. Crude mortality rates were found almost similar in the two areas i.e. 4.5/1000 population in the exposed and 4.1/1000 population in control areas. Mortality due to respiratory disorders in the exposed areas (1.6/1000 population) was nearly five times higher than the control (0.32/1000 population) areas.

Long term genetic effect(s) of methyl isocyanate (MIC) gas, if any, on the Bhopal population exposed in December, 1984

In this pilot study the cytogenetic status of 100 gas exposed and 100 unexposed individuals among those screened earlier under multi-centric genetic screening study of ICMR in Bhopal during 1986-1990 is being examined and compared with the previous genetic status to exclude the possibility of long term effects, if any, of MIC gas. Among the 800 retrieved pre-screened cases (543 exposed, 257 unexposed) only 267 were reportedly present in Bhopal of which 174 were contacted. Three generation pedigree of 147 consenting cases (127 exposed, 20 unexposed) was prepared. Cytogenetic analysis of limited number of samples (n=22) revealed cultures with low mitotic index (36% cases), assemblage of D/G group (>90% cases) and spontaneous aberration (22% cases).

Genetics and epigenetics of lung function among the victims of Bhopal gas disaster

This study aimed to find out the allele frequency, association and size effect of the selected gene polymorphism related to lung function among gas exposed people along with epigenetic studies in a subset of severely exposed people. 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 were identified. Further, from Bhopal Memorial Hospital & Research Centre (BMHRC), Bhopal, India database (between 2002-2008) 809 individuals were shortlisted. A total of 110 samples from subjects residing in the severely exposed areas were collected for genetic and epigenetic studies.

Cytogenetic analysis in methyl isocyanate (MIC) exposed population and their progeny

In this study the cytogenetic status of 1,800 gas exposed people aged >28 years belonging to six sub-groups and their progeny is being investigated. So far, a total of 1,027 eligible individuals have been enrolled in the study, counselling and pedigree analysis completed for 514 cases. Sample collection, processing and karyotyping has been completed for 106 cases.

To evaluate biochemical markers in cases of clinically stable stages of chronic obstructive pulmonary disease (COPD) in MIC affected population

This pilot study envisages to evaluate six biochemical markers of potential prognostic value of COPD in serum and induced sputum of 40 clinically stable mild, moderate and severe COPD cases among gas exposed persons. Biochemical markers evaluation for their expression and quantification in serum of 38 subjects (with COPD and no co-morbidities) have been evaluated.

Point prevalence morbidity of selected population/families with reference to drinking water

This study is examining the prevalence of morbidities in a sample population and its correlation with

reference to the use of drinking water sources in two areas *viz.* areas up to 1 km radius from UCIL boundary (Stratum I; sample size 1,092 families) and areas between 2.5-5.0 km radius from UCIL boundary (Stratum II; sample size 1,092 families). So far, 4,369 individuals belonging to 861 families in the two strata have been surveyed for morbidities and 3,296 individuals have been clinically examined.

Multi-centric study to find out prevalence of chronic kidney disease in adults in urban Indian population

This ICMR Task Force Study involving nine Centres, including Bhopal, is determining the prevalence of chronic kidney diseases in urban Indian adults. In this cross sectional community based study it is envisaged to survey 2,714 individuals above 18 years of age belonging to 1,300 households in 24 wards of Bhopal, selected through cluster randomized sampling technique. So far, work has been completed in three wards *viz.*, ward numbers. 25, 27 and 28 by enrolling 240 individuals.

BHOPAL MEMORIAL HOSPITAL & RESEARCH CENTRE (BMHRC), BHOPAL

Glutathione-s-transferase (GSTMI and GSTTI) haplotypes: Susceptibility to chronic obstructive pulmonary disorders (COPD) in smokers

The polymorphism in GSTMI genes was found to be associated with COPD. Individuals with GSTMI/non-null allele genotype were found to have enhanced susceptibility to oxidative stress-related pulmonary disorders *viz.* COPD.

Association of C-159T polymorphism in CD14 gene in patients suffering from pulmonary tuberculosis

The association between the CD14-159C/ T polymorphism and tuberculosis among the studied population showed CT genotype to be associated with enhanced susceptibility to MTB infection. However, significantly elevated serum sCD14, IgE and plasma N-GAL levels in patients reflect the importance of the mononuclear phagocytic system

activation in tuberculosis and may implicate that CD14 signaling as host response.

Effect of pesticide intermediate on *Escherchia coli* -A molecular approach

The study observed a mild bacteriostatic effect of NMNMC at the highest concentration of 100 pM, while agarose gel analysis of amplified *uspA* gene (PCR) revealed that no DNA damage has been induced by the analogue-NSNMC. The protein profile of cultures (con, iso, 10, 50, and 100 pM) processed at 24 hours of incubation revealed no change in band pattern. Whereas, a ~13 kDa band of *UspA* specific in *E. coli* was observed in all the cultures processed at 24, 48, 72 and 96 hours but not in the isolate cultures.

Cytogenetic changes in MIC exposed chronic kidney disease patients visiting BMHRC, Bhopal

This study evaluated cytogenetic changes in MIC exposed chronic kidney disease patients visiting BMHRC, Bhopal. The results showed significant increase in mitotic index, aneuploidy and chromatid breaks in Group-I. Moderate, linear and positive correlation was found between mitotic index and lymphocyte count of Group-I.

Cytogenetic study of patients with suspected genetic disorders in MIC gas victims of Bhopal gas tragedy with special reference to mentally challenged patients

The findings of this study suggest toxic gas may cause genomic changes in the exposed population having mental retardation. Chromosomal studies in mentally retarded patients may help in proper prognosis and diagnosis followed by genetic counselling and management rehabilitations, however, small sample size of the present study limits its merit.

Anticancer mechanism of curcumin and temozolomide on growth and inhibition of human brain tumor cell line

The comparative study of purified curcumin and chemotherapeutic drug temozolomide, singularly and in combination, against glioma cell line, LN-18; presented that the morphological studies

of LN-18 cells when treated with both the test drugs, showed potent efficacy against LN-18 cancer cells even at micro molar concentrations, but results with curcumin revealed that even at a lower dose (100pM) it is more effective than higher doses of temozolomide (200uM). The viability assays indicated the effectiveness of curcumin and temozolomide in a dose and time dependent manner. In both the groups the cell viability was significantly decreased and degree of inhibition increased with duration of treatment but 100uM curcumin was more potent than 200uM temozolomide. The curcumin and temozolomide treated tumour cells, singularly and in combination revealed the presence of pro apoptotic bodies, although apoptotic cell death was not induced. Also, Western blot results revealed that expression of *Wnt1* was down regulated on treatment with the test drugs indicating that curcumin can be used as a potential inhibitor of *Wnt-1* which may lead to inhibition cell proliferation and induction of apoptosis.

Trends of bacterial infections and their 'antimicrobial sensitivities among patients admitted in the intensive care unit (ICU)

The study assessed trends in antimicrobial susceptibilities among organisms isolated from patients admitted in the ICU. The results can be utilised to monitor antibiotic 'prescribing practices, as also provide the basis for targeted interventions to improve efficacy of prescribed antibiotics.

Prevalence of non tuberculous mycobacteria (NTMs) among AFB smear positive sputum samples

The study verified an increased awareness among the treating physicians, of the possible role of NTMs in clinical disease may help to decrease morbidity and mortality by timely initiating an appropriate therapy.

Airway device for patients undergoing elective laparoscopic surgeries under general anesthesia: I-gel (SAD) vs endotracheal tube (cuffed) – A comparative study

The study suggests that I-gel is easier to use, has smoother haemodynamic profile and it is as efficient as endotracheal tube in providing adequate

ventilation and oxygenation. But for the perilyngeal seal required, it is inferior to ETT. I-gel is a good alternative to ETT with added advantages. It is recommended that I-gel must be used for laparoscopic surgeries only by experienced users. The I-gel is an effective supraglottic airway and adds to the armamentarium of the anaesthesiologists.

A comparative study of intraperitoneal bupivacaine with tramadol vs intraperitoneal bupivacaine with magnesium sulphate for pain relief after laparoscopic cholecystectomy : A prospective randomized, double blind controlled study

The study established that the intraperitoneal instillation of tramadol with bupivacaine is an effective method for management of postoperative pain after laparoscopic cholecystectomy. It significantly reduced supplementary postoperative analgesic and with early patient mobility and discharge. But when compared tramadol plus bupivacaine with magnesium plus bupivacaine, tramadol had no synergistic effect with bupivacaine as compared to magnesium for reducing postoperative pain.

Observational study to analyse effect of institution wise educational program on implementation of surviving sepsis guidelines in severe sepsis patients and its effect on 12 outcome

The study suggests that the institute wide education program has significant impact on increasing compliance to both three and six hours bundles in Surviving Sepsis Campaign Guidelines but failed to show any significant impact on decreasing overall ICU and hospital mortality.

How useful is our pre-operative assessment of airway in predicting difficult intubation in obese patients - an observational study

The study suggests that the modified Mallampati test, thyromental distance, Delikan test are the better predictors of difficult intubation. In some patients IDS scoring was 25 who were not predicted to be difficult by routine preoperative tests. Therefore, studies aimed at identifying new predictors of difficult intubation in obese patients need be done. However, in obese patients skilled anesthetic assistance and a wide range of equipment to facilitate intubation should be available.

A descriptive study to assess the level of assertiveness among staff nurses working in BMHRC, Bhopal

The study advocates that graduate nurses and those who had professional experience of five years as also belong to rural area are non-assertive. Therefore, there is need for provision of in-service assertiveness skill training for this group of nurses in the BMHRC, Bhopal.

A descriptive study to identify the prevalence of co-morbidity among chronic obstructive pulmonary disease COPD patient seeking the treatment in OPD of BMHRC, Bhopal

This study observed that the COPD patients mostly had co-morbidities like hypertension, tuberculosis, cardiac diseases, cachexia, and respiratory problems.

XIAP gene expression in oral cancer

The study describes the potential of XIAP gene, involved in abnormal growth of oral squamous cells, as it belongs to human inhibitor of apoptosis protein family. XIAP is mainly coordinated through binding to effector caspase-3 and caspase-7, and down regulated apoptosis mechanism.

Caspase-3 expression in oral carcinoma

The study suggests that caspase-3 plays an important role in apoptosis pathway, any deregulation in Casp-3 gene may lead to failure of apoptosis resulting cancer, 54 per cent of our samples have not shown any implication of caspase-3, suggesting its role in OSCC. Furthermore, protein and RNA expression analysis of the caspase-3 may reveal interesting facts about caspase-3, which in turn may establish it as an important drug target for OSC.

Submission of study related documents for secondary prevention of coronary events after discharge from hospital "SPREAD"-A randomized, open trial comparing post discharge interventions to standard care in acute coronary syndrome (ACS) patients

The study evaluates prevention of reoccurrence of coronary events after discharge from hospital by lifestyle modifications and dietary intervention. The study was a double blind trial with positive effects

of dietary intervention and lifestyle modification to prevent reoccurrence of MI on coronary events after discharge from hospital *e.g.* cutting cigarette smoking, exercise, food regulations, medicine adherence, *etc.*

Utility of thromboplastin - plasma cell block technique and role of immunohistochemistry in serous effusions - A comparative study with conventional smear cytology

The study suggests that the cell block preparations act as a useful adjunct to smear preparations for categorization of malignant and benign. Plasma thromboplastin cell block technique is simple, requires less time and offers improved cytomorphological features as compared to conventional techniques. Plasma thromboplastin cell block method provides high cellularity, better morphological features and architectural patterns. It also offers an additional advantage for preservation of cell blocks for future molecular pathology. It is thus recommended that in all the samples of serous effusions that are received from patients of known clinical history of malignancy and in whom incidental suspicious cells are observed, cell block preparation is essential.

Extramural Research

Climate Change Project

A multidisciplinary, multi-centric and multi institutional study for generation of evidence of change in climatic conditions as well as on anopheline vectors and malaria so as to suggest adaptation measures for addressing the adverse impact of climate change. The study was undertaken at Nainital, Almora in Uttarakhand; Ramgarh and Ranchi in Jharkhand, Karbi Anglong in Assam and Kolasib and West Aizwal in Mizoram. Data on indoor/outdoor temperature, mosquito density, fever survey, detection of sporozoites from field collected mosquitoes, socio-economics of communities and current adaptation measures being taken by the health system were generated.

Based on generated daily data of temperature at all the study sites, it was found that over a span of three years, changes in temperature were noticeable in study areas of Karbi Anglong and

Almora districts, *etc.*, up to the tune of 2.69°C. Even decrease in temperature was also observed in Uttarakhand in winter months. Study provided insight for significance of using indoor temperature versus outdoor for determination of transmission windows and provided evidence for difference in temperature of breeding habitats (water body) and resting habitats of mosquitoes. Based on daily generated data of temperature, the degree days required for completion of sporogonic cycles could be determined which provided answer to high malaria endemicity in Ranchi (Jharkhand) against Bokajan (Assam). *Anopheles minimus* was encountered in Bokajan only on a few occasions; *An. culicifacies* was collected in high density (up to 82) from a few villages which were linked to deforestation. Detection of *Plasmodium falciparum* in the month of February from Nishola and Bhorra villages in Bhimtal PHC (hilly area) in Tadikhet (Almora), wherefrom malaria is not reported by local health authorities, provides evidence of knocking of malaria transmission in hilly areas. The projection of probable months of malaria transmission by mathematical model based on generated data of temperature in study villages and inputs from PRECIS model elicited that in 2030 and 2070 there is projected increase in TWs by 2-3 months. Based on Genetic Programming mathematical model, the density of *An. culicifacies* was predicted for some villages under Nainital with R² values from 0.7 to 0.94 between observed and predicted density. Socio-economic survey elicited through interview schedules (596) from two field sites revealed deficient knowledge, poor health seeking behaviour, non-acceptance of IRS and less use of bed nets *etc.* in all the sites. Dissemination workshops were conducted at Bhimtal, Ranchi and Bokajan to disseminate the findings of the project to concerned state government and delivered lectures for building the capacity of stakeholders with latest developments in malaria epidemiology and control.

The findings revealed that climatic conditions are changing as compared to 1960 and 1990 and hilly areas are showing evidence of malaria transmission even in the month of November and January when outdoor temperature is not conducive. Current adaptation measures in practice at three field sites were assessed and scope of capacity building and

strengthening of health system were identified for addressing the adverse impact of climate change.

Centre for Advanced Research on Environmental Health: Air Pollution

A total of 1285 women and 948 infants were followed up in the cohort for birth outcomes and acute respiratory infections, respectively. Two to four measurements of 24-hour concentrations of PM_{2.5} were also performed in multiple micro-environments (kitchen, living and near household areas) of participant households. The prevalence of low birth weight among rural and urban births was around 16 per cent with ~five per cent in both rural and urban arms reporting pre-term deliveries. In multivariate linear regression models, we observed a 4 g decrease per 10 µg/m³ change in 24-hour kitchen PM_{2.5} concentrations. In multivariate

logistic regression models, we observed a 2% increase in low birth weight per 10 µg/m³ change in PM_{2.5} concentrations.

Of the 1152 live births recorded in the M-C cohort, 20 children were lost on account of neonatal deaths (12) and early infant deaths (8). Of the 1132 children available for ARI assessments, continuous ARI assessments have been feasible on 948 (83.5%) children. We estimated that each child may have experienced ~10-13 episodes of ARI/year during the first two years.

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. Adult studies and modeling study are being completed.

NON-COMMUNICABLE DISEASES

ICMR's Institute of Cytology and Preventive Oncology and Preventive Oncology, Noida is engaged in research studies for prevention and early detection of cancer. The National Centre for Disease Informatics and Research, Bengaluru focusses on studies related to population and hospital based cancer registries, time trends in cancer incidence software development, etc. Besides, the Council is also engaged on studies in the areas of diabetes, neurological sciences, obesity and metabolic syndrome, *etc.* Highlights of studies undertaken by the ICMR in the area of non-communicable diseases during the year 2014-15 are given below.

Intramural Research

NATIONAL INSTITUTE OF CYTOLOGY AND PREVENTIVE ONCOLOGY (ICPO), NOIDA

Cancer prevention is one of the mandates of the institute. Following strategy is being followed at ICPO to achieve above objectives:-

1. Epidemiological studies and early cancer detection activities
2. Basic Molecular studies
3. Human resource development

CANCER PREVENTION ACTIVITIES

Early detection of cancer by population screening initiated

The ICPO has initiated program of cancer prevention by directly approaching the population in Gautam Buddha Nagar District of Uttar Pradesh with the aim to educate the population and screen them for early detection of Cervical, Oral and Breast cancer. This approach has become very popular and we are getting frequent requests from RWA's and *gram panchayats* for initiating screening programs. The screening programs conducted during the year are given in Table 1.

Glimpses of Cancer Screening Camps organized by ICPO: "A ray of hope for poor and for those having inhibition to approach expensive hospital services".

Table 1: Screening programmes conducted

| S. No | Site of the camp | Date | Total individuals screened | No of women screened for breast cancer | No of women screened for cervical cancer |
|--------------|-----------------------------------|-----------------------------|----------------------------|--|--|
| 1 | Community centre, sector 26 Noida | 13 th April 2014 | 76 | 25 | 12 |
| 2. | Amity University | 16 th April 2014 | 48 | 48 | 25 |
| 3. | Amity University | 07 th May 2014 | 51 | 51 | 33 |
| 4. | PHC Bisrakh | 11 th Sep 2014 | 50 | 50 | 38 |
| 5 | PHC Bisrakh | 16 th Oct 2014 | 104 | 104 | 84 |
| 6 | CHC, Badalpur | 1 st Nov 2014 | 57 | 57 | 46 |
| 7 | ICPO | 07 th Nov 2014 | 43 | 43 | 31 |
| 8 | CHC, Bhangel | 12 th Nov 2014 | 67 | 67 | 54 |
| 9 | IITF, Delhi | 14-27 Nov 2014 | 338 | - | 338 |
| 10 | CGHS Noida | 20 th Feb 2015 | 81 | 81 | 66 |
| 11 | PHC, Bisrakh | 19 th March 2015 | 17 | 17 | 15 |
| 12 | CHC, Bhangel | 30 th March 2015 | 38 | 38 | 36 |
| Total | | | 970 | 581 | 778 |



Web portal developed for information on cancer in India



Fig. 2. Web portal on Cancer.

ICPO has developed a web portal for providing information on different aspects of cancer. It has information in the form of pictorial/ cartoon, etc. for better understanding by the common man. It is made interactive to get more information

from experts from ICPO in respective field. This information portal will provide practical advice that can help save millions of lives (Fig. 2).

Validation study for Magnivisualizer developed at ICPO and launched by Government of India

ICPO developed a low cost magnifying device that can be used in field conditions both by clinicians and Paramedical staff for the detection of cervical and oral cancerous and precancerous lesions.

A non disclosure agreement and an agreement on technology transfer were signed between ICMR and M/S Smart Scientifics. ICMR is trying for its mass production.

Table 2. Variety of oral lesions detected and differentiated on the basis of colour, margins and contours by Magnivisualizer

| Type of lesions | With Torch | With Magnivisualizer |
|--------------------------------|------------|----------------------|
| Homo.Leukoplakia + OSMF | 5 | 10 |
| Homo.Leukoplakia | 5 | 24 |
| Non-Homo. Leukoplakia +OSMF | 2 | 5 |
| Non-Homo. Leukoplakia | 3 | 8 |
| Nodular Leukoplakia | 2 | 3 |
| Oral sub-mucous Fibrosis(OSMF) | 41 | 88 |
| Oral Lichen Planus | 1 | 3 |
| Erythroplakia | 0 | 2 |
| OSMF + Oral Lichen Planus | 0 | 1 |
| Suspicious for Cancer | 1 | 1 |
| Others | 2 | 8 |
| TOTAL | 62 | 153 |

Table 3. Lesion detected by torch and Magnivisualizer (Total no. tobacco users screened 1329)

| | Drivers and Conductors (n=553) | Below poverty line (n=178) | Villagers (n=598) | Total (n=1329) |
|---|--------------------------------|----------------------------|-------------------|-----------------|
| Lesions Identified with Tungsten bulb Torch | 66 (11.9%) | 12 (6.7%) | 26 (4.3%) | 104 (7.81%) |
| Lesions Differentiated with Tungsten bulb Torch | 40/66 (60.6%) | 8/12 (66.7%) | 14/26 (53.8%) | 62/104 (59.6%) |
| Lesions Identified with Magnivisualiser | 96 (17.3%) | 16 (9.0%) | 44 (7.4%) | 156 (11.7%) |
| Lesions Differentiated with Magnivisualiser: | 95/96 (98.9%) | 16/16 (100%) | 42/44 (95.4%) | 153/156 (98.1%) |

AV Magnivisualizer can detect the pre-cancer lesions of oral cavity more than two fold as compared to torch and it was 80-90% compared to cytology (Table 2 and 3).

Digital Magnivisualizer development:

After initial encouraging results and acceptance by the government of India as economical device that can be used for early detection of cancer and hence prevention, ICPO scientists have initiated the development of Magnivisualizer with attached digital photography system. It has been planned to make it possible to document and transmit the pictures through available mobile network to respective doctors for instant diagnosis. We hope to develop it within next two years.

Up scaling the Use of AV Magnivisualizer

Training workshops for up scaling the use of AV Magnivisualizer (VIAM) have been launched by the ICPO, NOIDA in collaboration with HRRC, ICMR and Field Units of NIRRH, Mumbai (PHASE-I). During this phase it is envisaged that the Medical professionals at the 30 field units of ICMR will be imparted training through the conduct of regional workshops of three days each. It is also envisaged that each trainee will train and evaluate at least five more medical personals under his/ her supervision. Furthermore, information will be gathered regarding the availability of cytology labs, colposcopic confirmation of lesions, availability of treatment of pre-cancerous and cancerous lesions. In case of non-availability of diagnostic/ treatment facilities, the feasibility of establishing a referral pattern to an established/ existing diagnostic/ treatment centre will be studied.

Demonstration, training and validation study of Magnivisualizer in different parts of the India Molecular Screening for Cervical Cancer.

The major activities and achievements in this field are that ICPO has been recognized as a National Referral Centre for HPV & Cervical Cancer Screening. ICPO has developed Multiplex PCR to detect various HPV types in one PCR reaction saving biological material, cost and time. It is proposed to develop kit for the detection of HPV DNA by PCR. It will contain all the reagents for DNA extraction and amplification of HPV DNA

and its detection. Detection of variant HPV types in Indian population is significant for the purpose of developing HPV vaccines.



HIV and HPV co-infection among female sex workers.

Cervical, oral and anal scrapes have been received at ICPO for 300 subjects. HPV 16 was the most prevalent genotype found in the samples analyzed. Multiple HPV genotypes detected. Work is currently in progress.

BREAST CANCER

Role of methylated CpG binding proteins and its involvement in the regulation of BRCA1, BRCA2 and p16 genes promoter in breast cancer cell lines

This study aims to observe the dynamic expression of BRCA1, BRCA2, p16 and MBD1, MBD2, MeCP2 proteins in cell lines of different hormonal status and its promoter regulation proteins. A phytochemical resveratrol will be used as a drug to destabilize the methylation-mediated silencing and its associated changes in MBD1, MBD2 and MeCP2 levels and regulation of BRCA1, BRCA2 and p16 genes.

ORAL CANCER

Molecular and Clinico-Epidemiological Studies on Oral Precancer and Cancer among Tobacco users in India:

Globally, over 3 Lakh new cases of oral cancer are diagnosed every year, where the majority of the cases are diagnosed in the advanced stages III or IV. This makes the oral cancer responsible for 3% to 10% of cancer mortality worldwide (The State of Oncology 2013, IPRI).

To identify the role of various cytokines (IL-10, IL-6 and TNFR-2), 50 cases and 50 controls were processed for identification of novel SNPs (Indian population) using denaturing high-performed liquid chromatography (DHPLC). The sequencing results showed one unknown mutation with hetro condition (C/T) in IL-10 was identified in one stretch. On analysis of the other starch of DNA we found only homoduplex peaks and some of the samples have peak shift. But on the analysis of the sequence of the sample for this DNA stretch, we did not found any novel mutation. But the observed variation in the peak pattern was for the already known mutation at -819. For further validation of these results; we need to increase the sample size and sequence more number of the samples. We also analyzed Exon 2 & Exon 4 of Tumor necrosis factor receptor II (TNFR II) based on chromatographic pattern noted on DHPLC, which shows a Hetero and Homo-duplexes peak pattern, and confirmation of novelty of the SNP will be determined by automated DNA sequencing.. This is first approach to scan full gene for novel nucleotide variations (not reported earlier) using DHPLC, the high throughput technology with reference to OSCC.

Studies on Genetic and Epigenetic alterations in head and neck cancers prevalent in north eastern region of India

Head and neck cancer is the sixth most frequently observed cancers The present study tries to identify the role of epigenetic mechanism in the process of oral carcinogenesis from the population of north eastern states of India as this population has unique genetic and dietary habits like eating smoked fish, increased usage of betel quid and tobacco products.

From the initial study with restricted genes using candidate gene approach with methylation specific PCR, it was found that alarmingly more than 70 % of cases have methylation mediated silencing in cell cycle regulatory genes and DNA repair genes. This not only brings to notice possible prevention strategy policies, but it also reminds for the inclusion of drugs that reverses the methylation patterns and treatment strategies.

Studies on Exosomal Components of precancerous oral cell lines and its implications in disease Management

Using tissue culture model system, the present study attempts to identify certain Exosomes proteins which are secreted differentially during the precancerous and cancer phase of the cancer development. Initial studies were undertaken to establish the protein components.

THERAPEUTIC

Development of Epidermal Growth Factor Receptor Inhibitor Database

EGFRIndb is a literature curated database of 4581 small synthetic molecular inhibitors of EGFR. It contains *in vitro* inhibitory activities either against EGFR or its different isoforms or various mutants. It will serve as a useful resource in drug discovery and provide data for docking, virtual screening and QSAR model development to the cancer researchers.

QSAR-Based Models against wild and mutant EGFR for Lung cancer

In this study, we have developed QSAR models for designing quinazoline derivatives that could inhibit wild EGFR and imidazothiazoles/pyrazolopyrimidines derivatives against mutant EGFR. In this study, three types of prediction methods have been developed to design inhibitors against EGFR (wild, mutant and both). In order to promote drug discovery, we have developed a web server for designing inhibitors against wild and mutant EGFR along with providing standalone version of software. These QSAR models will play a vital role in designing new anti-EGFR drugs.

DNA VACCINES AGAINST HPV

Development of DNA vaccine constructs of Indian specific Human Papillomavirus type 16 variants – Enhancement of Immunogenicity of L1 constructs and characterization of T-cell epitope based E6/E7 constructs.

In the previous study (Indo-German Task force project - ICMR, Ref. No. IG/7/07), we have identified sixteen (16) major variations (V1-V16) in L1, one in E6 in HPV-16 genome derived from cervical cancer tissue biopsy samples. We prepared a series of variant constructs after cloning to plasmid vectors for preparation of DNA vaccine constructs and evaluated their immunogenicity in animal model (BALB/c mice). In addition, there is a need to enhance the efficacy of these DNA based vaccine constructs, therefore using either genetic (IL-12 or TLR9 agonist) or non genetic adjuvant, work is ongoing to enhance the immunogenicity.

Multi-disciplinary approach to combat cancer by targeted drug delivery using intelligent polymeric nanoparticles. Funded by DBT; Duration 2010-2014.

The different Nanoparticles (PLA, Triblock 5000, Triblock 10000, Triblock 7500 and Multi-block NPs) synthesized and physical characterization at IIT, Delhi. The in vitro study of cellular uptake of the drug-targeted delivery of nanoparticles is being undertaken in breast cancer cell lines by flowcytometry.

Human Resource Development programs:

It is continuous program to train medicos, paramedics, graduates and post graduates in the various fields that will be helpful in the larger interest of cancer prevention. Following persons were trained during the year:-

| | |
|---|----|
| Cytopathology | 11 |
| M.D. Students guided | 4 |
| Two to 12 months Trainees (molecular division) | 64 |
| Short-term fellows from Indian Academy of Science | 11 |
| Post-doctoral / Pre-doctoral research (SRF/JRFs) | 7 |

Future Plans

- Development of hospital at the campus for keeping patients for early management before

they are transferred to other hospitals for the treatment.

- Cancer registry
- Development of new population screening strategies.
- Establishment of school of cytology

NATIONAL CENTRE FOR DISEASE INFORMATICS AND RESEARCH, BENGALURU

- The First Report of Development of an Atlas of Cancer in Punjab State for the years 2012-2013 gives an idea of the prevailing patterns of cancer by district in Punjab State. <http://icmr.nic.in/icmrnews/PUNJAB%20PCA%20-%20Chapter%20Complete.pdf>
- The Patterns of Care and Survival Studies in Cancer Breast, Cervix and Head & Neck for the year 2006-2008 is under publication. The main findings are - locally advanced cervical cancer, significant survival benefit observed when treated with a combination of radiation with cisplatin than radiation alone. The same observation was seen in patients with locally advanced cancers of the oro and hypo-pharynx. In breast cancer a high proportion of early stage patients had mastectomy with poorer survival compared to breast conserving surgery which is the usual practice.
- Development of Software Applications Programme with specific modules is a primary mandate of the NCDIR.
- The report of North East Cancer Atlas (other than areas where PBCRs exist) has been prepared.
- Population Based Cancer Registry at Patiala - The data for 2011-2012 has been published as a report.
- Making Cancer a Notifiable Disease-National Document - A draft document on the above has been prepared by NCDIR and has been sent to ICMR headquarters in August 2014 for further approval.
- Paper on retinoblastoma published.
- Visited Kerala State to select Model Rural Health Research Units

Network of NCDIR-NCRP**Cancer**

| | Network | Centres registered | | Data Sets |
|--------------|--|--------------------|------|------------|
| 1 | Population Based Cancer Registries | 29 | 300* | 29 |
| 2 | Hospital Based Cancer Registries including RCCs | 29 | | 24 |
| 3 | Patterns of Care and Survival Studies (inclu. 5 HBCRs) | 38 | | 102 |
| 4 | Cancer atlas NE | 17 | | 17 |
| 5 | HBCR-Data Management Software | 79 | | 52 |
| 6 | Punjab Cancer Atlas | 154 | | 58 |
| 7 | Haryana Cancer Atlas | 96 | | 0 |
| 8 | Pathology Software Module | 137 | | 29 |
| 9 | Radiotherapy Software Module | 17 | | 1 |
| Total | | 596 | | 312 |

**Sources of registration of PBCRs*

Diabetes, CVD and Stroke

| | Network | Centres registered |
|--------------|--|--------------------|
| 1 | 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 | 106 |
| 2 | Development of Stroke Registry in India | 55 |
| Total | | 161 |

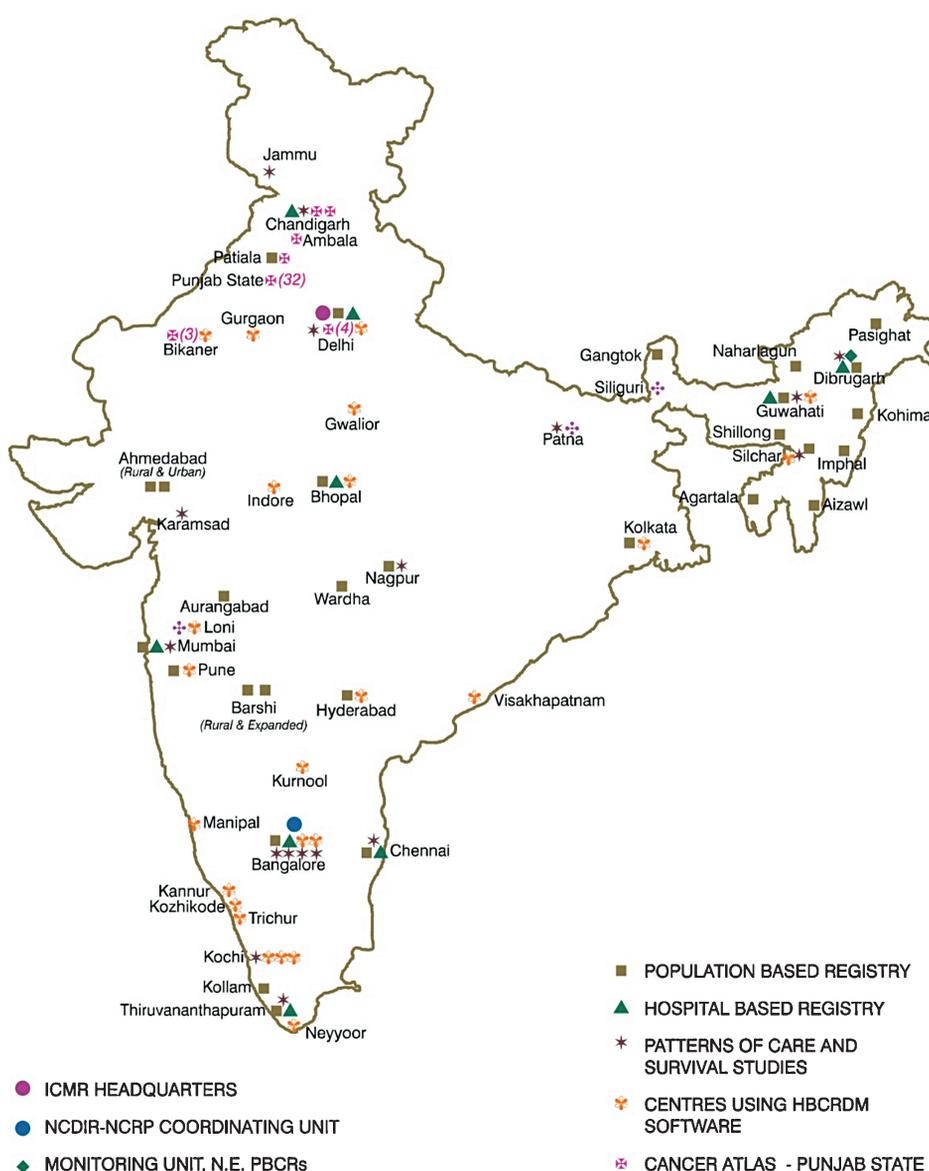
STATE WISE COLLABORATING CENTRES

| Sl. No. | State | PBCR | HBCR (incl. RCCs) | POCSS | HBCRDM Registered | PCA-Registered & Transmitting | NE Atlas | Total |
|---|--------------------------------|--|-------------------|-----------|-------------------|-------------------------------|-----------|------------|
| 1 | Jammu & Kashmir | | 2 | 3 | | | | 5 |
| 2 | Himachal Pradesh | | 1 | | | | | 1 |
| 3 | Punjab | 1 | | 2 | | 52 | | 55 |
| 4 | Chandigarh (UT) | | 1 | 1 | | 2 | | 4 |
| 5 | Uttarakhand | | | | 1 | | | 1 |
| 6 | Haryana | | 1 | 2 | 4 | 1 | | 8 |
| 7 | Delhi (UT) | 1 | 1 | 2 | 4 | 4 | | 12 |
| 8 | Rajasthan | | 1 | 1 | 2 | 1 | | 5 |
| 9 | Uttar Pradesh | | 2 | 1 | 2 | | | 5 |
| 10 | Bihar | | 1 | 1 | 3 | | | 5 |
| 11 | Sikkim | 1 | | | | | | 1 |
| 12 | Arunachal Pradesh | 2 | | | | | 2 | 4 |
| 13 | Nagaland | 1 | | | 1 | | 2 | 4 |
| 14 | Manipur | 1 | 1 | 1 | | | 1 | 4 |
| 15 | Mizoram | 1 | 1 | 1 | | | | 3 |
| 16 | Tripura | 1 | 1 | 1 | | | 2 | 5 |
| 17 | Meghalaya | 1 | | | | | 3 | 4 |
| 18 | Assam | 3 | 2 | 3 | 3 | | 6 | 17 |
| 19 | West Bengal | 1 | 1 | | 3 | | 1 | 6 |
| 20 | Jharkhand | | | | | | | 0 |
| 21 | Odisha | | 1 | 1 | 5 | | | 7 |
| 22 | Chhattisgarh | | 1 | 1 | | | | 2 |
| 23 | Madhya Pradesh | 1 | 2 | 1 | 2 | | | 6 |
| 24 | Gujarat | 2 | 1 | 2 | | | | 5 |
| 25 | Daman & Diu (UT) | | | | | | | 0 |
| 26 | Dadra & Nagar Haveli (UT) | | | | | | | 0 |
| 27 | Maharashtra | 7 | 2 | 3 | 3 | | | 15 |
| 28 | Telangana | 1 | 1 | 1 | 1 | | | 4 |
| 29 | Andhra Pradesh | | | | 2 | | | 2 |
| 30 | Karnataka | 1 | 1 | 5 | 25 | | | 32 |
| 31 | Goa | | | | | | | 0 |
| 32 | Lakshadweep (UT) | | | | | | | 0 |
| 33 | Kerala | 2 | 1 | 2 | 10 | | | 15 |
| 34 | Tamil Nadu | 1 | 2 | 3 | 6 | | | 12 |
| 35 | Puducherry (UT) | | 1 | | | | | 1 |
| 36 | Andaman & Nicobar Islands (UT) | | | | | | | 0 |
| Total | | 29 | 29 | 38 | 77 | 60 | 17 | 250 |
| <i>PBCR = Population Based Cancer Registries</i> | | <i>HBCR = Hospital Based Cancer Registries</i> | | | | | | |
| <i>POCSS = Patterns of Care and Survival Studies</i> | | <i>HBCRDM = HBCR Data Management Software</i> | | | | | | |
| <i>PCA = Dev. of an Atlas of Cancer in Punjab State</i> | | <i>NE Atlas = Dev. of an Atlas of Cancer in North East Regio</i> | | | | | | |

Apart from the above, 96 centres have registered under the project on Dev. of an Atlas of Cancer in Haryana, 137 centres have registered for Pathology Software Module, 17 centres have registered for Radiotherapy software Module, 55 centres have registered under Dev. of Stroke Registry in India and 106 centres have registered under 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.

NATIONAL CENTRE FOR DISEASE INFORMATICS AND RESEARCH
NATIONAL CANCER REGISTRY PROGRAMME - NETWORK
(Indian Council of Medical Research)



SOFTWARE DEVELOPMENT AT NCDIR

• Hospital Based Cancer Registry (HBCR) – Pattern of Care and Survival Studies (POCSS) Data Entry

Many of our Hospital Based Cancer Registries are involved in the project of Patterns of Care and Survival studies and the newly added Regional Cancer Centres are contributing data to both HBCR and POCS. To make it simpler and user friendly, it is better to have combined software for HBCR and POCS data entry. When a patient is registered for HBCR with the site of Ca Breast, Ca Cervix and Head & Neck cancer the same patient is getting registered for POCS also. Here a single patient is saved only once. Initially the HBCR staff can finish the data entry and POCS staff can complete the detailed information on treatment and follow ups.

Internet based software is developed and deployed in www.hbcindi.org website. RCCs and centres contributing data to both HBCR and POCS have been given login id and password to access the software.

• HBCR-HIV Data Entry

All India Institute of Medical Sciences, New Delhi wanted to capture patients having cancer and HIV for their research purpose. In this regard, the online HBCR data entry software had been modified for their data entry with special user id and password. Using this credential the registry can capture information on HIV. Export option is provided to get their data online.

• Young Onset Diabetes Registry data entry software.

Web based data capture software was developed for Young Onset Diabetes Registry as per the requirements given to NCDIR-NCRP from the technical coordinating unit of YDR, AIIMS, New Delhi.

• Independent modules –

- Pathology Data Entry with outputs/reports
- Radiotherapy Data Entry
- Surgical Oncology Data Entry
- Medical Oncology Data Entry

The software modules for Pathology, Radiotherapy, Medical and Surgical Oncology were developed to record details on cancer patients attending various departments in a hospital. These modules are available online through the NCRP portal (www.ncrpindia.org). Each of the above modules are cancer site specific and has been structured in three levels for data entry- Basic, Basic+ and Advanced, with option to feed information on patient's clinical history, diagnosis and treatment. These modules are independent, and have the facility to form linkages among the departments. Thus the data collected can be organized to create a hospital based cancer registry. The patient records can be recorded in a paperless manner with option to print copies. Administrative reports and tables for research are additional features.

Centres can register for the above modules online through www.ncrpindia.org/www.ncdirindia.org. Based on their request user id and password are generated and sent to centres for their routine use. As on now, 140 centres have registered and given access to pathology software. 13 of them are using for their routine work. 24 centres had registered for radiotherapy and only a single centre is using for the routine work. Few centres have registered for medical oncology and surgical oncology, but yet to start using.

• E-Monitoring

- a. Online Data status for RCCs and budget estimation
- b. Online Data Status - HBCRs, POCS, Pathology, Radiotherapy, etc.
- c. Online registration for independent modules
- d. Coreform stock
- e. HBCR File Maintenance
- f. QC Management
- g. Data Entry Operators daily data entry count

• Ongoing Software development

- a. Dynamic data entry
- a. JIPMER RT Module

• Admin Softwares

Application for NCDIR-Payroll for permanent staff

This application is used to maintain the payroll of the NCDIR staff. Using this application salary can be generated every month for all the employees, pay bills, Salary Bills, Salary Slip, etc. Bank letter can be generated to credit salary to the employees account automatically. Administrator can generate each employee's month wise yearly salary statement with earnings and deductions. Option to generate monthly NPS statement. DA arrears and NPS arrears statement can be generated whenever it is required. Each employee has the option to print salary slip using their credentials. This software has been functioning since July 2014.

- **File Movement for Administrative Department**

File movement application is used to track the current location of the physical file and its status. When the file moves from one user to another, a notification is executed to the person receiving the file from the person who sent the file. Through this application it is easy to find the pending file and its location. Daily email will be sent to administrator regarding the pending files. This software has been functioning since July 2014.

- **Annual Maintenance Contract (AMC) Management**

This module is used to maintain AMC details. Whenever a new AMC is placed it should be entered into the software. Notification will be displayed for the AMCs nearing to expiry nearly 4 months before on the home page of the Administrator.

- **Application for Biometrics Attendance Report Generation**

Application is developed for the employees attendance register with in and out timing. Monthly/ Periodical report can be generated for administrative purpose.

- Developed application module to maintain Inward and Outward information which is in the form of mails, posts etc. (To undergo testing)
- Payroll for Project Staff maintain payment information of all project staff (ongoing)

Population Based Cancer Registry

PBCRDM 2.1.1: New version of PBCRDM 2.1 had separate database tables for data entry and

processing section. This required the user to transfer data from data entry to the processing section every now and then. An improvement in the software was done to merge both the sections and eliminate this step. The hard copy of incidence proforma underwent changes to include 11 new fields. The new version PBCRDM 2.1.1 was created for registries and with the above changes in database, forms and reports. This version has been deployed at Bangalore, Sikkim and Bhopal PBCR for testing and suggestions so that the same can be deployed at the remaining 21 PBCRs using the offline version.

Data Entry Programme (www.pbcrintia.org): PBCRs having good internet speed have shown interest in using a web based application for the data entry and processing of their data. Hence a data entry Programme with basic quality checks was developed on www.pbcrintia.org. PBCRs needed to generate their own tables from the data submitted by them. Thus the Dynamic table generation was developed on www.pbcrintia.org.

Book Report Generator: While preparing the book report on 'Three year report of Population Based Cancer registries 2009-2011', it was felt that a program should be developed to generate the tables of all the PBCRs in the published format to reduce manual work in future. Thus an application to dynamically generate book report for use in the NCDIR Intranet has been developed.

Extramural Research

ONCOLOGY

Cancer Management Guidelines

The Task Force on Management of Cancers has been constituted to plan various research projects. Twenty three sub-committees were constituted to review the literature related to guidelines for management of various sites of cancers. The selected cancer sites are that of lung, breast, oesophagus, cervix, uterus, stomach, gall bladder, soft tissue sarcoma and osteo-sarcoma, tongue, acute myeloid leukaemia, ALL, CLL, NHL-high grade, NHL-low grade, HD, MM, MDS, and paediatric lymphoma. The consensus document for management of buccal mucosa cancer, colorectal, stomach, gall bladder and tongue cancers are available at ICMR website.

Molecular Basis of Genesis of Breast Cancer

The proposal entitled, “Comparative Study of Genetic, Clinical and Epidemiological Factors of Breast Cancer in Rural and Urban Area of India,” was initiated. At Dibrugarh centre, preliminary findings show the role of XRCC genes and family history in genesis of breast cancer. The project would be upscaled to rural and urban Delhi and Thiruvananthapuram.

Assessment of Cancer Occurrence in Punjab

The report of project on Cancer Atlas in Punjab was published. Oesophageal cancer in males and females was an important leading site in many districts. Some of the other sites of cancer that show higher or comparably high incidence rates with the highest seen in other PBCRs in India are: in males: penile cancer (in Faridkot and Bathinda), prostate (Jalandhar), urinary bladder (Kapurthala, Jalandhar) and brain (Mohali and Mansa); in females: breast (Bathinda, Mohali), vagina (Bathinda, Ludhiana), ovary (Mohali) and brain (Moga & Mohali). Report is available at ICMR web site.

Indo-University of Minnesota Collaboration

The ICMR has signed a Memorandum with the University of Minnesota, USA, to undertake collaborative research in the field of biomedical sciences.

HLA haplotypes frequency analysis within India: pre requisite for bone marrow donor registry and cord blood bank planning.

The objective of the study is to provide a detailed estimate of haplotype frequencies across India and in relevant regional populations; analyse existing HLA family typing to estimate HLA haplotype frequencies in Indian, national and regional populations. Collaboration with Indian BMT centres, renal transplant centres and cord blood banks were used to catalogue the frequency and regional diversity of HLA haplotypes. Using HLA haplotype frequencies, population genetic models that predict the likelihood of identifying a suitable adult donor or CBU for Indian patients in 14 regional groups were built. The match rates vary by regional groups largely due to the genetic diversity within

each group and the genetic distance or HLA sharing between regional groups. Overall the results show that at the current registry size in India (~25,000 donors), 64% of patients can find a 9/10 or better match if they search both India and the US-NMDP registry but only 30% have a match within India. Doubling the registry size from 25,000 to 50,000 and again from 50,000 to 100,000, appears to have a linear increase in match rate. This analysis shows that today patients in India are more likely to find a matched donor outside India and this will continue to be the case until the Indian adult donor registry size is over 100,000. Adult donor matching at the 10/10 level is difficult. These models show that even when the Indian registry grows to 1,000,000 donors, less than 2/3 of patients will have a 10/10 match. Although it is possible to use a 9/10 matched donor, outcomes with mismatched adult donors are associated with higher morbidity and mortality rates.

CARDIOVASCULAR DISEASES

Management for Acute Coronary Events (MACE) Registry: A Web Based National Network of Registry for Patients Hospitalized with Acute Cardiovascular Events

This registry can provide information regarding socio-demographic characteristics of ACS patients, risk factors, delay in presentation of these patients, door to needle time and door to balloon time, management practices and outcomes at 28 days and 6 months. A pilot study has been initiated in 2014 in 11 centres in the country and is planned to be scaled to 50 centres in the country. An electronic quality of care performance report has been developed and will be sent to all registries for improving the quality of care of ACS patients.

NEUROLOGY

Population Based Urban Stroke Registry

The annual incidence rate is 140/100,000 (95% confidence interval: 132.90, 147.09). The age adjusted incidence rate based on WHO standard world population is 181.67/1,00,000 (95% confidence interval (CI):172.44-190.89). The incidence rate was more using door to door survey (172/1,00,000; 95% CI: 164 to 179) as compared to the data collected by WHO STEPS methodology

(140/1,00,000; 95% CI: 133 to 147). There were 25% stroke patients in the 18-49 years category in this registry. Brain imaging was done in 95% patients in this study. Majority of patients (66%) had ischemic stroke.

Population Based Rural Stroke Registry

Incidence of first ever stroke to be 55.9 per lakh population. Sixty eight percent of the cases from the community were male. Only 8.5% of the cases were reported from hospitals/health centres/practitioners of the area. Allopathy, Traditional medicine, Ayurveda constituted first contacts of care in path way of care.

Centre for Advanced Research for Innovations in Mental Health & Neurosciences: Manpower Development and Translation Research” at NIMHANS, Bangalore

This centre was initiated in July 2014 and has 3 ongoing translation based projects and two PDF's per year in the area of 'Neurocritical Care'.

Under the project "Development of Neuroscience Educational Material for popularizing Neuroscience under Human Brain Bank", a Histological Atlas of the Common Infection of CNS, along with set of 48 histological slides depicting the pathological features and CD containing the text & photographs in the Atlas has been prepared. Forty five histological slides and more than 178 booklets with CDs were distributed among the post graduate students studying Pathology, Microbiology and Neurology in medical colleges. Nearly 17 posters have been prepared for public awareness and are being used in local exhibitions by schools/ colleges and Indian Epilepsy Association. A manual "Histological Atlas of Common Infection of Nervous system with Teaching Slides" has also been prepared.

Under the project "Translation research in ALS-Development of biomarkers for diagnosis, monitoring disease progression and evaluation of toxicity", presence of CHIT1, CHI3L1, CHI3L2 and Osteopontin was confirmed in CSF samples from ALS patients using ELISA technique. A ~26 fold increase in CHIT-1 levels and its high biological activity in ALS-CSF at < 6 months of disease suggests possible use of this protein as on early diagnostic marker.

Under the project, "Development of ELISA and evaluation of potential of 14-3-3 protein as a biomarker of neuronal injury/ neuro-degeneration with special reference to Creutzfeldt Jakob disease", a Recombinant Core 14-3-3 protein was designed and expressed in pMAL vector. Polyclonal antibodies were produced against this recombinant protein and were used for quantification of 14-3-3 protein in human CSF by dot blot & ELISA methods. A 10 fold difference in concentration of 14-3-3 protein was observed in CSF samples from CJD cases as compared to controls.

BIOMEDICAL ENGINEERING

DHR/ICMR-MHRD Medical Diagnostics And Devices Innovation Partnership Workshop on 19th and 20th Sep 2014: Around 85 participants from ICMR, IIT, DBT, DRDO, AEC, FICCI, ASSOCHAM, PHD Chamber of Commerce, Medical institutes, Industry/Startup companies participated. The salient recommendations of the workshop include building up human resource, setting up of virtual incubators, mapping unmet clinical needs, setting up testing facility in collaboration of ICAR and creating hungry systems.

ICMR Exhibition on "Innovations in Medical Devices and Biotechnology" was organized on 11.03.2014 at Rashtrapati Bhavan as part of Festival of Innovations organized by NIF. Around 46 technologies were exhibited at Rashtrapati Bhavan. Efforts are underway to upscale these technologies for public health.

MENTAL HEALTH

The Task Force Study on "Epidemiology of Substance Use and Dependence in the State of Punjab" has been initiated by PGIMER, Chandigarh. This study has three components (1) State of Punjab Household Survey (SPHS): The purpose of this exercise is to generate general population level using appropriate statistical procedures. (2) Punjab Drug Use Monitoring system (P-DUMS): The already existing Drug Abuse Monitoring system (DAMS) proforma will be used in the treatment centres. Further the system of creating and maintaining a centralized state-level register of persons undergoing treatment for substance use, with district and centre-wise listing is being

pursued presently, and this too will yield important data on treatment –seeking individuals; and (3) Rapid Assessment Survey for Indirect Estimation of Substance Use in Punjab (RAS): This is vitally important because direct prevalence of use of substances that are relatively rare and highly stigmatized is always an underestimation as per general household survey.

DISABILITY & REHABILITATION

National Disability & Rehabilitation Research Network (NDRRN) is planned to be created at 20 Bio-Informatics Centres across India. It has been proposed to involve 6 apex institutes under Ministry of Social Justice and Empowerment (MoSJE) and four institutes under Ministry of Health and Family Welfare (MoHFW).

DIABETES

Registry of People with Diabetes in India with Young Age at Onset

The phase I of Task Force project entitled, “Registry of People with Diabetes in India with Young Age at the Onset,” was completed. The initial findings indicate that there were more than ten variants of youth onset diabetes reported, in which Type 1 Diabetes Mellitus (T1DM) was the most prevalent (63.9%) followed by youth onset Type 2 Diabetes Mellitus (T2DM) (25.3%). Other forms of diabetes such as Gestational Diabetes (3.9%), Maturity Onset Diabetes of the Young (MODY) (3.1%), Chronic Pancreatitis (1.3%), Secondary Diabetes (1.0%), Latent Autoimmune Diabetes in Adults (LADA) (1.0%), Drug Induced Diabetes (0.3%) and Malnutrition Modulated Diabetes (0.1%) were also reported from the participating centres. The relative proportion of patients with T1DM and T2DM varied between centres. In all the diabetes categories, retinopathy was reported as the most common diabetes complication (3.6% in T1DM and 10.4 % in T2 DM). This was followed by nephropathy in T1DM and neuropathy in T2DM. The prevalence of complications increased with duration of disease. Retinopathy and neuropathy appeared to manifest within a short duration since diagnosis among T2DM patients compared to T1DM. Among those who have lived with diabetes for more than 20 years, the prevalence of coronary disease was more among T2DM patients (9.8%) compared

to those with T1DM (4.8%). 14.1 % of the YDR patients had at least one co-morbid condition such as hypothyroidism, dyslipidaemia, hypertension, tuberculosis, sepsis etc., with the most common co-morbid being hypothyroidism (3.1%). 20.8 % of the early onset T2DM patients were reported to have co-morbidities such as dyslipidemia (11.3%), hypertension (7.4%) and hypothyroidism (2.1%). Consolidated report is recommended for publication and is being processed for the same.

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 main objective of the study was to screen and analyze the genes implicated in Maturity Onset Diabetes of the Young (MODY) and neonatal diabetes in India. All MODY subjects included were diagnosed with type 2 diabetes before 25 years of age and negative for GAD antibodies. Overall, 371 MODY patients were screened for MODY1 (*HNF4A*) and MODY3 (*HNF1A*); 142 subjects for MODY2 (*GCK*); 135 for MODY4 (*IPF1*); 52 for MODY5 (*HNF1B*); 15 for MODY6 genes and 138 neonatal diabetes and 15 monogenic syndrome subjects were screened for various genes implicated for neonatal diabetes under ICMR funded projects. Patients with certain mutations in *KCNJ11* and *ABCC8* genes have been shown to respond very well to oral sulfonylurea. In this project such mutations were identified in 9 patients. This genetic diagnosis has made it possible to switch nine children from insulin treatment to oral sulfonylurea drugs, which is the most important translational aspect of this project. A website <http://www.neonataldiabetes.in> has been created. Through this website doctors can get a neonatal or a MODY case registered in this website and also request the MODY and NDM genetic testing for their patients.

ICMR-Indian National Diabetes Study (ICMR-INDIAB)-North East:

The study estimated prevalence of pre diabetes and diabetes among rural and urban population in north eastern region of the country. Four states have compiled their results. The overall weighted prevalence of diabetes in the four states was 5.4% in Assam, 5.7% in Mizoram, 5.2% in Arunachal

Pradesh and 8.6% in Tripura. The overall weighted prevalence of pre-diabetes was 11.8% in Assam, 5.8% in Mizoram, 12.8% in Arunachal Pradesh and 4.6% in Tripura. In all the four states, it was observed that the prevalence of diabetes was higher in urban compared to rural areas. The prevalence of hypertension in the urban and rural areas was (Assam: 38.3% vs. 30%, Mizoram: 31.1% vs. 23.5%, Arunachal Pradesh: 30.1% vs. 30.3% and Tripura: 36.1% vs. 26.7%). Similarly, the prevalence of dyslipidemia in the urban and rural areas of the four states was (Assam: 89.9% vs. 87.9%, Mizoram: 79.3% vs. 78.9%, Arunachal Pradesh: 80% vs. 79% and Tripura: 86.2% vs. 78.7%). The prevalence of hypertension was higher in urban compared to rural areas, whereas, dyslipidemia was similar in urban and rural areas of all the four states. The prevalence of coronary artery disease is higher among diabetic subjects compared to subjects without diabetes in Assam (Diabetes vs. No Diabetes - 8.6% vs. 1.2%), Mizoram (Diabetes vs. No Diabetes - 5.9% vs. 2.6%), Arunachal Pradesh (Diabetes vs. No Diabetes - 1.8% vs. 0.2%) and Tripura (Diabetes vs. No Diabetes - 3.5% vs. 1.1%) respectively. In terms of glycemic control in the rural areas, 25.6% of self reported diabetic subjects in Assam and 23.0% in Mizoram had poor glycemic control compared to 17% and 18.9% of the subjects in urban areas of Assam and Mizoram respectively. Similarly, 36% of self reported diabetic subjects in rural Arunachal Pradesh had poor glycemic control compared to 33.3% in urban areas whereas 15.5% of self reported diabetic subjects in rural Tripura had poor glycemic control compared to 21.6% in urban areas.

CHRONIC DISEASE HEALTH RESEARCH

As part of the ICMR-MRC collaboration on Noncommunicable Diseases, the following 4 collaborative projects are ongoing:

- Maternal Vitamin B 12, folic acid and homocysteine as determinants of gestational diabetes, fetal growth and inter-generational programming of diabetes.
- Phenotypic characterization of Non-Smoking Chronic Obstructive Pulmonary Disease (COPD).

- Promoting Sustained Lifestyle Changes to Prevent Type 2 Diabetes Using a Mobile Phone Intervention in India and the UK.
- Effects of a yoga-based cardiac rehabilitation programme (Yoga-CARE) on cardiovascular health: a clinical trial (India) and mechanistic study (UK).

ORTHOPAEDICS & DISABILITY PREVENTION

1. Osteoporosis:

Recent studies have shown that genetic effects on bone mineral density and bone turnover are related to allelic variations in several genes e.g. collagen type I, estrogen & vitamin D receptors Cbfa1 and various cytokines and growth factors.

2. Overuse Injury:

Growing number of young people participating in sports at an early age and competing at specialized or elite levels without consistent preparation has led to enhanced incidence of overuse injuries. Making the diagnosis of overuse injuries can be challenging besides identifying the causes. The contributions of alignment abnormalities to overuse injuries are unclear and will be systematically evaluated with special reference to epidemiology, anatomical location of injury, repetitive trauma, risk factors (physical activity) and treatment.

1. CKDu: At the request of MOH&FW, a team visited Srikakulam district of AP to assess the high prevalence of CKD. The project will have objective to determine prevalence of chronic kidney disease of uncertain etiology in rural areas of Srikakulam district (Andhra Pradesh) and compare them with that of villages in West Godavari (Andhra Pradesh) and Cuttack (Odisha).

GERIATRICS

1. A joint workshop with FORTE (The Swedish Research Council for Health Working Life and Welfare) was held in November 2014 to identify areas of collaboration between the two countries in Ageing and elderly care. A Memorandum of Intent between the ICMR and FORTE has been signed to collaborate in the field of ageing research and health during

the State Visit of President to Sweden in June 2015.

2. The Institute for Research and Ageing was proposed by ICMR as the first component of the National Burden of Non Communicable Diseases.

OTORHINOLARYNGOLOGY

ICMR's Task Project on Congenital Deafness in Dhadkai village of Doda district of Jammu & Kashmir

This study is a research cum intervention project which aims at assessment of hearing impairment in Dhadkai village, which is inhabited by large number

of deaf persons and identifying genes responsible for such deafness.

While the initial phase will help the affected people in terms of getting due attention for medical and rehabilitation support, subsequent analysis of genetic basis will help in developing and implementing counselling to prevent transmission of relevant genes so that the future generations could be saved. Further, a pedigree chart of entire village has been constructed and family groups with relatively higher number of hearing impaired have been identified. A sound proof room unit has been established at community health centre, Gundoh.

BASIC MEDICAL SCIENCES

During the year, the intramural research in the field of basic medical sciences was carried out by the National Institute of Pathology (NIOP), New Delhi, and the National Institute of Immunohaematology (NIIH), Mumbai and also at several other centres. The extramural research was undertaken in several areas *viz.* haematology, biochemistry, pharmacology, human genetics, nano medicines, stem cell research, biomedical ethics, *etc.*

Intramural Research

NATIONAL INSTITUTE OF PATHOLOGY, NEW DELHI

The thrust areas of research of National Institute of Pathology have been tumor biology, infectious diseases including *leishmaniasis*, *tuberculosis*, *leprosy* and *chlamydia*, stem cell biology and environmental toxicology. The scientists conduct both basic as well as translational research leading to development of Vaccines for prevention and Biomarkers for screening, diagnosis, prognosis and prediction of drug response/resistance for various diseases with mission to bring lab to bed.

TUMOR BIOLOGY

Breast Cancer

Gene expression and methylation profiles in early onset breast cancer

The study has revealed pathways like ABC transporters, Axon guidance, Cytokine cytokine receptor interaction pathway coming out differentially significant in early onset breast cancer while Fanconi anemia pathway, DNA Replication pathway, p53 signalling pathway were significantly deregulated in late breast cancer. Methylation profiling of breast cancer patients identified genes

like RASSF1, WT1 were commonly methylated in early and late breast cancer, while CD40, CD86, HOX genes were methylated in early cancer while GSTP1, CCND1 were found uniquely methylated in late breast cancer. The data was also merged to gain knowledge on epigenetic regulation of breast cancer by identifying certain genes which were showing good correlation between gene expression and methylation.

Micro RNA Signatures Associated with Breast Cancer Stem like Cells (CSCs) and their role in Drug Response

The study has been initiated to understand the molecular characteristics of CSCs by studying unique miRNA, gene expression profiles associated with CSCs compared to bulk tumor cells. Isolation of breast cancer stem (CD44+/CD24-) cells using CD44 and CD24 markers in 5 breast cancer cell lines, MCF7, MDA-MB-231, ZR-75-1, T47D, MDA-MB-468 has been done followed by expression profiling of miRNAs and gene expression profiling. The study aimed to identify novel genes up regulated by AR, upon DHT stimulation in breast cancer cell line MDA-MB-453. Identification of the new AR targets in breast cancer will reveal potential targets for targeting of breast cancer for the purpose of therapeutic intervention. Using MDA-MB-453 as the model for studying triple negative breast cancer cell line positive for AR expression, it has been shown that DHT (AR agonist) stimulation leads to proliferation of the breast cancer cells and this effect was abrogated by the addition of bicalutamide (AR inhibitor). Ten novel targets have been identified which are upregulated by AR in DHT-dependent manner. The data suggests that AR mediated effect can be reversed by AR antagonist like bicalutamide, therefore, the use of bicalutamide for the treatment of AR positive, triple negative breast cancer has been proposed (Fig 1).

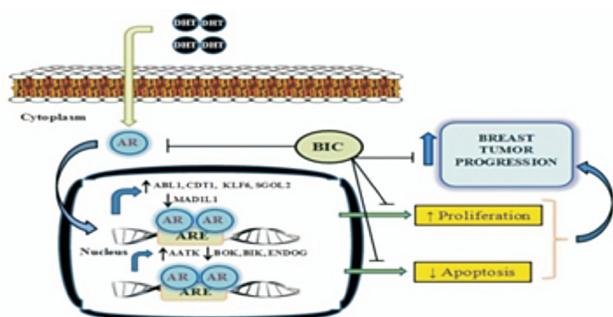


Fig. 1.

Prostate cancer

The identification of gene fusions of the 5'-untranslated region of TMPRSS2 (21q22.3) with the ETS transcription factor family members, like ERG (21q22.2), suggests a mechanism for overexpression of the ETS genes in the majority of prostate cancers. T2:ERG rearrangements in 58% of prostate cancer biopsy samples. Analyses of matched urine samples to assess the potential of T2:ERG as a non-invasive marker showed a concordance level of 95.2% between tissue and urine sample results. Sensitivity of TMPRSS2:ERG fusion marker was 58 % whereas specificity was 100% as none of BPH sample was found to have the fusion transcript.

Esophageal cancer in North East Region

Epigenetic Studies in Esophageal Cancer in High Risk Region of Northeast India include study of DNA methylation and histone modification. Study

of chromatin modification enzymes in ESCC showed higher expression of enzymes regulating methylation (DOT1L and PRMT1) and acetylation (KAT7, KAT8, KAT2A and KAT6A) of histone was found associated with ESCC risk. Tissue microarray study suggests the association of PRMT1 and KAT8 with esophageal cancer risk and their involvement in the transition process of low to high grade tumor formation. Differential methylation profiling of Tumor Suppressor Genes (TSGs) in tumor and corresponding normal esophageal tissue showed hypermethylation of OPCML, NEUROG1, TERT and WT1 and hypomethylation of SCGB3A1, CDH1, THBS1 and VEGFA gene. Integration of methylation data with microarray expression data showed 23 Integrome network enriched genes having relevance to tumor progression (Fig 2). Top 5 gene with highest Methylation Efficiency Index (MEI) were COL1A1, TAC3, SERPINA4, TNFSF13B and IL22RA2. The methylation and expression status of circulatory proteins involved in immunoregulation (IL22RA2 and TNFSF13B), extra cellular matrix remodeling (SERPINA4) and contraction of the circular muscle of human esophagus (TAC3) could be further explored as non-invasive biomarker for esophageal cancer. *In silico* analysis of copy number and gene expression profiling data from ESCC identified three putative molecular signatures from amplified region (*CCR1*, *ENAH* and *FGF12*), eight from deleted region genes (*MAPT*, *FGF18*, *UBE2E1*, *PARD3*, *SCP2*, *FGF14*, *PPP2CB*, *IL12A*). Further analysis was

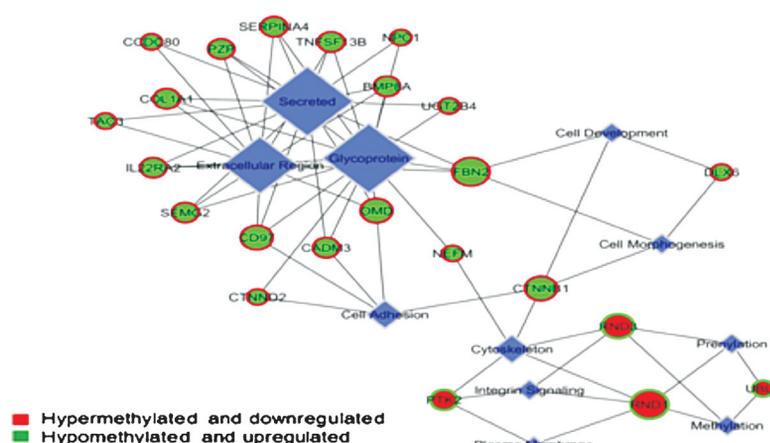


Fig 2. Network encompasses biological categories, differentially expressed and methylated genes that were significantly enriched. Green and red color circles representing upregulated genes with hypomethylation and downregulated genes with hypermethylation respectively.

done among the 22 type of cancer to know the mRNA level of these genes to find out suitable and appropriate esophageal cancer gene. FGF12 was the only putative marker showing upregulated expression in esophageal cancer in oncomine and Cancer Cell Line Encyclopedia (CCLE) databases. With these leads we did functional characterization of FGF12 by knockdown studies in ESCC cell line KYSE410 confirming the effects on functional assays like proliferation, colony formation, and wound healing assays.

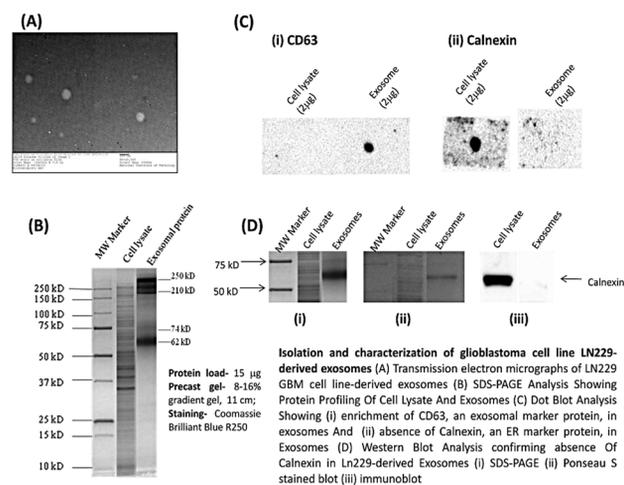
Nasopharyngeal cancer in Northeast India

The earlier studies have shown significant contribution of HLA class I region and EBV RNA sequences in susceptibility for Nasopharyngeal cancer (NPC) in NE region. To study additional genetic alterations in the entire HLA super locus (3.8 Mb regions) next-generation sequencing (NGS) was done. Analysis of results showed a high association of five SNP located in HLA region with the Nasopharyngeal cancer. Of these five SNPs, two SNPs were novel present in genes namely COL11A2 and MUC22 whereas three SNPs present in genes HLA DRB5, HLA-DPA1 and TAP2 were already known. Further the validation of these SNPs is undergoing in large sample size.

Neurological Cancers

Glioblastomas (GBM) are highly malignant brain tumours constituting 80% of all primary brain tumors arising in brain and spinal cord. Till date, the detection and monitoring of tumor during treatment is mainly based on CT-scan and MRI imaging techniques. Exosomes, secreted membrane vesicles ranging from 40-100 nm in size, being remarkably stable in body fluids have shown great promise for its use in prognosis, therapy, and biomarkers for cancer. In the present study, we have optimized the cell culture conditions for isolation of exosomes followed by isolation and characterization of exosomes from GBM LN229 cell line. Exosomes were isolated by differential centrifugation method and characterization was done by transmission electron microscopy, dot blot and/or western blot analysis for CD63, an exosomal marker protein and calnexin, an ER marker protein. TEM analysis showed exosomes with size range of 30-100 nm. Dot blot analysis showed enrichment of CD63 in exosomes. Dot blot and Western blot analysis of calnexin showed absence of calnexin in exosomes.

The exosomal proteins were subject to in-solution trypsin digestion for the sample preparation for mass spectrometric analysis to identify the exosomal protein and to further explore their potential as diagnostic biomarkers for the GBM (Fig. 3).



INFECTIOUS DISEASES

LEISHMANIASIS

Studies on miltefosine resistance in visceral leishmaniasis:

Increasing incidence of relapse in VL cases treated with miltefosine raised the concern for its immediate surveillance in the field to safeguard efficacy. The genomic microarray tools were used to study transcriptome profiling in clinical isolates of *Leishmania donovani* from pretreatment and relapse group. The study revealed important differences in gene expression pattern between relapse group and pre-treatment isolates. Approximately, 1800 genes comprising ~20% of total Leishmania genome were found to be differentially modulated of which 7.4% genes were up regulated while 12.4% were down regulated. The expression of selected genes by Q-PCR has been correlated well with the microarray results.

Data analysis using BLAST2GO, AmiGO and KEGG pathway led to classification of modulated genes into various functional categories including metabolic pathways, transporters, signal transduction pathway, nucleotide binding and cellular components. Transporters comprised the major category following unclassified proteins which include hypothetical proteins (proteins with unknown function (Fig. 4).

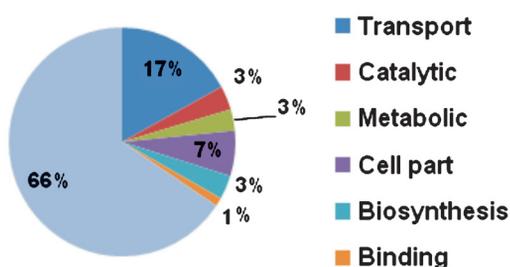


Fig 4. Distribution of differentially expressed genes in relapse group according to Gene Ontology (GO) function categories.

The Lipase precursor like gene, involved in lipid metabolic pathway, was consistently up regulated in parasite from relapse group as well as in experimental miltefosine resistant parasites. To investigate its role in establishing unresponsiveness towards miltefosine we transfected this gene into miltefosine sensitive *L. donovani* parasites and assessed growth kinetics as well as miltefosine susceptibility in transfected parasites. Parasites overexpressing lipase precursor (*LdLip*⁺⁺) showed better tolerance towards miltefosine pressure as compared to the sensitive parasite. There was a significant decrease in susceptibility towards miltefosine in *LdLip*⁺⁺ both at promastigote and intracellular amastigote stages. *LdLip*⁺⁺ showed more than 3 fold higher IC₅₀ value than the wild type parasite.

Mechanism of resistance towards paromomycin in *Leishmania donovani*

Paromomycin (PMM) is a new treatment option registered for the treatment of VL in India. Although no clinical resistance has yet been reported, it is crucial to understand the mechanism resistance towards PMM to ensure its long term effectiveness. In the present study, we induced PMM tolerance in three different field isolates to establish the mechanism of drug resistance in line with our earlier studies. All the three paromomycin resistant (PMM-R) isolates showed 6-7 fold reduced susceptibility towards the drug. The susceptibility of the PMM resistant and wild type isolates has been compared towards paromomycin in presence of inhibitors of ABC transporters. There was a significant increase (2 fold) in susceptibility towards PMM in PMM-R isolates in presence of verapamil, which is an inhibitor of MDR1 and

approx. 6 fold increase in presence of amlodipine, which is an ABCG2 inhibitor. A partial reversion of resistant property of PMM-R isolates in presence of verapamil and amlodipine established the role of ABC transporters in paromomycin resistance.

Role of CD8⁺ T cells in protection against *Leishmania donovani* infection in healed Visceral Leishmaniasis individuals

The study aimed to evaluate immunological mechanism associated with resistance to the disease in healed VL (HVL) individuals and further, the contribution of CD8⁺ T cells in the protective immunity. Peripheral blood mononuclear cells (PBMCs) from VL, HVL and naive groups were exposed *in vitro* to total soluble *Leishmania* antigen (TSLA). Significantly higher lymphoproliferation, cytokines and granzyme B levels have been observed in HVL group compared to naive or VL group. A strong association ($r_s = 0.895$, $P < 0.0001$) between proliferation index (PI) and granzyme B level has been found with a significant proportion of activated CD8⁺ T cells in HVL group. *Leishmania* immune group (HVL) exhibited durable and strong cellular immune response to TSLA in terms of lymphoproliferation as well as production of Th1 cytokines and granzyme B. Additionally, the elevated level of activated CD8⁺ T cells and stimulation of cytotoxic activity through granzyme B production, indicated a possible role of CD8⁺ T cells in resistance to *L. donovani* infection in the HVL group (*BMC Infect Dis* 2014, 14:653).

Application of new LAMP assay for diagnosis of VL and PKDL

The LAMP assay was applied to clinical samples for diagnosis of VL and PKDL. The assay gave high sensitivity of 96.4% for VL and 96.8% for PKDL with 100% specificity. Further, the assay was subjected to third party validation at RMRI, Patna, where it showed sensitivity of 98% and specificity of 97%.

TUBERCULOSIS

Signature Sequences: Novel Genetic Markers for Diagnosis of Tuberculosis (TB)

A rapid test capable of reliably detecting the presence of *Mycobacterium tuberculosis* is vital

for early detection and treatment of tuberculosis (TB). Therefore, need of the hour is to: a) identify more effective targets; b) targets which are unique to *Mycobacterium tuberculosis*, and c) use more than one target to increase detection accuracy. Our probes will not only permit rapid, cost-effective, high specificity and high sensitivity detection of bacillus in regular TB patients but these SS probes may be able to detect the bacterium from sputum/blood, also in cases where these could not be detected by GenExpert or other tests currently in the market (Fig. 5).

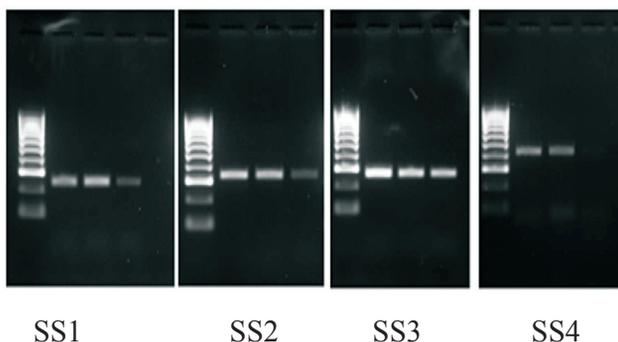


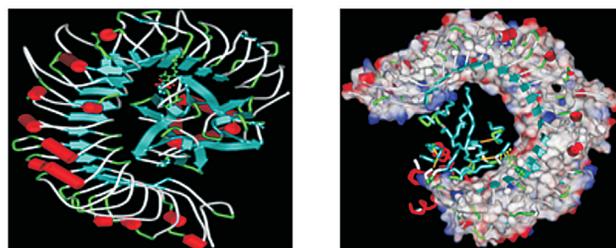
Fig. 5. PCR amplification of 4 different signature sequences probes and its sensitivity assessment.

Understanding the Underlying Mechanism of Macrophage Immune Modulation: Cross-talk between Inflammation and Cellular stress

TLRs are evolutionarily conserved pattern recognition receptors (PRR) and represent primary triggers of the innate immunity. They are responsible for sensing and responding to pathogen-associated molecular patterns (PAMP) of diverse invading organisms. Resistin, a member of the adipokine family, is known to be involved in the modulation of immune responses including inflammatory activity. The study has established the role of resistin in innate immunity by way of its interaction with TLR2 and activating/inactivating the downstream players leading to inflammation and anti-apoptotic pathway.

Resistin treatment induces both anti-inflammatory IL-10 (Fig. 6A) and the pro-inflammatory cytokines TNF- α and IL-6 (Fig. 6B). This reveals a complex network of signaling events in response to resistin.

The computational docking was performed to identify the possible docking interacting domain(s) of resistin and TLR2



Figs. 6A & B. Computational analysis predicts TLR2 as a strong interacting partner for human resistin. Schematic diagram showing TLR2 as molecular surface coloured with electrostatic potential and human resistin as ball and tube model. Position of interacting residues on the binding interface of resistin-TLR2 complex.

Corroborating above finding of resistin interaction with TLR2, the effect of resistin on the expression of TLR2 in human macrophage cells *in vitro* were investigated. FACS analysis shows a significant and proportionate increase in the expression of TLR2 on macrophage cell surface as compared to untreated cells. Interestingly, neutralization of cells with anti-resistin antibody or anti-TLR2 antibody abrogate the effect of resistin on TLR2 expression suggesting that the observed effect was indeed due to resistin. Given the role of TNF- α and TLR2 in inducing apoptosis in THP1 differentiated macrophage cells the downstream signaling effect of resistin on Caspase8 and 3/7 activation was elucidated. Resistin dramatically suppressed both Caspase8 and 3/7 activation; levels similar to that observed for the cells treated with *E. coli* LPS, (Serotype-O128:B12) a known inhibitor of caspases and thus apoptosis. However, neutralizing the cells with anti-TLR2 antibody apparently abrogate the inhibitory effect which suggests the involvement of TLR2 in down regulating the caspase activation (Fig. 7A). This antagonistic response of resistin is observed to be upstream of NF- κ B as inactivation of NF- κ B using PDTC fails to eliminate the inhibitory effect of resistin (Fig. 7B). These data, hence, satisfactorily demonstrates that resistin signals inactivation of Caspase8 and therefore Caspase3/7 and favours an anti-apoptotic state in the host cell via TLR2 (Fig. 7C).

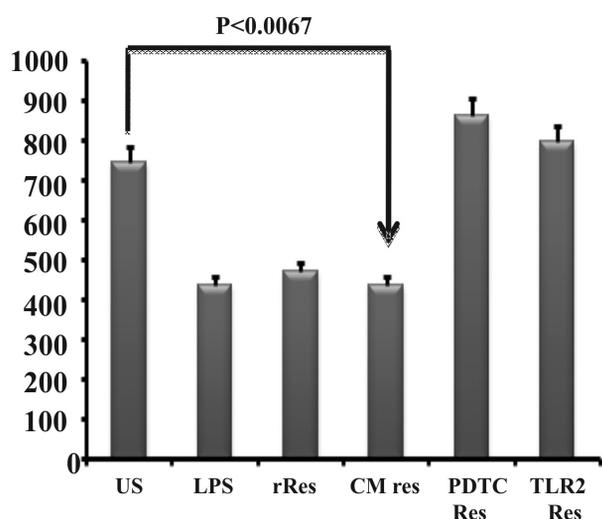


Fig. 7A. Resistin inhibits Caspase 8 activation in TLR2 dependent manner.

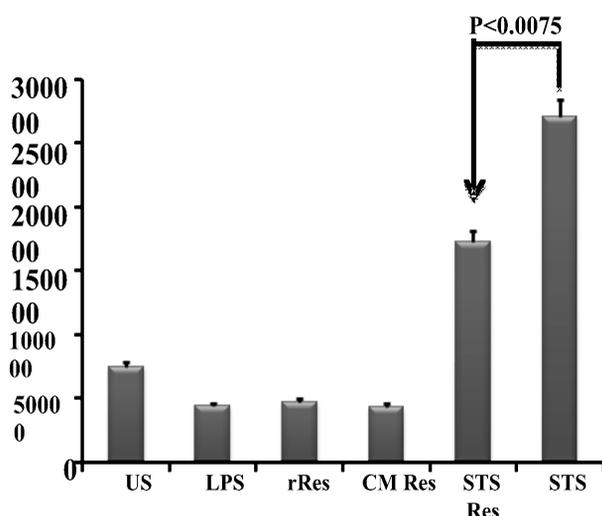


Fig. 7B. Resistin inhibits Caspase 8 activation.

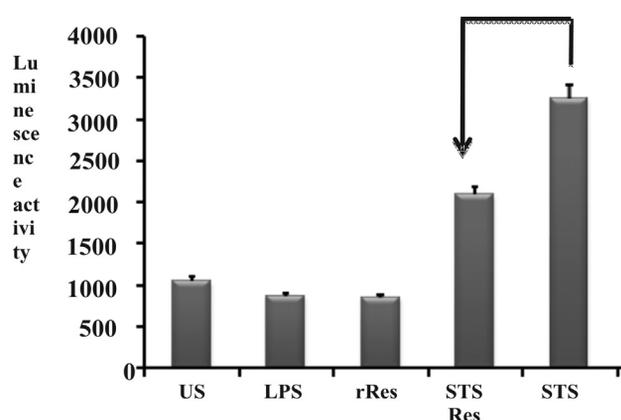


Fig 7C. Resistin inhibits Caspase 3/7 activation.

CHLAMYDIASIS

To understand the pathogenesis of *Chlamydia trachomatis* in Reactive Arthritis (ReA)/ Undifferentiated Spondyloarthritis (uSpA) patients, Th1/ Th2/ Th17 cytokines were estimated in Synovial Fluid (SF) as well as in serum. IFN-gamma was found to be significantly higher ($p = 0.0003$ versus control rheumatoid arthritis) in the SF of *C. trachomatis*-positive ReA/ uSpA patients in comparison to uninfected patients while IL-4 was downregulated in both SF as well as in serum. IL-17A was comparable in *C. trachomatis*-infected versus non-infected ReA/ uSpA patients, however, it was significantly higher ($p = 0.0001$ versus OA) than the control osteoarthritis patients. In SF, IL-6 was positively correlated to IFN-gamma ($r = 0.72$, 95% CI 0.23-0.91, $p = 0.007$).

Furthermore, level of IFN-gamma ($p = 0.04$) and IL-17 ($p = 0.0001$) cytokines were significantly upregulated than IL-6 in chlamydial heat shock protein 60-positive ReA/ uSpA patients, while IL-17 was also significantly higher ($p = 0.01$) than IFN-gamma in these patients. Comparison of cytokines and hsCRP in *C. trachomatis*-infected and HLA B27-positive versus *C. trachomatis*-negative and HLA B27-positive patients showed that hsCRP was significantly higher ($p = 0.007$) in the former group of patients while there was a decrease in IFN-gamma among HLA B27-positive patients ($p > 0.05$). Pro-inflammatory cytokine response was observed in patients with *C. trachomatis*-induced ReA with pleiotropic cytokine, IL-6 appearing as the key player.

ADULT STEM CELL BIOLOGY

Pre-clinical trial groundwork towards Cultured Epithelial Autograft application studies in burns patients.

A cost-effective method of growing cultured epidermis for application in burns has been standardized at our laboratory comprising of in vitro cultivation of epidermal sheets using commercially available human epidermal keratinocytes and SWISS 3T3 cells as feeders after growth arresting the feeders with low concentrations of Mitomycin C and a Prototype has been prepared. To translate

this technique into application in burns patients it is necessary to simulate large-scale production of Cultured Epithelial Autografts (CEA) from the human skin biopsy as the start up material employing the in-house technique. It is further proposed to perform Quality Control and Quality assurance issues. The chromosomal stability in the cultured keratinocytes of 3rd and 4th passages by G-banding suggested normal chromosomal structure and number. Studies have been initiated to grow non-xenogeneic CEA through the use of human dermal fibroblasts as feeders. The primary objective of dose derivation has been completed.

NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY, MUMBAI

The intramural research in the field of basic medical sciences was undertaken by the two institutes of ICMR viz. National Institute of Pathology (NIOP), New Delhi and National Institute of Immunohaematology (NIIH), Mumbai. The extramural Research was carried out in the areas like biochemistry, cellular and molecular biology, hemoglobinopathies, hemostasis, transfusion medicine, cytogenetics, autoimmune disorders and primary immunodeficiencies, etc.

Newborn Screening for Sickle Cell Disease in south Gujarat and Madhya Pradesh

Under the Newborn screening programmes for sickle cell disease ongoing in tribal populations in south Gujarat and Madhya Pradesh and around 90 babies with sickle cell disease are being regularly followed up and provided comprehensive care. Providing mobile phones to these families has enabled to monitor these babies more closely and provide comprehensive care and immediate attention in case of any complications even in remote areas. This will help us to understand the natural history of sickle cell disease in this region. The comprehensive care and follow up of the SCD cases will reduce the morbidity and mortality which will ultimately improve the survival of the patients.

This programme has also helped to avoid the further birth of sickle homozygous babies in the families at risk identified after newborn screening by offering genetic counseling and prenatal diagnosis.



Fig. Distribution of mobile phones to parents of sickle cell anemia.

Validation of the RBD Kit for detection of common β thalassemia mutations in Indians

The RBD kit for detection of the common Indian beta thalassemia mutations and abnormal hemoglobins developed by our Institute and prepared by Imgenex India Pvt Ltd for ICMR has now been validated by RMRC Bhubaneswar and our Institute. The next lot of kits will be validated at different centres in the country before being marketed. This kit will be useful for all centres involved in molecular and prenatal diagnosis of hemoglobinopathies in the country.

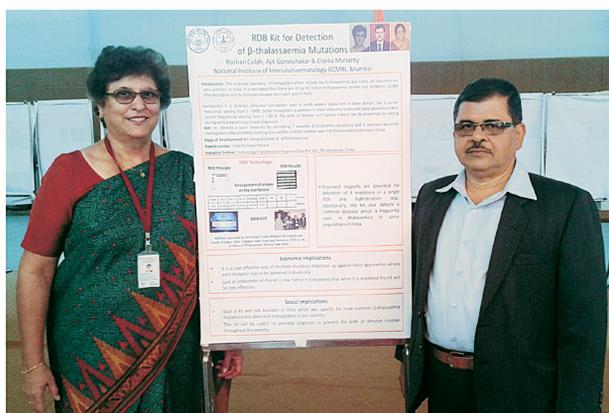


Fig. Poster on RBD Kits during “Innovations in Medical Science and Biotechnology” Exhibition at Rashtrapati Bhavan on 11th March 2015.

Newborn screening for HbE disorders and red cell enzymopathies at Tripura

A centre for screening and molecular diagnosis of hemoglobinopathies and red cell enzymopathies was established at Agartala Medical College where newborn screening was also undertaken for HbE disorders, HbS disorders, G6PD deficiency and pyruvate kinase deficiency. This will help us to understand the magnitude of the problem of these inherited red cell disorders in Tripura and their contribution to neonatal jaundice and hemolysis. The centre established in the north eastern region will be helpful for the diagnosis of common genetic disorders which in turn, will help in better management of these cases. The centre will also be developed for prenatal diagnosis facilities for genetic disorders which will ultimately help to prevent the birth of an affected child.



Fig. Onsite training for molecular diagnosis of hemoglobinopathies and enzymopathies at Agartala Medical College, Tripura.

Prenatal Diagnosis programmes at the Institute

Prenatal diagnosis for preventing the birth of babies with severe genetic disorders is a major activity of the Institute and last year prenatal diagnosis was offered to > 200 families at risk of a severe hemoglobinopathy, 85 families of severe hemophilias and other bleeding disorders and a few

families of severe paediatric immunodeficiencies. Prenatal diagnosis of Fanconi anemia has also been established. Training was also given to different centres in the country to establish additional centres.

Pattern of globin gene expression in an *in-vitro* stem cell culture system, exposed to the malarial parasite *Plasmodium falciparum*

Two tyrosine kinase inhibitors (TKIs) [Imatinib and Sorafenib] were tested *in-vitro* for their antimalarial activity and possible synergistic activity with existing antimalarial drugs for five different *Plasmodium falciparum* laboratory strains (3D7, Dd2, 7G8, MRC2, PKL9). Imatinib and sorafenib showed promising antimalarial activity with all the strains. These compounds caused dose dependent inhibition of parasite maturation. The isobologram analysis of the interactions of these TKIs with a standard antimalarial drug, artesunate revealed distinct patterns of synergism, additivity and antagonism at different ratios. Imatinib showed worthwhile synergism with artesunate indicating that imatinib and other tyrosine kinase inhibitors may have significant antimalarial activity and could be used in combination therapy.

Oxidative stress in hereditary chronic hemolytic anemia and the protective effect of the natural antioxidant-fermented papaya preparation: An *in-vitro* study

The role of natural antioxidants like fermented papaya preparation in reducing the reactive oxygen species, lipid peroxidation and phosphatidylserine externalization in patients with sickle cell disease has been shown in an *in-vitro* system.

National Inhibitor Survey in Haemophilia Patients

Development of inhibitors in severe hemophilia A patients results in increased morbidity and mortality. An on-going study in the Institute is a nationwide survey including over 4700 hemophilia patients which showed that 3 to 12 % of patients develop inhibitors. This information will help the Govt. to decide on the amount of various factor concentrates which will be required for management of these patients

Centre of Excellence for Primary Immunodeficiency Disorders

A Centre of Excellence for Primary Immunodeficiencies has been sanctioned at the Institute. Ours is one of the very few laboratories in the country providing investigations for diagnosis of these patients and offering possible management. Number of simple, rapid and cost effective assays have been developed for evaluation of these disorders and a patent has been applied for one of them.

Identified mutation in IFN γ R2 resulting in Mendelian susceptibility to mycobacterial diseases (MSMD) was identified for the first time in India in a family with three affected children with the help of next generation sequencing in collaboration with Institute of Bioinformatics Bangalore.

Proteolytic antibodies in immune mediated and infectious conditions : Role in health, disease and therapeutic implications (ICMR-INSERM Collaboration)

A set up for detection and characterization of proteolytic antibodies has been successfully established in diseases like Acquired hemophilia, Systemic Lupus Erythematosus, Scleroderma and Malaria and 112 cases have been studied. The effect of hydrolysing activity with the respective antigens such as FVIII, dsDNA and Topoisomerase were further confirmed by antigen specific assays in these diseases. Sequential follow up studies were conducted in these patients to look for the beneficial effects of the catalytic antibodies and to look for their immunoregulatory potential.

Understanding the molecular mechanism of oxidative stress and mitochondrial function in impaired Fanconi anemia pathway

Study on oxidative stress and mitochondrial function showed imbalance of mtDNA/nDNA ratio and elevated levels of ROS which suggests mitochondrial dysfunction. Molecular study of mitochondrial genes revealed a high frequency of mitochondrial DNA variations suggesting mitochondrial genomic instability in FA cells leading to alterations of biochemical pathways in FA cells.

Molecular analysis of telomerase RNA component (TERC) gene and telomerase reverse transcriptase(TERT) gene in aplastic anemia.

Study of telomere length and telomerase activity showed reduced levels of telomerase enzyme in aplastic anemia and the same was restored after immune suppressive therapy. This suggests that the telomerase enzyme can be used as a biomarker for the management of aplastic anemia.

Cytogenetic and molecular study of Myelodysplastic Syndrome (MDS)

Novel mutations in ASXL1 and TET2 genes were detected in Indian patients with MDS and were associated with the prognosis of the disease. Quantitative multiplex PCR of short fragments revealed DNA copy number changes in MDS which were associated with disease progression.

Study of defects in DNA repair pathways and mitotic cell cycle check points in Myelodysplastic Syndrome

Study on DNA repair gene defects showed a high frequency of XRCC1 gene polymorphisms (Arg280 His and Arg399Gln) in MDS suggesting disease association. The AA, AG genotypes identified in RAEB-I, RAEB-II subgroups of MDS can be considered as prognostic markers.

Study of Fucosyltransferases (FUT1, FUT2 and FUT3) haplotypes and their association with some autoimmune disorders

The FUT 1 and FUT 2 genes were analysed in 89 Bombay phenotype cases and the T725G missense mutation in the FUT 1 gene and a 10kb deletion covering the entire FUT2 gene were found as inactivating mutations in all the cases.

Studies on human neutrophil antigens (HNAs) and associated antibodies

No comprehensive study has been done on distribution of human neutrophil antigen (HNA) alleles and associated antibodies in the Indian donor population. A very high frequency (99%) of the HNA -2a antigen has been found among 170 blood donors in the Indian population.

Genetic determinants for expression of Duffy antigens on red blood cells and their interaction with *Plasmodium vivax*.

Molecular genotyping for Duffy antigens has been done in 900 individuals among rural populations of Chiplunin, Maharashtra and the prevalence of Fy(a+b-) was found to be only 10.4%. No Fy (a-b-) case have been detected so far. Cloning of *Plasmodium vivax* Duffy Binding Protein (PvDBP) along with Enhanced Green Fluorescent Protein (EGFP) is being done and will be used to study the pathogenesis of vivax infection by analyzing the interaction between PvDBP with RBCs of different Duffy genotypes.

Non-invasive fetal RHD typing

Non- invasive fetalRhD typing from maternal plasma provided a safer alternative for the management of RhD negative pregnant women. There was complete concordance between RhD grouping results performed by the non-invasive approach and the serological method used on cord blood samples in the 40 cases analysed.

Molecular genotyping of Rh, Kell, Duffy and Kidd blood group systems

Molecular characterization of common antigens of Rh, Kell, Kidd and MNS blood group system was done in 72 multi-transfused thalassemic patients and 285 donors. This will be useful in knowing the correct antigen status of chronically transfused patients and in selection of antigen negative RBCs for transfusion.

Pharmacogenomics of hematological toxicity of Zidovudine in HIV patients

The distribution of SNPs in the drug metabolic pathway among HIV patients on ART from India are being studied as SNP analysis would highlight correlation of any SNP with hematological toxicity of Zidovudine. SNP genotyping for drug metabolizing genes showed that UGT2B7* 1c allele with genotype AA – 18 and genotype AG – 7 was present in 25 HIV patients. ABCB1 was found to be triallelic.

Human Resource Development

In the field of Hematology and Transfusion Medicine various training programmes are organized in

hemoglobinopathies, hemostasis, cytogenetics, red cell serology and workshops on flow cytometry for diagnosis of pediatric immunodeficiency disorders are also conducted regularly.



Fig. Final Training and evaluation Programme for doctors and technicians from North Eastern states for blood group serology.



Fig. Annual training programme for bank officers and technicians.

Swacch Bharat Campaign

Under the *Swachchh Bharat* Campaign, lectures, quiz programmes, drawing competition for school children and essay writing competitions on innovative ideas to take the programme forward were organized.

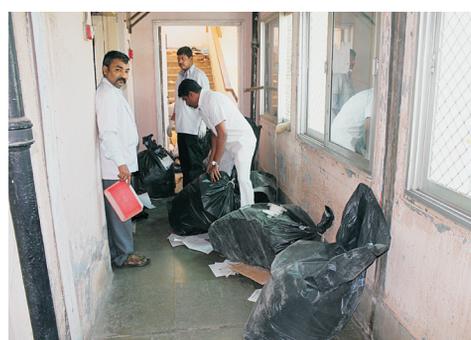


Fig. Swachchh Bharat Abhiyan.

Extramural Research

Anatomy

A study to estimate the age from teeth among North Western population using histomorphological and ultrastructural approach was undertaken at PGIMER, Chandigarh. Age estimation is an important facet for identification purposes in the field of forensic anthropology and becomes important where teeth are the only available material for identification. Single rooted teeth samples (400 (histology) +30 (ultrastructure)) of North- Western adult Indians of age 18-75 years were collected and studied. The present study revealed that tooth Cementum annulation (TCA) count was the most promising among all the histological techniques with an absolute mean error of ± 5.9 years. On the basis of gender differences, all the methods of age estimation were found to have no sexual differences in absolute mean error using t-test. Samples used in the study were extracted for Periodontal and non- periodontal reasons. When absolute mean error of these two groups of teeth was compared it was found that there was statistically significant difference in absolute mean error of age between two groups of teeth in case of TCA technique only.

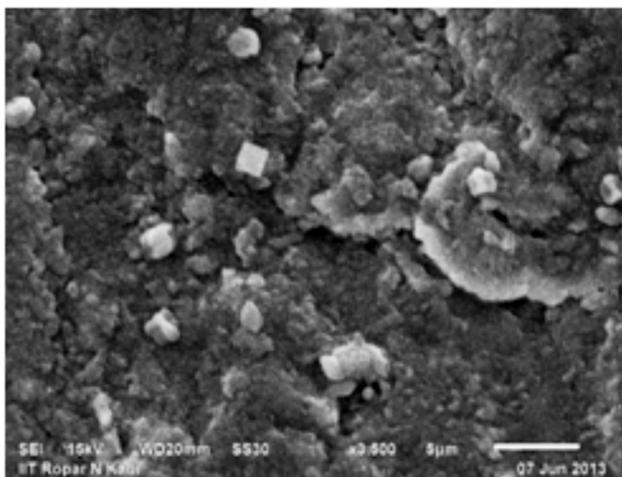


Fig. 1. Calcospherites in midroot region of tooth.

The results concluded that cementum annulation count was found to be most promising method for age estimation but periodontal status of the tooth has to be considered. Forensic scientists have long been facing a challenge for determining the age at death and study has a great importance where there is no acceptable proof of age (Fig 1).

A study on surgical anatomy of human cochlear and its applications during cochlear implantation was carried out at PGIMER, Chandigarh. Cochlear implantation (CI) is one of the greatest success stories of modern biomedical engineering, enabling the patients with sensor neural hearing loss to achieve hearing. The cochlea and round window differed significantly in their dimensions in each case, favouring the idea that each implant recipient should receive individual electrode array for their unique anatomy. The transverse diameter of cochlea is a significant predictor of basal turn length and can be used clinically for estimating the size of electrode. Unusual narrowing in basal turn of cochlea (Fig.2) found in some cases, may explain the difficulties experienced by surgeons to reach full insertion in such cases. Prior information of normal topography of cochlea in relation to adjacent neurovascular structures and their variations like impingement and abutment of carotid canal (Fig.3) on anterior cochlear wall, high jugular bulb and paper thin separation between cochlea and facial canal may assist in preoperative radiological assessment, selection of side and operative strategy for a traumatic cochlear implant insertion. The significantly smaller distance between cochlea and vascular structures in children calls attention to need of greater caution during CI in children. Veria technique is safe and reliable method for implant surgery in Indian population. Cochlear implantation should be planned early in congenitally deaf children and improvement in speech intelligibility are not immediate rather improve with time.

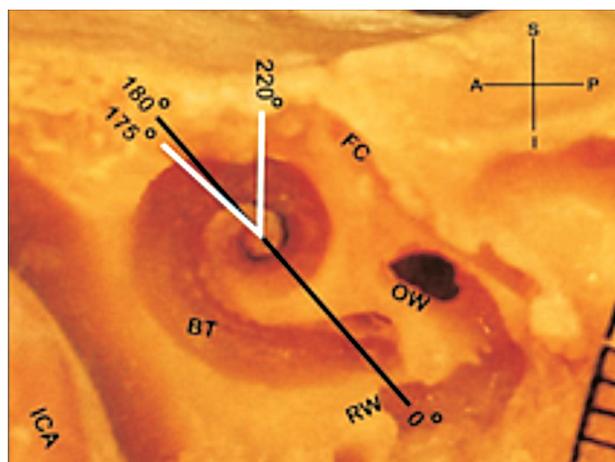


Fig. 2. Dissected cochleae showing narrowing between 175° and 220° segment of basal turn of cochlea; BT, basal turn of cochlea; RW, round window; OW, oval window; FC, facial canal; ICA, internal carotid artery; CC, carotid canal.

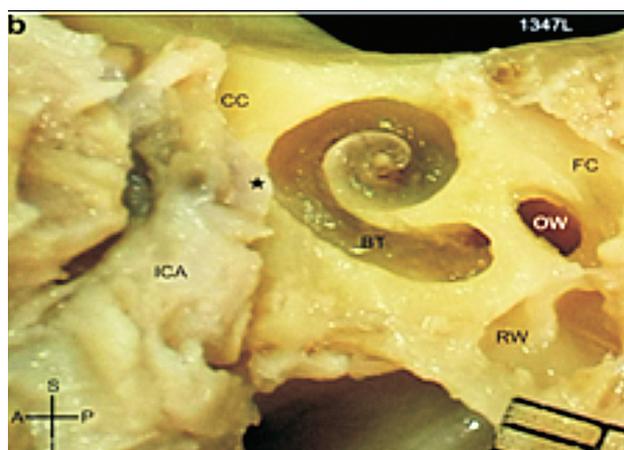


Fig. 3. Dissected cochleae showing abutment of carotid canal (asterisk*) on basal turn of cochlea (BT-CC=zero). BT- Basal turn of cochlea; CC- Carotid canal; ICA- Internal carotid artery; FC- Facial canal; OW- Oval window; RW- Round window.

Human Genetics

In a study on metabolome analysis of urine to detect disorders of amino acids, organic acids, sugars, purines and pyrimidines by gas chromatography/mass spectrometry in infants and children, the frequency of amino acid, organic acid and fatty acid oxidation defects and disorders of sugars, purines and pyrimidines in high-risk neonates and children was determined. A total of 822 suspected cases of inborn error of metabolism (age group 0-15 years) were enrolled and 87 cases were identified. Homocystinuria was found to be the most common metabolic disorder and 51 cases were diagnosed out of which 25 belonged to cobalamin defect/deficiency. Seven cases each of Biotinidase and Glutaric aciduria type I were identified. Apart from these diagnosis was done for- Carnitine Uptake Defect in 6; Methylmalonic acidemia in 3; Propionic acidemia in 2; Urea Cycle Defect in 2 and Carnitine palmitoyltransferase-1 deficiency in 2 cases. One case each of MSUD, PKU, SCHAD, Congenital lactic acidosis; respiratory chain defect and prolidase deficiency was also diagnosed.

Another study to identify the *genetic basis underlying Stevens–Johnson syndrome (SJS)* was conducted at AIIMS, New Delhi. SJS is a rare life-threatening, severe muco-cutaneous blistering disorder of the skin and mucous membranes commonly associated with infectious agents and inciting drugs. Acute cases reported are nearly 65% with a morbidity rate of 35% and mortality rate of 5-10%. Ocular complications in this disease

are common, with corneal damage being most severe long-term complications for survivors of SJS and result in permanent visual loss due to corneal scarring or vascularization. The present study reports details of molecular screening of the interleukin genes and levels of Granulysin, sFasL in these patients. Screening of IL-13 coding region revealed reported A/G polymorphism (rs20541) in 37% patients, IL-4 revealed reported T/C change (rs2243250) in 38%. IL-4R analysis identified reported A/G polymorphism (rs1801275) in another 34% of individuals. IL-13 promoter region was also screened and C/T change (rs1800925) identified in 29% individuals. Novel changes were detected in IL-13 in about 3% of the individuals. Apoptotic markers showed raised levels of Granulysin and sFas L in patients in comparison to healthy controls. The present case-control study adds to the repertoire of SNPs, especially the IL-13 and IL-4 promoter region showing novel as well as reported changes in the patients suggesting an important role of these interleukins. Raised levels of Granulysin and sFasL and clinical grading of ocular manifestations indicate the severity of disease. These may be used to identify at risk individuals based on their genetic makeup and may in future facilitate better management of this uncommon condition.

A study was carried out on the genetic association and correlation of Monoaminergic, GABAergic and glutaminergic pathway gene polymorphisms with brain imaging (PET/SPECT) in alcohol dependence at AIIMS, New Delhi. Environmental factors play a significant role in addiction and presence of genetic polymorphisms are reported in the development of AD. The findings of the study are indicative of the role of GG genotype in DRD1 rs4532 and TT genotype in DRD1 rs686 in alcohol dependence and the role of T allele in GABRA6 rs3219151 and G allele in Val158Met in conferring risk for severity of alcohol dependence in the patients.

Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death worldwide. In light of growing number of COPD cases in India, a study on association between smoking and genetic factors in the development of COPD was carried out to understand the factors that place smokers at the risk of developing the disease.

COPD exerts severe limitations on an individual's ability to perform his duties. The biochemical factors in the present study were selected to assess the contribution of oxidant-antioxidant imbalance theory in the etiology of COPD. Patients had higher levels of malondialdehyde and lower levels of antioxidants when compared with controls ($P < 0.01$). Significant increase in malondialdehyde and decrease in catalase activity and GSH level was observed with the progression of the disease. In controls, malondialdehyde showed significant positive correlation with lung function and glutathione peroxidase and negative correlation with glutathione. The development of COPD, upon exposure to tobacco smoke, is the cumulative effect of defects in several genes. Significant associations were found with MMP12, IL13 and for the first time in Indian population, with FAM13A, SERPINE2 and IREB2. SERPINE2 is a relatively new candidate gene in COPD, and both excess and deficiency of its protein contribute indirectly to the development of lung fibrosis and COPD respectively.

Lysosomal Storage Disorders (LSD) are a group of chronic, progressive, debilitating and life threatening conditions and ICMR has initiated a multicentric Task force collaborative study of the clinical, biochemical and molecular characterization of lysosomal storage disorders in India: The initiative for Research in Lysosomal Storage Disorders. The study aims to identify pathogenic mutations in LSDs, study genotype phenotype correlation and do molecular modeling for novel variants. The study is being funded by DHR at 10 Centres in India and will enable setting up a mechanism for the clinical, biochemical and molecular diagnosis as well as a network of genetic laboratories across the country for comprehensive diagnostics. Patients have been recruited and initial diagnosis based on clinical and radiologic features was confirmed by enzyme assay. In molecular studies, genes were analysed and common mutations were identified for various disorders. Mutations were detected in four cases in the GAA gene which is responsible for Pompe disease. Four mutations were also detected for Sandhoff disease. The genotype and the phenotype have been noted for some cases for correlations at a later stage of the study. Mutation analysis in other LSDs is in progress. The study will lead to better understanding of the mutation spectrum in Indian patients with LSDs.

Haematology

A study of genetic alterations in the disease progression of Chronic Myeloid Leukemia patients was conducted at Maulana Azad Medical College, New Delhi. The hallmark genetic abnormality in CML (RIZ1 a PR domain methyltransferase gene) is the reciprocal chromosomal translocation $t(9;22)(q34.1;q11.2)$, resulting in an oncogenic BCR-ABL chimeric gene. In this study, three different putative mechanisms for downregulation of RIZ1 gene expression in CML were investigated, i.e. epigenetic inactivation by promoter hypermethylation, loss of Heterozygosity (LOH) at Pro 704, and A563G mutation of RIZ1 gene (Fig 4).

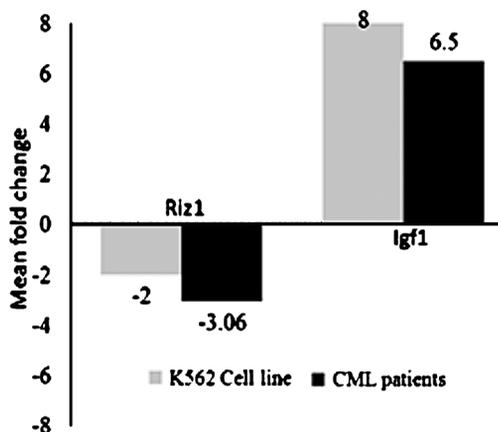


Fig. 4. A significant correlation of RIZ1 LOH at Pro 704 (del-del) was observed with advanced AP and BC phases of CML ($p=0.01$) and with minimal hematological response to Imatinib ($p=0.001$). The del-del genotype was also associated with increased susceptibility to CML, with odds ratio of 7.7(95%CI 2.1-27.2).

Hypermethylation of RIZ1 promoter was significantly associated with BC phase ($p=0.01$), loss of or no molecular response ($p=0.01$), and minor or minimal hematological response to Imatinib ($p=0.01$). RIZ1 expression was found to be 3.06 fold lower in CML patients as compared to healthy controls, and 2.0 fold lower in K562 leukaemic cell line on comparison with a nonleukemic cell line. There was a further reduction in RIZ1 expression, with progression of CML from CP to BC: 3.25 fold and 2.66 fold decline respectively. IGF-1 expression on the other hand, was upregulated 6.5 fold in CML patients and 8 fold in K562 leukemic cell line. There was a 7.3 fold increase in IGF-1 expression in BC and AP in comparison to 5.7 fold increase in CP, demonstrating a rising trend with CML disease progression. This study lends

besides India, 5 SAFHeR nations namely Bhutan, Bangladesh, Maldives, Nepal and Sri Lanka participated in the workshop. Fifty students and 4 faculty members participated in the workshop. The topics covered in the workshop were study designs, biostatistics, commonly used statistical software, data management, medical ethics, GCP and GCLP, clinical protocol development, RCT, reviewing research papers, drafting and submission of research proposals to funding agencies and communication skills.

Biomedical Ethics

The Biomedical and Health Research Regulation Bill -At present biomedical and health research apart from clinical trials with new drugs is largely unregulated. It is proposed to set up a ‘Biomedical & Health Research Authority’ for regulating ethics committees and research institutions involved in research in order to protect the safety, well being and rights of all research participants. The Draft “Biomedical and Health Research Regulation Bill, was prepared by the Central Ethics Committee on Human Research (CECHR) and submitted to the Department of Health Research for inter-ministerial consultation. The Bill was submitted to Ministry of Law for approval before submission to the Cabinet by the Department of Health Research.

Biomedical & Health Research Regulation Bill, 2014

| | |
|--|--|
| Finalization of The Biomedical and Health Research Regulation Bill, 2014: | <ul style="list-style-type: none"> Organised 27 subcommittee meetings under the Central Ethics Committee on Human Research (CECHR) involving stakeholders from different fields, engagement of Former Secretary, Ministry of Law for legal opinion and coordination of inputs from the subcommittee and finalization. |
| Finalization of the Note for the Cabinet | <ul style="list-style-type: none"> Preparation of Note for the Cabinet in coordination with DHR, inter-ministerial Consultation with 14 Ministries twice, consultation with subcommittee, preparation of replies to Ministries. |
| Coordination with DHR, ICMR Administration, and Engineering Deptt: | <ul style="list-style-type: none"> Requirements for setting up office of Authority- including space, office, infrastructure, meeting rooms, manpower, calculation of budget, salary structure, equipment, framework and organizational chart |
| Coordination with Deptt of Legal Affairs & Legislative Department at Ministry of Law | <ul style="list-style-type: none"> 8 Meetings with DS (Legal Affairs) and JS (Legis Deptt) for line by line finalization of the Bill, coordination regarding finalisation of the Note for Cabinet and Hindi translations. |

Capacity Building in Research Ethics -A number of initiatives and activities were pursued and ICMR bioethics cell is involved in providing information and guidance to researchers and Institutions from all parts of the country as well as involved in more than 20 ethics trainings and workshops organized by various Medical Colleges, Research Institutions. Some other activities pursued are as follows: Development of Draft Ethical Guidelines for Research in Children in collaboration with

AIIMS and Division of RHN (ICMR), Guidelines on Payment of Compensation in case of injury during research in ICMR funded research, Preparation of draft Framework Document on Payment of Compensation for injury during research, Consultation with Insurance companies, International SIDCER Recognition Program for Ethics Committee NIRT, Chennai, NIE, Chennai and NIRRH, Mumbai, Associated with CDSCO and the Quality Council of India in developing the Guidelines for Accreditation of ethics committees, Institutions as well as researchers involved in clinical trials under the Drugs and Cosmetics Act.

Centre for Advanced Research

Centre for Molecular Medicine: Emerging Areas in Molecular Medicine at Jawaharlal Nehru University, New Delhi

During the period under report the achievements of the Centre are as follows:

- Development of a liver cell line (using Pregnane & Xenobiotic Receptor promoter) for safety evaluation and screening of a wide array of drugs, herbals and xenobiotics.
- Preclinical evaluation of a novel radioprotector (benzimidazole) in radiotherapy against cancer patients with less cytotoxicity.
- Design and development of a novel peptide against ICAM that has the ability to block invasion of both *M. tuberculosis* and *P. falciparum* into host cells.
- Discovery of a novel peptide inhibitor against MDR *S. maltophilia* biofilms implicated in ventilator associated pneumonia (VAP) and highest mortality in critical care (in collaboration with NIPER Mohali).
- Establishment of role of epinephrine/norepinehrine as a direct regulator of cellular iron homeostasis explaining tissue iron overload detected in chronic stress.

Centre for Molecular Medicine at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow

During the studies at the Centre, the cytogenetic microarray (CMA) has identified rare copy number

variations in children with intellectual disability and autism. The diagnostic yield of CMA has been found to be about 10% and has been successful in providing genetic counseling and prenatal diagnosis to the families with such disabilities. The CMA is also being done on prenatal samples successfully and this experience is used to establish CMA based prenatal diagnosis [Translation into patient care] at the cost of Rs 25000 per test to make the test financially viable and to keep its continuity. This has been in accordance with the recent guidelines of American College of Medical Genetics to offer CMA as a first line test for evaluation of intellectual disability and autism and also to be incorporated in prenatal diagnosis.

In the project, sequencing for more than 40 genes is being done for rare monogenic disorders and this is reflected in the publications of novel mutations in Indian patients. This research work is contributing to the data of mutation spectrum of rare disorders worldwide and Indian patients. And it is also of great use in patient care and clinical services. Exome sequencing is another genomic technique and has been successfully utilized in identifying 3 novel genes and 5 novel mutations in known genes. Under the Thirteenth ICMR Course in Medical Genetics, 28 medical doctors have been trained in basic and clinical genetics including exposure to laboratory genetics.



Fig. Participants of Thirteenth ICMR Course in Medical Genetics & Genetic Counseling.

Establishment of a centre for excellence in Molecular Medicine- Advanced Programme in Basic and Applied Immunogenetics

During the year, additional primers and probes have been incorporated for HLA genotyping so as to cope

with the newly discovered alleles in the database. In addition, detailed SOPs for Luminex and sequence based HLA typing have been established. These are currently been employed for selection of histocompatible donors for patients requiring renal and hematopoietic stem cell transplantation (HSCT).

A major highlight of this year is the optimization of flow cytometry based cell surface and intracellular staining and sorting up to the extent of >98% purity using the Aria III flow cytometer. This technology has been offered to other investigators for immuno phenotyping, functional readouts and lineage specific sorting as a research tool for studies on infectious and autoimmune diseases.

Further, the ongoing Luminex based screening assays for detection of donor specific antibodies have been refined to greater degree of sensitivity. These assays are critical for detection of DSAs in patients requiring kidney transplantation in conjunction with the causative antibodies in patients to define those who could accept the graft.

The team has developed expertise for screening anti HLA class I and class II abs as well as those against the MICA molecules present on endothelial cells. In an attempt to evaluate the role of innate immunity in renal graft success, and analyzed functional dimorphism of MICA gene, which is known to affect its binding abilities with its receptor NKG2D, expressed on NK cells. The data revealed that patients homozygous at MICA 129 codon position for amino acid methionine (met) are more likely to develop antibody mediated rejection (AMR) episodes. Further, quantitative analysis of soluble MICA (sMICA) in patients undergoing renal transplantation revealed that those undergoing an eventful follow up showed a significant drop in their sMICA levels at variable periods post transplantation suggesting an important role for this system as a potential biomarker in solid organ transplantation.

Under the immunogenetic disease association models, evaluated the cytokine gene polymorphisms among HIV-1 infected patients and compared the same with healthy controls so as to determine the critical cytokines and their receptor gene variations influencing disease development.

A cohort representing each of the multiple HLA-DR3 haplotypes has been selected and is under genomic analysis. Additionally relative studies of various TagSNPs and microsatellites in the MHC and non MHC regions (e.g. PTPN22) among T1D patients and their comparative analysis have been performed. The data indicated that the genetic polymorphism in PTPN22 gene not only associate with disease susceptibility but also with the autoantibody positivity.

The centre has established the STR based chimerism assays for monitoring graftfunctioning following hematopoietic stem cell transplantation. A panel of indigenously developed STR markers has been standardized and this is being genotyped and resolved in an automated genetic analyser ABI3130xl and analysed using Gene scan and Genotyper softwares. Chimerism testing is being performed at ~ 15 days post HSCT to confirm engraftment followed by 3 and 6 monthly evaluations on regular basis. A formal report showing % donor chimerism is being issued based on which the further course of treatment is determined.

During the year the centre imparted training in the field of Transplant Immunology & Immunogenetics to ~25 young researchers and clinicians. Further, the quality assurance program (QAP) for HLA typing at national level was continued and percent concordance for different participating laboratories has been submitted with all details in the full report.

Biochemistry

Redox Proteomics approach to study of the oxidatively modified protein after Chronic Aluminum exposure in Rats resulted in oxidative damage to mitochondrial proteins and electron transport chain subunits leading to neuronal death. This study further lends support to the role of aluminium in etiopathogenesis of various neurodegenerative diseases.

The study on Role of Extracellular Matrix Proteins Vitronectin & Fibronectin in the Establishment of Staphylococcus Aureus Infection have demonstrated that Autolysin seems an appropriate target for developing new therapeutics as this bacterial protein also interacts with other extracellular matrix

proteins such as fibronectin, thrombospondin etc., and facilitating bacterial infection.

The study on Analysis of Structural and Mechanistic Basis of Novel Anti-thrombin Variants in Indian Families with Thrombosis demonstrated a novel point mutation at position 7549 A>G (T280A), highly conserved in serpin family is reported for the first time. Variant protein isolated from patient plasma indicated that loss of regulatory function was due to in-vivo polymerization. In conclusion, it is reported that a novel point mutation (T280A), a novel single nucleotide insertion (13363insA) in the background of known variants R47C, C4-stop and polymorphism of rs2227598, PstI and DdeI in AT gene of Indian population.

Investigation of Biochemical Changes Related to Initiation and Progression of Visual and Vascular Disturbances in Type-2 Diabetes Mellitus hypothesized that hyperglycemia mediated biochemical derangement is the early event, which might augment oxidative stress and influence increased production of AGEs in DR. Increased production of AGEs is the key factor that is associated with increased ROS production, which in turn activates NFκβ and up regulates the production of downstream growth factor VEGF in DR.

CELLULAR AND MOLECULAR BIOLOGY

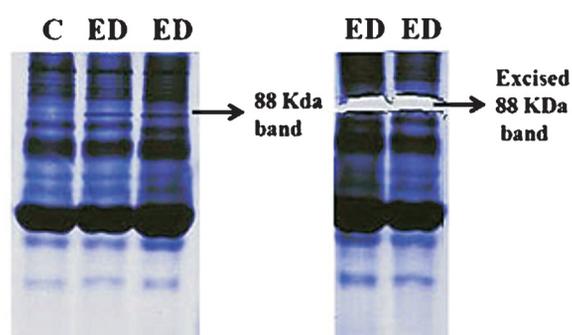
Molecular Cloning, Sequencing, Over Expression and Characterization of a Novel 88-KDa Protein Found in Patients with Eales' Disease at Vision Research Foundation, Chennai

Eales disease (ED) is an idiopathic inflammatory disease, which primarily affects the peripheral retinal veins. Etiology of disease remains poorly understood although disease is mostly associated with latent tuberculosis. Increased levels of iron in monocytes, oxidative stress, and inflammatory markers were reported in patients with ED. Previously a novel 88 kDa protein was reported in serum and vitreous of ED patients.

This protein was identified to be a complex of complement C3 (C3), Haptoglobin (Hp), Apo A1, Galectin-1 (Gal-1) by 2D liquid chromatography coupled mass spectrometry (2DLC-MS). The levels of protein expression were evaluated by ELISA in 20 number ED patients with age and sex matched

control subjects. By these results it was confirmed that Hp and its interacting partners were seen as 88kDa band in ED serum.

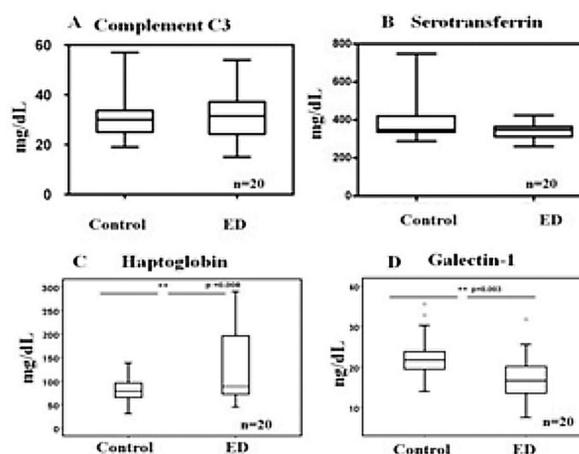
In addition to the above findings, presence of Mycobacterium tuberculosis proteins were also identified by the mass spectrometry in PBMC and in 88 kDa band ED patients. Possibly this observation is result of antimicrobial defense mechanism to deprive bacterium of iron. Presence of MTB proteins in ED increases Haptoglobin (Hp) synthesis, complement activation, alters iron acquiring proteins and other inflammatory proteins in serum. Hp is anti-inflammatory in nature and interacts with complement proteins to form 88kDa band in ED serum.



Native PAGE (7.5%) stained by Colloidal Commission stain. Lanes C: serum from normal healthy volunteers; lane ED: serum from patient with Eales' disease. The presence of 88 kDa protein in lane Eales' disease is indicated by arrow in the photograph. 50 μ g of serum protein was uniformly used for protein profile.

Box plots to compare between the levels of complement C3, serotransferrin, haptoglobin and galectin-1 in serum samples of ED patients and healthy control subjects (n=20). A. Levels complement C3 in control serum 330.21 ± 49.4 ; ED serum 340.1294 ± 42.48541 and difference among these groups is non-significant. B. Serotransferrin levels in control serum 31.6 ± 10.4 ; ED serum 31.9 ± 9.7 and difference among these groups is non-significant. C. The levels of haptoglobin in ED serum was found to be significantly higher in comparison to that of control subjects. (n =20, the levels of haptoglobin in Control = 80.72 ± 25.33 , ED = 131.12 ± 72.88 , $p < 0.008$). D. The level of Galectin-1 in Eales' disease serum was found to be decreased with

the statistical significance. (Control- 22.50 ± 5.15 ng/dL, ED- 17.25 ± 5.46 ng /dL, ($p < 0.003$).



Role of Htra2, a serine protease, in Mitochondrial Biogenesis and Aging in Mouse Model: Implications for Parkinson's disease

Htra2 is a serine protease localized in the inter-membrane space of mitochondria. Evidence highlights the importance of mitochondrial form of Htra2 in the progression of Parkinson's disease. The aim of this project was 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.

In conclusion, the studies identify 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. The current work will be communicated soon.

Delayed Vitamin K deficiency: Genetic profile of factors in vitamin K cycle and gamma carboxylation system at National Institute of Immunohaematology, Mumbai

Hemorrhagic disease of the newborn (HDN) or delayed vitamin K deficiency bleeding (VKDB) is an uncommon disorder with potentially devastating outcomes. The bleeding is most often intracranial, but can also be mucosal, cutaneous, or gastrointestinal.

They studied 47 infants who presented with vitamin k deficiency related bleeding between 5 days to 30 years. More than half of the patients had the 3 warfarin sensitive polymorphisms i.e. CYP2C9 *2

and *3 and VKORC1 G/A indicating that the same polymorphisms which are involved with warfarin sensitivity are associated with delayed vitamin K deficiency related bleeding. The bleeding episodes were not directly related to the age of presentation or the presence of polymorphisms. The diet was also not associated with delayed vitamin K deficiency related bleeding.

Role of Profibrogenic Cytokines on the Expression of miRNA-150 and miRNA-194 in Hepatic Stellate Cells
Chronic liver injury causes the apoptosis hepatocytes and the quiescent hepatic stellate cells get activated in response to the injury and proliferate as a wound healing response. The activated cells secrete the extracellular matrix proteins, which get deposited in between the hepatocytes and the blood vessels causing the fibrosis. The mechanisms that regulate the fibrogenesis are not known clearly.

In this study investigator has used the activated stellate cell line, LX2, for all the experiments to elucidate the role of miRNAs (miR-150 and miR-194) in modulating the profibrogenic cytokines. concluded that miR-194 is causing down-regulation of the rac, which in turn down-regulates the pathway involving AKT, pAKT and NFκB.

Targeted Correction of the Point Mutation of Sickle Cell Gene and Targeted mutagenesis of Nucleotide Associated with HPFH by RNA/DNA Oligonucleotides (RDOs)

Recent advances allow multiplexed genome engineering in *E. coli*, employing easily designed oligonucleotides to edit multiple loci simultaneously. A similar technology in human cells would greatly expedite functional genomics. Using an EBV transformed lymphoblast SC-1 cell line homozygous for the sickle cell mutation, demonstrated that relatively short single-stranded oligodeoxynucleotides (25–61 bases), homologous to the target sequence except for a single mismatch to the targeted base, are capable of correcting a single point mutation (A→T) in the mutant sickle cell gene in mammalian cells. ssODN with antisense orientation showed a higher frequency than sense orientation, indicating a possible influence of transcription on gene correction. These results show that a relatively short ssODN can make a

sequence-specific change in the target sequence in mammalian cells.

Mismatch Repair (MMR) machinery plays a significant role in recognizing and removing the alteration caused by the oligo incorporation event. It was observed that gene silencing of MMR gene with MSH-2 siRNA selectively increased targeting efficiency by blocking the mismatch repair machinery. The Taqman SNP genotyping assay showed a greater sensitivity for detection of mutation correction. The oligo-mediated targeting of human cells is currently limited by low targeting efficiencies and low survival of modified cells.

Biomarkers and Tumour Necrosis Factor-Alpha Gene Poly- Morphisms: A Relationship with COPD and its Progression
Chronic inflammatory disease, such as chronic obstructive pulmonary Disease (COPD) is multi factorial, determined by both environmental and genetic factors.

This study included 250 COPD cases and the control group which comprised of 230 healthy controls for polymorphism study and 204 COPD cases, 80 smoker controls and 50 non-smokers controls. The TNFα polymorphisms are found in a region of great polymorphic variation and they are in linkage disequilibrium with the HLA genes and with each other. This study provided substantial evidence to support the possible role of TNFα gene (+489 and -863 genotypes) in pathogenesis of COPD.

In COPD patients, elevated Serum levels of TNFα, CRP and IL-6 compared to healthy ever-smokers and non-smokers were found. Inverse relationship was observed between FEV1 (1% predicted) and serum TNFα, CRP and IL-6 in all subjects. The systemic inflammatory biomarker IL-6, CRP and TNFα were moderately repeatable over a 24 months period in COPD patients. They have also shown that a robust and repeatable association between IL-6 and CRP exists. These findings suggest that some of these biomarkers might be associated with the initiation and progression of COPD.

GENOMICS

Genetic Engineering of Vegetable and Legume Crops with Oxalate Decarboxylase Gene for Removal of Oxalic Acid on Antinutritional Stress Factor for Better Human Nutrition

Oxalic acid, a two-carbon dicarboxylic acid present ubiquitously as an inert end-product of carbon metabolism in increasingly recognized as a food toxin with negative effects on human nutrition. Oxalates in animals, including human, mostly originate from diet and excess ingestion of oxalate result in a variety of kidney-related disorders, besides neurolathyrism and coronary disease. Decarboxylative degradation of oxalic acid is catalyzed, in a substrate-specific reaction, by oxalate decarboxylase (OXDC), forming formic acid and CO₂. Genetic engineering of FvOXDC in different food crops showed up to a 90% reduction in oxalate content, which correlated with concomitant increases in calcium, iron and citrate. Comparative proteomic analysis revealed a spectrum of OXDC-regulated proteins and pathways previously unknown as OXDC targets and suggested that metabolic remodeling was associated with the decrease in oxalate content. Collectively, the study provides insights into OXDC-regulated metabolic networks and may provide a widely applicable strategy for enhancing crop nutritional value.

Nano-medicine

Synthesis and characterization of Ultra-Fine Gold Nanoparticles, their Utility and Safety as a Novel X-ray Contrast Agent: An in-vivo study in Animal Model Highly stable and monodispersed Gold Nanoparticles (GNP) of three different sizes ultrafine ≤ 2 nm, and fine (5 \pm 1 nm, 10 \pm 2 nm) were prepared and characterized by UV-Visible spectroscopy, DLS and TEM. The surface charge distribution of GNPs inhibits the particles to be aggregated and which stays stable for long time.

Dose, time and size dependent study to evaluate the cytotoxicity of GNPs in renal and liver cells following three parameters MTT assays, PI and DAPI staining and cyto-morphology did not reveal cytotoxicity at high concentration (4.0 \pm 1.5 μ g/ml) after 24 hours. All the three sizes produced mean of 92 \pm 5% viability, no necrosis and 1.5-3% of apoptosis, without changes in cellular morphology in renal and liver cells in vitro. Evaluation of fate and toxicity of GNPs was done by in-vivo mice model study.

Dose, time and size dependent in-vivo acute and chronic bio-distribution and sequestration study

in vital organs showed that the sequestration and excretion varies with size and time. The accumulation of all three sizes of GNPs in vital organs varies with time and maximum accumulation noted at 6 hours whereas in chronic study it dropped to a very low level at 90 days with urinary excretion as documented by ICPAES study or urine.

Renal sequestration of 2 nm was least as compared to other fine size of GNPs. With all 3 sizes of GNPs there was no significant abnormality observed in blood hematology, serum biochemistry including renal and liver function tests, organ weight and histopathology of all organs after 30 days.

To utilize the GNPs as radio-enhancer for early diagnosis of cancer or using as better X-ray contrast agent, the dose dependent in-vitro micro-CT imaging showed marked enhancement of tumor in EAT tumor model in mice and other also organs just after 2 minutes of GNPs administration intravenously.

Thus this in-vitro and in-vivo study proves the concept of using fine size GNP as a safe radio contrast agent with marked radio enhancing capability.

Biocompatible Nanocomposites Based on Nanohydroxyapatite for Bone Tissue Engineering Applications

In this study, different bio-nanocomposite loaded with various loadings of nanohydroxyapatite (nHA) by using both synthetic and biopolymer for bone tissue engineering was developed. The first composite is made up of biopolymer chitosan/starch along with various loadings of silane treated nHA (0% to 15%) and very small amount of carbon nanofibre. A small amount of biocompatible material i.e. 2-hydroxyethylmethacrylate (HEMA) has been used to enhance the interfacial bonding. The tensile, compressive and flexural strength of the composite showed a sustained increase with increasing nHA loading up to 10% for tensile strength and up to 8% for compressive and flexural strength respectively. XRD and FTIR analyses clearly confirm the presence of dispersion and interfacial bonding interactions between the filler and matrix. The second composite consists of synthetic, bioinert-biocompatible PAEK-PDMS along with various

loadings of nHA (0% to 10%) and reinforced with silane treated nano carbon fibre. SEM micrographs revealed that the nHA particles were uniformly dispersed in the PAEK/PDMS matrix and no phase-separation was observed. XRD and FTIR analyses clearly confirm the presence of dispersion and interfacial bonding interactions between the filler and matrix. Cytotoxicity test confirms that two aforesaid composite are cytocompatible.

The two above mentioned bionanocomposite demonstrated excellent ability of inducing apatite formation in simulated body fluid, which is promising to be used for load bearing bone substitutes. Therefore, the bionanocomposites may be potentially used in bone tissue engineering applications. The third composite is bioelastomeric copolymer synthesized by using sebacic acid, glycerol and HEMA, along with various loadings of nHA (0% to 8%) and reinforced with nano cellulose fibre. The optimal mechanical properties for this nanocomposite have been found to be at 7% nHA loading. XRD analysis and SEM micrograph revealed efficient dispersion of nHA throughout the matrix as particles and as well as agglomerates. The mode of degradation of this bioelastomer is via erosions and the structural integrity is maintained till the end in spite of in vitro degradation. Cytotoxicity test confirms that the developed composite is cytocompatible. Apatite formation of these nanocomposites showed excellent biocompatibility, thus showing potential as promising soft tissue repair and replacement applications.

Development & Evaluation of Nanofibre Based Myocardial Patch for Functional Regeneration

The reconstruction of ischemic cardiac muscle tissue into healthy tissue after acute myocardial infarction requires multidimensional tissue engineering approach. This can be achieved through the integration of physical cues (topography) with chemical cues (growth factors), yet the right combination of both remains a huge challenge in cardiac tissue engineering. By fabricating a scaffold mimicking the cardiac tissue architecture and strength, have shown for the first time the occurrence of heterogeneous fibres by electrospinning the physical blend of two synthetic polymeric systems.

The heterogeneous scaffold was in resemblance with the fibrils of collagen I and III of left ventricular heart. By tuning the ratio of two polymeric systems, the level of fibre heterogeneity can be controlled during fabrication process, which was reflected in their physical and functional properties, in terms of strength, elasticity and contact angle. These heterogeneous fibrillary structures also influence the cellular adhesion, proliferation and differentiation. The cellular differentiation at a molecular level was confirmed through gene expression analysis by RT-PCR. In order to provide chemical cues through the nanofibrous scaffolds, dual growth factors VEGF and bFGF were loaded into the heterogeneous fibrous scaffold during fabrication and characterized for the encapsulation efficiency, release kinetics and bioactivity of released proteins. The growth factors loaded nanofibrous patch were tested in an in vitro ischemia mimicking hypoxic cell culture model.

The regenerative potential of the growth factor loaded nanofibrous patch was tested in vivo in an acute myocardial infarction rabbit model. The non-invasive functional assessment of heart after patch implantation was monitored through ECG and Echocardiography. The evidence for functional repair and possibility of regeneration was confirmed through histopathological and immunohistochemical analysis. In conclusion they have successfully generated a bioactive nanofibrous patch for the functional regeneration of cardiac tissue.

Stem Cell Research and Therapy

Study of Biology of Leukemic Stem Cells Over a period of 3 years, total 216 acute leukemia patients were characterized immunophenotypically, out of which 108 were observed to be Acute Myeloid Leukemia. About 63% of total AML cases had CD34 positive blots, out of these 28 cases were further evaluated for LSC characterization and cell signaling studies followed by correlation in disease outcome and treatment group.

Leukemic stem cell characterization panel consists of 13 markers and was divided into four categories based on functional and original properties of each protein in vivo. LSC specific markers involved:

CD44, CD90, CD96 and Wilm's tumor-1, Receptor subunits involved: CD123, CD25, CD116 and CD135, Epithelial related markers which were evaluated in the present study were CD32, CD18 and CD24 and lastly, kinase subunits included Hematopoietic cell kinase and Adenylate kinase-5. Also, four cell signaling pathways were studied in the present study namely: JAK/STAT, MAP/ERK, PI3/AKT and Wnt/beta catenin. Mutations in FLT3, NPM1, NRAS, C-KIT and WT1 were evaluated by PCR amplification followed by sequencing.

Multi parametric flow cytometry was used for marker expression analysis and cell signaling studies and using statistics of receiver operating characteristic curve it was found that the average sensitivity of the assays is 80% and specificity is almost 95%. However, to further validate the data a larger sample population is required. Frequency of LSC population was independent of total blast population and it was not gender biased which was seen in total acute leukemia cases where almost 70% diagnosed cases were male. Highest frequency of LSCs is found to be associated with AML-M0 FAB subtype and largely quiescent. Total 29 normal HSC samples from 19 normal marrow samples, 6 ITP and 4 cord blood samples and 28 AML LSC samples were further evaluated for antigen expression and signal transduction.

Comparison of marker expression in Lineage negative, CD38 negative and CD34 positive population in normal and leukemic samples revealed that six out of thirteen markers were significantly differentially expressed which are: CD44, CD90, CD96, WT-1, CD123 and CD135. Interestingly, when compared in survival groups CD90, WT-1, CD25, CD32 and AK-5 differentially over expressed on LSCs of patients with survival less than 6 months with constitutive expression of pAkt, pErk and pStat-5. However, Only 8 out of 18 patients received standard 7+3 chemotherapy. It was seen that expression of markers like CD90, CD25, CD24 and CD32 was very less on LSCs of patients who achieved a remission. It was observed that mutation in FLT3 induces constitutive activation of pAKT which is a major signaling molecule in PI3/mTOR/Akt pathways involved in propagation of anti-apoptotic signals.

The basic hurdles in achieving a larger sample population is unavailability of consent, enough and fresh marrow from normal individuals undergoing hip or cardiac surgeries. Also, only 35% of AMLs show a distinct population of LSC which again is a rate limiting step for this study. Since a higher LSC percent and constitutive activation of pathways belong to a poorer outcome; putting up LSC identification tubes with CD44, CD90, CD123 and CD135 along with cell signaling tubes one unstimulated and one with inhibitor cocktail might help in defining overall response to treatment or patient specific prognosis at the time of diagnosis. This study might help in defining prognostic values for acute myeloid leukemia.

Study of Stem cell therapy in patients with myocardial infarction and persistent total occlusion of infarct related artery [Cell therapy in Occluded Arteries Treatment (COAT)]

The current standard of care for late presenting myocardial infarction (>24 hrs) is conservative management and majority of studies have not been in favor of opening the infarct related artery unless there is evidence of ongoing ischemia in the form of angina or evidence on stress imaging. The preliminary study would suggest that even in the absence of ischemia, opening the artery late after infarction followed by injection of stem cells between one and six months following the index event, results in a significant improvement in left ventricular function and functional capacity. This benefit has become evident at three months and maintained till one year. This benefit was not seen in the control arm which underwent just opening up of the infarct related artery without injection of stem cells.

Centre for Advanced Research in DNA fingerprinting and diagnostics of Medicinal plants from Eastern and North-Eastern India, Bose Institute, Kolkata

The Centre was set up in collaboration with Manipur University and Botanical Survey of India, Kolkata with aim to the development of Species Specific Markers from AFLP based analysis of DNA fingerprint, development of a Database, Human Resource development among young researchers aiming towards transfer of technology

to laboratories in Universities and Research Institutes of the North Eastern States and raising awareness for Sustainable Utilization of Plant Genetic Resources.

The study is carried out on six medicinal plants which were collected and authenticated by Botanical survey of India (BSI) for the research studies described below. Species specific markers have been developed for three species of Zingiber genus viz. *Zingiber officinale*, *Zingiber zerumbet* and *Zingiber montanum*. The studied landraces of *Z. officinale* (Medicinally most potent species within *Zingiber* genus) showed variation in their medicinal potential (6-gingerol and antioxidant content) and the landraces showed trait related clustering with AFLP derived phylogenetic tree. Trait Related AFLP markers for 6-gingerol (medicinally most significant compound found in *Z. officinale*) have been established. To explore the mechanism responsible this variation in active principle Gene expression study of Chalcone Synthase (i.e the rate limiting enzyme of 6-gingerol producing Phenyl Propanoid pathway) have been undertaken and data showed high CHS gene expression in high 6-gingerol containing landraces and vice versa. SNP analysis of CHS gene among these contrasting genotypes showed total seven SNPs among which two SNPs are responsible for amino acid change in the CHS enzyme. One of the Amino acid change is located within the catalytic triad of CHS enzyme and this is presumably the reason for the variation in medicinal potential among the contrasting genotypes of *Z. officinale*. b) In India, Podophyllotoxin (a non-alkaloid lignan is useful for production of anticancer drugs etoposide, etopophos and teniposide) is mainly obtained from rhizomes and roots of *Podopyllum hexandrum* Royle. It contains Podophyllotoxin and the amount varies from 2.91%- 4.77% on dry weight basis. Molecular characterization of *P. aurantiocaule* has been done by AFLP for possible exploitation and utilization of this plant as an alternative source of Podophyllotoxin in immediate future. Evidences prove that there is so much confusion regarding the taxonomical position of this particular genus and specifically *P. bipinnatifidus* was reported as polyphyletic in nature. There are references that the *P. bipinnatifidus* and *P. assamicus* those were earlier treated as different species are the two

varieties of *P. bipinnatifidus*. DNA fingerprinting based on AFLP technique was extensively studied for these above two plants and no such species specific alleles have been found suggesting *P. bipinnatifidus* and *P. assamicus* are not appears to be distinct species which is in agreement with earlier reported molecular studies. Species Specific markers have also been developed for *Panax assamicaus*, *Panax bipinnatifidus*, for molecular authentication and documentation. This would be also useful for supplementing conventional drug assessment protocols in authentication for resolving adulteration-related problems often faced in herbal medicine production. d) *Zanthoxylum acanthopodium* and *Zanthoxylum oxyphyllum*, found in North East India, are commercially important due to its edible fruits and leaves containing essential oils used in cosmetics and perfume industries. These species have also been used by local population as ethno medicine. We used amplified fragment length polymorphism (AFLP) to produce DNA fingerprints for two *Zanthoxylum* species viz. *Z. acanthopodium*, and *Z. oxyphyllum*. Twelve collections (eight of *Z. acanthopodium*, four of *Z. oxyphyllum*) were used in the study. Six selective primer pairs were found to detect polymorphism. A total of 483 alleles were produced. Species-specific markers were identified in the two *Zanthoxylum* species (23 for *Z. acanthopodium*, and 13 for *Z. oxyphyllum*). The dendrogram showed species specific clustering of two species. The AFLP markers developed in this study could be used to authenticate two *Zanthoxylum* species to resolve adulteration-related problems faced by pharmaceutical industries to supplement conventional drug assessment protocols. e) Extensive study based analysis of a wide collection of *Curcuma* sp have revealed absence of species specific alleles.

Assessment of the free radical scavenging assays gives a clear picture that *Zingiber montanum* has the highest efficacy in terms of free radical scavenging in DPPH assay and also showed comparative inhibition of curcumin depletion by sulfur free radicals generated by γ -irradiated glutathione (GSH). A relative comparison of free radical scavenging capacity showed *Z. montanum* > *C. longa* > *A. calamus* > *P. pseudoginseng*. In the sulfur free radical scavenging assay, all the

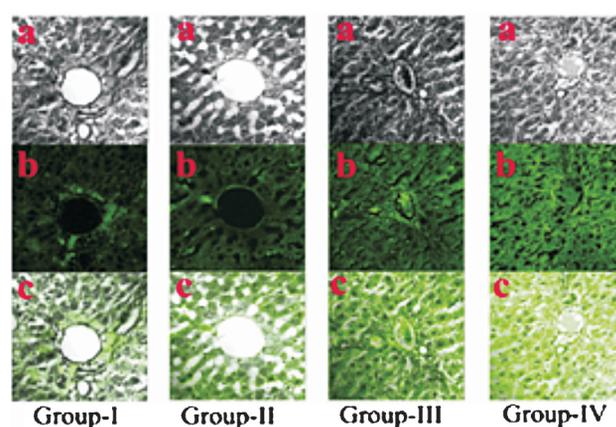
candidate plants showed considerable inhibition of curcumin depletion by GS[•] radicals but, *Z. montanum* exhibited the highest capacity. Assessment of radioprotective potentials of *Z. montanum* rhizome extract was carried out using *in vitro* and *in vivo* models through conventional and molecular cytogenetics. The observed values indicate that this particular antioxidant rich plant have tremendous potentials of protecting the bone marrow cells which are extremely sensitive to ionizing radiations. It is evident that *Z. montanum* has profound radioprotective properties and can protect the haemopoietic progenitor cells from γ -radiation induced genomic instability in albino rats. Evidences from *in vitro* DNA experiments and findings from cytogenetic analyses suggests that rhizome extract of *Z. montanum* have tremendous potentials in protecting genomic entities from possible instabilities caused by gamma ionizing radiations. Micropropagation of *A. calamus* was carried out *in vitro* using MS medium supplemented with plant growth regulators.

Four Workshop cum Hands –on –Training for young researchers on various methods of DNA fingerprinting and assay of medicinal potential have been organized.

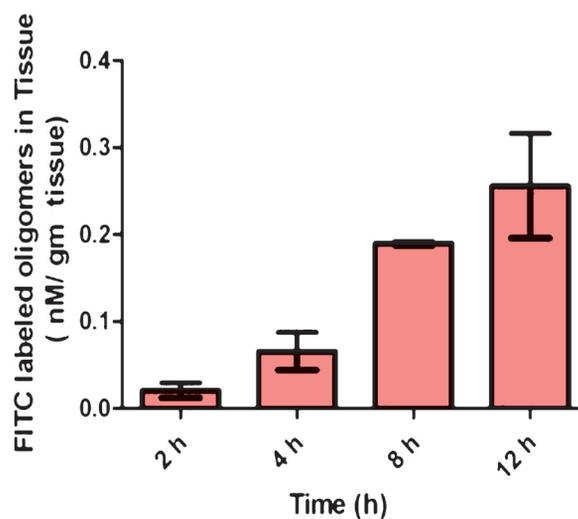
PHARMACOLOGY

Insulin like a growth factor II (IGF-II) Phosphorothioate antisense oligomers search for a potential future drug of liver cancer at Jadavpur University, Kolkata. Hepatocellular carcinoma (HCC) is a multistep complex process, caused by genetic alteration. Insulin-like growth factors and their receptor have been widely implicated to HCC. Insulin-like growth factor-II (IGF-II) is a mitogenic polypeptide, found in various fetal and neonatal tissues of humans and rats and expresses in HCC. The anticancer potential of phosphorothioate antisense oligonucleotides (ASOs) was investigated against three coding exons (exon-1/exon-2/exon-3) of IGF-II messenger ribonucleic acid in rat hepatocarcinogenesis model. During diethylnitrosamine and 2-acetylaminofluorene induced hepatocarcinogenesis, rats were treated with ASOs. Various biochemical and histological studies were conducted. About 40% of carcinogen treated rats, which received two oligomers (against exon-1 or-3) did not show any hepatic lesion, hyperplastic

nodule or tumor and remaining 60% of those rats showed lesion incidence and had about 59% and 55% reductions in the numbers of hepatic altered foci, respectively. Reductions in the total lesion-area when compared with carcinogen control rats were 64% and 53%, respectively for the animals treated with carcinogen and received the ASOs against exon-1/-3. Fluorescein isothiocyanate-labeled ASO reached in the hepatocytes in 2 h. No predominant IGF-II overexpression was observed in case of rats treated with the two ASOs. Treatment of the antisense IGF-II oligomers in carcinogen treated rats show better hepatocellular integrity along with several preneoplastic/neoplastic marker isoenzyme/enzyme modulations.



(A)



(B)

Fig. Hepatic localization of FITC-labeled IGF-II antisense oligomers and quantification of FITC-labeled oligomers in experimental rats. (A) Confocal microscopic image of liver section of rats treated with FITC-label IGF-II antisense oligomer $\times 400$. a: Tissue alone, b: FITC -oligomer alone, c: FITC-oligomer in liver tissue. (B) Tissue content of FITC-labeled oligomer in liver tissue of experimental animals.

In conclusion, two of the three antisense oligomer-types effectively controlled IGF-II over expression, causing the delay of the development and/or progress of hepatic cancer in rats. The study resulted in four publications.

In a study conducted at the V P Chest Institute, Delhi experimental animal models of chronic predictable (restraint) stress (CPS) and chronic unpredictable stress (CUS) were used and the effects on neurobehavioral (anxiety), biochemical and immune parameters were studied. Further, NO-ergic mechanisms were also investigated during such stressors. Upstream and downstream signaling pathways were pharmacologically modulated and biochemical and behavioural markers were assessed during both forms of stress. NO-cGMP—phosphodiesterase V inhibitor, NMDA-NO, and NFkB selective agents were administered (sildenafil, memantine, PDTC and specific NO synthase inhibitors). The results of the study suggest the differential behavioural and biochemical effects of modulation of NO-ergic pathway in rats, depending on the type and duration of stress i.e. CUS or CPS and associated role of ROS-RNS interaction.

A study on evaluation of some newer antiepileptic drugs on biochemical markers of bone turnover and bone mineral density and an investigation on preventative treatment for some conventional antiepileptic drugs in rodents was completed at Jamia Hamdard University Delhi. The effect of raloxifene, in comparison with calcium and vitamin D3 (CVD) supplementation, on phenytoin, PHT and sodium valproate, SVP- induced alterations in bone in mice and to unravel the role of estradiol and TGF- β 3 in mediation of bony effects by either Antiepileptic Drugs (AEDs) or raloxifene. The effect of raloxifene on seizures and on the antiepileptic efficacy of PHT and SVP was also investigated. Swiss strains of female mice were treated with PHT (35mg/kg.p.o) for 120 days to induce bone loss as evidenced by reduce bone mineral density (BMD) and altered bone turnover markers (BTMs) in lumbar bones (alkaline phosphatase ALP, tartarate resistant acid phosphatase TRAP, Hydroxyproline HxP and urine (calcium). The bone loss was accompanied by reduced serum estradiol levels and bone TGF- β 3 content. Preventive and therapeutic treatment with raloxifene ameliorated bony alterations and

was more effective than CVD. It also significantly restored estradiol and TGF- β 3 levels. Deprived estrogen levels (that in turn reduced lumbar TGF content) following PHT and SVP, thus, might represent one of the various mechanisms of AEDs-induced bone loss. Raloxifene preserved the bony changes without interfering with antiepileptic efficacy of these drugs, and hence raloxifene could be a potential therapeutic option in the management of PHT and SVP-induced bone disease if clinically approved.

In another set of experiments, PHT and SVP (but not LTM) significantly lowered BMD of lumbar vertebrae (L2-L4) as evaluated by DEXA scan. While both PHT and SVP reduced alkaline phosphatase, ALP and hydroxyproline, HxP in lumbar vertebrae, and elevated tartrate resistant acid phosphatase, TRAP and urinary excretion of calcium, LTM did not affect any of these markers of bone turnover, indicating that the drug might be a safer option in female epileptic patients prone to bone changes.

In conclusion, as there are no consensus guidelines available for addressing the effect of AEDs on bone health even though it is advisable to screen all patients on chronic AED therapy for early diagnosis of possible effects on bone health and to prescribe CVD supplements in all patients susceptible to bone loss (though latter has also been questioned in some cases).

The study provided the first experimental evidence on the potential role of raloxifene in preventing and ameliorating PHT and SVP-induced bone loss without affecting the antiepileptic efficacy of these AEDs. The study extends previous reports of adverse effect of AEDs on bone and report the same in Swiss strain albino female mice for the first time. Further, the lack of bony effects observed following LTM treatment suggests that the same could be a better alternative to PHT or SVP in female epileptic patients or those having a risk factor for osteoporosis.

The findings of the study suggest that alterations in estrogen mediated TGF β 3 content could account at least partly for the bony deficits induced by PHT and SVP. RLX, being an SERM having tissue selective estrogenic action on bone and TGF- β agonistic

action, reverses the bony changes induced by these AEDs without affecting the antiepileptic efficacy of these drugs.

Gene expression and protein profiling of Group A Streptococcus upon treatment with quorum quenching agents from marine bacteria was carried out at Alagappa University, Karaikudi. The aims and objectives of the study were as follows: Screening of bioactive compounds from marine bacteria for anti-quorum sensing (QS) and antibiofilm activity against Group A Streptococcus (GAS). Molecular characterization of active principles against GAS and Comparison of the transcription and protein profiles of GAS grown with and without the anti-quorum sensing and antibiofilm biomolecules. In the present study coral associated bacteria were screened for their ability to inhibit biofilm formation by Streptococcus pyogenes. Out of the 10 isolates screened, Al (*Bacillus subtilis*) was found to have greater potency in inhibiting the biofilm of *S. pyogenes*. Ethyl acetate extract of Al cell free culture supernatant was purified by TLC, gel filtration chromatography and HPLC and then the partially purified fraction was subjected to GC-MS analysis. GC-MS analysis of the active fraction revealed the presence of 5-hydroxy-2-furancarboxaldehyde (OHFCA) as one of its major ingredients. The compounds, 5-hydroxy 2-furancarboxaldehyde (OHFCA), its parent compound 2-furancarboxaldehyde (2FCA) and its isomer 3-furancarboxaldehyde (3FCA) were assessed for their antibiofilm activity against *S. pyogenes*. 3FCA was more efficient than OHFCA. The antibiofilm efficacy of 3FCA was evaluated against GAS, which showed a concentration dependent increase in the activity. The obtained results clearly suggest that 3FCA possesses specific antibiofilm activity against *S. pyogenes*. Growth curve assessment and XTT assay showed an insignificant difference among control and treated samples confirming 3FCA as an ideal antibiofilm agent against GAS with no antibacterial effect. Light microscopic analysis showed reduction in biofilm -covered surface area in the 3FCA treated wells and CLSM analysis revealed decrease in the thickness of biofilm. The SEM micrograph of treated cells showed an abnormal morphology, which was confirmed by studying the morphology of GAS grown in solid media in the presence of

3FCA and its behaviour in liquid media. The result of scanning electron microscopy, SEM analysis was corroborated by the mucoidal colony forming units, CFUs seen in tryptose agar plates supplemented with 3FCA. Even in liquid media, treated cells were found to be grown in clumps and floating contrary to control samples. The difference in the cell surface was also analysed with Transmission electron microscopy, TEM which clearly showed mucous layer surrounding the cells.

To corroborate the results, GAS was treated with healthy human blood, since hyaluronic acid capsule and surface associated M protein play a crucial role in evading opsonophagocytosis. The results showed down regulation of *covR* gene, and up regulation of *covS* and *hasA*. *ciaH*, another important TCS which controls about 120 genes and involved in oxidative stress and acid tolerance was found to be down regulated. *srtB* gene which plays crucial role in auto aggregation in *S. pyogenes* was found to be down regulated.

The toxicity of 3FCA was assessed primitively, with the help of eukaryotic model organism *C. elegans*. The unaffected growth of *C. elegans* in the presence and absence of the compound confirms that 3FCA does not have any lethal effect on the growth of *C. elegans* and hence can be primitively considered as a nontoxic compound. The increased hyaluronic acid production and increase in adherence of treated GAS were expected to help their internalization in the *C. elegans*, which was confirmed with the CFU assay.

It is speculated that the 3FCA targets the proteins involved in cell wall biogenesis for its antibiofilm activity, which is also supported by the SEM results. Moreover, the proteins involved in amino acid biosynthesis specifically arginine could play a crucial role in biofilm inhibition, since arginine possesses an antibacterial activity against *S. mutans*. The result of proteomic analysis goes well in hand with that of *covR* mutant *S. mutans* wherein the proteins involved in amino acid production, ABC transporter and cell wall biogenesis were found to be differentially regulated.

A exploratory study on extent and determinants of costs of acute poisoning in Tricity of Chandigarh – was done at PGIMER, Chandigarh to ascertain (i)

the epidemiological profile of acute poisoning cases in Tri city of Chandigarh; (ii) the annual direct cost because of acute poisoning in Tri city of Chandigarh; (iii) the annual productive cost of acute poisoning in Tri city of Chandigarh; and (iv) the determinants of such cost in Tri city of Chandigarh. The study area was Tricity of Chandigarh and the source of data was police /hospital records and information was obtained regarding the victims of acute poisoning from Tricity of Chandigarh. The period of the study was from 1.12.2011 to 30.11.2014. A structured questionnaire was used and medical records and bills available were reviewed. The analysis of data was done using IBM Statistics package (SPSS 18). Cost of acute poisoning was calculated under the three categories: Direct cost; Productivity cost and Morbidity cost.. The particulars of 1910 poisoning victims were collected from police stations/hospitals. 1799 Persons were eligible. After finalizing the list the victims were traced. 1238 Cases were traceable whereas 561 were untraceable. 1126 Persons gave consent . 1042 Persons are alive and 84 are dead. Out of 1042 Persons, victim were 398, family included 447 residents & relative were 197. The analysis of distribution of cost showed direct cost Rs.51,89,792/, indirect cost Rs.8,43,635/- and productivity cost Rs.35,27,91,964.5/- .The total cost involved was Rs.35,88,25,392.5/- .The total acute poisoning victims were 218 in the year 2011, 406 in 2012 and 503 in 2013. The detailed data was presented.

The results showed that 78% of incidence of acute poisoning was in nuclear family. Students (28%) followed by house wives (20%), employee (19%), businessman (8%), skilled workers (7%) were the most common victims. In majority of instances place of procurement and exposure was at home. Most of incidence acute poisoning were between 18 to 24 hours followed by 12 to 18 hours. In 96% instances, no medical help was available at the site of exposure. Doctor was available at first site of treatment in most instances (98.5%). In most instances (84.4%) relatives/family members were informed from site of exposure to acute poisoning.

In silico design, synthesis and pharmacological screening of some quinazolinones as DHFR inhibitors for anticancer activity was conducted at Sinhgad Technical Education Society's Smt.

Kashibai Navale College of Pharmacy, Kondhwa (Bk.), Pune .The research study describes *in silico* screening of quinazolines/ quinazolinones and quinazolinone metal complexes, their chemical synthesis and *in vitro* and *in vivo* pharmacological screening for as dihydrofolate reductase, DHFR inhibitors for anticancer activity. The first batch of *in silico* prioritized quinazolinone and quinazolinone metal complexes were synthesized, characterization of metal complex was done by AAS, ESR, IR, TLC and X-Ray diffraction. The prioritized quinazolinones were synthesized and were evaluated for TLC, ¹H-NMR and ¹³C-NMRs. Molecules complied with the spectral assignments. The compounds were then evaluated on set of 10 cancer cell –lines for *in vitro* cytotoxicity assay. In preliminary cancer cell line cytotoxicity screenings, compounds SS-43, SS-44, SS-49 and MS-01 were found to be cytotoxic and active as compared with Methotrexate , as per National Cancer Institute, Bethesda and guidelines from ACTREC Centre Navi Mumbai at ≤10 µg/ml concentration. The compounds were evaluated in L1210 mice model where they did not show promising activity. These are in further process of evaluation for *in vivo* anticancer activity by K562 xenograft model where all the four compounds are active in *in vitro* anticancer assay. Further a second batch of prioritized compounds was evaluated having Platinum metal complexes/chelates and quinazolinone moiety, for *in vitro* cytotoxicity assay. The study is in progress and according to recent report one compound is found to be active. The study resulted in two publications.

MEDICINAL PLANT

A study on evaluation of anti-plasmodial potential of plant derived alkaloids was completed at Haffkine Institute for Training, Research and Testing, Mumbai .The bio-rational approach of the project in combination with an alkaloid focus provided evidence for the rational of exploration of medicinal plants as a source of antimalarial agents. The objectives of the study were as follows: Selection of medicinal plants reputation for use of traditional (Popular, indigenous, folkloric) medicine; Evaluation of anti-plasmodial activity of crude extracts of selected medicinal plants to confirm and verify scientifically the antimalarial

potential of these plant; Plants with promising antiplasmodial potential to be processed for isolation, purification and identification of alkaloids (the active antimalarial compounds); To assess the antimalarial potential of purified alkaloids and identify new antimalarial lead compounds. In all 16 traditional medicinal plants of 12 different families were collected taxonomically identified, extracted and tested for biological activity against two strains of *Plasmodium falciparum*: 3D7 (chloroquine-sensitive strain), 3D-7-CQ-R (chloroquine-resistant strain) so as to evaluate scientific validation for their traditional use. Some of the plants showed good anti plasmodial activity thus, justifying their traditional use. *Argemone mexicana* has the best activity *in vitro*, both for the extracts in polar solvents and aqueous extract. The efficacies of berberine-azadirachtine and berberine-piperine combinations prolong survival of *P. berghei* infected mice and confer complete protection up to forty days observation period. Hence, may be considered for trail in human. The *in vivo* IC₅₀ of berberien was 25.16 mg/kg. The crude alkaloidal extracts of *A. mexicana* also demonstrated promising activity *in vivo* giving 51% inhibition with 50 mg/kg body weight in mice against *Plasmodium berghei*. The results indicate that the plants *Argemone mexicana* and *berberien* the alkaloid isolated from this plant has a promising anti-plasmodial activity against *Plasmodium berghei*, which upholds the earlier *in vitro* findings as well as its folkloric use. The bio-rational approach of the project combined with an alkaloid focus provided evidence for the rational exploration of Indian medicinal plants as a source of antimalarial agents.

A Survey of north Indian medicinal plants for their larvicidal activity against malaria and filarial vectors completed at Dr B.R.Ambedkar Centre for Biomedical Research, Delhi. The objectives of the study were (i) Extensive survey of procuring the different species of medicinal plants known for their anti malarial activity from various localities in North India; (ii) Evaluation of larvidical activity, using plant extract/fraction of selected plant species against the larvae of mosquito vectors (e.g. *Anopheles spp.*, *Culex quinquefasciatus*) ; and (iii) Selection of plant/plant parts showing optimum larvicidal activity and conservation of elite germplasm. Certain medicinal plants from north

India such as *Spilanthes spp.*, *Artemisia annua* and *Vernonia anthelminitica* were surveyed and selected to test their efficacy against larvae of *A. stephensi* and *C. quinquefasciatus*. Crude extracts of these plants were prepared in various solvents like ethanol, acetone, chloroform, methanol and hexane. All plants have shown a strong larvicidal activity against both of these vectors. An important bioactive compound spilanthol was isolated from *S. acmella*. Similarly, when crude extract of *A. annua* when separated through column chromatography using petroleum ether- ethyl acetate gradient (0-100%) yielded 76 fraction which were pooled into three different active fractions A, B and C on the basis of same or nearly similar R values. The aforesaid pooled fractions when assayed against the larvae of *A. stephensi* too reported a strong larvicidal activity. The respective marker compound purified from the individual fractions A, B and C, were Artemisinin, Arteannuin B and Artemisinic acid, as confirmed and characterized through FT-IR and NMR. The project aims at exploring the medicinal plants that can be used as the alternative for eliminating and bio-controlling the vectors of carious deadly diseases like malaria and filarial. The development of alternative herbal natural drugs (products) seems to be imperative to control the vectors. The results could be applied on a large scale to eliminate the larvae of different mosquito vectors at their source level *i.e.* stagnant water bodies, storage water tanks etc. This green technology is eco-friendly in nature and could be very useful as a bio-larvicidal without polluting the environment. The extract of *Artemisia annua* which till now was being used against malaria parasite can also be used against malaria and filarial mosquito vectors at the lowest concentrations and has a very strong larvicidal potential reported so far. Bioassay driven isolation proved beneficial for screening the marker larvicidal compounds from the active fractions, which were further isolated and characterized through NMR.

A study to evaluate the immunomodulatory properties of *Withania somnifera* leaf extract in visceral leishmaniasis was carried out at University of Hyderabad . The objectives were as follows: To determine the immunomodulatory effects of *Withania Somnifera* leaf (WSL) extract in case of *L. donovani* infection; To evaluate the upregulation

of Th1 & Th2 cytokines in the presence of WSL ; Separation of WSL and testing immunomodulatory properties of each compounds. The compound was partially purified using silica gel column chromatography and identified the compounds present in the F5 and F6 using LC- MS/MS analysis. The results showed that these fractions induce potent morphological and biological changes in promastigotes stage of the parasite via apoptosis-like death.. The *withaferin A* was found to inhibit the pteridine reductase 1 enzyme of parasite. It was also have shown that these fractions can modulate the immune response in the parasite infection towards the protective side. Both the fractions and *withaferin A* can suppress the expression of IL-10 (Th2) cytokine, which is important for the parasite propagation in macrophages. There was no change in NO production in treated samples compared to control, but there is production of reactive oxygen species in treated samples compared to control. A mice model was used to mimic the visceral leishmaniasis, which were treated with both the fractions F5 & F6 and *withaferin A*. The parasite load in the spleen and liver, were reduced as compared to untreated mice. Using real- time PCR to determine the immunomodulatory role of fraction F5 and F6 in spleen mRNA. It was found that there was suppression of Th2 (IL-10, IL-4, TGF-B) cytokines and upregulation of Th1 (IL-12) cytokine. While, there was no significant difference between the IFN- γ expressions in treated and untreated mice. But the ratio of IFN- γ : IL-10 is higher in the treated as compared to untreated mice, which indicates the parasite clearance from the spleen tissue.

TRADITIONAL MEDICINE

A study on lifestyle intervention through naturopathy and yoga for curtailing progression of Rheumatoid Arthritis was completed at Bapu Nature Cure hospital, Delhi. The study aimed to evaluate the effect of Naturopathy and Yoga intervention on regression of Rheumatoid Arthritis (RA). A total number of 134 AR patients were recruited between the age Group 25-60 years in the study,. These patients were divided into three Groups for the study in which Group-1 (48 patients) included yoga, naturopathy and allopathic medicines. Yoga comprised of pawanamuktasana part-I, shavasana

and pranayama (bhramari, kapalbati, deep breathing and nadi sodhana for 20 min., Naturopathy included massage therapy (30min.) and hot & cold fomentation (21min) . Group-2 (44 patients) was provided 'yoga' and 'allopathic' medicines. Group-3 was the controlled Group of 42 patients and was provided only allopathic medicines. The study parameters were morning stiffness, tenderness, fatigue, visual analog scale, sleep, grip strength finger squeeze test, ROM, DAS28 score, functional capacity, inflammatory markers (CRP, TNF-alpha & ESR) and immunological markers (ACCPA, RF&ANA).

The study was conducted for three years in which intervention Group-1 & Group- 2 received 80 sittings of yoga and naturopathy (first two months thrice a week, next four months twice a week and last six months once a week) over a period of one year. These parameters were recorded at baseline and thereafter at 1st, 3rd, 6th and 12th month level. Immunological markers were assessed at baseline and at twelfth month.

On comparison of all the three Groups, at baseline patients showed non-significant results and after 1st, 3rd , 6th and 12th months treatment, patients showed significant difference with decrease in morning stiffness ($p < 0.01$), tenderness ($p < 0.01$), fatigue ($p < 0.01$), visual analog scale ($p < 0.01$) and increase in sleep ($p < 0.01$). There was also a significant difference in the increase in grip strength ($p < 0.001$), finger squeeze test ($p < 0.001$) and ROM ($p < 0.01$).

A significant difference was also found with decrease in DAS28 score ($p < 0.01$), increase in functional capacity (HAQ) ($p < 0.01$), decrease in inflammatory markers [CRP ($p < 0.05$), TNF-alpha ($p < 0.05$) & ESR ($p < 0.05$)], immunological markers [ACCPA ($p < 0.001$) & RF ($p < 0.001$)] but the study could not find significant difference in Anti-nuclear Antibodies. The study concluded that yoga and naturopathy when combined with modern medicines provide important additional physical and psychological health benefits for RA patients. 'Yoga' and 'Naturopathy' can be used effectively as an adjunct therapy in rheumatoid arthritis because there is apparently significant effect of yoga and naturopathy in curtailing the progression for RA.

REGIONAL MEDICAL RESEARCH CENTRES

Regional Medical Research Centres (RMRCs) are specifically meant to address local/regional health problems in remote, inaccessible and tribal areas of the country. Currently there are six RMRCs located at Belgaum, Bhubaneswar, Dibrugarh, Jabalpur, Jodhpur and Port Blair. Recently, RMRC, Jabalpur has been upgraded to a full-fledged Institute and renamed as National Institute for Research in Tribal Health (NIRTH). These Centres are engaged and working closely with the local/State health authorities for control and management of different disease conditions. In addition to providing support to state health authorities and addressing local health needs, RMRCs are also engaged in high quality research on the disease prevalence, outbreak investigations and undertake interventions as needed. During the year under report, studies were carried out on traditional medicine & screening of medicinal plants, malaria, filariasis, leptospirosis, cancer, haemoglobinopathies and other health problems of the local populations.

REGIONAL MEDICAL RESEARCH CENTRE, BELGAUM

TRADITIONAL MEDICINE/ MEDICINAL PLANTS

Elucidating anti-arthritic potential of selected medicinal plants and their fractions in Wistar rats

The study was aimed to develop a safe and cost effective anti-arthritic agent to treat arthritis based on the traditional herbal knowledge. Two plants, namely *Plumbagozeylanica* Linn. (roots) and *Holopteleaintegrifolia* (Roxb.) Planch. (bark), were selected. The extracts (petroleum ether, acetone,

hydroalcoholic) were screened for their anti-arthritic activity in both in vitro and in vivo models. The active extracts were fractionated further and screened for their anti-arthritic activity.

PZHY#1 (*Plumbagozeylanica* Hydroalcoholic extract Fraction-1) and HIHY#1 (*Holopteleaintegrifolia* Hydroalcoholic extract Frction-1) were selected for further isolation and characterization from their respective active extracts, based on their safety (acute toxicity study), efficacy studies (Carrageen and complete Freund's Adjuvant induced inflammations). Five compounds have been isolated and characterized from the active fraction of *P. zeylanica* (PZHY#1) viz. Plumbagin, Lupeol, Lupeol acetate, β -Sitosterol and P#5. Similarly, five major compounds from HIHY#1 have been characterized as Fridelin, β -Sitosterol, Stigmasterol, β -Amyrin and H#5. The compounds P#5 and H#5 are being reported for the first time from these plants. The study resulted in providing the scientific data on safety and efficacy of the selected plants, which are being used in the traditional practice (Fig 1).

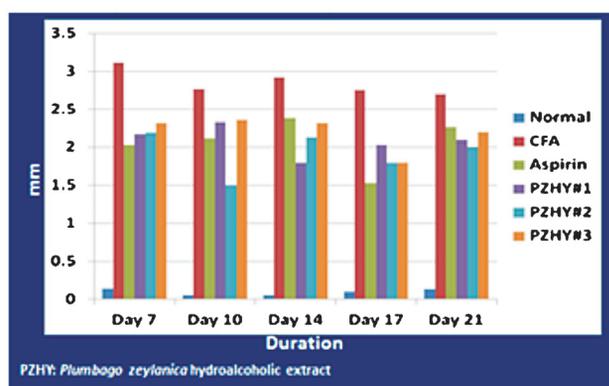


Fig. 1. Effect of fractions from hydro-alcoholic extract of *Plumbagozeylanica* L. on ankle joint circumference.

Production of shikimic acid from *in vitro* cultures of selected medicinal plants from Western Ghats

Shikimic acid is a precursor of many alkaloids, aromatic amino acids, and indole derivatives. The world's demand for shikimic acid is met from fruits of Chinese star anise- *Illiciumverum*. Efforts are on to search new sources of shikimic acid from Indian plants. Meantime, there is a need of alternative strategies for the production of shikimic acid. *In vitro* culture holds tremendous potential in production and elicitation of bioactive compounds. Present proposal aims at identification of cell lines for eminent production of shikimic acid from *in vitro* cultures of selected medicinal plants from Central Western Ghats.

So far, shikimic acid content was estimated in 58 angiosperms and gymnosperms belonging to 25 families. Among the 18 families of angiosperms, only Clusiaceae, Ranunculaceae, Anacardaceae and Simaroubaceae accumulated higher levels of shikimic acid. The results revealed that the highest content of shikimic acid in *Mammeasuriga* (family- Clusiaceae) (29.34 mg/g) followed by the *Calophylluminophyllum* (18.20 mg/g) and *Garciniamorella* (8.52 mg/g) of the same family. Further screening and identification of suitable species for tissue culture studies are in progress.

Documentation of genetic and phytochemical variations of *Gymnemasylvestre* (Retz.) R.Br. & S. & newly described species *Gymnakollimalayanum* Ramach. & M.B. Viswan, from Eastern India and Western Ghats

Gymnema sylvestre (Retz.) is a well-established anti-diabetic plant. Despite extensive use in diabetes and several other conditions in Ayurveda and other traditional (non-codified) practices, little is known about the extent of variations in the chemo profiles and genotypes of these plants within populations and between populations. Therefore, in the present study, it is aimed to estimate the variations in chemoprofiles and genotypes of *G. sylvestre* populations in Western Ghats and Eastern India.

A total of 118 plant samples were collected from Western Ghats and Eastern Himalayas. All the

samples were subjected to ISSR and RAPD based DNA fingerprinting analysis. The genetic distance between the samples from Eastern Himalayas and Western Ghats was approximately 65% which indicates the existence of two geographically distinct populations. In cluster analysis, populations of Eastern Himalayas formed a separate cluster from those of Western Ghats. Even within populations of Western Ghats there were 4 clusters which indicated the existence of geographically distinct populations. This shows that different population might have emerged from different ancestors in both the regions that diverged away from each other in evolution.

In the phytochemical studies, the quantity of gymnemagenin and deacylgymnemic acid, the marker compounds for *Gymnema* as well as established active constituents of the species was estimated in all collections. The variation of gymnemagenin and deacylgymnemic acid was high from 1.21 to 23.95 µg/g and 0.6 to 4.1 µg/g respectively in the collected plant samples.

The study indicated that there is high genetic differentiation and low gene flow in this species, which suggests that conservation should emphasize on preserving the populations. The variation of gymnemagenin and deacylgymnemic acid was also found to be high in the collected plant samples and the details generated would help industry and conservationists in selection of the right germ plasm for cultivation/ reforestation strategy (Fig. 2, Table 1).

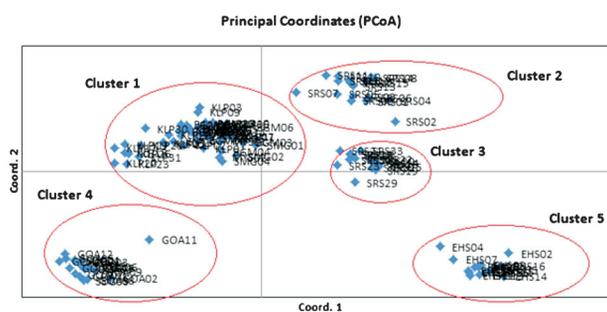


Table 1. Various genetic diversity parameters analysed in *G.sylvestre* based on Nei, 1987.

| Population codes | | na | ne | <i>h</i> | <i>I</i> | <i>Ht</i> | <i>Hs</i> | <i>G_{ST}</i> | <i>N_m</i> | PPB% |
|------------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|-----------------------|----------------------|--------------|
| ISSR | EH | 1.5849 | 1.2703 | 0.1677 | 0.2618 | 0.1546 | 0.1127 | 0.2708 | 1.3462 | 58.49 |
| | WG | 2.0000 | 1.4781 | 0.2863 | 0.4393 | 0.2662 | 0.0996 | 0.6257 | 0.2991 | 100 |
| | Species | 1.9708 | 1.4573 | 0.2740 | 0.4212 | 0.2660 | 0.0791 | 0.7027 | 0.2116 | 97.08 |
| RAPD | EH | 1.8305 | 1.3036 | 0.2019 | 0.3286 | 0.2116 | 0.1550 | 0.2676 | 1.3686 | 83.05 |
| | WG | 2.0000 | 1.5223 | 0.3139 | 0.4769 | 0.2936 | 0.1369 | 0.5336 | 0.4371 | 100 |
| | Species | 1.9846 | 1.4284 | 0.2636 | 0.4096 | 0.2612 | 0.1041 | 0.6014 | 0.3314 | 98.46 |

na: observed number of alleles, ne: expected number of alleles, *h*: Nei's gene diversity at population level, *I*: Shannon's index of genetic diversity, *Ht*: heterozygosity at the polymorphic loci, *Hs*: average heterozygosity, *G_{ST}*: degree of genetic differentiation, *N_m* gene flow

Evaluation of effect of shodhana procedures on Vatsanabha (*Aconitum heterophyllum* Wall. Cat.)

The present study is intended to evaluate and revalidate the Shodhana procedures used for detoxicating of Vatsanabha (*Aconitum heterophyllum* Wall. Cat.). The authenticated sample of plant drug has been obtained. The SOPs have been prepared for the Shodhana procedures and the crude drugs have been subjected to shodhana process with Cow urine (Gomuthra), Cow milk (Godugdha), Sheep milk (Ajadugdha) and water. The preliminary results of the toxicity studies in Wistar rats indicated the high toxicity of untreated drug with 100% mortality and safety of treated (Shodhita) aconitine with 100% survival rate. The further phytochemical and chronic toxicity studies are in progress.

PHYTOCHEMISTRY

Chemoprofiling of the medicinal and aromatic plants from Western Ghats region of North West Karnataka

The chemoprofiling of medicinal and aromatic plant RMRC-BM IP_156, which is used traditionally for treatment of diabetes, is under progress. The volatile secondary metabolites were analyzed by using GC-FID and GC/MMS. The results revealed that out of twenty-five samples, five plants had the same signature or marker compositions with phenyl derivatives were dominated in all collection in a year, while remaining twenty samples were completely different composition with no marker compositions and containing monoterpene hydrocarbon, oxygenated monoterpene and

oxygenated sesquiterpene rich constituents in different seasons of collection. This investigation revealed that, among the all collected samples, two groups were identified viz., phenyl derivative and terpenoid groups, despite being morphologically identical plants.

Translational Research

Sensitization of multidrug resistant ABCB1 overexpressing oral carcinoma KB cells to paclitaxel by glaucarubinone from *Simarouba glauca* DC.

Multidrug resistance (MDR) is one of the most common causes of relapse in cancer chemotherapy. Inhibition of ABC transporters to reverse MDR is a prominent approach to enhance the efficacy of cancer chemotherapy. The modulatory role of Glucarubinone (GLU) from *Simarouba glauca* DC on Paclitaxel (PTX) sensitization in MDR resistant human oral cancer cells (KB) was investigated.

In silico molecular docking studies revealed that GLU possesses greater binding affinity with transmembrane domain region of P-gp. In line with this GLU pre-treatment significantly enhanced PTX anti-proliferative effect in ABCB1 over-expressing KB cells. It was observed that the anti-proliferative effect on KB cells showed 100% (cell death) at 400 nM/mL of GLU and 70 nM/mL of PTX, respectively. Based on these findings 200 nM/mL of GLU and 23.42 nM/mL of PTX (the IC₅₀ values) were chosen for chemosensitizing experiments. In combination treatments KB cells were treated with GLU (200 nM/mL) 1 h before PTX (23.42 nM/mL) showed superior anti-proliferative activity when compared with PTX or GLU treatment alone. The Rhodamine

123 drug efflux studies revealed that there was a significant transport function (ABC) inhibition by GLU plus PTX treatment. Furthermore, the combination treatment showed significant ROS production and apoptotic morphological features in ABCB1 over-expressing KB cells. Interestingly, it was also found that this enhanced anticancer efficacy of GLU was associated with PTX-induced cell arrest in the G2/M phase of cell cycle. The characteristic apoptosis was also further confirmed in GLU-PTX by determination of the DNA ladder which is a result of DNA fragmentation and indicative of late stage of apoptosis in ABCB1 over expressing KB cells (Fig. 3).

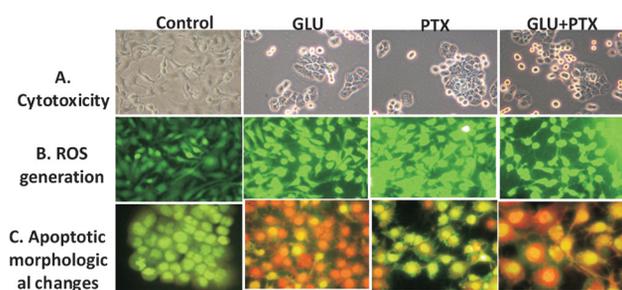


Fig. 3. Effect of GLU, PTX and GLU + PTX on cytotoxicity, ROS generation and apoptotic morphological changes in ABCB1 over expressing resistant KB cells.

Further, it was seen that GLU treatment at concentrations (0-600 nM) has not impacted the % viability of normal lymphocytes at the end of 24 h incubation. Particularly, GLU alone treatment at concentrations 200 nM/mL has not impacted the % viability of lymphocytes. PTX treatment at conc. 23.42 nM/mL showed a significant toxicity in normal lymphocytes. The combination treatment (GLU-PTX) did not show any significant change in lymphocyte viability. Furthermore, the combination treatment showed that there was no significant ROS production and apoptotic morphological features in human blood lymphocytes. This study thus has revealed the MDR reversal activity in cancer cells and safety of Glucarubonine and the combination treatment.

Pilot study on enhancement of L-DOPA production using various bio-systems

L-DOPA (3, 4-dihydroxy- phenyl-L-alanine) is a drug that is being used for the treatment of Parkinson disease (PD) for over past 30 years. It is very useful drug for reducing the tremors and other symptoms

of PD during the early stages of the disease and allows to extend their period of time of normal and productive lives. A novel system for enhanced and stable production of L-DOPA needs to be developed with ease of purification to meet the growing demand by rising geriatric population. Thus a joint venture between RMRC, ICMR, Belgaum and IIT, Mumbai has been initiated for the enhancement of L-DOPA production using various bio-systems. The project aims at developing a novel system for production of L-DOPA.



Fig. 4. In vitro seed germination, (b) rapid shoot multiplication and (c) callus induction in *Mucunaprurens*.

Mucunaprurens seeds are known to be a good source of LDOPA and possess tyrosinase activity. Hence, rapid shoot multiplication and callus cultures were established using seed as explants for detecting presence of L- DOPA in vitro. Murashige and Skoog medium supplemented with BAP (2.0 mg L⁻¹) for germination, BAP (2.0 mg L⁻¹) + 2, 4-D (0.5 mg L⁻¹) for rapid shoot multiplication and 2, 4-D (2.0 mg L⁻¹) with glutamine (500 mg L⁻¹) was used for callus initiation and proliferation. Callus obtained were used for liquid batch cultures to study their growth and production of LDOPA. Further studies on its optimization are under progress (Fig.4).

National Hospital Based Rotavirus Surveillance Network (NRSN) (West Zone- Regional Level)

The study is carried out as part of the NRSN Network under the West Zone. Two hospitals viz., KLE's Dr.Prabhakar Kore Hospital under Jawaharlal Nehru Medical College, Belgaum and Krishna Institute of Medical Sciences Hospital, Karad are the clinical recruitment sites under the RMRC, Belgaum. The prevalence of rotavirus observed in last 11 months (Nov, 13 – Sept, 14) in the two CRS was 31.8%. GARV positivity was

found to be high in the month of November and December in JNMC, Belgaum and KIMS, Karad respectively. Majority of the cases enrolled in the study belong to the age group of 7-12 months at 34.3% and 42.7% for JNMC, Belgaum and KIMS, Karad respectively. The GARV positivity was also found to be high in the age group of 7-12 months at 38.3% and (47.4%) for JNMC, Belgaum and KIMS, Karad respectively. In JNMC, Belgaum, male (60.7%) accounted for majority of the samples collected and GARV positivity was found to be 29.2% and 32% in male and female respectively. In KIMS, Karad male (65.2%) accounted for majority of the samples collected and GARV positivity was found to be 34.4% and 29.7% in male and female respectively. Majority of GARV positive cases were associated with mild or some dehydration.

Immunoepidemiology of Lymphatic Filariasis (LF) Identification of biomarkers

A study is being undertaken with the objectives (1) Identification of markers (antibody/antigen) for measuring the exposure to LF, (2) Identification of markers of filarial pathogenesis in lymphedema, and (3) Development of immunoassays for exposure and filarial lymphedema. During the period 3 genes of *Wuchereria bancrofti* were identified for the study and cloned, expressed and the recombinant proteins purified. Antigenic peptides of an antigen identified through bio-informatics analysis and synthesized. The recombinant proteins and peptides were investigated for sero-reactivity and appear to be better markers for measuring the exposure.

Tribal Health Research Unit/ Model Rural Health Research Unit

Tribal Health Research Unit at Regional Medical Research Centre was established in January 2015. Three tribal villages (Mallapur at Devdurga Taluk, Raichur District; Bommanahalli cluster at Haliyal Taluk Uttara Kannada District; Ningyanahatti at Belgaum Taluk, Belgaum District) have been identified to initiate the activities. It is proposed to look for specific, prevailing health issues in these villages and find the sustainable interventions to address those issues. As the baseline, house to house survey has been carried out in these villages and the socio demographic data has been collected. The data analysis is in

progress. Apart from this, the sampling and data collection for the other project on G6PD deficiency among tribal population in Karnataka has also been initiated in association with THRU (Fig.5).

A Model Rural Health Research Unit (MRHRU) is being established at Sirwar PHC complex, Sirwar, Raichur District in Karnataka. A temporary laboratory has been set up at PHC Sirwar (Fig.6).



Fig. 5. Tribal Health Research Unit Fig.6 Inauguration of MRHRU staff and physicians interacting with a tribal man.



Fig. 6. Inauguration of MRHRU.

Services to State Health Authorities in infectious diseases

RMRC has been providing identification & characterization services with respect to various infectious diseases/pathogens to various State/ Govt. hospitals, District Health Units and Medical Colleges. The Centre is strengthening State health delivery and services. In one such studies, *Shewenella* algae was found as an etiological agent of acute gastroenteritis. The Centre also extended its services in outbreak investigation of Cholera in rural areas of North Karnataka and of JE in urban as well as rural areas of Karnataka state. The Centre also participated in the 'Transmission Assessment

Survey' for lymphatic filariasis in UDUPI dist at the request of Karnataka and NVBDCP, New Delhi.

REGIONAL MEDICAL RESEARCH CENTRE, BHUBANESWAR

Lymphatic Filariasis

In order to support 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 transplacental transfer of filarial antigens occurs in 34.8% of CFA positive mothers and higher adult worm load in pregnant mothers has been predicted to be one of the factors that influence the transplacental transfer of filarial antigens from mother to cord blood. The qualitative assessment of IgG3 and IgG4 by western blot analysis indicates that IgG3 level in cord blood and mothers were approximately similar. But transplacental transfer of filarial specific IgG4 and filarial antigens occurs more when the adult filarial worm burden was high in mothers. This indicates that in-utero exposure of the developing fetus to filarial antigens causes altered immunity of the children making a long-term hyporesponsiveness to filarial antigen (Fig 1).

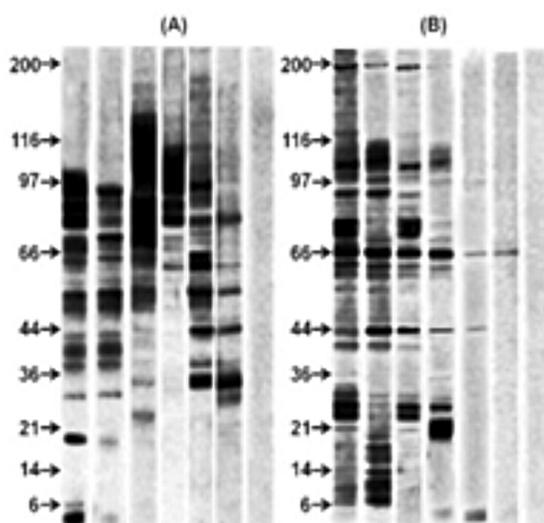


Fig. 1. Recognition pattern of anti-filarial IgG3 (A) and IgG4 (B) antibody responses against WSA by immune blotting in maternal blood sera and cord blood samples of all the three groups. Lane 1-2, mother and cord blood of M+C+ (Both mother and cord CFA +ve) group; Lane 3-4, mother and cord blood of M+C- (mother CFA +ve and Cord CFA -ve) group; Lane 5-6, mother and cord blood of M-C- group (Mother and cord CFA negative); Lane 7, Non endemic normal serum (control). Standard molecular weight markers are indicated on left margin.

Further attempt has been made to evaluate the extent to which maternal filarial infection influences the humoral responses induced in children born to mothers immunized with TT and BCG. It has been observed that IgG antibodies to BCG are not significantly different between filarial uninfected and infected mothers and decreased levels of IgG antibodies to BCG detected in cord samples born from infected mothers compared to uninfected mothers indicates down regulation of IgG response in cord blood of infected mothers.

Malaria

The centre has undertaken a project mainly to find out the cause of persistence of malaria in Kalhandi district in spite of implementation of EDPT and distribution of LLIN for several years. The preliminary surveyed carried out during 2013-14 reveals the presence of three major vectors viz., *An. annularis* in foothill, *An. culicifacies* in riverine and *An. fluviatilis* in hilltop ecotype, which are biologically and bionomically different from each other. Hence a different approach may be needed for vector control and stop transmission (Fig 2).

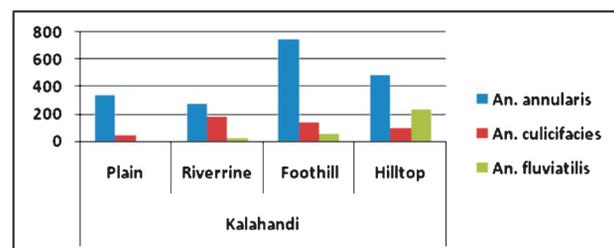


Fig. 2. Vectors encountered in Kalahandi districts, Odisha.

Diarrhoeal Disorders

The centre is continuously monitoring to identify the bacterial pathogens causing diarrhoea and their drug susceptibility pattern to help the local health authorities to reduce morbidity and mortality due to diarrhoea especially cholera. An epidemic outbreak in Norla Block of Kalhandi district was investigated that reveals that the causative agent was *V. cholerae* O1 ogawa biotype El Tor and strains were sensitive to ciprofloxacin, norfloxacin, tetracycline, doxycycline and ofloxacin; but were resistant to ampicillin, gentamicin, furazolidone, nalidixic acid, erythromycin and chloramphenicol. The MAMA-PCR assay indicated that all *V. cholerae* were El Tor variant of *ctx B* gene of classical strain belongs

to Haitian variant. This is the 1st report from the state causing large cholera outbreak due to Haitian variant of *Vibrio cholerae* of O1 strains. Early reporting helped for the treatment and management of cholera patients in this region.

Tuberculosis

The RNTCP approved tuberculosis drug susceptibility testing laboratory has been providing supervision and monitoring activity for quality TB diagnosis in 10 states namely Odisha, West Bengal and 8 North East states. Out of 1362 sputum specimens collected during the period under report, 283 yielded positive culture on LJ medium. The smears examined by LED FM detected AFB in 20% specimens in contrast to 16.5% by ZN microscopy. The results shows that compared to ZN microscopy, LED FM is more sensitive in detecting AFB. The LED FM can be utilized in programmatic mode with short one week training to the laboratory technicians. The LED FM should be implemented in programmatic condition which will enhance AFB detection, thereby curtailing the spread of infection in the society. Further out of 141 suspected MDR TB patients tested by Line Probe Assay belonging to tribal districts of Odisha, one was found to be MDR and another rifampicin mono-resistant.

The diabetes has a major impact on the epidemiologic dynamics of tuberculosis and poses several challenges to control of TB in a resource-poor country like India. Diabetes/TB burden can be brought under control by timely diagnosis of TB among patients with diabetes by intensified case finding, by adequate and effective treatment of detected cases and preventive therapy. For the first time we had screened 220 patients with Type2diabetes for active TB in Odisha and one of them was found to be positive (1/220,0.4%). Similarly amongst 220 patients with active TB registered in RNTCP, 54 (24%) were having pre-diabetes and 32 (14%) having diabetes. These observations raise concerns since T2DM is a silent progressive disease and in the absence of appropriate treatment and life style changes, it would lead to debilitating complications. Hence, screening for T2DM in TB patients could improve TB-specific treatment outcomes.

Virology Network Laboratory

The Centre is continuing surveillance and outbreak investigation of important viral diseases in the region through the established Grade-I viral lab as a part of network of viral research and diagnostic laboratories in India. Diagnostic services have been provided to more than 24000 patients during last four and half years covering more than 50 viruses important for public health use. During the period under report the centre has received around 4,449 samples from 40 different hospitals, 6 medical colleges and outbreak investigations from various districts of Odisha. The viruses investigated 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. 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.

Enteric viruses

Among enteric viruses Rota antigen was detected in 40% of cases. Genotype G1, G2, G4 and G9 (G Type) and P4, P8 and P9 (P Type) were detected as major genotypes. G1P8 was the most common combination found in 15% of antigen positive cases. Hepatitis A Virus was detected in 28% and Hepatitis E Virus was detected in 36% of cases.

Heaptitis Viruses

Among the cases of jaundice screened for hepatitis virus infection, HBV and HCV were detected serologically in 23% and 1.8% respectively and genotyping was done in 16.5% and 3% of cases respectively where the major was HBV genotype D along with A and C and HCV genotype 1b were identified as the genotypes circulating in this region.

Respiratory Viruses

Viral respiratory infection was another important disease which was covered for laboratory diagnosis. Through Real Time PCR assay, respiratory viruses like, 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 analyzed. The viruses those detected were Flu A 22%, H1N1 18.6%, Rhino 13.8%, Para influenza 20% and Adeno in 30.6% of cases. Emerging viruses like Boca, HMPV and Parecho viruses were detected with low prevalence.

Air borne Viral Diseases

Among air borne diseases, Measles IgM was detected in 35% of the cases the circulating genotype was identified as D8. Varicella IgM was detected in 52% of cases. Rubella IgM was detected in 3.5% whereas in 70% of cases IgG antibody was detected.

HPV infection in Women

A total 104 no. of cases with chronic cervicitis or suspected cervical discharge were enrolled during this period. 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 50-85 yrs, 9 cases are associated with HPV infection.

Encephalitis

Viruses that cause encephalitis were also investigated and viral etiology established in 12.8% of suspected encephalitis. Herpes simplex virus was detected in 15%, Herpes Virus II in 6% and Japanese encephalitis was detected in 9% of cases. Other encephalitis causing viruses detected in low prevalence were Enterovirus, WNV, Dengue, Measles from. Investigations have been made both in pig and human population in Malkangiri to establish the prevalence and source of infection of JE/AES.

AES causing death in children due to JE virus was reported during 2012 in Malkangiri district after 2 decades of no JE report/record in the state. During 2013 and 14 similar outbreaks of AES affecting mostly children were reported from Malkangiri, Keonjhar and Jajpur district. Investigation confirmed JEV infection in the outbreaks from Keonjhar (28/100) and Jajpur (05/16). However the outbreak at Malkangiri remained inconclusive with regards to possible viral etiology. But blood samples from pig shown JEV antibody by PRNT from Malkangiri district. The entomological survey conducted in the above three affected areas showed higher density of *Culex vishnui* group of mosquitoes (Fig 3). This is showing persistence of JE virus in animal host. The investigation supported public health measures.

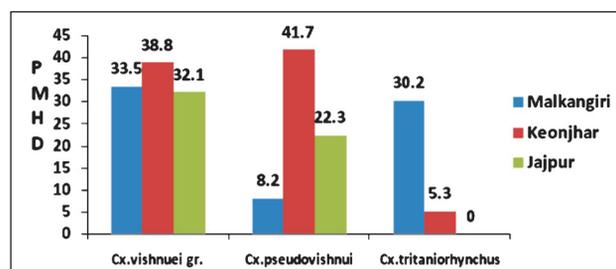


Fig. 3. PMHD of Japanese encephalitis vector in Odisha.

Outbreak investigations

The major outbreaks investigated along with State Health Departments from April 2014-March 2015 are summarized below

Jaundice outbreak in Cuttack

During last week of Sept 2014 outbreak of jaundice was reported from Cuttack municipality area. The team surveyed a population around 300 residing in 28 households. A total of 175 individuals were examined. Clinical features included mild to moderate grade of fever, yellowish discoloration of urine and sclera (Fig 4).

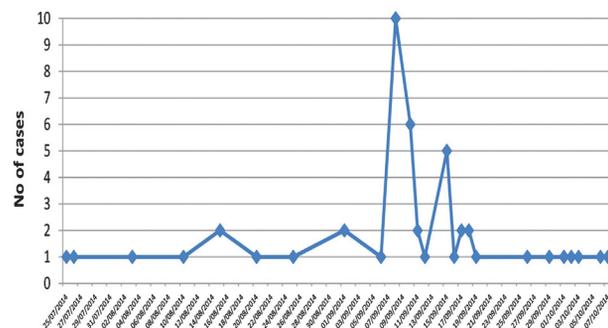


Fig. 4. Day wise onset of jaundice cases from 25th July to 7th Oct, 2014.

From 53 symptomatic individuals, blood samples were collected and tested for HEV IgM and in 47(89%) individuals antibody were detected. The clinical manifestation and the serological investigation findings confirm the illness is due to Hepatitis E virus infection. Possible cause of outbreak is due to spread of infection by faeco-oral route transmission.

Survey Report on Jaundice Outbreak at Sambalpur

During last week of Sept 2014, outbreak of jaundice was reported from Sambalpur municipality area and surveyed a population around 4820 residing in 290 households. A total of 290 individuals were examined and 49 blood samples were collected and tested for HEV and HAV IgM. In 35 individuals (71.42%) HEV antibodies were detected. The clinical manifestation and the serological investigation findings confirm the illness is due to Hepatitis E virus infection. Possible cause of outbreak is spread of infection by faeco-oral route transmission. The DNA sequence data showed that the strains belonged to Genotype 1, subtype A, which is the predominant type is circulating in India.

Investigation of AES Cases in Malkangiri, Odisha

AES cases were reported from Malkangiri district during Nov-Dec, 2014 from Potrel, Kodiguda, Drumaguda, Urumaguda, Sandodiguda, Gomphakonda village. 34 serum samples from symptomatic, asymptomatic and household contacts and 17 blood / CSFs samples were referred to RMRC lab by the State Health Dept. Samples were investigated for JE, Chandipura, Entero, HSV, Dengue JE and Chandipura. All the samples were negative except one positive for HSV and one positive for Dengue.

Investigation of AES cases in Mathurapur village, Korei CHC, Jajpur district

During the survey blood sample were collected from 16 individuals. In 5 cases antibody for JE virus was detected. All were found negative for Dengue IgM. The risk factors like paddy fields surrounding the village and rearing of pigs in nearby villages may be the reason for circulation of the virus.

Outbreak investigation of chickenpox & Measles in Puri district

Out of the 6 samples tested for suspected Chickenpox, all were varicella IgM positive. The report has been communicated to the concerned authority.

Investigation done in 2 villages namely Lekapai (total population 410), block K. Singhpur and village J. Totaguda (Total population 80), block Derigaon of Raygada district for suspected Measles outbreak. The samples were tested for Measles IgM and PCR looking at the duration of illness. Out of the 29 samples tested 11 were Measles IgM positive and 5 samples were PCR positive (3 throat swab and 2 urine samples). The report has been communicated to the concerned authority.

Tribal Health

A study was undertaken to evaluate the adolescent reproductive and sexual health (ARSH) programme in Odisha and identify gaps in implementation of advocacy strategies. Formative research carried out in selecting two blocks (one sector covering 3 sub-centres) in Kalahandi (Dharmagarh and Junagarh) and Raygada (Jamadehipentha and Gunupur) districts covering 2087 adolescents. The baseline information revealed very poor knowledge, attitude and behaviour on ARSH issues by the beneficiary and quality of care at facility (Adolescent Friendly Health Clinics) level for its accessibility and utilization of quality health care services. Advocacy strategies implemented for one year in two study sectors at quarterly intervals covering adolescent males and females and married adolescents independently through social mobilization through peer group education, counseling sessions on adolescent health issues, availability and accessibility of health care services. Interventions include empowerment of adolescents and community through education (awareness generation) using logistic supportive 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. Orientation training imparted at sector and sub-centre levels for stake holders (ANM, Anganwadi Workers, ASHA) about quality of care at community and facility levels. 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 delivery of service providers levels. This study will help to strengthen the programme for effective implementation of quality care and coverage of ARSH services (Fig 5).



Fig. 5. Training and community mobilization for ARSH.



Intensive 4-days training was imparted to field survey teams (health supervisors and health investigators of two outsourced surveyed agencies on measurements of health indicators including height and length on stature meter and infantometer respectively, weight on digital weighing balance, and test for iodine content in the salt used by households, blood pressure on digital BP monitor, blood glucose on glucometer and haemoglobin (Hb) estimation. They were trained about quality control measures so as to measure these parameters accurately and consistently across personnel and place. A total 125196 dry blood samples received from two survey agencies analysed in laboratory ensuring internal and external quality checks.

The survey will help to improve and health and nutrition status by planning district based on magnitude of problem in different districts so as to reduce the gap between districts, states and segments of population.

Translational Research

The multiplex PCR developed to monitor 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 has been validated and training was imparted to District VBD consultant and Malaria Technical Supervisors held at Kalahandi Field Unit (Fig 6).

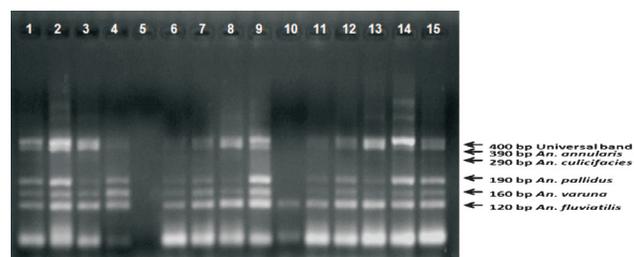


Fig. 6. PCR based identification of mosquito species.

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

Cancers

During this year a total of 3 studies were pursued on stomach, breast, hepatocellular and oesophageal cancers including cancer registry. A study to investigate the risk factors of hepatocellular carcinoma (HCC) in Sikkim and Arunachal Pradesh and pattern of Survival and Quality of Life of the oesophageal and stomach cancer patients in North-Eastern Region of India were initiated during the present year. Estimation of cancer disease burden through 12 *population based cancer registries in north-east India* was continued. Highest AAR among both males and females was seen in Aizawl, Mizoram. East Khasi Hills in Meghalaya had the highest relative proportion of tobacco associated cancers among both males and females. Meghalaya state as a whole showed the highest AAR in cancers of the oesophagus (in both males and females) and of the hypopharynx and larynx in males.

Hypertension and Cardiovascular diseases

A community based study to assess the impact of community based dietary salt restriction in the reduction of blood pressure among tea garden workers was carried out. The community based intervention by dietary salt restriction brought down daily salt consumption to 6 gm/day. In the intervention garden, mean SBP changes from the baseline value of 139.5 mmHg to 135.0 mmHg and DBP changes from 84.4 to 76.7 after IEC intervention & follow up after 8-10 months. Salt consumption could be reduced physically by market survey and could be corroborated with laboratory documentation of lower urinary sodium. The prevalence of hypertension also decreased from the initial 52.4% to 39.3% after 8-10 months of follow up with intensive IEC activities.

Diabetes

The NE component of ICMR INDIAB study is presently being carried out in Meghalaya and Manipur. In Assam the study was completed and the overall type 2 diabetes mellitus (T2DM) prevalence was 5.4% & pre-diabetes was 11.8%

with a higher prevalence in urban compared to rural areas. Overall ratio of known to newly diagnosed T2DM in Assam was found to be 1:0.8 while the ratio in urban & rural areas was found to be 1:0.5 & 1:1 respectively. The study was completed in Mizoram too and the prevalence of diabetes & pre-diabetes was found to be 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

Mosquito borne diseases

Studies on malaria drug development, prevention, pathogenesis, drug resistance, immune response and outbreak investigations were pursued. Collaborative projects with Dibrugarh University & Guwahati University were undertaken for screening of indigenous medicinal plants and synthetic hybrid compounds for antimalarial activity. Out of the 19 compounds screened against chloroquine sensitive 3D7 strain 5 compounds showed some appreciable antimalarial activity. Blood samples have been collected from suspected cases to study the 'Nutritional factors and severity of malaria' and Folate metabolism pathway gene polymorphism. The *molecular epidemiology study revealed* some novel point mutations have been detected in five drug resistance markers viz. *Pfmdr*, *Pfdhps*, *Pfdhfr*, *ATPase6* and *Pfcr1* studied. Four biomarkers (*MSP1-block2*, *Pfscp*, *Pfs25* and *GLURP*) have been investigated for genetic diversity of *Plasmodium falciparum*. The *Plasmodium falciparum* population of NE India has been found to be highly diverse based on *MSP1-block2* gene and *GLURP*. The *MSP1* of *P. falciparum* from NE India has been found to be of 3 variants (R033, MAD20 and K1) and *GLURP* has been found to have 9 alleles. A NIH funded study on *Malaria Evolution in South Asia (South Asia-ICEMR)* in collaboration with University of Washington, USA collaborative project was initiated to assess variation in the ability of freshly isolated parasite populations to change genetically when challenged and to study the prevalence of hemoglobinopathy and malaria in a selected population of Assam and their association.

Japanese encephalitis (JE)/Acute encephalitis syndrome (AES)

The *Study on Eco-Epidemiological Perspectives of Emerging West Nile Virus in Assam* was continued. 225 AES clinical serum/cerebrospinal fluid were collected from AES patients from Dibrugarh of which 20.44% (46/225) was positive for WNV. All the positive cases were found to cluster around the months of June to August 2014 and males irrespective of the 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). Of the available convalescent serum of the WN IgM positive samples 2 of them showed a rise in neutralizing antibody titer. A total of 32,991 mosquitoes were collected from 4 WN reporting areas of which 328 pools of mosquito have been processed. Six pools were found positive by RT-PCR and belonged to *Mn. uniformis*, *Cx. vishnui* and *Cx. quinquefasciatus*. Cross protection by live attenuated JE vaccine SA 14-14-2 studied in Swiss albino mice revealed that SA 14-14-2 showed 100% protection against locally isolated as well as standard Indian JEV strains. A study on *Surveillance of Chikungunya virus activity in Assam and Meghalaya* was initiated during the present year.

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 142 suspected fever cases were screened during 2014 by rK39 diagnostic kit and 2 were found positive for kalaazar. Active surveillance done by house to house visit in 2014 reported new cases of Kala-azar and two new PKDL cases 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, different immunodiagnostic antigenic fractions have been identified for *P. westermani* type 1. Temporal Variation in the pattern of immuno-reactivity was determined in experimental rats. Immunoblot analysis of human IgG response of confirmed human lung fluke cases to different *P. westermani* type 1 antigens were determined. The 2-D proteome of adult lung flukes was probed with infected rat & human sera to identify important antigens having potential to be used for diagnostic and other applications. A study entitled *Molecular typing of Taenia solium/cysticerci and comparative analysis of human and animal isolates from North and North eastern India* was initiated during the year

Bacterial diseases

In the study to find out the *Genotyping diversity and drug susceptibility and mutation patterns among Mycobacterium tuberculosis complex from Assam*, the prevalence of drug resistance of 426 isolates in 'new and previously treated' smear positive pulmonary tuberculosis patients against first line of anti-tubercular drugs was determined. The population structure of *M. tuberculosis* complex by 24-loci MIRU-VNTR typing and spoligotyping revealed two new clades of *Mycobacterium tuberculosis* related to CAS1_Delhi and EAI family. 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. A total of 53 bacterial isolates from neonatal septicemia cases were received from AMCH, Dibrugarh and *Klebsiella pneumoniae subsp. Pneumonia* was the predominant bacteria identified. ESBL was detected in 30% of *Klebsiella* isolates. A total of 67 (22.1%) samples were positive for pathogens among the total 303 CSF samples processed (52 were culture positive: 52; 16 S rRNA gene PCR: 15). Gram negative organism were predominant (n=32, 48%). The most frequent Gram negative bacteria were *Acinetobacter baumannii* (n=12, 18%), *Klebsiella* (n=8, 12%), and *Pseudomonas* (n=6, 9%) and Gram positive were *Enterococcus* (n=10, 15%) and coagulase-negative *Staphylococcus* (n=11, 16%). Group B Streptococci and *Escherichia coli* were found on one occasion each (n=1). About 63% of the gram positive isolates were sensitive

to amikacin, 57% to netilmicin, 38% to ampicillin/sulbactam and 14% to piperacillin/tazobactam. About 42%, 22% and 19% of the gram negative isolates were sensitive to piperacillin/tazobactam, netilmicin and amikacin respectively. About 83% of the gram positive and 79% of the gram negative isolates tested were resistant to cefotaxime. RMRC is investing the *Epidemiology of scrub typhus in selected areas of Assam and Arunachal*. During the reporting period blood samples were collected from suspected patients. All the collected sera samples were screened for the presence of *Orientia tsutsugamushi* antibody. Of the clinically suspected ST (scrub typhus) cases, 31.5% (24/76) were found to be ST IgM positive, whereas, 17.6% (39/221) of the AES cases accounted were positive for ST IgM antibodies. A follow up of ST reporting areas were carried out during which, a total of 770 human blood samples were collected of which 32.6 % (185/567) were sero-positive against ST and 5 have been found to be PCR positive for *Orientia* 56 kda gene. Among the 301 ST negative samples screened, about 12.2% (37/301) and 11.2% (34/301) were found sero-positive for SFG (Spotted Fever Group) and TG (Typhus Group) respectively. Ten samples were found to be PCR positive for *Rickettsia* 17kda gene. Three new studies were initiated during this year (i) A study to find out the *prevalence of bacterial pathogens colonizing the genital tract of pregnant women and to compare with those causing early onset neonatal sepsis* (ii) *Epidemiological typing of Burkholderia cepacia complex Stenotrophomonas maltophilia isolated from septicaemic patients in North East India* and (iii) *Study of interepidemic survival of V. cholerae in outbreak prone areas of Assam*.

Viral diseases

In the ongoing *Phase II multisite monitoring of human influenza viruses* study, 545 nasal/throat samples were collected from 3 PHCs and a referral hospital in Dibrugarh district. Influenza virus detected by PCR in 166 samples (106 *Type A/H3N2*; 12 *H1/2009/Swine* & 44 *Type-B*) and 43 numbers were isolated (*Type A/H3N2*: 29 & *Type B*: 14). 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. Rotavirus prevalence (2014-15) was 43.5% (95% CI: 37.3% - 45.9%) n=208/478). Prevalence was highest in infants (51.9%) followed by 13-24 months (44.7%) and above 25 months age group (32.7%) (p-value =0.01). In G and P typing the most common subtype of rotavirus A detected were G1 P[8]-80%, G9P[4]-10% rest 10%. The infrastructure development of four 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* (iii) *Establishment of a network of laboratories for managing epidemics and natural calamities* (iv) *DBT-ICMR animal house facility for Biotechnology Research in North-eastern Region* are in progress. The process of infrastructure development is in progress. During the period diagnostic facility for 22 viruses has been developed. A total of 540 samples were processed for diagnosis of different viruses. Five different outbreaks were investigated including outbreak of HFMD (enterovirus) in Sept 2014, JE outbreak in July 2014, two Influenza A H3, Chicken-pox in Feb 2015. 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 continued. Under the *genotyping study* 105 new cases of Hepatitis B infections were identified and enrolled. Acute viral hepatitis due to HBV, HAV and HEV were found to be 39%, 36.4% and 3% respectively. Hepatitis B core antibody positivity was recorded in 2.85% of 70 health care workers recruited and anti HBs was detected in about 51% (20/39) of them. Among the 87 blood donors enrolled, anti HBc (total) was found in 10.34 % (9/87) and Anti HBs >10mIU/ml was observed in 42.85% (24/56) cases. HBV genotyping revealed genotypes C, D and I in 13 HBsAg positive samples. In the *host innate immunity and hepatitis B persistence* study a total of 189 subjects have been recruited (control group: 37; study group: 152). Low viral load (<1000 IU/mL) was detected in 48.8%, intermediate (1000-20,000 IU/mL) in 17.1% and high (> 20,000 IU/mL) in 34.1% of the cases. The gene expression of 26 innate genes was analyzed for 73 samples. Gene upregulation was seen in BTLA in HBeAg and high viral load cases compared to HBeAg

negative or low viral load cases. Overall, TLR1, TLR2, and TLR4 and TLR6 are downregulated in CHB compared to normal subjects. A multi-site epidemiological and virological survey of Nipah virus with special emphasis on North East Region of India has been initiated.

Outbreak investigations

The Centre investigated an outbreak of acute hepatitis / jaundice in Sibsagar district of Assam which affected more than 20 subjects mostly adults. Out of the blood samples collected 88.9% (8/9) (88.9%) were found positive for HAV IgM ELISA and 2 were positive for HAV nucleic acid. An outbreak of acute respiratory tract infection in two villages under Tengakhat & Khowang PHC of Dibrugarh District, Assam which included a total of 43 cases in the 01 month to 66 years age group were investigated. Out of the collected samples 53.5% (23/43) were found positive for *Influenza Type-A/H3* and 2.3% (1/43) found positive for *Influenza Type-B*. The Centre investigated diarrhoeal disease outbreak in a Koilamari TE. Twelve of the 20 rectal swabs collected showed the growth of *Vibrio cholerae* O1 El tor serotype Ogawa. The isolates were sensitive to ciprofloxacin, gentamicin, doxycycline, tetracycline and chloramphenicol and resistant to ampicillin, trimethoprim. The investigation report was shared with the state health agencies for taking the control measures. The Centre investigated outbreak of maculopapular rash in soles, palms and ulcers in mouth among pediatric and adult population in Dibrugarh and Sonitpur District which was confirmed as Hand foot and mouth disease (HFMD) due to enterovirus. An outbreak of bilateral lower limb swelling and weakness affecting adults in two adjoining villages (Soripora) of Sibsagar District was investigated by the centre. Even though the aetiology could not be confirmed, CPK was raised 3-4 times in representative samples which led to a provisional diagnosis of polymyositis for investigation.

Others Areas

A study has been initiated to assess the health related quality of life and functional status of community dwelling elderly persons in rural settings of Assam. A cluster randomized controlled trial has been initiated to assess the effectiveness of

diet and lifestyle intervention through Information Education Communication (IEC) tools with Angan Wadi Centres (AWCs) as the centre of knowledge dissemination for Non-communicable Disease risk reduction. The data collection for the study titled Health systems preparedness for interventions for diabetes, hypertension, chronic respiratory diseases, cardiovascular disease and cancers and deaths due to non-communicable diseases among the tribal population in India was recently completed. Among the population the highest deaths (70.4%) were found due to non communicable diseases followed by infectious diseases (11.8%) and other causes (17.8%). Majority of deaths were reported among the OBC population (41.33%) followed by ST (39.78%), SC (8.00%) and General (10.89%). Most of the deaths were reported at home (57.78%) followed by District hospital (15.11%), other health centre (14.44), Pvt. hospital (6.89%), PHC/CHC/ Rural hospital (2.67%), on the way to the health facility (2.44%) and Medical college/Cancer hospital (0.44%). 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.

REGIONAL MEDICAL RESEARCH CENTRE FOR TRIBALS (RENAMED AS NATIONAL INSTITUTE FOR RESEARCH IN TRIBAL HEALTH (NIRTH), JABALPUR

TRIBAL HEALTH RESEARCH UNIT

To determine the association between malaria and sickle cell, 2520 patients were screened for malaria, sickle cell trait and disease during the period under report. Prevalence of sickle cell carrier (AS) and sickle cell disease (SS) was 16% and 2.4% respectively. *Plasmodium falciparum* (330) was the predominant species, 49 patients had *P. vivax* and 5 cases showed mixed infections. It was observed that the prevalence of sickle cell carrier and sickle cell disease among malaria patients were 5.7% and 0.6% respectively which is significantly lower than patients without malaria (n=1939), parasite densities were significantly lower in malaria patients with sickle cell than malaria patients without sickle cell. During the study, the Centre also detected for the

first time in India, sympatric distribution of two species of *P. ovale curtisi* and *P. ovale wallikeri* in Chhattisgarh on the basis of molecular diagnosis. Further analysis revealed that the prevalence of sickle cell carrier among tribal population was higher (13.7%) than general population (10.3%).

New initiatives under the Tribal Health Research Forum (THRF)

Micro mapping of G6PD deficiency among the tribals of India and its importance for antimalarial therapy

Multi-centric study on “Micro mapping of G6PD deficiency among the tribals of India and its importance for anti-malarial therapy” was initiated partnering NSCB Medical College, Jabalpur. During the period 470 tribals including school children have been screened in tribal district Dindori. Among these 25 (5.3%) samples were found to be deficient for G6PD enzyme activity. Three samples were completely lacking enzyme activity. All deficient samples are being characterized for their molecular make up and mutation.

Vector borne diseases

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

A study was undertaken to evaluate the *pfhrp2* and *pfhrp3* gene variations / gene deletions in *P. falciparum* samples from malaria-endemic states of India. Study sites were selected in 8 states (North East, Orissa, Madhya Pradesh, Chhattisgarh, Jharkhand, Maharashtra, Gujarat and Rajasthan). Two sites (one high transmission and one low transmission) were selected from each state. Screening of malaria parasite was done by microscopy and mono infection of *P. falciparum* positive samples were collected. Plasma samples separated from erythrocytes were stored for serological and molecular analysis. *P. falciparum* infection was confirmed by species specific nested PCR for *pfhrp2*, *pfhrp3* and their flanking genes. Out of 23,125 subjects, 12% were found positive for malaria parasite with 74% *P. falciparum*. A total 1521 *P. falciparum* infected cases were enrolled for the study. All samples were tested by RDT and

PCR. Among these 3% were found RDT negative. RDT negative samples will be further analyzed for *pfhrp2*, *pfhrp3* gene deletion.

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

The study was carried out in 2 malarious districts *i.e.* Bastar and Korea of Chhattisgarh state. Two CHCs from the district and 4 villages in each CHC were selected for this study. The anopheline fauna of the villages in both the districts consisted of mainly *Anopheles culicifacies*, *An. subpictus*, *An. fluviatilis*, *An. annularis* and *An. vagus* in indoor resting collections. The mean density of *Anopheles culicifacies* caught per man hour during the year was 6.42 in Bastar district and 8.99 in Korea district. Susceptibility of *An. culicifacies* to diagnostic dose of deltamethrin (0.05%) and Alphacypermethrin (0.1%) revealed that the corrected mortality in Korea district was 90% to deltamethrin (VR) and 99% to Alphacypermethrin in different localities. In Bastar district, the corrected mortality was 74% to deltamethrin and 77% Alphacypermethrin. These results indicate that the species is resistant to Deltamethrin and Alphacypermethrin in Bastar. A total of 2637 *Anopheles* mosquitoes were assayed by PCR for malaria parasite, of which a single *An. culicifacies* was found positive for *P. falciparum* from Bastar district.

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 anti-malarial drugs (ACT) in uncomplicated *P. falciparum* malaria in Balaghat district of Madhya Pradesh. Therapeutic efficacy was carried out with oral ACT over a 3-day period. Genomic DNA of the parasite was used to amplify the drug resistance genes (*pfdhfr* and *pfdhps*) using gene specific primers. Three hundred fifty five patients were screened in Balaghat district, out of which 137 were positive for malaria, 113 were *P. falciparum* positive of which 86 enrolled were enrolled. Eight cases had *P. vivax* and 16 cases mixed infections of *P. falciparum* and *P. vivax* were also detected. The therapeutic efficacy

outcome was determined for 85 patients. Overall a therapeutic efficacy was 100%, *ie* adequate clinical and parasitological response. Out of 86 cases, 80 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 37.5% with wild type. Majority of the parasite population were having double mutations (44%) with double mutant *pfdhfr* A₁₆N₅₁R₅₉N₁₀₈I₁₆₄ followed by single mutant *pfdhfr* allele A₁₆N₅₁C₅₉N₁₀₈I₁₆₄ (16%) and 2.5 % was triple mutant *pfdhfr* allele A₁₆N₅₁C₅₉S₁₀₈P₁₁₈. In *Pfdhps* gene, wild type was predominant (85%) followed by single mutant (15%).

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.

A study was formulated to execute a need based IEC intervention in Saharia tribal dominated areas having high prevalence of PTB and to assess the risk factors for pulmonary tuberculosis among them was initiated in 3 phases. Baseline survey (Phase I) and Phase II (IEC intervention) have been completed. The findings of the baseline survey showed alarmingly high TB disease prevalence of 3003 per 100,000. The findings also highlighted poor knowledge about various aspects related to tuberculosis. Major risk factors for tuberculosis were identified. Based on the findings of the baseline survey, IEC intervention activities were designed and executed in the study villages. End-line survey (phase III) comprising of TB disease and KAP survey including risk factors for PTB is in progress.

Virology

Virus research and diagnostic laboratory

Virus Research and Diagnostic Laboratory (VRDL) is currently providing diagnosis for 15 different viruses using molecular and serological approaches. During the period more than 8700 samples were tested. Highest request for testing the presence of Dengue and H1N1pdm09, were received (2657 and 3575 respectively). Thirty percent samples were

found to be positive for dengue and 43.9% were positive for Influenza A H1N1pdm09.

Dengue outbreaks in Narsinghpur, Mandla, Bhopal and Shivpuri districts were investigated. Molecular studies conducted on the samples revealed that 3 serotypes [DEN 1, 2 & 3] were circulating in Madhya Pradesh this year. For other Hepatitis viruses a total of 1786 samples were tested out of which 18 % samples were positive for viral infections (HAV, HBV, HCV and HEV) leading to hepatitis. Respiratory Syncytial Virus (RSV) and 17 cases were positive among 213 samples. The study conducted to understand sero-protection of rubella at tertiary care hospital revealed that about 40% pregnant women are susceptible to rubella.

National Hospital based Rotavirus Surveillance Network

Rotaviruses are a major cause of acute gastroenteritis in the case of human and animals. This study was initiated to generate a nationally representative data on rotavirus burden and strains circulating in India and to understand the trends and pattern of diarrhoea attributable to rotavirus among children < 5 years of age seen at in-patient facilities. Hospitalized Children with acute gastroenteritis (age group of 0-60 months) were included in the study. The samples were screened by the Premiere Rotaclone EIA kit that detects Group A rotaviruses. All aliquots of positive samples were stored at -20°C for further characterization. A total 202 samples were collected and of these 56 (27.72%) were confirmed for rotavirus group A by ELISA (Rotaclone). Further work is in progress

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

This is an ongoing study to assess the impact of the intervention to improve maternal and child health services. The IEC activities were carried out in each intervention village by forming village level committees, conducting of health education camps, group discussion, banner display and distributing

pamphlets. This year in Phase-II of the study work is in progress to evaluate ANC coverage, TT vaccination, IFA consumption, utilization of natal & post natal services and immunization of children.

Outreach Activities

- ✓ A workshop on “Tuberculosis: Tribal Perspectives” was organized during 21-22 June 2014. The interactions of researchers and policy makers provided an opportunity to understand research priorities in tuberculosis, particularly among tribal population in the country.
- ✓ Two workshops on Ethics were conducted
 - Ethical issues in biomedical and health research on 25-26 July 2014 where 40 participants were present along with NIRTH staff and students.
 - Good Clinical Practices 1st Oct. 2014 where 40 participants were present

REGIONAL MEDICAL RESEARCH CENTRE, PORT BLAIR

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

Persistence of microfilaraemia is evident after six rounds of mass drug administration with DEC (MDA), prevalence of infection remains at 3.3%, with antigenemia of 3.2% in 2-4 age class and 2.9% in 6-7 age class. It is evident that MDA alone is unlikely to achieve elimination, administration of DEC fortified salt was considered as an additional measure. The project has been initiated for hastening the process of elimination in collaboration with the Directorate of Health Services, Directorate of Tribal Welfare (A&N Administration) and VCRC, Puducherry

Estimation of tuberculosis among marginalized and confined population of Car Nicobar Island- an attempt to understand the influence of socio-cultural factors on the disease and its treatment

In 2001-02, the annual risk of tuberculosis infection (ARTI) and prevalence of smear-positive

pulmonary tuberculosis among the Nicobarese of Car Nicobar were 2.5%, and 735.3 per 100,000 respectively. The RNTCP was introduced in A & N islands in 2005 and DOTS+ strategy in 2011. The current study estimated the prevalence of sputum positive pulmonary tuberculosis as 241.6/100,000 population and that of MDR tuberculosis was 9.7/100,000. Prevalence of MDR TB among isolates subjected to DST was 10%. This is probably the combined result of the socio-economic progress, appropriate public health interventions and an improved environmental sanitation in the island. The declining trend in tuberculosis prevalence presents an opportunity to sustain and intensify the intervention so that the prevalence could be brought down to elimination levels.

Observations on malaria situation among the Nicobarese in an isolated island, Car Nicobar- Working towards elimination

In Car Nicobar island, malaria has declined since tsunami. However, malaria cases have been reported locally, which shows that transmission still persists, albeit at low level. In Car Nicobar island, 381 malaria cases were reported in 1989, with an API of 19.4/1000 population. Over the years, with a better understanding comprehension of the transmission dynamics of malaria and introduction of bioenvironmental control strategy, the API came down from 19.4/1000 in 1989 to 4.3/1000 in 2002. Since 2006 there has been a gradual decline to 1.21 (2014). In view of this, it is planned to carry out a study to eliminate malaria in this island and accordingly a proposal entitled “Operational Feasibility of malaria elimination in the Car Nicobar Island of Andaman and Nicobar archipelago” is envisaged.

Surveillance of diseases-Hospital/Community based

Viral Research and Diagnostic laboratory (Grade I) network at RMRC, Port Blair

The centre is providing diagnostic services to the entire UT of A & N Islands, for diseases suspected of viral aetiology. The surveillance detected 58 cases of Chikungunya and 109 cases of dengue. Under this initiative, strengthened viral disease diagnosis and surveillance and detected infection

with an array 24 viral pathogens through the viral diseases surveillance programme of the Grade-I virology network laboratory. In addition to referral samples, two surveillance teams have been constituted, that visit the PHCs in South Andaman and referral hospital in Port Blair. Reports based on the laboratory investigations are accomplished within 2-4 hours and are communicated to the concerned medical officer, either telephonically or through SMSs.

National hospital based Rotavirus surveillance study for paediatric inpatients and outpatients

A total of 476 possible cases of Rotaviral diarrhoea admitted to the wards of the selected hospitals were enrolled. Rotaviral aetiology of the diarrhoea was confirmed in 169 (35.5%) cases. The most frequent G/P genotype combinations detected were G1P[8], G2P[4], G12P[8] and G9P[4] respectively. G1P[8] remains the commonest among the genotypes detected.

Antibiotic resistance patterns and distribution of genetic markers of drug resistance among the *Escherichia coli* mediated on diarrhoea/urinary tract infections in Andaman and Nicobar Islands

Stool samples were collected from 696 paediatric patients with diarrhoea and processed for isolation of bacterial enteric pathogens. From these samples, 67 (9.62%) enteric bacterial pathogens were isolated, which include diarrhoeagenic *E. coli* (EAEC, EPEC, ETEC), *Shigella* sp. (*S. sonnei*, *S. boydii*, *S. flexneri*), *Salmonella* sp.

National nutrition monitoring bureau - diet and nutritional status of the individuals and prevalence and determinants of non-communicable diseases

A total of 4052(1850men & 2202 women) participants were analysed for their 'fasting blood sugar (FBS)levels', among them 454(11.2%)were found to be diabetic (FBS levels >126mg/dl) and 341(8.4%)were observed to be pre-diabetic(FBS levels 110-126mg/dl).A total of 3688(1691men & 1997 women)participants were analysed for their blood pressure(BP)levels, among them it was observed that 444(12.0%) were hypertensive(BP >140/90mmHg) and 1060(28.7%)were pre-

hypertensive(BP 120-139/80-89 mmHg).A total of 1784(968men & 816women) participants were analysed for dyslipidaemias based on their lipid profiles.Among the 1784 participants analysed, 76(4.3%) were found to behaving hypercholesterolemia(TC levels >240mg/dl) and 235(13.2%) were observed to be having their TC levels above optimal range(borderline 200-239mg/dl).

Setting up of Tribal Health Research Units at Port Blair

This unit was established with objectives of assessing the nutritional status and health profiling of the particularly vulnerable tribal groups (PVTGs) of the A & N islands. Besides, undertaking studies on ethno-medicinal health care practices prevalent among the tribal communities of these islands also forms the objectives of the unit. Till date assessment of nutritional status health profiling for the Onges and Andamanese have been accomplished. The nutritional status of the school aged Andamanese children appeared to have improved since the last survey when 77% of the children below 19 years of age were undernourished. The nutritional problems among the adults seem to be that of over nutrition with about 66% (21 of the 32) of the adults being either overweight or obese. Traditional practices among the Nicobarese, Onges and Karens have been elicited through interactions with the Traditional Knowledge Practitioners (TKPs), which has been incorporated in the Community Biodiversity Registers (CBDRs). Overall information on 254 plants is available in these CBDRs. Of these 254 plants, 37 were studied for anti-microbial and 15 for anti-malarial potentials, of which 22 plants showed anti-microbial and 8 showed anti-malarial potentials respectively.

BASIC AND APPLIED RESEARCH

Development of DNA vaccine for Leptospirosis infection

Currently there are no successful vaccines for prevention of leptospiral infection. Therefore, efforts were undertaken towards development of a candidate vaccine. The efficacy of a synthetic consensus DNA plasmid vaccine has been developed against the *Leptospira* membrane lipoprotein LipL45. It was found to elicit both cellular and

humoral responses. The efficacy of the levels of protectiveness conferred by the DNA construct is under progress.

Second Phase of Task Force “Biomedical Informatics Centre’s of ICMR”

Diversity of intestinal microflora of the accessible ethnic communities of Andaman and Nicobar Islands as a part of the activities under Second Phase of Task Force ‘Biomedical Informatics Centre’s

Studies were conducted for identification and quantification of various intestinal microbiota among the three cohorts viz. remote (cohort 1), rural (cohort 2), urban (cohort 3) population of Nicobarese, with the overall objective to study the immunity markers and their association with the diversity of intestinal microflora, the association of nutritional status and various disease prevalence.

16S amplicon sequencing of 3 faecal samples (one from each cohort) yielded 15,75,976 sequences corresponding to 1496 Operational Taxonomic Units (OTUs) in subjects from remote group. 1434 OTUs in rural and 1331 OTUs in urban group were detected. Core microbiome is represented by 301 OTUs with *Faecalibacterium* as the predominant genus. UniFrac Analysis shows that there is a significant variation between Remote-Urban and Rural Urban pair-wise cohort comparisons.

ECOLOGY AND ENVIRONMENTAL MICROBIOLOGY

Ecology of leptospirosis – role of biofilms in pathogenesis and transmission dynamics of leptospirosis

Studies were conducted to demonstrate environmental biofilm formation by leptospira with other bacteria and the ability of leptospira to survive environment. *Azospirillum brasilense* was found to be high intensity biofilm forming bacteria with Leptospira in the paddy field environment. Self aggregating strains of Leptospira were found to form more readily than other strains. The biofilm formation starts by formation of aggregates between Leptospira and *Azospirillum* cells, which adhere with each other finally resulting in mature biofilm. SEM 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*. It is clear that Leptospira is benefited from this interaction by acquiring the ability to survive under harsh environmental condition. Thus biofilm formation may play an important role in transmission dynamics of leptospirosis. This study would help in developing strategies for the control of leptospirosis.

DESERT MEDICINE RESEARCH CENTRE, JODHPUR

VECTOR CONTROL

Surveillance of pyrethroid resistance in *Anopheles stephensi* strains of Rajasthan and studies on genetic and biochemical mechanisms of pyrethroid resistance in *An. stephensi*

The current susceptibility status of *An. stephensi* was determined in the study villages against DDT, malathion, alpha-cypermethrin, cyfluthrin and permethrin and a larvicide – temephos. The experiments of *An. stephensi* against alpha-cypermethrin in village Baitu of Barmer district and Bhanipura in Bikaner district, against cyfluthrin in villages Thob of Barmer district and Tejpala of Jaisalmer district exhibited intermediate resistance in the species, which warrants further verification of the results.

Use of insecticide treated nets (ITNs) in alternative forms for the protection against malaria transmission in the desert

The survey has been carried out in the six villages of Jaisalmer district during post monsoon period (September 2013) and in winter season (January 2014). Five species of *Anopheles* were reported from the study villages including *Anopheles stephensi* and *Anopheles culicifacies*. The malaria cases of the villages where intervention has been made in the form of insecticide treated bed nets were significantly decreased than the control villages. Mosquito density in the control villages

were increased during the study period, however in the intervention clusters the density has been decreased after intervention..

Impact of irrigation change on the prevalence of malaria in arid and non-arid parts of Rajasthan

Study was initiated to study the impact of irrigation change on the ecological conditions with respect to vector prevalence and malaria incidences as well as to determine key factors influencing vector/malaria prevalence and distribution using RS and GIS. Two ecologically different districts, Jaisalmer and Banswara, have been considered to carry-out the studies.

Current status of insecticide resistance among mosquito vectors in Rajasthan State – a collaborative study

The studies have been carried-out in Bikaner and Nagaur districts. In Both the districts, *An. stephensi* exhibited intermediate resistance against DDT in all the study villages Jamsar, chawandia and Alay, however, against synthetic pyrethroids, alpha-cypermethrin and cyfluthrin, the species was found totally susceptible. The study in other districts is in progress.

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)

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.

VIROLOGY

Comparative study of Gene characterization of Influenza A pandemic (H1N1) 2009 viruses from virus isolates of 2009 pandemic and 2012 re-emerging viruses in western Rajasthan:

Phylogenetic analysis and molecular characterization

Throat swab samples as collected from the suspected patients of Swine flu by the OPD staff of Medical College hospital of Jodhpur were used for the present study. The history of the patients as Serious, Recovered or Death case was taken as the basis of selection of sample. The samples found positive (as per hospital records) for the three Pandemic Influenza genes viz; Inf A, Sw A, Sw H were selected for the detailed study of genomics. The diagnostic tests were performed as the repeat study to confirm the virus positive status of the sample. Real Time PCR was employed as per the protocol notified by the WHO.

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 ionize and rifampicin using liquid culture media

The project is to standardize and evaluate rapid method for culture and sensitivity of *M. tuberculosis* and to support RNTCP with culture of *Mycobacterium 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 new 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 randomized controlled trial

Under this task force study on hypertension, Kotra Tehsil, of Udaipur district was selected, which comprises more than 90% of tribal population. Based on coverage area of an AWW centre, 12 clusters were selected and divided randomly in intervention and control groups equally. So far 3600 individuals have been interviewed with collection of blood sample from 956 individuals. In six clusters intervention would now be given.

NUTRITION

Nutritional status of elderly rural population and development of appropriate intervention model using existing health system

Study was initiated in Luni Tehsil of Jodhpur. The project aims the clinical assessment, anthropometric measurements, assessment of micronutrient deficiencies, *viz.*, Iron, Zinc, Vitamin A and E *etc* and the assessment of the dietary intake of the elderly population. Base line survey of 400 household has been completed. Two training programmes were also conducted. .

National Nutrition Monitoring Bureau Unit

The survey under NNMB unit was conducted in Jodhpur, Jaipur and Kota cities and is ongoing in Dholpur city. The preliminary analysis in Jodhpur city, indicated that around 12.7 % of adults are diabetic, 14.53 % are hypertensive and 17.4 % are hypercholesterolemic.

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 in Rajasthan

Under this multi centred task force study, ICMR is financially supporting four districts

namely Chittorgarh, Churu, Jodhpur, and Kota in Rajasthan. The Objective of the study is to improve health and nutritional status of vulnerable segment of population by implementing multi-component health and nutrition education intervention, focusing on dietary counseling and modification keeping in view the cultural and socio-economic status of population. Sample size estimated at village level was 96 individuals including 24 pregnant women, 48 under 5 years age children and 24 adolescent girls. Out of 40 villages (10 villages from each district), 38 villages have been covered for base line data. The intervention will now be given.

As one of the activity of MRHRU, Jaipur this project is taken in Jaipur with the same objective as in 3 above. All the 10 villages are covered as to collect the base line information. The intervention will be started soon.

OTHER AREAS

Facilitation of establishment of Model Rural Health Research Unit in Rajasthan by DMRC

Department of Health Research (DHR) has taken initiative for establishment of Model Rural Health Research Units (MRHRU) during the 12th Plan period. On 1st February, 2014, one of the MRHRU was inaugurated at Bhanpur Kalan CHC, District Jaipur, Rajasthan by Dr. V. M. Katoch, Secretary, DHR and DG, ICMR. The medical college identified to be associated with Bhanpur Kalan MRHRU by the Government of Rajasthan is S.M.S Medical College, Jaipur. Three research projects have been initiated at MRHRU. Among these, one is on nutrition and the others on use of contraceptive and Active surveillance of tuberculosis.

Annual Health Survey- Clinical Anthropometric and Biochemical Component (CAB)

Under this survey, 102000 blood samples were received which were analyzed for haemoglobin content.

Biomedical Informatics Centre

The broad objectives are, to identify genetic loci associated with diseases of national interest, to develop solution for controlling pathogens causing diseases such as H1N1 (2009) pandemic influenza

viruses, Dengue viruses, Tuberculosis and Malaria through determining pathogen gene sequences and their analysis in genomic database and designing surveillance system and developing approaches for controlling drug resistance by malaria parasite in Rajasthan. Data mining was done on various scientific data bases to develop knowledge stock of risk factors associated with Type II diabetes.

Rajasthan Conclave-2

Rajasthan Conclave-2 was organized from 21st to 23rd September, 2014. The main objective of the conclave was to bring together the medical faculties and professionals, programme managers and policy makers of state health department, university teachers and young research scholars, to explore the ways and means to strengthen and consolidate the collaborative research efforts made since Rajasthan Conclave-1, through discussions and interactions and to identify researchable issues in view to formulate more and more collaborative research programmes related to public health for the immediate benefit and wellbeing of the local people. A total no. of 176 participants attended the conclave, which included the faculty of 08 state medical colleges viz., SMS medical college, Jaipur, SN Medical College, Jodhpur, SP Medical College, Bikaner, JLN Medical College, Ajmer, RNT Medical College, Udaipur, Govt. Medical College, Kota, Jhalawar Medical College, Jhalawar and RUHS College of Medical Sciences, Jaipur, 03 institutions from private sector viz., NIMS Medical University, Jaipur, MG Medical University, Jaipur and Geetanjali Medical College, Udaipur including AIIMS, Jodhpur and Ayurved University, Jodhpur, involved in medical practices and research; and from universities viz., JNVU, Jodhpur, MLSU,

Udaipur, MDSU, Ajmer, MGSU, Bikaner and Amity University, Noida. Faculty and Research Students, involved in basic and applied research participated in the conclave presented their work and contributed whole heartedly during the Interactive Sessions and Panel discussions. The experts and members of Scientific Advisory and Ethics Committees of DMRC were also present during the deliberations of the event.



Fig. Dr.V.M. Katoch, Secretary DHR (GOI) and DG (ICMR) inaugurating Rajasthan Conclave-2.



Fig. Dr. G. S. Toteja, Director, DMRC and Dr. R.S.Chhipi, Additional Director (Rural Health),Govt. of Rajasthan, signing the MOU between DMRC, Jodhpur and DMHS, GOR.

SUPPORTING FACILITIES

During the reported period, statistical assistance was provided to all ICMR institutes by the National Institute of Epidemiology (NIE), Chennai, and the National Institute of Medical Statistics (NIMS), New Delhi. Several new projects have been undertaken, which have been discussed in detail.

NATIONAL INSTITUTE OF EPIDEMIOLOGY, CHENNAI

National Hospital Based Rotavirus Surveillance Network Project

A multicentric surveillance system was initiated by ICMR in 2005 to estimate the burden of rotavirus diarrhoea in the country. To build on the success of this network, and as per the recommendations of the National Technical Advisory Group on Immunization (NTAGI), ICMR has extended the surveillance activities in the country. Laboratory QA/QC was coordinated by CMC, Vellore, while the data management for the project was done by NIE.

The phases I, II and III of the surveillance were launched in September 2012, September 2013 and July 2014, respectively in various institutes (fig. 1).



Fig. 1. Rotavirus surveillance sites

A total of 13164 children were enrolled and 12123 stool samples were collected in the study during September 2012 – March 2015. Out of 12123 stool samples screened, 40.7% samples were positive for rotavirus. The region-wise rotavirus positivity rates are shown in Fig. 2. The major genotypes found were *G1P*[8], *G2P*[4], *G9P*[4], *G12P*[6], *G9P*[8], *G1P*[6], *G12P*[8].

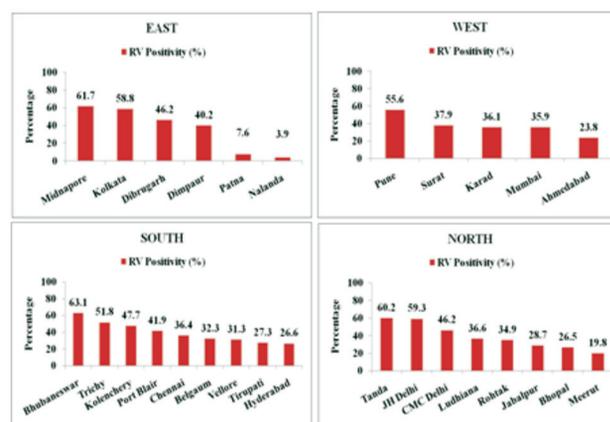


Fig. 2. Distribution of rotavirus positivity by region.

WHO/TDR multicentric trial on ‘Uniform multi-drug therapy (U-MDT) regimen for all types of leprosy patients’

Uniform therapy for all leprosy patients will simplify treatment through primary health care and general health services. A WHO-TDR supported multicentric trial evaluated six-month MDT regimen currently recommended for multibacillary (MB) patients as U-MDT for both types of leprosy. In U-MDT, clofazimine is added for paucibacillary (PB) patients and treatment duration is reduced from 12 to six months for MB patients. The primary objective of the trial was to determine efficacy to prevent cumulative level of 5% relapse rate at the end of five years. NIE was the international coordinating centre for the trial.

The open design trial enrolled newly detected, previously untreated PB and MB leprosy patients in six sites in India (Gaya, Kanpur, Pune, Rohtas, Tiruvannamalai and Villupuram) and two sites in China (Guizhou and Yunnan). Clinical improvement was recorded post-treatment completion and during annual follow-up visits. Clinically confirmed relapse was defined as occurrence of one or more new skin patches consistent with leprosy, without evidence of post-treatment reactions. The primary outcome of relapse as rate per 100 person years (PY), cumulative risk over five years and secondary outcomes in terms of acceptability, compliance and safety was analyzed.

Out of the total 3437 (98.6%) patients, 3389 (PB=2091; MB=1298) were screened for the trial. Of the PB patients, the mean age was 29.3 ± 15.1 years and 54% of them were males. At the time of recruitment, 33% of the PB patients had nerve lesions and 55 (3%) patients had grade 2 disability (G2D). Two of the PB patients developed clinically confirmed relapse (PY=8780; rate=0.023/100 PY; cumulative risk: 0.11%). During treatment period, eight had suspected adverse drug reactions (ADR) (PY=1009; rate=0.79/100 PY). Rates of occurrence (per 100 PY) of new lesions on account of reactions, neuritis, type 1 and type 2 reactions were 0.24 (n=23), 0.39 (n=37), 0.54 (n=51) and 0.03 (n=3), respectively. Of the 1298 MB patients, mean age (SD) was 35.3 ± 16.1 years and 66% were male. G2D was present in 5% and 63% had nerve lesions. Four MB patients had clinically confirmed relapse (PY=5379; rate: 0.07; risk:0.37%). Sixteen MB patients reported suspected ADR (PY=605; rate: 2.64). The occurrence of new lesions on account of reactions, neuritis, type 1 and type 2 reactions were 1.34 (n=76), 1.37 (n=78), 2.01 (n=114) and 0.49 (n=28) per 100 PY, respectively. All PB and MB patients, opted for U-MDT and compliance was 99%. Skin pigmentation due to clofazimine was of short duration and acceptable.

Almost all the new patients recruited for the trial completed the regimen in nine months. At fifth year post-U-MDT regimen, low relapse rates (<5%) among PB and MB patients were observed. ADR and other adverse events were minimal and clofazimine related pigmentation was acceptable. Hence, the evidence supports introduction of the regimen in the national leprosy elimination programmes.

Socio-cultural features and stigma of leprosy for treatment & control in general health services in India: Cultural epidemiological study

Despite leprosy treatment being offered through general health services, it remains to be determined whether and how the social 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, NIE coordinated a multicentric cross-sectional study in six Indian sites (Fig. 3) to clarify the relevance of socio-cultural features of experience and meaning of illness and stigma between leprosy and other health problems. After obtaining consent, patients were interviewed on the awareness, help-seeking and treatment experience; while the community members were interviewed about support, stigma and community participation and health providers on integrated care and treatment services. Further interviews were conducted for 608 leprosy and 560 non-leprosy (tuberculosis=313; malaria=118 and skin=129) patients and 176 providers (public=119; private=57) and 197 community members.



Fig. 3. Participating sites.

Leprosy patients were slightly younger and had more women than non-leprosy patients. Awareness about causes for leprosy and tuberculosis needed more attention. There was no major problem in primary health care experience. All were respected and given equal time while seeking help for other health problems. However, almost one-third mentioned about not receiving any explanation about their illness. Among the non-leprosy patients, mostly

tuberculosis patients reported stigma features as compared to others. Across the sites, experienced stigma was higher among non-leprosy group than in the leprosy group whereas the reverse was true for anticipated stigma.

Health system preparedness for management of non-communicable diseases (NCDs), Koraput, Odisha

Health care facilities in India are inadequately equipped to address the increasing burden of NCDs. A cross-sectional survey of primary and secondary health care facilities including the providers and patients in Koraput district, Odisha was conducted. Overall, 131 patients were surveyed of whom most were females, 30% above 60 years of age. Tobacco use was prevalent among half of the patients. Among these patients, 97 had hypertension while 34 had diabetes. Median duration of treatment of diabetes or hypertension in the previous year was 6 months. Median time taken to reach nearest government health facility was 30 minutes. Nearly 37-44% patients did not take treatment or took irregular treatment for hypertension and diabetes. Most (58-71%) of the patients reported lack of availability of drugs in the public sector facility. The median out of pocket expenditure for one time purchase of drugs was ranged from Rs. 500–800 for various NCDs. Only one fourth of the patients had their blood pressure under control (Table 1).

Fourteen community health centres (CHCs), one district hospital and one sub-district hospital were surveyed. All facilities had doctors, however, there was a lack of training of doctors for the management of NCDs. There were no clinical management protocols for outpatient or inpatient treatment of NCDs. There was poor availability of essential drugs for both hypertension (12/14 CHCs and 2 GH but quantity inadequate) and diabetes (9/14 CHCs and one district hospital). Nineteen doctors were surveyed, among whom 14 were MBBS and five had Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH) qualifications. Most were providing treatment for hypertension, diabetes and chronic respiratory disease, however, only a few provided treatment for ischaemic heart disease (IHD)/stroke. Most (12/19) knew the correct definition for hypertension diagnosis, but only five knew correct definition for diabetes diagnosis.

Primary and secondary care facilities should be strengthened in terms of trained manpower, laboratory facilities, adequate quantity of essential drugs and patient information systems to deliver good quality NCD patient care. Community should be sensitized regarding modification of behavioural risk factors and need for regular treatment should be emphasized.

| Table 1: Fact sheet: Patient survey | |
|---|--------------|
| Demographic characteristics (%) (N=131) | % |
| Age >60 years | 31 |
| Females | 59 |
| Never attended school | 57 |
| Labourer (skilled/unskilled/ agricultural) | 17 |
| Risk factors | |
| Ever smoking among males (N=54) | 56 |
| Ever smokeless tobacco use | 52 |
| Ever used alcohol | 33 |
| BMI below 18.5 kg/m ² | 29 |
| BMI ≥ 23 kg/m ² | 36 |
| Treatment characteristics | |
| Median duration of diabetes, hypertension treatment in the previous year | the 6 months |
| Place of treatment: CHC(%) | 44-67 |
| Place of treatment: Private doctors (%) | 9-12 |
| Counselling on diet or physical activity lifestyle modification by government medical staff (%) | 63 |
| Median time taken to reach nearest government health facility | 30 min |
| Hypertension patients (N=97) | |
| % | |
| Availability of prescription | 18 |
| Prescribed amlodipine | 7 |
| Prescribed atenolol | 5 |
| Treatment not taken/Irregular treatment in previous one month | 37 |
| Lack of availability of drugs in public sector facility | 58 |
| Achieved BP control (Systolic <140mmHg, Diastolic <90 mmHg) | 22 |
| Median out of pocket expenditure for one time purchase of drugs (N=90) | Rs 500 |
| Diabetes patients (N=34) | |
| % | |
| Availability of prescription | 26 |
| Prescribed glibenclamide | 21 |
| Prescribed metformin | 12 |

| | |
|--|--------|
| Treatment not taken/Irregular treatment in previous one month | 44 |
| Lack of availability of drugs in public sector facility | 71 |
| Median out of pocket expenditure for one time purchase of drugs (N=29) | Rs 500 |

Cause of death in tribal areas, Koraput, Odisha

Mortality data is useful for prioritizing the health problems and to appropriately allocate health resources. A cause of death survey in Koraput district, Odisha was conducted that had more than 50% Schedule tribes (ST) population. A community-based cross-sectional survey was also conducted, of 440 deaths that occurred in previous one year among adults more than 15 years of age using verbal autopsy in rural Koraput. Overall, 52% of the deceased were above 60 years of age, proportion being higher for males. Majority (86%) never attended school and were below the poverty line (BPL) card holders (90%). Three fourth belonged to ST. Home was the most common place of death (83%). Nearly half the deceased had history of smoking while 64% had history of alcohol use, proportion being higher amongst males as compared to females. Chronic respiratory disease was the most common pre existing disease (22%) followed by hypertension (19%) and tuberculosis (13%).

Overall, non-communicable diseases accounted for more than half of the deaths (55%) followed by infectious diseases (28%) and other causes (17%). Cardiovascular diseases (25%) followed by

neoplasm (10%) were the leading cause of death due to NCDs. Age standardized cause specific death rate (CSDR) was highest for NCDs, among both males and females. Among the infectious diseases, nearly half of the deaths were due to tuberculosis (Table 2).

Non-communicable disease control should be one of the health priorities for Koraput district. Intersectoral interventions that target tobacco and alcohol use should be initiated in the district. Primary care facilities should be strengthened to provide screening and treatment for hypertension and chronic respiratory disease to prevent mortality due to the complications of these diseases. Revised National Tuberculosis Control Programme (RNTCP) in the district should be strengthened for enhance case detection and treatment compliance to reduce mortality due to tuberculosis.

HIV Sentinel Surveillance (HSS)

Antenatal Clinic (ANC) attendees in the HSS centres are considered as the surrogate group for general population in the age group of 15-49 years. HSS undertaken by National AIDS Control Organisation (NACO) is an ongoing systematic collection, collation, analysis and interpretation of data periodically, which helps to calculate HIV load in the country and to take appropriate action within stipulated time. HSS carried out by NACO with the guidance of Tamil Nadu State AIDS Control Society (TANSACS) generated data to improve tracking of HIV trends, as well as to understand the epidemic's characteristics and its level of proliferation across

Table 2: Fact sheet for Cause of Death

| | | Male (N=264) | Female (N=176) | Overall (N=440) |
|---------------------------------|--------------------------|-----------------|-------------------|--------------------|
| Demographic characteristics (%) | Age \geq 60 years | 49 | 57 | 52 |
| | Never attended school | 78 | 96 | 86 |
| | Availability of BPL Card | 91 | 89 | 90 |
| | ST | 71 | 75 | 73 |
| | Place of death- Home | 79 | 88 | 83 |
| Risk factors (%) | Smoking | 67 | 29 | 52 |
| | Alcohol consumption | 77 | 45 | 64 |

| | | | | |
|--|---|-----|-----|-----|
| Pre existing disease (%) | Chronic respiratory disease | 24 | 20 | 22 |
| | Hypertension | 20 | 18 | 19 |
| | Tuberculosis | 17 | 7 | 13 |
| Deaths in three main categories (%) | Non-communicable diseases | 56 | 53 | 55 |
| | Infectious diseases | 30 | 26 | 28 |
| | Others | 14 | 21 | 17 |
| Age standardized cause specific death rate per 100, 000 population (All age group) | NCD | 715 | 427 | 562 |
| | Infectious diseases | 377 | 209 | 288 |
| | Others | 183 | 163 | 172 |
| Age standardized cause specific death rate per 100, 000 population (30-70 years) | NCD | 891 | 587 | 749 |
| | Infectious diseases | 484 | 279 | 385 |
| | Others | 261 | 196 | 231 |
| Major causes of death (%) | Circulatory system (I00-I99) | 26 | 23 | 25 |
| | Infectious and parasitic diseases (A00-B99) | 28 | 23 | 26 |
| | Neoplasm (C00-D48) | 6 | 15 | 10 |
| | Respiratory system (J00-J99) | 7 | 7 | 7 |
| | Digestive system(K00-K93) | 9 | 2 | 6 |
| | External causes (injuries/suicides) (S00-Y98) | 4 | 3 | 4 |
| | Mental and behavioral disorder (F00-F99) | 2 | 2 | 2 |

the geographical areas of Tamil Nadu. The HSS has been carried out by NIE since 2006. In the current round of surveillance, a total of 97721 blood samples were collected from the States of Tamil Nadu, Andhra Pradesh, Telengana, Karnataka, Kerala, Orissa and Puducherry. The same were entered into SIMS, an online data management package. The provisional HIV positivity rates are provided in Table 3.

Table 3: HIV Sentinel Surveillance: provisional HIV positivity rates

| State | No. Tested | No. Positive N (%) |
|----------------|------------|--------------------|
| Telangana | 9995 | 39 (0.39) |
| Karnataka | 24712 | 90 (0.36) |
| Andhra Pradesh | 15095 | 50 (0.33) |
| Tamil Nadu | 28773 | 77 (0.27) |

| | | |
|--------------|--------------|-----------|
| Orissa | 12752 | 31 (0.24) |
| Pondicherry | 800 | 1 (0.13) |
| Kerala | 5594 | 3 (0.05) |
| Total | 97721 | |

Integrated Biological and Behavioural Surveillance (IBBS)

This surveillance was mainly focused on high risk groups (HRG) especially on female sex workers (FSW), men who have sex with men (MSM), injecting drug use (IDU) and Migrants. Since epidemic in India is classified as of a concentrated nature, control strategies on HRGs will bring down the HIV prevalence. Trainings were given to the staff of concerned States (TN, AP, Kerala & Puducherry) on the methodology of IBBS. In the current round of surveillance, a total of 17233 blood samples were collected from the States of Tamil Nadu,

Andhra Pradesh, Kerala, and Puducherry. The same were entered into TABLETS and simultaneously transferred into the central server located at NACO. The provisional HIV positivity rates among various high risk groups (district-wise) are provided in the following tables (Table 4a, 4b, 4c, and 4d).

Table 4: Integrated Biological and Behavioural Surveillance: provisional HIV positivity rates among various HRGs (district-wise)

Table 4a: Kerala

| Domain Name | Typology | No. Tested | No. Positive N (%) |
|----------------|----------|------------|--------------------|
| Thrissur | FSW | 141 | 3 (2.13) |
| Kozhikode | FSW | 293 | 2 (0.68) |
| Pathanamthitta | FSW | 400 | 0 (0.00) |
| Ernakulam | IDU | 299 | 3 (1.00) |
| Kozhikode | IDU | 270 | 0 (0.00) |
| Alappuzha | IDU | 311 | 0 (0.00) |
| Trivandrum | MIG | 426 | 0 (0.00) |
| Ernakulam | MIG | 938 | 2 (0.21) |
| Ernakulam | MSM | 263 | 7 (2.66) |
| Kasargode | MSM | 346 | 0 (0.00) |
| Kollam | MSM | 308 | 4 (1.30) |
| Kollam | TG | 245 | 6 (2.45) |

Table 4b: Tamil Nadu

| Domain Name | Typology | No. Tested | No. Positive N (%) |
|----------------|----------|------------|--------------------|
| Thiruvavur | FSW | 352 | 2 (0.57) |
| Chennai | FSW | 370 | 1 (0.27) |
| Madurai | FSW | 387 | 8 (2.07) |
| Erode | FSW | 345 | 7 (2.03) |
| Tiruppur | MIG | 1172 | 0 (0.00) |
| Thanjavur | MSM | 379 | 14 (3.69) |
| Tiruvannamalai | MSM | 385 | 7 (1.82) |
| Sivaganga | MSM | 400 | 1 (0.25) |
| Dindigul | MSM | 350 | 32 (9.14) |
| Namakkal | MSM | 329 | 6 (1.82) |
| Chennai | TG | 363 | 22 (6.06) |
| Coimbatore | TG | 388 | 9 (2.32) |

Table 4c: Andhra Pradesh

| Domain Name | Typology | No. Tested | No. Positive N (%) |
|---------------|----------|------------|--------------------|
| Nellore | FSW | 310 | 2 (0.65) |
| Chittoor | FSW | 388 | 15 (3.87) |
| Adilabad | FSW | 300 | 4 (1.33) |
| Mahabubnagar | FSW | 389 | 50 (12.85) |
| Guntur | FSW | 360 | 28 (7.78) |
| Nellore | IDU | 338 | 9 (2.66) |
| Hyderabad | IDU | 276 | 13 (4.71) |
| Krishna | MIG | 877 | 29 (3.31) |
| Chittoor | MIG | 1130 | 19 (1.68) |
| Hyderabad | MIG | 674 | 3 (0.45) |
| East Godavari | MSM | 396 | 20 (5.05) |
| Anantapur | MSM | 300 | 31 (10.33) |
| Warangal | MSM | 388 | 60 (15.46) |
| Guntur | MSM | 386 | 43 (11.14) |
| Hyderabad | TG | 198 | 26 (13.13) |
| Krishna | TG | 294 | 21 (7.14) |

Table 4d: Puducherry

| Domain Name | Typology | No. Tested | No. Positive N (%) |
|-------------|----------|------------|--------------------|
| Pondicherry | FSW | 391 | 4 (1.02) |
| Pondicherry | MSM | 378 | 9 (2.38) |

Health impact of quarry works in Suburban areas of Chennai

The main objective of this study was to measure the health impact of quarry works among the residents within 5km radius from the quarries and to measure the environmental parameters in the study area. The study was initiated during February 2014 with a total number of 2299 samples including 1973 from the community residing near the quarries and 326 workers working in the quarries. X-ray was done for all the participants whereas the sputum collected only for the symptomatic and the cases with X-ray positive. Spirometry test was done for selected participants identified by random selection method. The number of positive TB cases as confirmed by laboratory diagnosis was 2/1973 from the community and 3/326 from the quarry workers with an average rate per 100,000 of 101.4 and 920.2 for each of the groups respectively. Data entry and verification are presently complete and

some important preliminary tables are presented as follows (Table 5a and 5b).

| Group | Male | Female | Total |
|--|-------|--------|-------|
| N | 904 | 1069 | 1973 |
| Mean age (yr) | 42.5 | 41.4 | 42.9 |
| Mean BMI (kg/m ²) | 23.76 | 25.48 | 24.7 |
| Abnormality both in X-ray and symptoms | 5.4 % | 3.7 % | 1.5 % |

| Group | Male | Female | Total |
|--|-------|--------|-------|
| N | 250 | 76 | 326 |
| Mean Age (yr) | 37.7 | 41.5 | 38.6 |
| Mean BMI (kg/m ²) | 21.78 | 22.37 | 21.93 |
| Abnormality both in X-ray and symptoms | 17.6% | 14.5% | 16.9% |

The above analysis shows that *TB* and *lung function abnormalities* are more prevalent among the quarry workers. Further, the environmental parameters are presently being collected by Sathyabama University separately (one of the collaborators of this study). After completion of collection of environmental parameters, the desired spatial risk model will be developed.

Prevalence and intensity of soil transmitted helminths among the government primary school children in Madhya Pradesh and Bihar

Soil transmitted helminths (STH) are one of the most common infections worldwide and affect the poorest and most deprived communities. Although several Indian States are conducting school-based de-worming programmes, reliable estimates of STH prevalence and intensity are needed before making large-scale periodic treatment as well as to monitor the performance of these programmes. Surveys were conducted in Madhya Pradesh and Bihar to estimate the prevalence and intensity of STH. In Madhya Pradesh, 7522 children were surveyed studying in 264 schools in 44 blocks of 21 districts covering 11 agro-climatic zones of Madhya Pradesh during September – October 2014. Stool samples were collected from 5,767 children, of

which, 5619 were examined for presence of STH, using the WHO recommended Kato Katz method. In Bihar, 65 sentinel government primary schools were selected from 15 districts covering the three agro-climatic zones of the state and stool samples from 3250 were examined. The overall weighted prevalence of any STH in the State of Madhya Pradesh was 12.2% (95% CI: 7.1-17.4). The prevalence in different agro-climatic zones ranged from 0.5% (Malwa plateau) to 26.9% (Jhabua hills). Hookworm was the most prevalent STH, with a weighted prevalence of 12.0% (95% CI: 6.8-17.1). In Bihar, the weighted prevalence of any STH was 35% (95% CI: 24-45). Roundworm and hookworm were the most prevalent STH infections. Majority of the STH infections were of light intensity. The STH prevalence was higher in the northern, northeastern and northwestern districts of Bihar, which corresponds to North west and North east alluvial plains respectively (Fig. 4).

The WHO recommends annual treatment in areas where STH prevalence is between 20 and 50%, and bi-annual treatment in areas with prevalence rates of over 50%. In Bihar, continuation of single dose mass de-worming was recommended in schools in all the districts of the State with good documentation of its coverage and periodic surveys in sentinel schools to monitor the performance of deworming programmes. In Madhya Pradesh, although the prevalence of any STH in was >20% in only one agro-climatic zone, in view of risk of re-infection, it was recommended that annual mass-deworming campaigns should be conducted in schools in all districts.

HEALTH SYSTEMS RESEARCH (HSR)

AD-HOC PROJECTS

Adolescent health care

Various health systems related issues have been covered under ad-hoc projects. Some of these have addressed various health problems of adolescents while others have studied the services of government health system. A study from Karnataka focused on adolescent health issues pertaining to males, which were neglected in most studies. This qualitative study brought out several issues pertaining to reproductive

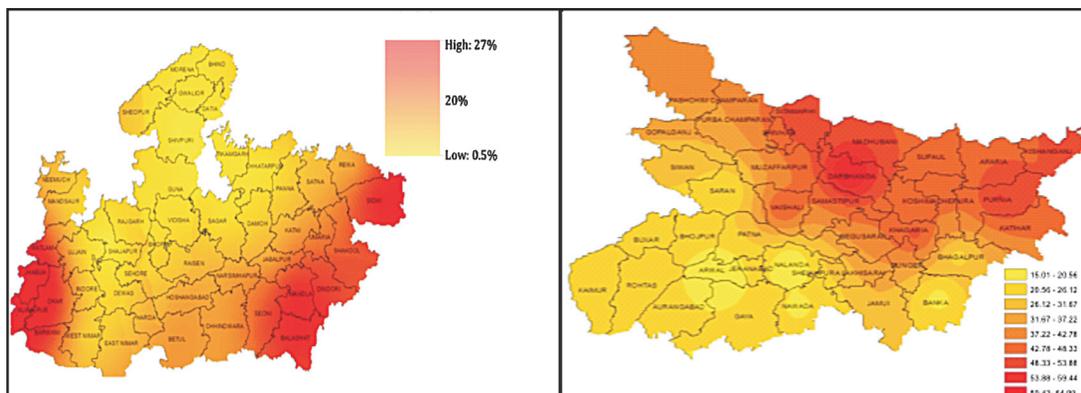


Fig. 4. Predicted prevalence of STH in Madhya Pradesh (left) and Bihar.

health, emotional changes and premarital sex from the perspective of adolescent boys. It was found that boys were aware of the physical and emotional changes that they undergo during adolescence. Boys in the age-group of 16-19 years were aware of premarital sex, rather than the younger group of 13-15 years. All adolescents involved in this study agreed that premarital sex is socially unacceptable. The study noted that the mothers were aware of various issues of adolescence but were hesitant to discuss regarding sexual health matters with their sons. However, the awareness regarding adolescent health issues was low among fathers. Teachers were hesitant to discuss these issues with students. Also, the teachers group informed that there was no sufficient training for the teachers to guide their students. The teachers accepted that they were not comfortable to discuss these issues in school and also expressed concern that the students would misinterpret these talks. Sex education and its importance of being provided in schools were stressed by the teachers. During discussion with the adolescent boys, it was found that none of them were aware of any service or helpline to solve their doubts regarding adolescence related issues. Most of them agreed that they would avail services from doctors and other health care providers, who could guide them in a friendly manner. They had mixed opinions about peer-counselling, but they did highlight their need for discussing their health queries.

Another study that is in progress in the Karnataka State reports the reproductive and health problems of adolescent girls. This study is ongoing and is documenting the access and utilization of various adolescent health programmes in two

districts while another study also from Karnataka, recently completed the development of tools, and is currently studying the prevalence of smokeless tobacco use and its risk factors among adolescents in Karnataka.

Gender perspectives of health

Understanding gender perspective of health and health care is another area of interest of HSR. A study from Rajasthan has developed a competency framework for mainstreaming gender-responsive health services. It assessed the health care providers' understanding and perspective on gender equality and equity with a focus on adolescent health care services. The competency framework is being finalized for providing gender-responsive services by various levels of health care providers, including quality control, standard operating procedures using structure, processes and outcome framework of quality. This study is based on data collected from different levels of health care providers from six districts of Rajasthan.

Health care delivery and access

Studies pertaining to various issues of health care delivery and access have been undertaken. Health insurance being an important issue, efforts to achieve universal health care are presently underway in Karnataka State. One such study reported the awareness and coverage of health insurance and other related issues related to the acceptance of health insurance by the community. It was found that about 72% of rural households in southern Karnataka were aware of health insurance. Furthermore, they obtained related information and

also availed the health insurance through women self-help groups. Government insurance schemes were popular among them and also adopted by these households. A significant proportion of households (about 30%) expressed their willingness to pay upto 500-1000 as a yearly premium. Also, a majority of these people preferred government hospitals to private hospitals for major illnesses. Some studies are also devoted to understanding health care access issues, particularly among vulnerable population groups. One such study from Kerala, studied the maternal and child health care issues among the scheduled tribes with a special focus on health infrastructure and manpower deployment. This study initially highlighted the health and nutritional status of children in Wayanad tribal district. The results revealed that the undernourished status of the *Paniya* tribal children was high compared to other groups in the region. The higher levels of wasting, stunting and underweight indicated chronic malnutrition. Other diseases like skin diseases, atopic dermatitis, vitamin deficiencies and bronchial asthma were also reported to be high. The overall health and nutritional status was very poor, mainly due to lack of awareness in the community, in addition to non-availability of health services and poor infrastructure in the existing health facilities. Another study from Mumbai has been initiated recently and is being undertaken as a geo-spatial analysis of accessibility of health care services by migrant pockets spread across the city. This study is based on geographic information system (GIS) technology.

Other HSR issues

HSR Division has funded some other areas of interest to health systems research. A study undertaken in a nursing college in Karnataka documented the impact of supervisory support training programme on the performance of health workers posted at PHCs. This study reported the level and problems related to supervision in PHCs. A supervisory support training module is also being developed for the use by the health assistants. Furthermore, it is also evaluating the impact of supervisory support training programme in terms of supervisory satisfaction among health assistants and health workers. Another study has been initiated recently in Coimbatore district of Tamil Nadu for developing patient friendly IT-enabled tools for selection of desired health care services. Multiple

criteria that influence the selection of desired health care services have been finalized and data collection is under progress. Another study has been initiated in Uttarakhand to develop a model for vital registration and communication among health care providers through mobile technology. A study aimed at understanding the reasons for low utilization of health care services for treatment of sexually transmitted infections in Bhopal city of Madhya Pradesh has also been undertaken, while a study from Chandigarh, initiated during this year is aimed at assessing the approaches and factors in increasing attraction and improving retention of physicians in rural and remote areas.

Multi-centric task force intervention projects for improving migrants' health care and health system's responsiveness

A study on the community-based interventions to improve the health care access among internal migrants living in 13 cities was completed during the current year. Of the 13 cities, six were metro cities (Delhi, Kolkata, Mumbai, Hyderabad, Bengaluru and Lucknow) and seven were fast growing smaller cities (Aligarh, Bhubaneswar, Imphal, Jaipur, Ludhiana, Nasik and Visakhapatnam).

Based on the findings of a comprehensive formative research, the interventions were implemented. The interventions were implemented through local health system in systematically selected clusters along with an equal number of control clusters. All these clusters were inhabited by recent (<10 years of migration) migrants. The key factors of this intervention were the partnerships with State/municipal health officials, non-governmental organization and industries. The supply-side issues were also addressed by these partners. Community mobilization activities were also undertaken to improve the demand for health care. These activities were undertaken by the local health institutions in collaboration with local non-governmental organizations and community-based organizations. The data collection of the impact evaluation is presently complete and data from all 13 centres are to be collated for combined analysis. The preliminary observations, based on the centre-wise reports received so far revealed that both responsiveness of the health system and access to migrants improved in intervention clusters, are compared to the control clusters. There were considerable differences

between pre- and post-intervention data, related to some health care indicators. Geographical coverage as well as the quality of services were also improved in some clusters. Qualitative data revealed various dimensions of partnership building and community mobilization.

INNOVATION AND TRANSLATIONAL RESEARCH

Non – invasive rapid diagnostic test for kala-azar (Visceral leishmaniasis) using urine samples

Approximately 200 million persons in the Indian subcontinent are at risk for visceral leishmaniasis (VL). It is usually reported from Bihar, Jharkhand, West Bengal and Uttar Pradesh, and the northeastern States of the country. Of these, the State of Bihar contributes >80% of Indian VL cases, where 33 out of 38 districts are endemic. A non-invasive method was developed at Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, using urine as a medium for the diagnosis of VL. The technology was released in September, 2014 by the then Hon'ble Health Minister. In this non-invasive method the diagnosis is made by the use of rK39 immunochromatographic strip test using urine samples. The buffer used for the test has been prepared indigenously. Sodium azide (NaN_3) is being used as the buffer in this method. It is a preservative and also acts as a non specific binding agent. Urine can be collected easily from VL patients in the field, hence, the acceptance of providing urine samples will be high as compared to blood. The technology has been independently validated with its sensitivity and specificity 90 and 100% respectively in parasitologically confirmed cases of VL. This diagnostic kit can be implemented in the kala-azar elimination programme.



Non – invasive rapid diagnostic test for kala-azar (Visceral leishmaniasis) using oral fluid samples

Another indigenous non – invasive rapid diagnostic kit for detection of visceral leishmaniasis (VL) was developed at RMRIMS, Patna and was released in September, 2014 by the then Hon'ble Health Minister. The commonest method for diagnosis of VL in the field is by rK39 immunochromatographic strip test for screening and clinical correlation. For this purpose blood or serum is required. The confirmatory method for diagnosis is by demonstration of amastigotes of *Leishmania donovani* in splenic / bone marrow aspirates. A diagnostic kit has been developed where rK39 immunochromatographic strip test is used in sputum (oral fluid) as a medium instead of blood /serum for the diagnosis of VL. It is a simple, rapid, easy-to-perform and purely non-invasive method and needs no special equipment or sophisticated technology. The results can be read visually within 15 minutes. Sputum is easy-to-collect and, therefore, acceptance from the affected community will be high. The technology has been independently validated and its sensitivity and specificity is 99 and 100%, respectively in parasitologically confirmed cases of VL.



Affordable technologies transferred to Industry for commercialization

(i) The first indigenously developed “Japanese encephalitis vaccine (JENVAC)” under Public Private Partnership (PPP) was developed. The indigenous virus strain was isolated and characterized by National Institute of Virology (NIV,) Pune, which was transferred to Bharat Biotech, New Delhi,

for further vaccine development. In September, 2015, an MoU has been signed between NIV and HLL biotech, Chennai for transfer of JE strain for manufacturing JE killed vaccine. (ii) An Indigenous “Thalassemia Detection Kit” developed at National Institute of Immunohaematology (NIIH), Mumbai has been transferred to Imgenex India Pvt. Ltd, Bhubaneswar, with contract manufacturing. (iii) An Indigenous affordable technology “PCR Based Food Borne Pathogens Detection Kit” was developed at National Institute of Nutrition, Hyderabad in collaboration with an Industry Bioserve Biotechnologies Pvt. Ltd., Hyderabad. (iv) Monoclonal antibody to *Chlamydia trachomatis* was developed at National Institute of Pathology (NIOP), New Delhi. This is a monoclonal antibody based indigenous diagnostic assay for detection of *C. trachomatis* in women and is licensed to M/S Accurex for commercialization. (v) Urine based immunodiagnostic kits for assessment of infertility (for EIG, PdG, LH and FSH) developed at National Institute for Research in Reproductive Health (NIRRH), Mumbai was transferred to HLL Lifecare Ltd, Trivandrum. (vi) A process for the preparation of mosquito larvicidal formulation from *Bacillus thuringiensis* var. *israelensis* was developed at Vector Control Research Centre (VCRC), Puducherry. During the year, the technology has been licensed M/s Fine trap, Yavatmal (10th licensee).

Following technologies have been transferred to M/s Soothe Healthcare, Greater Noida

(i) An indigenous device “AV Magnivisualizer” for screening and detection of Cervical Cancer developed at Institute of Cytology and Preventive Oncology (ICPO), Noida; (ii) Simple and affordable technology for “Glucose monitoring devices and testing strip- QuickcheQ” developed at BITS, Hyderabad; (iii) An indigenous affordable technology “Elisa kit for Ferritin estimation” developed by National Institute of Nutrition, Hyderabad; (iv) An Indigenous affordable technology “Dried Blood Spot (DBS) collection kit for Vitamin A analysis” developed by National Institute of Nutrition, Hyderabad; (v) Recombinant protein based assay for diagnosis of Hepatitis E developed at National Institute of Virology (NIV), Pune; (vi) Personal Cooling Garment system

developed at National Institute of Occupational Health (NIOH), Ahmadabad; (vii) Test/reagents for lung fluke useful for differential diagnosis from lung TB developed at Regional Medical Research Centre (RMRC), Dibrugarh.

Technologies ready for release

The following affordable ICMR technologies are ready for release:-

(i) IgG ELISA diagnostic kit for paragonimiasis (lung fluke infection) developed at RMRC, Dibrugarh; (ii) Recombinant IgM ELISA for the diagnosis of Leptospirosis developed at RMRC, Port Blair; (iii) Latex based agglutination technique for the detection of leptospiral antibodies during acute stage of the disease developed at RMRC, Port Blair; (iv) PCR based diagnostic kit for *Chlamydia trachomatis* developed at NIRRH, Mumbai; (v) Personal Cooling Garment (PCG) system developed at NIOH, Ahmedabad; (vi) Resazurin Reduction Test (RRT) Kit to Assess Sperm Quality in Men developed at NIRRH, Mumbai; (vii) Urine based immunodiagnostic kits for assessment of infertility (for EIG, PdG, LH and FSH) developed at NIRRH, Mumbai; (viii) Recombinant protein-based assay for diagnosis of hepatitis E developed at NIV, Pune; (ix) Diagnostic kit for *C. trachomatis* in reproductive tract infection developed at NIOP, New Delhi.

SOCIAL & BEHAVIOURAL RESEARCH

During the reporting period of 2014-2015, 12 projects were completed, 11 new projects in the priority area of gender and health and two fellowships were funded.

A community based approach in designing a model TB sensitization programme for self help groups (SHGs) – A study from South India

Tuberculosis (TB) continues to be a major public health problem, innovative community-based TB prevention and intervention strategies aimed at TB control are crucial. A powerful community task force has gained momentum in many districts in South India by way of self help groups. These groups could be involved in TB control activities considering their reach in communities especially at the grass root level. A community driven TB

sensitization model based on participatory action approach using self help groups in TB control activities was, therefore, explored.

This was an experimental study done in three phases and involved a quantitative and qualitative design. The first phase was the qualitative phase to test the acceptability of SHGs in the community and the level of awareness of TB. The second phase was to develop a TB sensitization intervention strategy through a community based approach. The third phase was a randomized control trial to test the intervention through an experimental design. The sample included 1560 SHG representatives (796 in the intervention and 764 in the control group). Participants were assessed at 0, 3 and 6 months.

At the baseline, assessment regarding their involvement in health in general and their willingness in getting involved in TB control activities in particular was determined. It was found that 84% in control and 88% in intervention groups had never been involved in any health related activities. In both the groups, more than 95% of the members were willing to get involved in TB control activities.

The involvement of SHGs in TB control activities was analyzed at both 3rd and 6th month assessments using three key parameters namely spreading awareness of TB, identification and referrals of chest symptoms and registration as directly observed treatment short-course (DOTS) providers which was compared in both groups. It was observed that at the end of 3rd month, a significantly higher proportion of members in the intervention group had involved in TB advocacy, identified symptoms and referred them to the primary health centres (PHCs) when compared to their counterparts in the control group. However, at the end of the sixth month, it was observed that a significant difference was only observed in terms of identification and referral of symptoms and not in other activities between the intervention and control group members.

A community driven TB sensitization model based on participatory action approach using self help groups in TB control has proven to be an effective strategy in TB control. Once sensitized, they can be considered a powerful task force in promoting TB awareness, identification and referral of

chest symptoms and also as community DOTS providers.

Intervention to enhance acceptance of contraceptive use among couples by reducing domestic violence from husband

A community level intervention study was planned in a Mumbai slum community to enhance the contraceptive use by reducing domestic violence among women of age group 18-39 years, having at least one child, living with husband and reporting an unmet need for family planning. A total of 1136 women were selected using systematic random sampling and a structured questionnaire was administered. The information regarding contraceptive behaviour and domestic violence was collected at baseline or pre-intervention survey. Two groups were formed, group A, women reporting unmet need for family planning and domestic violence in the past 12 months and group B, women reporting unmet need for family planning and no domestic violence. According to the intervention model, family planning counselling was given to all women. Additional marital communication skill counselling was provided to women belonging to group A, post-intervention survey showed that there was a reduction in prevalence of domestic violence, and increase in awareness of contraceptive methods. About 60 per cent women were using contraceptive methods at the time of post-intervention survey indicating decrease in unmet need for family planning. The study clearly demonstrated the reduction in domestic violence, increase in awareness of contraceptive methods and increase in contraceptive use through an effective intervention model.

Effectiveness of integrated treatment for alcohol use and intimate partner violence in men with problem drinking in South India

Alcohol use is a growing public health problem with immense ramifications to the individual and society. Heavy drinking episodes are associated with various high risk behaviours with acts of violence against one's spouse being an important consequence of alcohol abuse. Intimate partner violence that encompasses acts of physical violence, emotional and sexual abuse is frequently encountered in the context of heavy alcohol use.

This randomized controlled trial looked at the impact of culturally adapted enhanced treatment module compared to standard intervention in reducing intimate partner violence and reducing depression and anxiety among wives and children of male alcohol dependent patients. Results revealed that enhanced treatment was superior to standard intervention in reducing acts of spousal abuse and violence and improving mental health status of spouses of alcohol dependent subjects. In addition, the improvement was sustained upon three months follow up. In both standard intervention and enhanced treatment groups the severity of alcohol use decreased significantly. Another important finding relates to prevailing gender norms that contributed significantly to acts of spousal abuse. Thus, treatment approaches that specifically target spousal abuse in the context of heavy alcohol use needs to be incorporated as part of intervention offered to individuals and families of patients with harmful use of alcohol.

Quality of life (QoL) of tribal people: Development of household and village based multi-factor indices

Quality of life refers to the overall wellbeing of an individual, community or country. It has important implications in individual health care, as an indicator of health in regional and national levels as well as for international comparison vis-a-vis political and economic development. There is a need to develop culturally relevant indices to measure the QoL in tribal communities in India. This study was done to develop a multi-level (household and village level) multi-factor (including 9 important factors of QoL) QoL index to assess the QoL in the Malayali tribal community in Tamil Nadu. After a thorough review of literature and primary situational analysis in the tribal areas, a comprehensive index was developed including nine domains namely, demography, health, economic condition, work participation, infrastructure, social capital, recreation, self perceived QoL and development index. The index was piloted and checked for face, content and linguistic validity. Multi-stage systematic random sampling was done using probability proportion to size method to select a total of 3985 individuals from 1096 households of Malayali tribes from Salem, Namakkal districts of Tamil Nadu. The data

were collected using palm top computers on to Epi Info package version 3.2.5. The data were analyzed using simple descriptive statistics and multilevel factor analysis to check construct validity. The survey revealed that the average family size was four, with predominance of nuclear family type. The prevalence of any morbidity in the past one month was about 15%. Treatment seeking behaviour was high and they predominantly sought treatment from private health facilities. Antenatal registration, antenatal care, immunization coverage were very high. Institutional delivery rates were low at 58% despite high antenatal coverage. Illiteracy was high at 55% and unemployment was also high at 30%. The tribal communities were deprived of access to safe potable water and sanitation facilities. Social capital was high in terms of membership in women's SHGs. The dominant form of recreation was television watching, chatting, taking a nap and spending time with friends. Traditional games, music and dance were not common. Self perceived QoL was reported to be high in the domains of social relationships, cultural values and environmental condition but said to be poor in terms of education, employment and income. Using these factors, a multilevel multi-factor index was developed by standard techniques. The index was analyzed using an exploratory factor analysis using a multilevel model, one at household level and one at the village level. It was found that the construct of QoL in the household level grouped into three main factors namely health, socio-demographic and economic factors. At the village level the construct of QoL grouped into three factors two of which included economic factors and one indicated a good perceived QoL despite poor infrastructure. The QoL of the sample was measured using this index. It was found that about 30% of the surveyed households had poor QoL and 4% had excellent QoL. At the village level about 37% of the villages had poor QoL. This multi-factor multi-level index can be used to measure the QoL at the household and village levels among tribal communities. It should however, be validated on a larger scale using predictive and concurrent validity methods. It should also be adapted and developed at a larger scale to cover other tribal communities in India. The index can be useful for future planning, delivery and evaluation of tribal development programs.

Community-based brief intervention vs simple advice for women tobacco users

Women in this community were predominantly (94%) smokeless tobacco users. The commonest smokeless tobacco products used by women were *gul* and *paan* (20% each). The mean age of initiation of tobacco was 26 years. The most commonly reported reason for consumption of tobacco products was dental problems followed by peer pressure. Most women (81%) were aware that tobacco use was harmful and majority reported that they were very interested in quitting tobacco use. A substantial proportion (40%) reported cancer as one of the major harms of tobacco use. However, awareness of other harms was very low.

About 46% of women had never previously attempted to quit tobacco use and the mean number of quit attempts among those who tried to quit was very low (1.34 ± 0.793). A quarter of women reported concerns about health as a reason for quitting attempt, followed by family pressure to quit. Most women tried to quit using self-help alone. Only 6% sought medical help for quitting tobacco. The median duration of abstinence during a quit attempt was 10 days. Dental problems were the most common reason reported for relapse to tobacco use. Self-help alone (57%) and advice and guidance only (52%) were perceived as adequate interventions to quit tobacco use amongst these women.

The subjects in the brief intervention were twice as likely to stop tobacco use as compared to individuals in the simple advice group. The odds of stopping tobacco use among subjects in the brief intervention was 2.236 (95% CI: 0.962-5.197).

Stress, anxiety and depression among adolescents attending coaching institutes: Kota (Rajasthan) – The coaching hub of India

The present study was accomplished to examine the level of stress, anxiety and depression faced by adolescents who were taking coaching for pre-medical and pre-engineering exams in Kota, Rajasthan. A total of 400 students seeking coaching for medical college and 400 for engineering college, almost equally divided between males and females, were included in the study. The key findings on the variables of stress supported the pre-generalized

notion that adolescents preparing for pre-medical competitive examinations were more stressed than those preparing for pre-engineering competitive examinations. The anxiety level of adolescents was measured on four constructs: autonomic arousal, skeletal muscle effect, situational anxiety, and subjective experience of anxious effect. The major findings suggested that about one third of the adolescents reported moderate level of anxiety, which was followed by normal, severe, extremely severe and mild level of anxiety. A substantially large number of students (approximately 30 per cent) were found to be depressed at moderate level. The girls were found to be more depressed and having the feeling of hopelessness, sadness, decreased level of enthusiasm, anhedonia, inertia, etc. The final analysis of stress, anxiety and depression revealed that extremely severe cases were found more in medical students rather than in engineering.

NATIONAL INSTITUTE OF MEDICAL STATISTICS, NEW DELHI

COMPLETED STUDIES

Knowledge Network Project on HIV/AIDS

A Knowledge Network was formed to review the data gaps in the global literature on research and programmes involving truckers and clients of female sex workers and collaborate with the *Avahan* partners to document the programmatic learning. The consortium included the National Institute of Medical Statistics (NIMS), New Delhi; National AIDS Research Institute (NARI), Pune; St. John's Medical College Research Institute, Bengaluru; Institute of Economic Growth, Delhi; Tata Institute of Social Sciences, Mumbai; and Family Health International, Delhi. The activities included participation in training and mentoring workshops on scientific writing; work on different papers and disseminate programmatic learning by way of presenting research findings in national and international conferences / seminars / workshops / peer-reviewed journals. The study published six research articles in journals of national and international repute.

A study on the potential gain in life expectancy after elimination of specified causes of death in selected States of India

In this study an attempt was made to construct, (a) cause of death life tables and estimate cause-specific death rates for selected States of India namely Bihar, Maharashtra, Rajasthan and Tamil Nadu representing different geographical regions of the country and Kerala as a model State and, (b) to estimate potential gain in life expectancy after partial and complete elimination of certain causes of death by preparing cause deleted life tables like, (i) infectious and parasitic diseases, (ii) diseases of the nervous system, (iii) diseases of the circulatory system, (iv) diseases of the respiratory system, and (v) pregnancy, childbirth and the puerperium.

Ongoing Studies

Following are the ongoing studies:

(i) The Clinical Trials Registry – India (CTRI): The CTRI has been established at the Institute for registering all clinical trials on health products that are drugs, devices, vaccines, herbal drugs, etc. This has created complete, authentic, public and readily available data including ongoing and completed clinical trials. The existence of such a registry provides a corrective system against “positive results bias” and “selective reporting” of research results to peer-review publications. It also increases awareness and accountability of all the participants involved in clinical trials and increase awareness among general public.

(ii) Research on HIV/AIDS epidemiology: The Institute continues to collaborate with the National AIDS Control Organisation (NACO) and National Institute of Health & Family Welfare (NIHFW) for HIV Sentinel Surveillance (HSS) and other serosurveys in the country and has been acting as the nodal Institute for the modelling, estimation and projection of HIV burden in India and its States. As a part of surveillance, the NIMS is acting as the regional coordinating Institute for the conduct of NACO’s National Integrated Biological and Behavioural Surveillance (Chhattisgarh, MP and Odisha). It is aimed to generate evidence on risk behaviour among high risk groups by collecting biological and behavioural data to support planning

and prioritization of programme efforts at district, State and national levels.

(iii) Acceptance level, knowledge, attitude and practice of Indian System of Medicine in North-East: The study was taken up with the objective to measure the level of knowledge, acceptance and practice of the Indian System of Medicine amongst the people of North-East areas for common diseases. It also aimed to study the association between socio-demographic characteristics and satisfaction and/or trust of the Indian System of Medicine.

(iv) Baseline and end-line household malaria survey in World Bank project districts/States: This study is with collaboration with the National Institute of Malaria Research with the objective to estimate the key indicators related to coverage and care seeking in the malaria control programme.

(v) Malaria burden study to validate the assumptions of mortality estimates: The Institute is collaborating with the National Institute of Malaria Research for the study. It aims to determine malaria incidence and deaths due to malaria in districts representing zones of high, moderate and low risk of malaria in India.

(vi) Burden of disease due to cancer in India: The study was aimed to assess the magnitude of cancer in the country. The information generated in the study will support the planners in allocation of resources for the cause of cancer control.

(vii) Exploratory geo-spatial analysis to study the utilization of RCH services in North-East States: The study was aimed at visualizing data on utilization of RCH services, and spatial statistical analysis on utilization of RCH services.

INTELLECTUAL PROPERTY RIGHTS (IPR)

The mandate of IPR unit is to provide technical, legal and other support on IPR-related issues to all ICMR supported research (intramural and extramural) and to facilitate technology transfer, licensing and commercialization under IPR policies which needs regular monitoring from time to time. During the period under review, the highlight of all the activities are as follows:

During this year a total of 17 patents were filed, 11 in India – six from intramural research and

five from extramural research. One patent granted during this period belonged to VCRC, Pondicherry. Four Patient Cooperation Treaty (PCT) applications have been filed, two from extramural research done at AIIMS, New Delhi and BITS, Pilani, and two from intramural research done at NIV, Pune, and ERC, Mumbai. Also two patent applications (in National phase) were filed in the United States of America. Besides patent filing, steps have been initiated towards a transparent process seeking collaboration by private/public sector companies through website advertisement and the criteria of selecting the collaborators have been finalized.

A robust, transparent and inclusive guidelines for PCT filing have been prepared to have a framework for the standardization and monetization of PCT filing as the PCT filing and subsequent identification of countries for filing and maintenance is an expensive process. The IPR unit continued its training of women scientists under the Women Scientist scheme of TIFAC/DST.

The ICMR's technologies were exhibited at the second edition of Pharmac South 2015, at Chennai Trade Centre on 3rd and 4th July, 2015 which was considered relevant for industrial partnering. It was co-organized by Orbitz exhibitions Pvt. Ltd. and Indian Drugs manufacturers' association-Tamil Nadu and Puducherry State Board (IDMA-TNPSB) and was poised to bring pharma vendors, pharmaceuticals and nutraceuticals manufacturers, contract manufacturers, packaging solution and encapsulation providers, under one roof for effective collaborations for technology commercialization.

The core theme of the fourth edition of PHARMAC SOUTH 2015 was to provide a common platform to all the companies involved in the pharmaceutical, machinery, packaging to promote their products. The Indian Council of Medical Research has participated with an idea that newer technologies need to be developed and used in sectors of health care. The ICMR showcased several healthcare technologies, developed and patented nationally and internationally. Also various publications of ICMR viz. May 2015 issue of Indian Journal of Medical Research, Annual Report of DHR were displayed whereas ICMR's Intellectual Property Rights policy and book on ICMR's Technologies for commercialization-2011 were distributed free

for increasing the reach of ICMR's technologies.

Health professionals appreciated the poster presentation and range of research and various comments were received on the healthcare related technologies exhibited in the conference.



INTERNATIONAL HEALTH DIVISION

The International Health Division (IHD) in ICMR liaises with the biomedical research collaboration of India with other countries, as also with national and international agencies such as Ministry of Science & Technology, Indian and foreign missions and WHO etc. There are a few specific agreements signed by the Ministry of Health and Family Welfare (MoHFW) with other countries and rest are those signed directly by ICMR/DHR with international organizations/ institutions such as INSERM in France, German Federal Ministry of Education and Research (BMBF) and Helmholtz Association (HGF) in Germany; National Institutes of Health (NIH) in USA; University of Minnesota USA; International AIDS Vaccine Initiative (IAVI), USA; Canadian Institutes of Health Research (CIHR) in Canada; University of Sydney, Australia; London School of Hygiene and Tropical Medicine (LSHTM) and Medical Research Council (MRC) in UK; ICMR- Foundation for Innovative New Diagnostics (FIND), Switzerland; Academy of Finland (AF) in Finland; Global Alliance for Chronic Diseases (GACD); Research Council of Norway (RCN), Norway; Russian Foundation for Basic Research (RFBR), Russia. The Department of Health Research (DHR) has signed a Memorandum of Understanding with National Institute of Health & Care Excellence (NICE), UK.

The purpose of these Memoranda of Understanding (MoU) and Joint Statements has been for exchange of scientific information, scientists/technicians, joint execution of scientific projects and organization of joint scientific meetings, seminars, workshops and symposia in identified areas of cooperation. 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.

In addition the IHD has also represented ICMR in various bilateral/multilateral Joint Committee Meetings (JCM) coordinated by Ministry of External Affairs (MEA), Department of Science and Technology (DST) and MoHFW, Government of India (GoI) for cooperation with various countries.

The following JWG/JSC meetings under various MoUs and Joint Statements have been held:

- (i) First JWG meeting on April 29-30, 2014 between India and Saudi Arabia at MoHFW, New Delhi;
- (ii) First JWG meeting between India and United Kingdom on Health on September 3, 2014 at MoHFW, New Delhi;
- (iii) Seventh meeting of JWG under the Indo-Swedish MoU on cooperation in the field of Healthcare and Public Health held at Stockholm, Sweden on September 18-19, 2014. (Fig. 1)
- (iv) Seventh meeting of Indo-Russian JWG on Science & Technology at New Delhi on September 23, 2014;
- (v) Third Indo-US Working Group meeting on Health at New Delhi on November 14, 2014.
- (vi) Indo-US JCM at New Delhi on November 17, 2014.
- (vii) Tenth meeting of Indo-German Committee (IGC) on Science & Technology on November 20, 2014 at New Delhi.
- (viii) Indo-Sweden Bilateral meeting between Honorable Ministers at New Delhi on November 24, 2014. (Fig. 2)

The International Workshops/ meetings held under Bilateral/multilateral programmes are:

- (i) Indo-Canada Mental Health Experts' Group Meeting held on September 29, 2014 at ICMR, New Delhi;
- (ii) ICMR-FORTE joint workshop on Ageing and Health held during November 24-25, 2014 at ICMR, New Delhi;
- (iii) SAFHeR Foundation workshop in Clinical & Laboratory Medicine Research between February 9-12, 2015 at NIOP, New Delhi.

Memoranda of Understanding (MoUs) signed

During the reported period, the MoUs signed included, addendum – three for GACD Secretariat funding at UCL, London signed in June, 2014 under existing ICMR-GACD MoU; MoU between the Research Council of Norway and ICMR on Health Research, signed on October 14, 2014 in Norway; MoU between ICMR and IAVI on Cooperation in AIDS Vaccine Development signed in October/November, 2014 in New York and ICMR headquarters New Delhi respectively. (Fig. 3); MoU between the ICMR and the Russian Foundation for Basic Research on Cooperation in Health Research was signed on December 11, 2014 at Hyderabad House, New Delhi. (Fig. 4 & Fig. 5); MoU between ICMR and University of Sydney, Australia for collaboration in Health Research signed in January/March, 2015.

Dialogues initiated and MoUs forwarded to GoI for approval of Indo-US MoUs on Maternal and Child Health; NIAID, USA and ICER, Chennai; ICMR-NHMRC, Australia; ICMR and Drugs for Neglected Diseases Initiative (DNDi), Switzerland; MoU between DHR and ICAV, Canada; ICMR-Bill & Mellinda Gates Foundation (BMGF), USA; ICMR-London School of Hygiene and Tropical Medicine (LSHTM), UK; MoU between ICMR & FORTE, Sweden. Approval of GOI is awaited. International travel of Indian scientists involved in approved bilateral collaborative research projects under various MoUs and Joint Statements with other countries is also synchronized by IHD. A total of 23 exchange visits of scientists / officials to and from India were coordinated under various international collaborative programmes / projects in the reported year.

Health Ministry's Screening Committee (HMSC)

Research projects by Indian investigators which involve foreign assistance and/or collaboration in biomedical/health research are submitted to ICMR for approval by the GoI. The IHD acts as the Secretariat for HMSC which takes a decision on various projects peer-reviewed by the concerned Technical Divisions at ICMR. During the year 2014-2015, four HMSC meetings were organized, wherein 81 projects were considered and out of which 59 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. Of these two projects are co-funded by ICMR.

International Visitors / Dignitaries

The IHD also organized various visits to ICMR from foreign visitors (countries / agencies) such as High Commissioner of Australia, European Union; INSERM, France; NICE, UK; MSF & LSHTM, UK; Department of Health, UK; University of Oxford, UK; University of Sydney, Australia; University of Minnesota, USA; FORTE, Sweden; IAVI, USA; NIH, USA; RFBR, Russia; CDC, USA, HGF, Germany, DNDi, Geneva, CNRS, Paris, RCN, Norway, etc.

The ICMR International Fellowship Programme for Indian biomedical scientists is also orchestrated by the IHD. This programme aims at augmenting the capacity strengthening of institutions involved in basic, applied, epidemiological and clinical sciences through exposure of Indian researchers to the latest international advancements to better understand diseases and find strategies for their prevention and cure. The ICMR International Fellowships have been awarded to six senior and twelve Young Indian scientists during the year 2014-2015, of which 12 Young and five senior scientists have undertaken the fellowship. The reports of these ICMR International Fellows have been placed on ICMR website.

Joint Call for Proposals

The Joint call for proposals under ICMR-MRC, UK (Mental Health) & Indo-US Joint Statement on Diabetes were uploaded on the ICMR website. Announcement made for submission of applications for transfer of human biological material for commercial purposes and/or research and development of commercial products with deadlines as April 30, July 31, October 31, 2014 and January 31, 2015 and 230 cases were considered by the Committee in its four meetings held so far on May 28, August 25, November 27, 2014 and February 25, 2015, Of which 132 cases were approved.



Fig. 1. IHD's participation in the 7th Indo-Swedish Joint Working Group meeting under Indo-Swedish MoU for cooperation in the field of Healthcare and Public Health held during 18-19 September, 2014 at Stockholm, Sweden.



Fig. 2. A meeting between Hon'ble Minister of Health & Family Welfare, GOI, Shri J.P. Nadda & Hon'ble Minister for Health Care, Public Care and Sports, Mr. Gabriel Wikstrom, Government of Sweden held on 24th November, 2014 at New Delhi. Also present at the function were Sh. Lov Verma, Secretary (HFW) & Dr V M Katoch, Secretary (DHR), H.E. Mr. Harald Sandberg, Ambassador of Sweden to India, Dr Jagdish Prasad, DGHS and other senior officers of the Ministry of Health & Family Welfare and ICMR along with members of the Swedish delegation..



Fig. 3. Signing of MoU between ICMR and International AIDS Vaccine Initiative (IAVI), USA on cooperation in AIDS Vaccine Development in November, 2014 at ICMR Hqrs New Delhi.



Fig. 4. Dr. V.M. Katoch Secretary Department of Health Research, MOH&FW and Director General, ICMR from India and Academician Vladislav Ya PANCHENKO, Chairman of RFBR from Russia signed the MoU between ICMR and RFBR on cooperation on Health Research at Hyderabad House, New Delhi on December 11, 2014 in the presence of the Hon'ble Prime Minister of India and H.E. President of Russia.



Fig. 5. Academician Vladislav Ya PANCHENKO, Chairman of RFBR, Russia & Dr. Alexander N. Sharov attended a discussion meeting on implementation plan of ICMR-RFBR MoU on December 12, 2014 at ICMR Headquarters, New Delhi.

HUMAN RESOURCE DEVELOPMENT

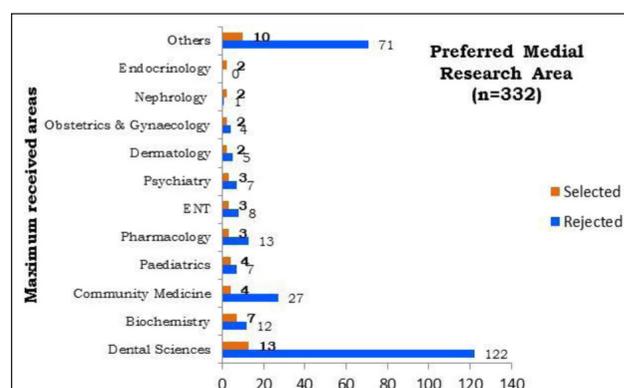
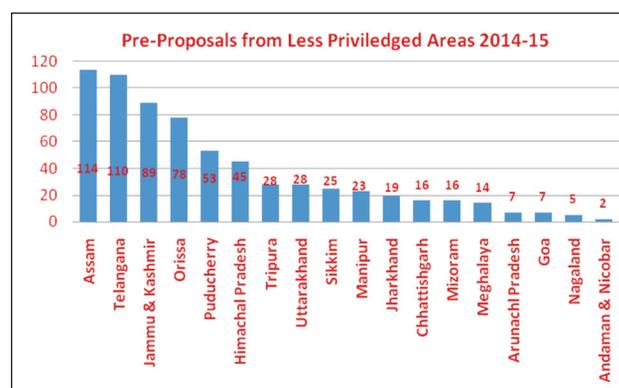
1. Junior Research Fellowship

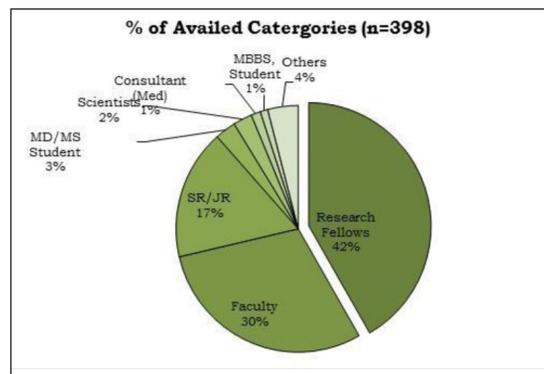
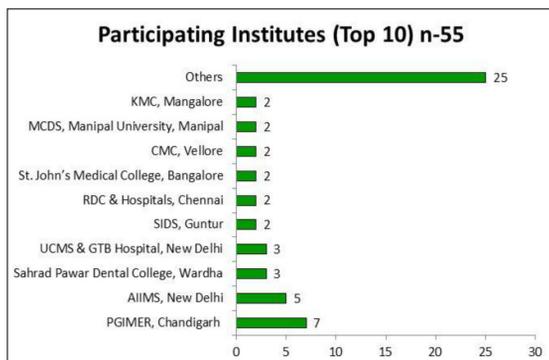
During the period under report the Council conducted 14th National Level Examination for

Selecting JRFs to augment Biomedical Research in the country. Every year 150 JRFs (i.e. 120 for Life Sciences and 30 for Social sciences including biostatistics) are selected for doing Ph. D. in Biomedical Sciences in different institutions. Number of candidates appeared in the year 2014-15 was approx.10,000. The examination was conducted at 12 centres (Bangaluru, Bhopal, Bhubaneswar, Chandigarh, Chennai, Delhi, Guwahati, Hyderabad, Kolkata, Mumbai, Sri Nagar and Varanasi). A total of 643 JRFs is on-going (2010-2014) at various national level institutions. The value of existing fellowships is at present Rs.25, 000/- p.m., The annual contingency grant is Rs.20, 000/- p.a. + HRA.

2. Financial assistance to MD/MS/DM/MCH thesis in Biomedical Research.

Financial assistance of Rs.25, 000/- is provided to MD/MS/DM/MCH students who are in the 2nd year of MD/MS course. The Selection Committee recommended financial assistance to a total of 558 MD/MS/DM/MCH thesis out of 2084 proposals received so far. Out of 332 -thesis protocol 55 protocols/candidates awarded financial assistance during reporting period (Fig 1-3).





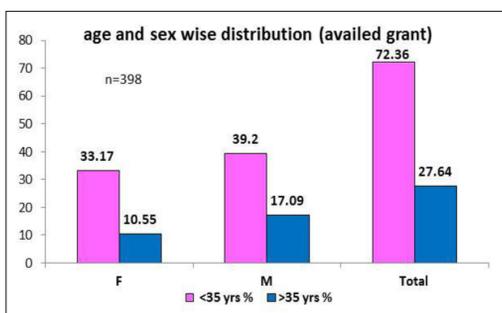
3. MD, Ph. D. Programme 25 slots/ year:

Programme was revived to identify young medical graduates with brilliant academic record for pursuing post-graduation & later to absorb them in its research cadre. The candidate who passes all MBBS examinations in the first attempt with 60% or more aggregate marks is eligible for the examination. Under this programme selected medical graduates are provided financial assistance for 4 to 5 years. The eligible candidates were selected through national level examination.

Programme is on-going at three universities i.e. King George University, Lucknow, NIMHANS, Bangalore & Sri Ramachandra Medical College, Chennai. During 2014-15 out of 15 allotted slots 10 were selected. So far 93 candidates have joined the MD/PhD programme. 36 candidates have submitted their thesis in various areas.

4. International Conference/ Workshops to Non ICMR scientist Support Scheme:

One of the major mandates of the Council is capacity building of biomedical scientist of the country by providing them financial assistance for participating in International Conference/ Training programmes/ Workshops etc. Out of total 1760 applications 624(35.45 %) applicants were supported during reporting period.

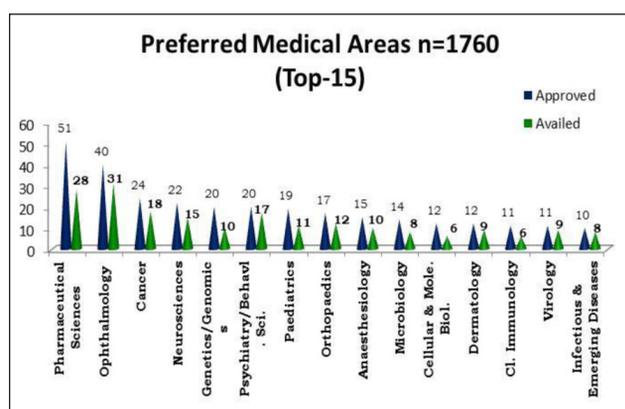
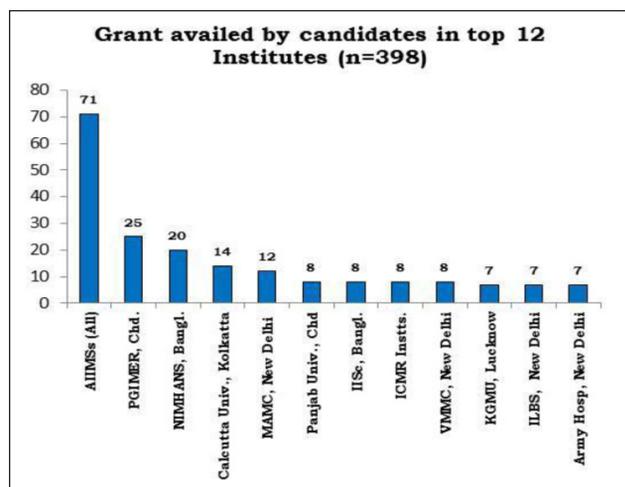


An analysis of the availed 398 (out of 1760) applications shows that, 12.73 % i.e. 224 applications were male. Out of 224 male majority of the applications i.e. 156 (39.20%) are young people i.e. < 35 years of age. Similar trend is observed in the female availed applications (174), i.e. 132 (33.17%) are < 35 years of age. At an overall level (males and females together), similar trend is seen i.e. 288 (72.36%) applications are from the < 35 years of age group & 110 (27.64%) > 35 year age group (Fig.4).

Designation wise analysis of the applications that were availed (398), reveals that maximum percentage of Research Fellows (JRF-SRF) i.e. 41.71% were benefitted from this scheme. Faculty (Lecture/Reader/Assistant/Associate Professor/ Addl Professor/Professor Medical/Principal/Vice Chancellors/Dean) also were benefitted from the scheme and out of 398 apps, 29.65% were from this category followed by Senior/Junior Residents 17.09%, MD/MS/Mch Student 2.76 %, Scientist (B to G) 1.51%, Consultant Medical 1.26 %, & MBBS – Student & Directors 1.01% (Fig.5)

AIIMs emerges as the top most institute (Received -250, Approved-108, Availed-71) from which people availed this scheme, followed by PGIMER, Chandigarh- (Received -82, Approved-42, Availed-25), NIMHANS Bangalore-(Received -35, Approved-24, Availed-20), University of Calcutta, Kolkata-(Received -54, Approved-18, Availed-14), MAMC, New Delhi-(Received -26, Approved-13, Availed-12), Panjab University, Punjab /Chandigarh-(Received-29, Approved-13, Availed-8), ICMR, Institutes-(Received -23, Approved-12, Availed-8), IISc, Bangalore-(Received -27, Approved-11, Availed-8), VMMC, New Delhi-(Received -18, Approved-9, Availed-8),

KGMU, Lucknow-(Received -24, Approved-9, Availed-7), ILBS, New Delhi-(Received -22, Approved-9, Availed-7), Army Hospital, New Delhi-(Received -11, Approved-7, Availed-7) etc (Fig.6)



An analysis of Research areas reveals that Pharmaceutical Sciences emerges as the top-most area for which this scheme was availed followed by Ophthalmology/Vision, Cancer, Neurosciences, Human Genetics/Genomics, Psychiatry/Behavioural Sciences, Paediatrics, Orthopaedics, Anaesthesiology/Critical Care, Microbiology, Cellular & Molecular Biology, Dermatology, Clinical Immunology, Virology, Infectious & Emerging Infectious Diseases etc. (Fig.7).

A state wise analysis indicates that New Delhi is most active with most number of applications received, approved and availed from New Delhi only. Details of participating states is given in Table-1.

5. Post Doctorate Fellowship Programme(50/yr):

To identify and support young Ph.Ds. for the conduct of research using ICMR Institutes working for priority areas of Health Research. So far 255 applications were received by the ICMR, 230 applications were short listed for personal discussion and 112 were selected. Out of total 112 selected PDF's 83 have joined different ICMR institutes, 50 PDF's completed their studies, 8 PDF's discontinued and 25 PDF's still continue their study for the year 2014-15. However, a total of 41 new PDF's proposals were received by division during 2014-15. Out of 41 proposals 12 were approved for funding and 8 PDF's were ongoing.

Status of PDF programme

6. ICMR Awards:

A total of 215 applications were processed for evaluation through different committees for selection for the year 2011 & 2012. A total of 45 awards were declared.

7. Grant-in-aid for Organising Seminars/Symposia/Workshops:

To update knowledge transfer from lab to land at national and international level in the concerned area Conference/CME/programmes/ Workshops etc. scheme is ongoing and out of total 940 applications 459 applicants were approved during 2014-15.

PUBLICATION AND INFORMATION

Publication, information and communication are integral part of any research organization and so is with Indian Council of Medical Research. This functions as a bridge between ICMR and its research and development programmes on one hand and scientists of other organizations, Planners, Policy Makers, Administrators as well as general public on the other hand. ICMR continued its multifaceted activities in the field of publication, communication and information dissemination with the sole objective of reaching to different target groups. Various initiatives undertaken under this programme are summarized as under:

PERIODICAL PUBLICATIONS

Indian Journal of Medical Research

The Indian Journal of Medical Research (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 interest. Other regular sections such as Editorials, Commentaries, Student IJMR, Research Correspondences, Viewpoints, Perspectives, Clinical Images, Systematic Reviews with Meta Analysis, *etc.* were also continued to be published.

The IJMR was continued to be covered by all global abstracting and indexing services. It is available full text free on the net with a searchable menu. IJMR archive is available at <http://ijmr.in> with full text of articles available free in pdf format since its inception (July 2013).

During the year 2014-2015, a total of 283 articles were published under various categories/ sections; 47% were original research articles, followed by 6.7% review articles. Correspondences accounted for 14.5% and Clinical Images 9.9% (Fig.1). The quality of peer review was maintained by involving

reviewers from both India as well as from foreign countries. Overall, during the period under report a total of 1360 reviewers peer reviewed for the IJMR and of these 19% were from countries other than India (Fig.2).

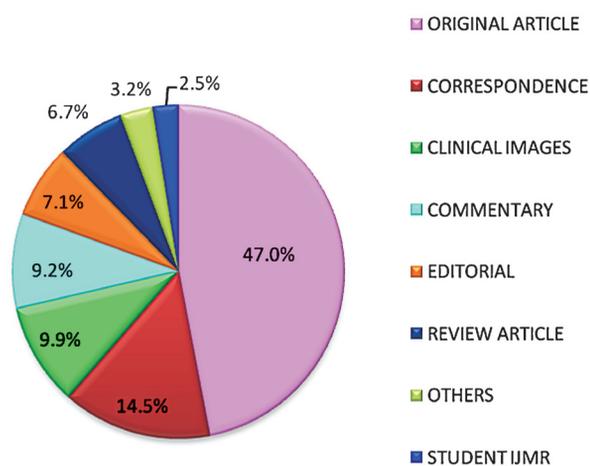


Fig. 1. Articles published in IJMR under different categories (2014-2015).

During 2014-2015, a total of 2139 articles were submitted to the IJMR for consideration for publication. Of these, 35% were contributed from countries other than India (Fig. 3).

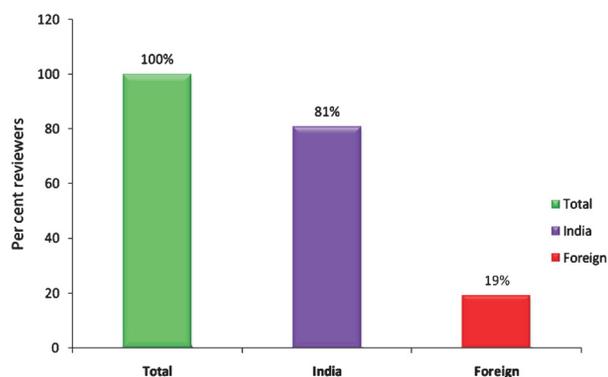


Fig. 2. Percentage of Indian and Foreign Reviewers of IJMR (2014-2015).

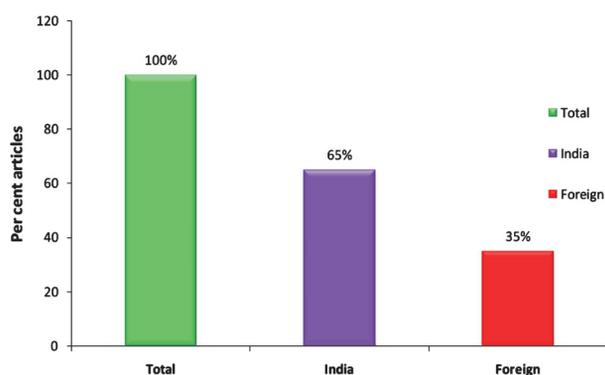


Fig. 3. Percentage of Indian and Foreign Submissions (2014-2015).

A supplementary issue on “Reproductive Health & Strategies for Family Planning” was brought out in November 2014 with Dr RS Sharma as Guest Editor. A total of 16 review articles contributed by renowned experts in the field and three original research articles were published in this issue.



Annual Report

The Annual Report of Director General (both English and Hindi Versions) for the financial year 2013-14 was brought out and tabled in the Parliament. The ICMR also collaborated in the publication of Annual Report of the Department of Health Research for the year 2014-15. Both these report 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 mostly Hindi Bulletin was continued. The articles published on various health topics included:

Bancroftian filarial rog, Electromagnetic Vikiran ke Khatre, Bharat mein Vitamin A Sampooran Karyakram, Bharat mein Vaccines aur Tikakaran ka Sankshipt Itihas, Atiraktadab prabandhan, Haemophilia ki sthiti aur Samajik Prabhav, Swasth Hriday ke liye Anukool Parivesh, Madhumeh : Sanket aur Lakshan, Schizophrenia, Tambakoo Nasha Unmulan : Ek Swasthya Prathamikata.

Non Periodicals

Hindi version of two popular books published by NIN, Hyderabad were brought out during the year viz., ‘Dietary Guidelines for Indians- A Manual’ as ‘*Bhartiyon ke liye Aahar Sandarshika- Ek Niyamavali*’ (May, 2014), and “Diet and Heart Disease” as “*Ahar aur Hridaya Rog*” (Feb., 2015).

Monographs on Indian Medicinal Plants

Medicinal Plants Division of ICMR Headquarters brought out following monographs:

Quality Standards of Indian Medicinal Plants:

The programme on preparation of Monographs on Quality Standards of Indian Medicinal Plants is in progress at various national laboratories/institutions in the country. These monographs are on the pattern of WHO guidelines and focus on the diagnostic features and phytochemical studies, including markers besides having information on pharmacological, clinical, toxicological aspects along with dosage, adulterants/substitutes, etc.

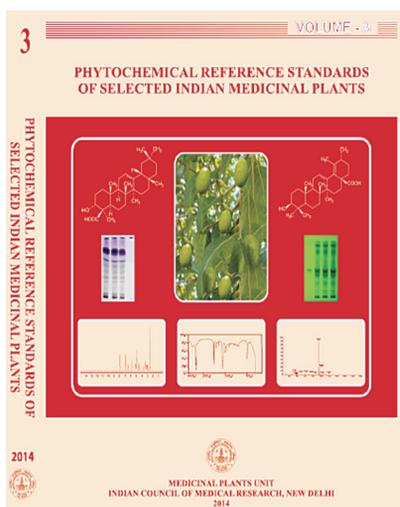
During the year the The Quality standards on 35 medicinal plants were developed, monographs prepared, finalized, technically reviewed and published as Vol. 12 as part of series on “Quality Standards on Indian Medicinal Plants”.

Generation of Phytochemical Reference Standards and Development of Repository of Reference Phytoconstituents of Important Indian Medicinal Plants:

Phytochemical reference standards (PRS) are ideally those compounds in the plant which are therapeutically active. However, in many cases the therapeutic activity of the plant is attributed to a number of phytoconstituents present in the plants. Under such circumstances any compound that is unique to the plant or the major compound or the

major chemical constituent can be regarded as the PRS.

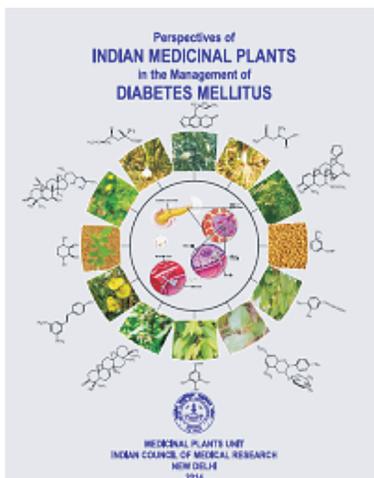
The third volume of Phytochemical Reference Standards of Selected Indian Medicinal Plants containing monographs of 30 PRS was published during the year.



Medicinal plants monographs on diseases of public health importance :

The activity aims at integration of leads/scientific information on diseases (including etiopathogenesis) and plant drugs as given in the ancient texts (ISM) and Allopathic system of medicine on one hand and the multidisciplinary research data generated on these plant remedies with focus on pharmacological, toxicology, clinical, phytochemical, pharmacognostic, on the other.

The monograph on Perspectives of Indian Medicinal Plants in the Management of Diabetes Mellitus was published during the year.



Development of a website exclusively dealing with Medicinal Plants

A website has been developed exclusively on the activities of the Division and hyperlinked with the ICMR’s main website.



▲ Quality Standards of Indian Medicinal plants

1. Captain Srinivasa Murti Drug Research Institute for Ayurveda and Siddha, Chennai, Tamil Nadu
2. Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala
3. Institute of Chemical Technology, Matunga (E) Mumbai
4. Indian Council of Medical Research, New Delhi

● Phytochemical Reference Standards of Selected Indian Medicinal Plants

1. Natural Remedies, Bengaluru

★ Adhoc Project on Herbal Drug Interaction

1. Natural Remedies, Bengaluru
2. AIIMS, New Delhi

BIOMEDICAL INFORMATION & COMMUNICATION

Scientometric Studies

Annual Research Output of ICMR Institutes

The annual document ‘2013 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 684 papers were published by the ICMR institutes during the calendar year 2013. The National Institute of Cholera and Enteric Diseases (NICED), Kolkata topped the tally with 79 papers followed by National Institute for Research in Reproductive Health (NIRRH), Mumbai (66), National Institute of Nutrition (NIN), Hyderabad (62), National Institute of Immunohaematology (NIIH), Mumbai (47), National Institute for Research in Tuberculosis (NIRT), Chennai (47), National Institute of Malaria Research (NIMR), New Delhi (46) and National Institute of Virology (NIV), Pune (42). Of the 684 papers, 464 (67.863%) papers were covered by *Journal Citation Reports(JCR)/ Science Citation Index (SCI) 2013*.

Of the 376 journals used for publishing 684 papers, 199 journals had an impact factor (IF) equal to or greater than 1.000. The publishing journal *New England Journal of Medicine* had the highest IF (54.42).

The average IF/paper of the Council for the calendar year 2013 was 3.103.

Human Resource Development in Biomedical Communication

As per memorandum of understanding signed between ICMR and Central Council for Research in Unani Medicine (CCRUM), New Delhi, two workshop- one on Research Project Writing and another an Scientific Paper Writing were organized for scientists of CCRUM during the year under report.

ICMR Awards for Popular Medical Books in Hindi for Biennium 2012-13

A total of 9 books received were scrutinized by the Expert Committee in its meeting held on 13th August, 2014. The book entitled “*Vigyan ki Vikalangata par Vijay*” by Shri Vinod Kumar Mishra was adjudged for the First Prize of Rs 1.00 lakh. Sh T. S. Jawahar, Sr DDG (Admn) gave away the Certificate and Prize money to the winner during a function organized on 12th March, 2015.

Hindi Day Lecture

A popular lecture in Hindi was organised at the ICMR Hqrs on 13th Oct., 2014. Prof S. K. Sharma,

Head, Deptt of Medicine, AIIMS delivered the lecture on the topic “Nidra evam Swasthye (Sleep & Health)”.

Dissemination of Biomedical Information

ICMR carried out large number of education and extension activities during the year in different parts of India to disseminate the activities and achievements of ICMR at various platforms as well as to enhance the outreach of ICMR. A brief description on the same is as follows:

12th Agriculture Science Congress Expo

ICMR participated in the 12th Agriculture Science Congress Expo held at National Dairy Research Institute (NDRI) from 3-6 February, 2015 at Karnal, Haryana and displayed attractive and informative posters for dissemination of ICMR activities and achievements. The Exhibition was inaugurated by Hon'ble Governor of Haryana Shri K S Solanki. Large number of participants and local people visited the ICMR Pavilion and interacted with ICMR Scientists to gather useful information.

Science Expo at Pravasi Bharti Diwas and Vibrant Gujarat

ICMR participated in Mega Science Expo organized as part of Pravasi Bharti Diwas and Vibrant Gujarat in Gandhinagar, Gujarat during 7-13th January, 2015 and showcased its activities and achievements through 97 attractive and vibrant posters on the major research activities and achievements of public health importance. These posters highlighted the major research activities of the ICMR in the area of Nutrition, Environmental and Occupational health, malaria, leprosy, tuberculosis, cancer, cholera and enteric diseases, HIV/AIDS and regional health problems.

The significant feature was the display of ICMR indigenously developed affordable technologies that include the Magnivisualizer for detection of cervical cancer, kit for beta thalassemia, non-invasive procedures for kala-azar detection, ferritin estimation, Cooling Jacket as developed by NIOH, Ahmedabad for persons working under heat stress conditions, etc. Scientists who developed these technologies were also present to answer the queries of the visitors.

Other important feature of the exhibition was the inclusion of live demonstration of malaria parasite, aquatic and adult stages of vector mosquitoes, insecticide treated bed nets, larvivorous fishes like Guppy and Gambusia.

Video films on the activities and achievements of the ICMR and its institutes were also screened for visiting public.

Pride of India Mega Science Expo during 102nd session of the Indian Science Congress

ICMR participated in Pride of India Mega Science Expo organized as part of 102nd session of the Indian Science Congress at in Mumbai from 3-7th January, 2015 and showcased its activities and achievements through 125 attractive and vibrant posters on the major research activities and achievements of public health importance. These posters include the posters on Nutrition, Environmental and Occupational health, malaria, leprosy, tuberculosis, cancer, cholera and enteric diseases, HIV/AIDS and regional health problems developed on the basis of the inputs received from different ICMR Institutes. There were also posters on different indigenous and affordable technologies developed by the ICMR Institutes, ICMR Institutional network, policies and programmes, guidelines/bills, human resource development, Video films on the activities and achievements of the ICMR and its institutes were also screened throughout the period in Hindi as well as in English. Union Minister of Science & Technology, Dr Harsh Vardhan, visited the ICMR Pavilion on 3rd January, 2015, appreciated the efforts and shared his experiences with Secretary DHR & DG, ICMR, Dr V.M. Katoch. Dr R. Mashelkar, Former DG, CSIR, Prof S.B. Nimse, President, Indian Science Congress and many other dignitaries also visited the ICMR Stall. Many scientists, academicians, media persons, students and local people visited the ICMR Pavilion and interacted with ICMR scientists. ICMR Pavilion was adjudged as the Most Informative Pavilion.

Kangda Avishkar Expo-2014.

ICMR participated in Kangda Avishkar Expo-2014 held at Kangda, Himachal Pradesh during 12-14 December, 2014. The exhibition was inaugurated by DG, CSIR Dr P S Ahuja. Posters on the important activities and achievements of ICMR were displayed

and a lecture on '*Paushtik Ahhar Swasthye Jeevan Ka Adhar*' was organized specially for the students for creating awareness for healthy diet. Video films were also screened to disseminate information on ICMR activities and achievements.

Health Pavilion as part of India International Trade Fair

ICMR had put up an exhibition in the Health Pavilion designed by Ministry of Health & Family Welfare, Govt. of India during India International Trade Fair (IITF), 2014 held at Pragati Maidan from 14-27th November, 2014 and displayed informative posters for general public in the area of maternal & child health, tuberculosis, nutrition and general information on ICMR.

Science Expo at Solan, Himachal Pradesh

ICMR participated in a mega Science Exhibition held at Solan, Himachal Pradesh during 18-20 September, 2014 organized by a voluntary agency SANSA and displayed important activities and achievements of various ICMR Institutes. Large number of students and local people visited the exhibition.

Infra-Educa 2014 held at Pragati Maidan, New Delhi

ICMR also participated in an information campaign entitled "12th Infra-Educa 2014" held at Pragati Maidan, New Delhi from 21-22nd June, 2014 organized by Friendz Exhibition and Promotions for dissemination and promotion of scientific culture among the students and displayed informative posters on various activities and achievements of ICMR. Students visiting the ICMR Pavilion were briefed on various training programmes and courses conducted by ICMR in the area of biomedical and health research.

Rajasthan Science Congress

ICMR Participated in 2nd Rajasthan Science Congress held during 1-3 May, 2014 at Dr K N Modi University, Niwai, Rajasthan and showcased its scientific activities and achievements through informative posters and audio-video programmes. Many dignitaries including former Director General, ICAR Dr R S Paroda, many other

scientists, academicians, students and local people visited the ICMR Pavilion.

World Book Fair

ICMR participated in World Book Fair held in New Delhi during 14-22nd February, 2015 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.



Fig. ICMR Participation in Kangda Avishkar Expo-2014.



Fig. Pride of India Mega Science Expo during 102nd session of the Indian Science Congress held in Mumbai from 3-7th January, 2015.

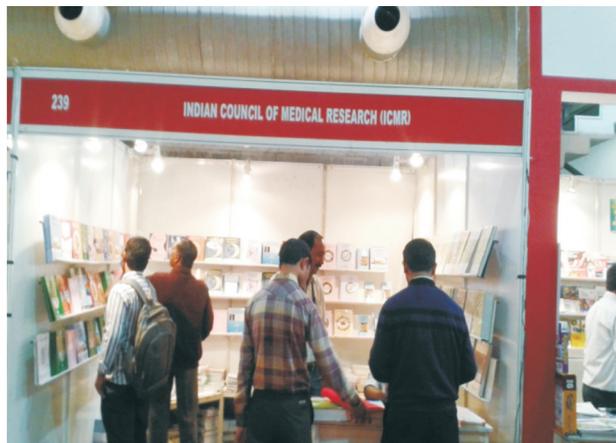


Fig. Pravasi Bharti Diwas and Vibrant Gujarat in Gandhinagar, Gujarat during 7-13th January, 2015.

Fig. ICMR participated in World Book Fair held in New Delhi during 14-22nd February, 2015.

Science and Agriculture Expo at Udaipur, Rajasthan

ICMR also participated in Science and Agriculture Expo as part of 6th Vision Rajasthan- a Science & Technology Exposition held at Udaipur, Rajasthan from 1-3 March, 2015. ICMR stall also featured inspirational nutrition messages. The visitors included rural women, farmers, teachers, student community, and school children. Shri. Arjun Lal Meena, Member of Parliament from Udaipur, visited the stall. The IEC material on Dietary Guidelines for Indian developed in Hindi were distributed to all the visitors. The awareness lectures for visitors of different age groups were followed by on-the-spot Quiz competition and the winners were given token prizes. ICMR's display was adjudged as the best display.



National Databases of Indian Medical Journals

ICMR funded project “National Databases of Indian Medicals Journals” produces two resources by indexing (IndMED) as well as hosting full text of Indian Medical Journals (MedIND). IndMED indexes over 118 peer reviewed journals selected by a Journal Selection Committee. It indexes each citable article published in journals covered by it by creating bibliographic records. MedIND now hosts full text articles of 68 Indian Medical Journals. IndMED and MedIND websites were completely revamped through re-designing of their layout, content enrichment, color schemes, inclusion of logos of the stakeholders, images, search boxes etc. to make them more attractive and user friendly. The criteria for selection of new journals was reviewed and modified with a suggestion that all journals should comply to the guidelines of IJMJE, Vancouver System and IMRAD Paper Format. A brain storming session was organized by inviting experts representing various stake holders (user community and medical journals) along with ICMR authorities on 20th February 2015 to do a comprehensive review of the project. Participants strongly recommended continuation of IndMED and MedIND along with new initiatives.

Training Cum User Awareness Programmes were organized at NIC HQ as well as different parts of the country to create awareness and promote usage of IndMED and MedIND databases for the benefit of the medical community.

LIBRARY & INFORMATION SERVICES

The subscription to core bio medical e-journals like Lancet, Science, Nature, and NEJM for all ICMR Library & Information Centres has been continued under ICMR e- consortia. Subscription for full text electronic data base ProQuest Health & Medical Complete, which covers more than 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 12,228 Publishers. It presently has a massive database of journal literature, indexed from 44,717 e-journals with links to full text at publisher

sites. J-Gate offers two types of products/services (i) J-Gate Portal: a. table of contents (TOC) for 44,717 e-journals. b. Database - A comprehensive searchable database with 47,084,440 articles, with 10,000+ articles added every day. (ii) 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) - JCCC 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 online by the institutional members.

- Union Catalogue of Books (2.037 crore bibliographic records)
- Union List of Current Periodicals (36.9 thousand records)
- Union Catalogue of Periodicals (20.23 thousand records)
- Database of Periodical articles (9.12 lakh records)
- CD-ROM Database (22.2 thousand records)

Training Programmes

Training programme on J-Gate Plus has been organized on 23rd July 2015 for southern region of ICMR institutes at RMRC, Port Blair. Training programme for ProQuest Health & Medical Complete and J Gate plus was organized for western region of ICMR institutes at NIOH, Ahmedabad on 27th August 2015 and for central/northern region of ICMR institutes at NIREH, Bhopal on 23rd September 2015. Training programmes for J Gate Plus and ProQuest for north-eastern region was organized at RMRC, Dibrugarh on 29th October 2015 and for J-Gate plus for eastern region of ICMR institutes at RMRC, Bhubaneswar on 27th November 2015. These training programmes were organized with the sole purpose of promoting the usage of digital library resources among the scientists and librarians of ICMR institutes.

Bioinformatics Centre

Bioinformatics Centre (BIC) was established as Integrated Research Information System (IRIS)

in 1983 under the Division of Publication & Information (P&I) at ICMR with a mandate to computerize activities of ICMR. It was renamed as Bioinformatics Centre in 1999 with an extended mandate to nucleate and support informatics in medical research. Recently, BIC has been designated as independent unit. Bioinformatics Centre is providing wide range of services to the scientific fraternity as well the administration and finance. During the period 2014-15 BIC was primarily involved in (i) Revamping of ICMR Network, (ii) managing Task-Force ‘Biomedical Informatics Centres of ICMR’, (iii) Management of Extramural Research in the area of Bioinformatics and Medical Informatics and providing services to ICMR, (iv) ICMR Website, (v) Time Bound Reports and other Services to the Council, (vi) Management of ICMR Servers and Network, (vii) Videoconferencing Service.

Revamping of ICMR Network

BIC has initiated revamping of ICMR Local area network for higher speed internet connectivity and to resolve the issue of network saturation. The revamped network will be fully compliant as per the new rules of government and can work well with IPV4 as well as IPV6 Protocol.

Task-Force Projects

Modern Biology tools and techniques (Genomics, Proteomics *etc.*) are revolutionizing medical research. Important applications of modern biology tools in medical research include (i) understanding role of genetic and/or environment in disease progression, (ii) disease modelling and outbreak prediction, (iii) exploring pathogenesis at molecular level, (iv) identifying prognostic and diagnostic markers, (v) identifying genetic loci associated with susceptibility or resistance to diseases, (vi) developing prophylactics and therapeutics and (viii) developing databases of clinical and biomedical information.

Though the modern biology tools and techniques are being used world-wide in medical research, these are not being used as widely in India. Primary reasons for limited use of modern biology tools and techniques in Indian medical research include (i) lack of awareness among clinicians/physicians (ii) lack of availability of interdisciplinary expertise

and (iii) lack of appropriate infrastructure for collection, storage, analysis, interpretation and visualization of medical data.

In order to address these issues ICMR initiated task-force ‘Biomedical Informatics Centres of ICMR’ in 2013. Under the task-force ICMR established 20 Biomedical Informatics Centres in medical colleges and medical research institutes across India with the mandate to nucleate and support informatics in medical research by creating awareness through workshops and training programs, providing data management and analysis services and initiating large-scale collaborative projects with medical professionals from host institute and regional medical colleges.

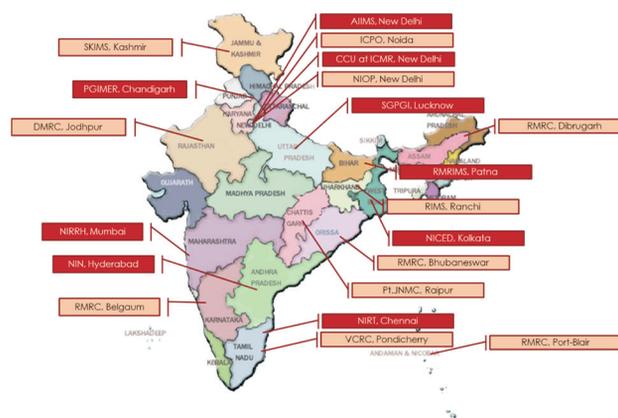


Fig. Biomedical Informatics Centres of ICMR.

During the year these Centres conducted 16 workshops and training programs on diverse themes; from basic bioinformatics and medical informatics to advance big data analytics for disease research. The workshops were attended by more than 200 medical professionals and researchers from regional medical colleges and medical research institutes. Few important workshops include:

- January 18-22, 2015, Workshop on NGS data analysis in Indo-US Symposium on Big Data Analysis and Translation in Disease Biology
- March 24 – 28, 2014 National Level Workshop on Biostatistics and its Applications in Biomedical Research
- February 3-6, 2015, National Level workshop on Bioinformatics in Drug Discovery
- March 25, 2015, brainstorming session entitled Base-pair to Bedside- Genomics and Cancer Prevention

Services to medical researchers

The Centres provided consultation in developing 8 large-scale proposals. The Centres also provided data analysis and interpretation services such as NGS data analysis, Metagenomics data analysis, molecular modeling, Phylogenetic analysis, Docking and design and developing databases of clinical and biomedical data.

Collaborative projects

This year the Centres initiated 24 collaborative projects related to identifying disease targets through comparative genomics, protein modeling and analysis of identified target structures, docking and designing leads, in-silico ADMET modeling of identified leads. Significant contributions include demonstration of critical virulence factors of *Salmonella typhi* which are being followed up as potential vaccine candidates. Other important achievements from collaborative projects include:

- Identification of (i) two potential active compounds MRSA, (ii) broad spectrum anticancer agents, (iii) potent antiplasmodial agents, (iv) potent inhibitor of Cholera toxin, (v) NCI524121, CAP332797, NCI524136 and ZINC00518462 leads against *Listeria monocytogenes*, (vi) inhibitors of dapE encoded N-succinyl-L,L-diaminopimelic acid desuccinylase against *Helicobacter pylori* infections, (vii) pentamidine, 1,3-dinitroadamantane, (viii) acyclovir and their analogues as potent inhibitors of hypoxanthine-guanine phosphoribosyl transferase (HGPRT) a novel drug target against Leishmaniasis, (ix) ZINC 3370091 as potential inhibitor of NS5 RNA methyltransferase of dengue virus, (x) ZINC38636720 as potent inhibitor of mitochondrial encoded cytochrome C oxidase subunit I (MT-CO1). MT-CO1 has been associated with several neuro-degenerative diseases.

These leads can be developed further.

- Identified novel SNPs in upstream promoter of uridine diphosphoglucuronate-glucuronosyltransferase family polypeptide 1 (UGT1A1) gene associated with Gilbert Syndrome (GS). These SNPs are likely to affect mRNA splicing

- Identified adenosine-to-inosine RNA editing sites using lung, hippocampus and liver transcriptome sequences. The technique was also applied on liver and kidney transcriptome sequencing data
- Identified novel mutation through sequencing in PFIC1-3 associated with progressive familial intrahepatic cholestasis (PFIC)
- Identified linkage between ectodermal dysplasia (ED), a developmental disorder with immune disorders and cancer through interactome analysis.
- Role of methyl CpG binding protein (MeCP) 2 in pathogenesis of Rett syndrome through structural analysis
- Identified and associated multiple point mutations in dhps and dhfr genes with high drug resistance of *Plasmodium falciparum* in Arunachal Pradesh
- Predicted functional effects of three novel mutations in CARD domain of AIRE gene in patients with autoimmune polyendocrinopathy syndrome, type-I.
- Identified specific microbiome associated with juvenile idiopathic arthritis, novel loci associated with type 2 diabetes through meta-analysis of GWAS studies.
- Identified 579, 501 and 442 differentially expressed glycogens (DEGGs) for brain vs liver, brain vs muscle and liver vs muscle groups respectively. DEGGs have been shown to be associated with drug resistance of various cancers.
- Network analysis of DEGGs identified PEMT and HPXN genes in Brain vs lung; IGF2 and NID2 genes in brain vs muscle; and STAT6 and FLT1 genes in lung vs muscle samples as hub proteins with highest degree. Further work is required to confirm role of hub proteins in cancer tissue
- Network analysis of DEGGs identified genes have shown that these are hub proteins with highest degree.
- Comparative analysis of gut-microbiome has shown that enterobacteriaceae and bifidobacteriaceae are significantly associated with Juvenile idiopathic arthritis.

- Identified two classes (Delta-proteobacteria in phylum proteobacteria and RF3 in phylum tenericutes) significantly associated with liver cirrhosis. Further studies are required to correlate significance of these changes with disease causation, manifestation and progression
- Comparative analysis of gut flora from Indian population with American population showed that Indian population has higher abundance of bacteroidetes and reduced abundance of firmicutes than American population. Further studies are required to associate this difference with food habits and obesity.
- Proposed that lactulose administration for treatment and prevention of hepatic encephalopathy and minimal hepatic encephalopathy has no effect on gut microbiome composition
- Developed SickCell Patient Data Management and Analysis Software for storage, management and analysis of clinical and biochemical data of sickle cell disease patients.
- Developed comprehensive cholera portal containing genomic, proteomic, phenotypic, epidemiological, literature, meeting reports, seminars, symposium abstracts as well as recent diagnostics and therapeutic information on cholera
- Developed database of genes involved in infertility containing information about polymorphism/mutations in genes.
- Developed repository on genetic polymorphism and affected pathways associated conditions in polycystic ovarian syndrome
- Developed registries of glomerular disease, drug induced liver injury and community acquired acute febrile illness containing data on general examination, laboratory investigations and medications.
- Developed India Battles Cancer, a portal that provides information on the leading cancers in India with major focus on awareness, prevention and treatment of these cancers. The portal currently focuses on information for the general public and patients.
- Developed database of BCR stereotypy associated with chronic lymphocytic leukemia (CLL). Developed a webserver for prediction of CLL stereotypy.
- Developing repository of data on nutrition, malarial infection, diarrhoea, measles and acute respiratory infection collected from rural, tribal and primitive tribes of Kalahandi and Rayagada districts of Odisha. It is an ongoing program of the Institute.
- Developed and updated database of drug resistance tuberculosis containing socio-economic, patient, clinical, biochemical and molecular profile of patients with multidrug resistance (MDR) and extensive drug resistance (XDR) tuberculosis.
- Developed Leishmanial Metabolome database containing comprehensive curated collection of Leishmania metabolites and metabolism data. The data was compiled from MALDI and LC-MS experiments as well as from the literature.
- Developed database of genes involved in oral cancer. The data is collected from public resources and literature. It is a comprehensive resource on Oral Cancer.
- Developed NucleoProtDb: Database containing experimentally determined 3D structures of diverse types of protein-adenine/guanine phosphates complexes
- Developed national food and nutrient database using books and data published by National Institute of Nutrition.
- Developed a diet calculator with complete recipe dictionary and recommended dietary guidelines for Indian citizens.
- Developed national food borne diseases surveillance portal containing detailed data on food borne outbreaks collected from different sources including media
- Developing clinical data base for surgical gastroenterology containing patient details, reports, diagnosis, reports of patients admitted to SKMIMS since 1999

ICMR Computational Genomics Centre

High-throughput technologies are transforming the medical research from single gene based studies to analysis of entire metabolome and metagenomes.

Analysis and interpretation of high-throughput data requires expertise and infrastructure, beyond the capacity of single laboratory. In order to provide a centralized resource for ICMR research laboratories and medical research community of India, BIC prepared a proposal for establishing a centralized ICMR Computational Genomics Centre with a mandate to provide consultancy, services, expertise and infrastructure to medical researchers in analysis and interpretation of high throughput genomics data. The proposal has been accepted by ICMR and DHR.

Research Management

During the year BIC processed 16 adhoc projects and 44 fellowships in the area of Bioinformatics and medical informatics. BIC is taking initiatives to promote projects on medical informatics and projects involving use of primary patient data.

ICMR Website

ICMR Website is a platform to publish announcements related to ICMR headquarters and its institutes. It is updated on daily basis. Regular updation includes employment opportunities, call for proposals, workshops and conferences, courses, results, official circulars, recruitment rules, RTI information, tender links, report of non-ICMR scientists visiting abroad to present paper, tour reports of ICMR international fellowship programme for Indian biomedical scientist, IJMR monthly issue, STS information, press release *etc.* Database is also updated regularly for publications detail, scientist profile, extramural projects. A link and space is provided to host NAC-SCRT. Average page view of ICMR website for the year 2014-15 is 16401. International visits are 38.47% and visits from India are 60.38% (As per Web Analytical Software designed by NIC)

Time Bound Reports and Other Services to the Council

Parliament queries and other time bound queries from ministry are answered with the help of extramural project information system. BIC is handling the nodal point for ePublishing of tenders on the CPPP Portal for ICMR and its institutes, Headquarter emails are checked and forwarded to corresponding divisions.

Management of Servers and Network

The internet and intranet are provided by BIC to ICMR Headquarters and its Institutes. Database server for Short Term Studentship (STS), Management of Acute Coronary Event (MACE) Registry, National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT), OPA (File Tracking System) Payslip and GPF is managed by BIC.

Video conferencing facility

Video conferencing facility is managed and provided by BIC to ICMR Headquarters and its institutes. It is used to conduct interviews and meetings in the country as well as abroad.

WEB-BASED EXTRAMURAL PROJECT MANAGEMENT SYSTEM

The web-based Extra Mural Project Proposal Processing and Management System developed by ICMR is now fully functional. The system has started functioning w.e.f. January 2012. The system encompasses the complete life-cycle of a proposal funding beginning with the submission of project proposals to its processing, sanction, funding till it's completion through web-based system only.

During the year, a new module has been designed and developed for PRG recommended budget and 'Finance' which includes 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. A fully operational online system has brought about transparency and differential access to the system provided to the concerned technical heads and the Director General.

There has been an increase in the number of proposals received by ICMR using this system.

On an average, 259 pre-proposals are received every month. In the year April 2014-March 2015, ICMR received 3108 pre-proposals using e-PPS. These proposals were reviewed and of these, 870 pre-proposals 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.

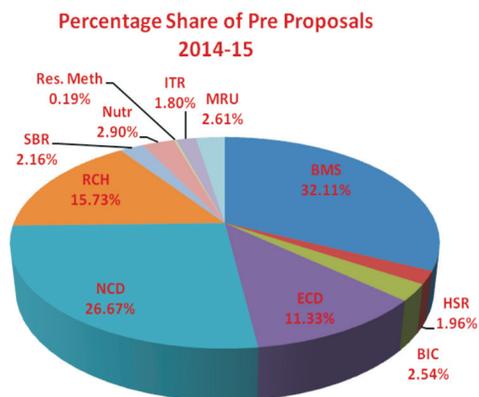
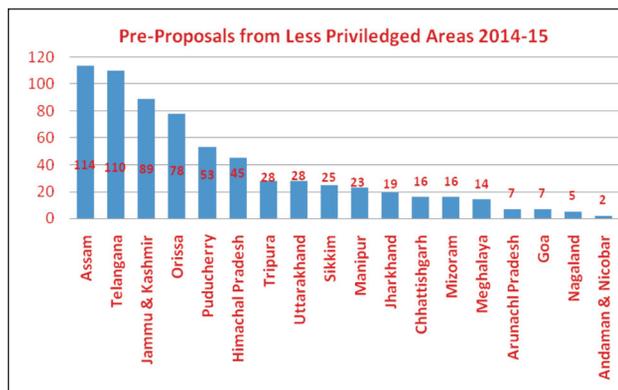
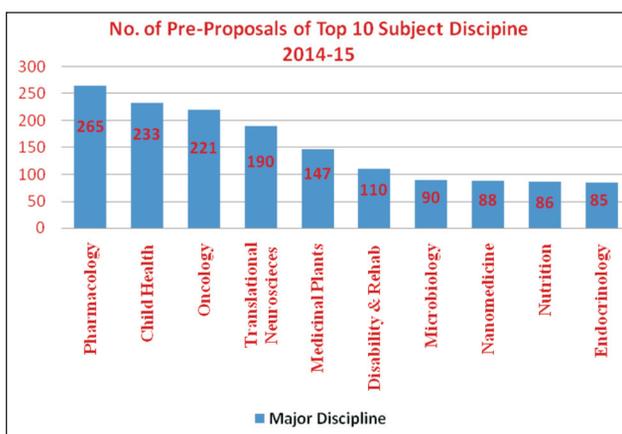


Fig. Percentage share of pre-proposal (Division wise).

Research Methodology, Innovation and Translational Research and Multidisciplinary Research are the major areas that were introduced during the year.



The top most subject discipline was Pharmacology which was followed by other important areas of Health Care. last year (2013-14) Oncology topped the list and pharmacology was on 3rd position.

During the year under report, a total of 866 full proposals were received and marked to respective Technical Divisions for processing. During the year, recommendations of Experts/Project Review Committees pertaining to a total of 1016 detailed proposals were communicated online. Of these, 188 projects were approved by PRC for further processing, 70 projects were technically approved with clarifications on certain issues and rest were not found suitable for funding.

Online project processing and management scheme has been designed and developed for DHR under “GIA scheme for Inter-Sectoral Convergence & Coordination for Promotion and Guidance on Health Research”. The portal became operational *w.e.f* August 1, 2014 and a total of 432 Pre Proposal were received on the web portal of DHR.

An initial analysis of the data culled out from the system for ‘ad-hoc’ proposals being submitted from the different parts of India, has clearly indicated change of productive institutions, subject areas being covered by investigators, the pattern of Cities and the ‘Major Discipline’ being chosen by investigators. Some of the remote cities, which have entered in the ICMR Extramural Project Scheme, are Amrawati, Anglong, Barpeta, Bhimavaram, Cachar, Gorimedu, Idukki, Una, Nanded, Palakkad, Palampur, Sangli and Rewa.

ICMR INSTITUTES/CENTRES

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2. National Institute of Occupational Health
Meghani Nagar
Ahmedabad 380016
3. National Institute of Epidemiology
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Tamil Nadu Housing Board
Ayapakkam
Chennai 600077
4. National Institute for Research in Tuberculosis
No. 1 Sathiyamoorthy Road
Chetput
Chennai 600031
5. National Institute of Malaria Research
Sector 8, Dwarka
New Delhi 110077
6. National Institute of Nutrition
Jamai Osmania, Tarnaka
Hyderabad 500007
7. Food and Drug Toxicology Research Centre
National Institute of Nutrition
Jamai-Osmania
Hyderabad 500007
8. National Centre for Laboratory Animal Science
National Institute of Nutrition
Jamai Osmania
Hyderabad 500007

9. National Institute of Cholera and Enteric Diseases
P-33, CIT Road Scheme XM
Beliaghata
Kolkata 700010
10. Centre for Research in Medical Entomology
4, Sarojini Street
Chinna Chokkikulam
Madurai 625002
11. Enterovirus Research Centre
Haffkine Institute Campus
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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 Multistoryed 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
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25. National Centre for Disease Informatics and Research
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Poojanhalli Road, Off NH-7
Adjacent to Trumpet Flyover of BIAL
Kannamangla Post
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27. Regional Medical Research Centre
Nehru Nagar
National Highway No. 4
Belgaum 590010
28. Regional Medical Research Centre
Nandankanan Road
P.O. Chandrasekharapur
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29. Regional Medical Research Centre
N.E.Region, East-Chowkidinghee
Post Box No. 105
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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
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Port Blair 744101

ICMR CENTRES FOR ADVANCED RESEARCH

1. Advanced Centre For Newborn Health Research,
All India Institute of Medical Sciences,
New Delhi.
2. Centre for Evidence Based Child Health Advance Pediatric Centre,
Postgraduate Institute of Medical Education and Research,
Chandigarh.
3. Centre for Advanced Research on Environmental Health:
Air Pollution, Sri Ramachandra University,
Chennai.
4. Emerging Areas In Molecular Medicine, Jawaharlal Nehru University,
New Delhi.
5. Centre for Molecular Medicine, Sanjay Gandhi Postgraduate
Institute of Medical Sciences,
LuckNow.
6. Centre of Excellence In Molecular Medicine, All India Institute of, Medical Sciences,
New Delhi.