Annual Report 2013

Regional Medical Research Centre
(Indian Council of Medical Research)
Bhubaneswar, Odisha

Annual Conference of LSI Organised by RMRC Bhubaneswar

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Regional Medical Research Centre

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CONTENTS

Contents

From the Director's desk...

The Centre has focussed its activities in areas of research in both communicable and non-communicable diseases, human resource development programme and in establishing strong linkage with State Health Department in finding solutions to the regional health problem. Besides basic and applied research, translational research has been taken up in operational mode. Communicable research programme includes studies on lymphatic filariasis, malaria, diarrhoeal disorders, tuberculosis and diseases of viral origin. Non communicable diseases include nutrition, sickle cell disease, hypertension and diabetes.

Besides establishing the field units at Rayagada and Kalahandi in collaboration with Govt. of Odisha, the centre has established a new Bio-informatics Cell with support from council. Research on diabetes and hypertension has been added as new dimension added to research area of the centre from this year. Activities to be undertaken at Rayagada and Kalahandi will address issues on improving health related parameters pertaining to diseases of public health importance. Preparedness and prevention of diarrhoeal disorder, malaria, control of IMR, MMR, reduction childhood morbidity and mortality, burden of tuberculosis and augmenting RNTCP through strategy development in two field units like improving nutrition in under 5 areas are the key elements of activities initiated in the said units. Transfer of Technology, training and capacity building are included to ascertain sustainability of the programme.

During the period, 24 scientific projects were undertaken by this Centre of which 17 are extramural in nature, funded by ICMR Task Force, DST, DBT, NVBDCP or Gates Foundation. Of these, 19 are on-going including three translational and 8 studies got completed.

During the year 2012-13, till date 25 papers are published with average impact factor of 2.46. All publications are indexed, published mostly in reputed International Journals.

The Center's library subscribes 53 print journals (Foreign & Indian) for the year 2013 along with online subscription of JCCC@ICMR through ICMR consortia and ICMR E- Journal Consortia. The Institute is connected with 100 MBPS NKN Leased lines which is operational round the clock for networking. The Centre's library is WiFi enabled. This year the Centre's library has started a Daily Article Service to all ICMR scientists through mail which provided full text of a current research article on a specific research topic on all working days.

The Centre has generated 10.20 crore through sponsored research in year 2012 & 13. Human resource activity focused on imparting training to M.Sc students sponsored from various reputed Universities in the state and outside to complete their 6 month dissertation work. During 2013, 31 students completed their course work. Pre-Ph.D course has been introduced this year under Utkal University. University sponsored 17 students are regularly attending classes at this Centre from June to December 2013. Ph.D scholars sponsored from UGC and ICMR under fellowship as JRF / SRF

altogether 12 candidates are continuing Ph.D programme on date. Ph.D were awarded to 8 Ph.D students during 12-13. Ph.D thesis submitted by 5 students working under the scientists of the Centre as guide. One Postdoctoral fellow (ICMR)is also working in this Centre. Training was imparted to around **50** staff engaged by Registrar General of India on blood test (Hb%) and anthropometry for use in field to estimate national data on anaemia. State Govt. staff were trained on entomological attributes that can help identification and estimation of infection to assist vector control in malaria. At Kalahandi and Rayagada field units, technology transfer on ELISA & PCR for diagnosis of dengue and culture to diagnose diarrhoeal disorder has been completed and regularly monitored.

The Centre has established linkages with other ICMR and non-ICMJR institution like State Govt. of Odisha, NVBDCP, Delhi, RGI, Gates Foundation, DBT, AIIMS, Delhi, IVI, DST in upgrading the expertise, sharing scientific information and collaborative research programme. Training on vaccinology was undertaken by our scientist at International Vaccine Institute, Seoul, Korea, SGPGI, Lucknow and other reputed institutes. Scientists were deputed outside to present their scientific output in International forum like ASTMH Conference held at Atlanta, USA in Nov. 2012. Collabor5ation with State Health Department was strengthened in form of consultancy, undertaking evaluation of health programme, referral diagnostic services, epidemic investigations and disaster management.

With total staff strength of 103 at present 90 are in position and all vacant positions are in active process being filled up shortly after the selection is over. Out of sanctioned strength of 19 scientists, only 15 scientists are in position.

The Centre has organised several workshops, seminars and meetings including animal and human ethical committee, two Pre-SAC reviews and SAC meeting.

During the year several developmental activities were undertaken by the Centre. The laboratories were upgraded to modular lab to facilitate; more working space to accommodate expansion in research areas and more students. Both field units were furnished with furniture and equipment to make it an advanced lab facility. OPD was constructed through CPWD. Renovation of staff quarters and institute premises, horticulture, guest house and hostel facilities carried out with support from ICMR. Foundation stone of BSL3 facility was laid down by Secretary DHR and DG, ICMR that was processed for construction during the year. Construction for separate administrative building adjacent to the main lab building and traning facility for Ph.D scholars, M.Sc students were processed for construction during the period. Bio-informatics Cell was modernised with equipment and other required gadgets.

Several cultural functions and Annual Function of the Centre was organised as a Staff Welfare activity. For the staff, gymnasium facility has been provided Besides, playground and canteen facility were processed for construction. I sincerely thank scientists and staff for their endeavour and contributions. I am also thankful to the State Health Department and other agencies, collaborating Institutes and experts of SAC, ethical and other technical committees for their assistance, support and co-operation. I extend my deep gratitude to DG, ICMR and Council for their continuous support, guidance and encouragement. With all round support, the Centre can continue its endeavour to achieve its goal.

DR.S.K. KAR
DIRECTOR

HIGHLIGHTS OF RESEARCH ACTIVITIES: 2013

During this year the centre has made attempt to address some of the most important health challenges like vector borne diseases diarrhoeal disorders, bacterial meningitis, encephalitis, tuberculosis, and non-communicable diseases like under nutrition, diabetes, hypertension and sickle cell disease. Most of the studies are sponsored either by ICMR Task Force DST, DBT, Gates Foundation, NVBDCP and MoH&FW. The network established with the State Health Department, Medical Colleges and Hospitals of the region for referral investigation of sporadic cases and outbreak investigation of viral and other bacterial infections has been further strengthened. In order to strengthen the manpower of this region the centre is undertaking pre PhD, PhD, MSc dissertation (six month) and various training courses. Two field units, one at Raygada District Head Quarter Hospital, Raygada and other at District Head Quarter Hospital Bhawnipatna of Kalahandi district have been established in collaboration with the state health department and started functioning by way of providing diagnosis for diarrheal disorders, drug resistant TB, nutritional assessment and transfer of technology to the state health services. Here are highlights of important activities ranging from basic to translational research that have been undertaken during this year by the scientists with an aim to solve some of the public health problems.

The National Health Policy of Government of India aims to eliminate lymphatic filariasis from the country by 2015. Evidence suggest that filarial infection largely affects young population showing 25.30% infected among children below 5 years of age. In contrary,clinical signs of filariasis occurs in later age group, during adolescence or adulthood. To explore any sub-clinical pathology in prolonged silent phase that leads to fulfledged disease later, 102 children between 5 to 18 years of both sexes with evidence of infection (either Microfilaremia or circulating filarial antigen) with or without symptoms were enrolled in to the study. Lymphoscintigraphy examination detected abnormality in the lymphatic scan among 74(72.5%) enrolled children at the baseline. While ultrasound examination using 11 megahertz probe indicated presence of adult parasite in same side lymphatic pathology detected. Symptomatic subjects demonstrated higher frequency (80%) of pathology. Amongst the enrolled children 52 were assigned randomly to annual and 50 to biannual dose of DEC + Albendazole and followed up 6 monthly for two years at different time points. Result shown improvement in lymphatic pathology in 71%, 94% and 85% of children at 6th, 12th and 18th month period respectively.Total reversal of Pathology was observed in 65% of individual at 24^{th} month. MF clearance and adult worm clearance at 24^{th} month (n=50) was 95% & 60% respectively. This indicates that it can serve as an effective tool for advocacy to improve the compliance rate of MDA in children as well as a tool to prevent morbidity by reversing early lymphatic pathology amongst asymptomatic cases in the community. Even the cases with early lymphoedema exhibited reversal.

Although host genetic polymorphism and other environmental factor(s) may influence susceptibility to infection and disease, filarial infection in mothers has been considered a risk factor for increased susceptibility to infection in the off springs. Elevated levels of IL-10 in anti-sheath negative cord blood and IFN-ã levels in anti-sheath positive blood, cord irrespective of infection status of mothers provides an evidence that presence / absence of anti-sheath antibodies with the

association of cytokines in the neonates skewed the filarial specific immunity to either Th1 or Th2 responses and can decide the natural history of filariasis during childhood. Another study has indicated increased population of apoptotic T cells among infected individuals compared to endemic controls along with increased Fas-L expression on the surface of B1 cells. Since FasL expressing B-1 cells are important mediators of CD4+ T-cell apoptosis, they may be responsible for hypo responsiveness in microfilariaemic individuals.

As a participating Centre, under National network for genotyping human filarial parasites, alllele frequency of different gene loci (beta-tubulin, ALT-2, ITS or r-DNA in W.bancrofti parasite population of endemic areas of Odisha was studied. This will be useful for the future planning for LF elimination programme. L3 stage specific RT-PCR assay for detection of infective stage *W.bancrofti* in vector was evaluated as a part of multi-centric project. The assay has been found to be useful for monitoring LF elimination programme.

Malaria survey conducted using molecular tool in Badampahar CHC (MayurbhanjDist) and Ghatgaon CHC (KeonjharDist) has revealed P malariae monoinfections in 11.6% of cases while mixed infections were 14.2% in Ghatagaon and 6% in Badampahar. Under the current scenario of screen and treat strategy, presence of P malariae poses a difficulty, as the screening is performed by RDT alone. Further in the present study the species specific RDTs were not able to detect neither the monoinfection nor the mixed infections of P malariae. Therefore the molecular method can be used as a tool for surveillance to overcome such problems.

Since polymerase Chain reaction may not be feasible in field condition, an alternative tool i.e. LAMP assay has been developed to detect different malaria parasite (*P.falciparum*, *vivax* and P malariae). Primers have been designed and reaction conditions standardised. The efficacy has been shown to be comparable to PCR. It will be validated in field condition and observer variation will be checked before recommendation.

Treatment seeking behaviour, LLIN use and acceptance of IRS by the tribal communities was studied. Even though acceptance was seen to be high (around 90%) and 92% of the households were willing to purchase ITN / LLIN at subsidised cost, the API was seen to be high (more than 14) in the studied villages of the two blocks of phulbani district. Hence, other factors need to be addressed. Distribution of sibling species of malaria vectors and their role in transmission is being studied. Genetic sequence of ribosomal D3 and mitochondrial COII region of Anopheles mosquitoes were submitted to gene bank, NCBI; to which accession numbers assigned for use in public domain.

To determine the feasibility, acceptability and costs associated with the introduction of the modified killed whole cell oral cholera vaccine in India a public health setting has been assessed by instituting, a pilot study has been instituted in Satyabadi block of Puri district during May –June 2011. The 1st dose of mass vaccination was received by 31551 and 2nd dose by 23751 individuals. The first dose coverage, based on population census was around 61% with a drop-out rate of 25%. A total of 46% of population received two (complete) doses of oral cholera vaccine during the mass vaccination campaign. The post vaccination surveillance is being undertaken continuously. A matched case-control study is being conducted in the above population to assess the individual protection level of Oral cholera vaccine following implementation. Cholera positive cases are being identified

through facility-based surveillance and enrolled with matched controls in 1: 4 ratio. Information on vaccination status and other exposure variables from both cases and controls were obtained. A total of 1913 rectal swab samples from the acute diarrhoeal cases attending selected health facilities were investigated identifying 32 cases and 128 controls. After completion of the scheduled cases and controls as per the protocol odds ratio will be analysed, that will show the individual level protection of the vaccine. Community level protection also will be analysed to give the comprehensive effectiveness of the vaccine at community level.

Etiology of diarrhoea in 3 tribal districts of Odisha was investigated. Bacterial pathogens their molecular characterization and anti-biogram was described and reported to district public health system along with water sources identification of infection. Prompt investigation and early reporting could support public health action in preventing epidemics and diarrhoea case fatality. Further the centre has carried out outbreak investigations of severe diarrhoea in tribal dominated areas; Mohana, Laxmipur, Dasmantpur and Kashipur blocks of Gajapati, Koraput and Rayagada districts from October, 2012 to June, 2013.Out of total 107 rectal swabs collected , 81 were culture positive (75.7%) from which 48 (59.2%) were E. coli, 16 (19.8%) were Vibrio cholerae O1 Ogawa, 11 (13.6%) were Shigella spp. and 6 (7.4%) were Aeromonasspp, while no V. cholerae were isolated from water samples. The early reporting of cholera had helped the health authorities of the district to take adequate control measures which could check the spread of cholera epidemic in this region.

As a part of the preparations for the phased introduction of a pentavalent vaccine (DPT-Hep.B-Hib) by Government of India in selected states of the country in Universal Immunization Programme(UIP), a 24 hour sentinel surveillance unit has been established at SVPPGIP, Cuttack to find out the burden of the disease. During the period under report, 543 CSF and 250 blood samples were investigated from suspected meningitis cases between 1 to 59 months of age. Amongst them 16 samples were found to be latex positive (six for Hib, ninefor S.pneumoniae and one for group B streptococcus), 3 CSF were culture positive for Staphylococcus aureus and 1 was positive for Salmonella typhi and 2 blood samples were culture positive for Klebseillapneumoniae and 4 were positive for Pseudomonas aeruginosa. Real time PCR identified 21 cases of Streptococcus pneumonia and 4HiB. Antibiotic sensitivity testing supported case management in the hospital.

Investigation on viral diseases is on-going under ICMR virology grade 1 network laboratory project. From Jan to October 2013, a total number of 5412 samples from sporadic / referral cases were received from different Govt. and Private Hospitals of Odisha for investigation. Amongst the total cases majority were with clinical suspicion of a viral diseaseiefor hepatitis (n=919), Viral diarrhea (n= 546) , Rubella (n=444) , Encephalitis (n=442), Dengue(n=103) , Respiratory infection (n=97) , Measles (n= 78) , Chickenpox (n=72) , Human Papilloma Virus (n= 17) , CMV (n=11) , Mumps (n=6) , Fever and rash (HFMD, Parvo) (n= 4) and EBV(n= 1) etc. In addition during this period 1 outbreaks of jaundice, 4 chikenpox, 4 measles, 2 rubella and 1 encephalitis out breaks covering 11 districts have been investigated along with state health department from these samples total 39 different types of viruses have been detected .They were HSV I, HSV II, JE Virus, Dengue, CHIK, Rota, Astro, Adeno(Enteric), Noro G1, Noro G2, Coxackie, Measles, Varicella, Mumps, Rubella, Entero HAV, HEV, HBV, HCV, HDV, HPV, EBV, CMV, Adeno, Influenza A(FluA), FluA(H1N1), Flu B, HMPV

A / B, Rhino, Para influenza 1, Para influenza 2, Para influenza 3, Para influenza 4, RSV A / B, Corona viruses(Cor63,Cor229,Cor43, HKU1), Parecho virus, Boca Virus(HBoV) and EV. The enteric virus, Rota, found during this year mostly belongs to G1, G9, G10, and G12 types and P8, P10 types. HAV infection were observed in 6-15 years of age group (42.8%) of patients while, HEV infection was high in adult population (56.8%). Amongst the respiratory infections Adeno Virus was most common (22.2%) followed by Human Boca, Rhino and Corona Virus. Neurotropic viruses causing acute encephalitis admitted to different hospitals were investigated and clinical manifestations described. HSV, Measles, Dengue and Varicella zooster virus were seen as the major causes of viral AES either as single or co-infection.

During 2010 Dengue serotype II was prevalent whereas in 2012-13 all four serotypes were found to circulate with serotype II as the dominant type. In the northern region serotypes were I, II and III, whereas in sourthern regions serotype II and IV were reported. CHIKV circulating in Odisha, seen to be of IOL (Indian Ocean Lineage) strain within ECSA (East Central South Asian) genotype, originating probably from the Kenya 2004 strain (predecessor) and being transmitted by Aedesalbopictus. It was observed that the recent outbreaks of chikunguniya in Odisha have been caused by IOL group of ECSA-Genotype, which was postulated to be transmitted by Albopictus, this is the most abundant vector in Odisha.

Under surveillance of drug resistance TB in Raygada district, 634 sputum positive tuberculosis samples were subjected for drug susceptibility testing with the four first line drugs, where mono resistance was seen in 35. Out of this isoniazid in 14 cases, streptomycin in 21 cases and 3 isolates showed resistance to isoniazid and rifampicin (MDR). However MDR was not observed in any of the newly diagnosed TB cases (n=577).

The study undertaken in Kalahandi (Dharmagarh and Junagarh) and Rayagada (Jamadehipentha) districts to assess the knowledge, attitude and behavior on reproductive health problems of adolescents, quality of care at Adolescent Friendly Health Clinics, accessibility and utilization of health care services by adolescents so as to devise plausible ways and intervene with package of services for improving utilization of adolescent health services indicates that adolescent females of Rayagada(21.3-31.8%) are experiencing more premenstrual complains syndrome than the adolescent females of Kalahandi district. In both areas about 50% of the girls are maintaining menstrual hygiene practices, but only 4-12.7% are using sanitary pads. Similarly knowledge on RTI / STI and HIV / AIDS and level of awareness about contraception and family planning methods is greater in Kalahandi as compared to Rayagada district.

With respect to the access and responsive of the health system towards migratory population, the centre has found that migrant community usually experience common health problems like common cold, fever, gastritis, diarrhoea, abdominal pain, vomiting, dysentery, asthma and T.B. Around 45 % availed regular health services from government health facilities. The husband or elder person like mother in law / aunt are the decision maker or health issues. Among the pregnant women 80% opted for institutional delivery and 56% of them had gone for 3 visits of ANC and 90% have received more than 2 doses of injection TT in urban slums of Bhubaneswar MC. The individual component of primary MCH services indicates a good coverage. But when all components combined

together, the coverage is very low (around 12.9%) only. Therefore an interventional package combining the approaches of inclusive partnership with government and non-governmental providers, CBOs, community participation and community mobilization for better health service utilisation has been developed and is being implemented.

Neonatal screening for sickle cell disease was undertaken in Kalahandi district from March 2013. This included 635 cord blood samples. 110 cord blood samples were found to be SCD positive of which 8 were homozygous for the disease. A three monthly door-to-door follow-up and investigation of parents of 2 homozygous and 9 heterozygous SCD neonates confirmed their SCD status and information on parental morbidity pattern using a pre-designed questionnaire was collected. The percentage of SCD homozygous cases was found to be highest in M. Rampur block of Kalahandi district. No SCD related morbidity was detected in the newborns followed till date.

Under translational research the centre has taken effort to develop two PCR based tools for public health use. One is to monitor the information of vector prevalence, incrimination of vector for malaria transmission, identification of the sibling species of vector and chloroquine (CQ) sensitivity of the parasite ingested by the vector. This technique has been internally validated and the practical aspects of the technique has been demonstrated to the researchers of NIMR and programme personnels of the state NVBDCP in workshop held during 4th to 8th February 2013 at Regional Medical Research Centre, Bhubaneswar . The other tool developed by the centre is to detect all different serogroups of V. Cholera causing cholera in a single PCR test. In-house validation of the technique has been done. Applicability of both the tools are now being field tested.

As a service component the centre is providing outpatient facility to patients of lymphatic filariasis and haemoglobinopathy at Capital Hospital, Bhubaneswar. The facility is being utilized for referral investigation, diagnosis management of suspected cases of filariasis in haemoglobinopathy from different parts of the state. Besides, the facility is providing treatment to acute and chronic filarial disease including decompression therapy for filarial lymphedema reduction.

Chronic kidney disease with high case fatility in two blocks of Cuttack district was raised as a public health concern by the district administration. Baseline investigation was undertaken in collaboration with district health system, SCB Medical College, Cuttack and NIN, Hyderabad. The initial survey indicates chronic tubule-interstitial disease, Possibility of heavy metal poisoning effect of indiscriminate use of analgesic or any other toxins have been planned to be evaluated.

Under tribal health research forum a study is being undertaken to develop a morbidity management strategy for febrile illness through syndromic approach in tribal population of Rayagada district. Baseline morbidity survey undertaken in 5800 population covering 21 villages from 3 adjacent sub-centres has revealed that the prevalence of febrile illness was 9.5% and 1.8% in winter seasons . Total morbidity including non-febrile disorders was 21.9% and 6.44% of the population during above seasons. Among the febrile illness, acute respiratory infections (62%), malaria (22%) and diarrhoeal disorders (10%) were common during rainy season, while in winter, URTI was commonnest. Both the extremes of ages (<5 yrs& more than 60 yrs) were predominantly affected. Bacterial infections (S.pneumonae, H.influenzae& Staph aureus) were identified as the cause of ARI in 75% cases followed by viral infections (Corona & Para influenza) in 20% of ARI cases. P. falciparum was the dominant

(87%) malaria parasite. Diarrhoeal disorders were associated with E.coli in 30% & Rotavirus in 10% of cases. Under nutrition was found among 71.4% of children below 5 years of age. The population demography showed a distorted pyramidal structure with low population in higher age group (>55yrs) compared to normal population. To answer the above distortion, cause of death was analyzed by verbal autopsy procedure, covering 52 deaths in past 1 yr. The common causes of death noted were cardiovascular diseases, pulmonary tuberculosis, liver diseases and malaria.CVD deaths were corroborated with hypertension prevalence of 24.6% in adults.

Baseline assessment on functioning of the existing health system, capacity of grass-root level health providers and knowledge/awareness/proactive of the community has been undertaken to frame a strategy for intervention. Implementation of this strategy has been initiated in a pilot mode for pre-testing and results being analysed for modifying the strategy for intervention and evaluation.

Analysis of host genetic factors associated with essential hypertension among the natives of odisha has revealed that the CYP11B2 -344T/C, eNOS VNTR 4a/b, ANG G-6A polymorphisms are associated with hypertension in males and AGTR1 A1166C, eNOS VNTR 4a/4b and eNOS E298D polymorphisms are associated with hypertension in females.

Two field units in tribal dominated districts (Rayagada and Kalahandi) established in collaboration with State Health Department are operating with the goals to improve the health parameters of the region with emphasis on tribal population. Technology transfer, alternative health care strategy and application of advanced diagnostic techniques at district headquarters level are being focused to achieve the goal.

Annual Report 2013

On Going Studies

Contents

On Going Studies

Contents

Translational Research

1. A study of Sub-clinical Lymphatic Manifestation in W. bancrofti Infection.

Principal Investigator	:	Dr. S.K. Kar
Co-Investigators	:	Dr. B. Dwibedi, Dr A.S. Kerketta
Starting date	:	October 2009
Closing date	:	April 2014
Funding	:	Extramural (GATES Foundation, USA)

Background

Several reports from filarial endemic regions globally including Odisha indicated that while most of the endemic children (25-30%) below 5 years of age get infected, overt clinical disease appears later in life, ie. late adolescence or adult hood. It is not clear about any pathology that develops following infection till the clinical signs appear. Lymphoscintigraphy evidences suggest sub-clinical lymphatic abnormality in mf carriers who does not show any clinical signs. Study on *B. malayi* infected children (3-15yrs) has shown evidence of sub clinical lymphatic pathology in form of lymphatic obstruction.

It was proposed to undertake an observational study to find out any sub-clinical lymphatic pathology in filariasis infected children and adolescents in *W. bancrofti* endemic area of the state; and to observe the effect of MDA with DEC and Albendazole (alb) on the lymphatic abnormality.

Objective

1. Prevalence of sub clinical lymphatic pathology in population between 5-18 years with *W. bancrofti* infection in defined endemic community.
2. Effect of single annual and biannual dose of DEC plus Albendazole on lymphatic pathology in the identified group.

Progress of work

Screening and Enrollment

102 subjects have been enrolled to the study after confirming the eligibility criteria, of which **52** subjects were assigned randomly to annual and **50** to biannual dose (DEC + Albendazole) group. Out of **102** subjects, **50** were symptomatic and rest of the children were asymptomatic, but with detectable mf and/or antigenimia.

Out of **52** asymptomatic children **32** were mf -ve and antigen positive where **20** were positive for antigen and mf. In **50** symptomatic children clinical signs or symptoms of filariasis were observed in form of presence or history of lymphadenitis, lymphedema, testicular enlargement or hematuria. Out of these children **10** were positive for mf.

Baseline investigation and follow up

All the subjects enrolled at baseline **(n=102**) had undergone Lymphoscintigraphy and ultrasound examination. They were given dose of DEC + Albendazole in the dosages prescribed for their age and study arm (annual or biannual). Till date **101** children completed 6 month follow up, **100** completed follow up for 12 month, **100** completed 18 month follow up and **53** subjects have completed 24 months follow up. All the investigations were repeated 6 monthly. The lymphatic abnormality noted at baseline was compared with the subsequent follow up results which is outlined below.

In the enrolled subjects, the initial microfilaria (mf) count ranged from **2 to 1540 mf/ ml** (GM=**208.75**) The Og4C3 titre in the Baseline was **182 to 15107 units (Mean=5108).**

Lymphoscintigraphy of both upper and lower limbs was carried out by expert in nuclear medicine using radio labeled sulphur colloid. The procedure was standardized before initiating the study. Effect on lymphatic pathology was evaluated by comparing

the scintigraphic observation made at the follow up visit with the pretreatment (baseline) findings. The scintigraphic image showing visualization of lymph nodes and lymphatic channels on both the limbs and the tracer uptake ratio from the distal end of the limb was compared with the baseline observations in the same limb, to interpret on the lymphatic flow/ pathology and improvement if any.

Out of **102** subjects **73(71.5%)** had shown some abnormality in the lymphatic scan at baseline. The earliest age showing lymphatic pathology was 6 years among the studied children. Ultrasonography has shown filarial dance sign (FDS) of adult worm in **9** subjects.

All the enrolled children were given first dose of DEC plus Albendazole supervised by a physician and they were followed for any side reactions. Among them **10 (9.8%)** children reported to have side reactions like fever, headache, and leg pain, nausea, head reeling and cough. All were mild in nature and managed at home. 4(3.96%) had side reaction at 6th month and 3(3.19%) at 12th month and 2(4.25%) at 18th month & no side reaction was recorded at 24th follow up period.

Out of the **102** children **(50** Symptomatic & **52** Asymptomatic) enrolled at baseline **101** children completed 6 month follow up, **100** completed follow up for 12 month, **70** completed 18 month follow up and **16** subjects have completed **31** months follow up. Result of repeat lymphoscintigram at these time point compared with the baseline status has shown improvement in lymphatic pathology hence lymphatic flow in 72/101 (**71.2%**), 71/100(**71%**), 71/100 (**71%**) and 38/53(**71.69%**) of children who had baseline abnormality and followed at 6th, 12th, 18th and 24th month period respectively.

Subsequent Plan

As enrollment of all subjects is over so now follow up activity is going on which is expected to be completed by April 2014.

2. Identification of markers to detect the early on set of infection in children during their postnatal exposure to fillariasis.

Principal Investigator	:	Dr. M.S. Bal
Co-Investigators	:	Dr. S.K. Kar ,
		Dr. A. K. Satapathy
		Dr. N. N. Mandal
		Dr. P. K. Sahu
Starting Date	:	July 2013
Duration	:	Three years
Funding	:	Intramural

Background

Maternal infection has been considered to be a risk factor for filarial infection in offspring. Since the first exposure of an individual to filarial antigen takes place in-utero, maternal filarial infection presumed to play an important role in the outcome of infection. In our hypothesis susceptibility to filarial infection may be due to transfer of filarial antigen and subsequent intra uterine sensitization that modulates the outcome of infection in children. To prove the hypotheses the following project has been undertaken.

Objectives

(i) to follow-up the children born to filarial infected and non infected mother for observing parasitological, antigenical and clinical out come

(ii) to determine the influence of maternal infection on subsequent B cell response (antibody isotype) to filarial antigens among follow-up children

(iii) to find out the extent of modulation of parasite specific cellular reactivity and cytokine production in children during their natural exposure to infection.

Progress

The study has been initiated from July 2013. Based on our previous records and the hospital records necessary steps have been taken to identify the infected /non infected mothers. Then the corresponding study villages of infected and non

infected mother have been identified in the different CHC/PHC area of the Khurda district. A detailed clinical history of each enrolled/ study individual as well as their children with regard to filarial infection will be recorded. The study is in progress to collect blood samples from mothers and their corresponding children for detail analysis.

3. The Epidemiology of malaria with special reference to *P malariae* in two tribal blocks of Odisha.

Principal Investigator	:	Dr. M.R. Ranjit, Scientist-E
Co-Investigator(s)	:	Dr. S K Kar, Scientist-G & Director Dr A S Kerketta, Scientist-D Dr. A.S.Acharya, RA Dr M M Pradhan, Dy Director (Malaria), DHS, Govt of Odisha MO I/C of Ghatgaon & Badampahar
Starting Date	:	1 / 3/2012
Closing Date	:	28 / 2 /2014
Funding	:	EM: ICMR (Concept/8/ 2010- ECD-II Dated 5/2/2012)

Objectives

(i) to find out the incidence of *P malariae* along with *P falciparum* and *P vivax*.

(ii) to analyze the intra-species diversity of *P malariae* among the clinical isolates.

(ii) to investigate the association of *P malariae* with severe clinical malaria particularly renal failure.

Background

Every year at least 0.4million people in Odisha are reported to be slide positive for malaria parasites and more than 200 deaths are being reported due to it. Even though the tribal dominated forested districts are known to contribute substantially more malaria than the non-tribal districts, the exact cause of persistence of malaria in those areas is not known. According to the NVBDCP malaria report the *P falciparum* accounts >80% of the malaria cases in the state followed by 10-15% of *P vivax*. However, we have observed as high as 44.6% of *P malariae* by PCR in some selected tribal and forested areas of Orissa compared to 8.3% by microscopy. Although, the reason for such unexpected hike in *P malariae* occurrence is not known, the apparent shortage of *P malariae* prevalence by light microscopy could include morphologic variations that may contribute to misdiagnosis, but the seasonal incidence of the high prevalence cannot be ruled out. Moreover *P malariae* can remain long in blood circulation and cause chronic nephritis. The increased hospitalization of severe malaria cases with multi-organ failure in recent years and growing incidence of malaria attributed renal failures in the tribal districts of the state may be due to misdiagnosis of *P malariae* infection (both mono and mixed) as mono infection of *P falciparum* that needs to be evaluated. Therefore in the proposed study we will do a systematic investigation on the incidence of *P malariae* along with *P falciparum* and *P vivax* and its association with clinical outcome of severe malaria particularly nephrotic syndrome in Odisha.

Progress of Work

During the period (From 1/3/2012 to 28/2/2013) under report about a total of 1589 fever cases (Ghatgaon: 974 and Badampahar: 615) suspected to be malaria were screened by bivalent RDK for malaria in selected sub-centers and patients attending malaria clinic at CHC hospitals. Out of the total 974 fever cases screened in Ghatgaon CHC area 103 (10.6%) were found to be found to be RDK positive. Amongst these RDK positive samples 77 (7.9 %) samples were found to be PCR positive and 70 (7.2%) are microscopically positive (Table 1). Out of total 77 PCR positive samples 30(29.12%) were found to be positive for *P falciparum*, 19(18.4%) were *P vivax* and 9(8.7%) with *P malariae* monoinfections. Most interestingly by PCR about 19(24.67%) cases were found to harbor mixed infections as out of them 11 were with *P malariae* .Most significantly microscopy only 1(1.29%) was found to have *P malariae* monoinfections . The present study indicates that the mixed infections are more common than it was expected. The prevalence of *P falciparum*

is more common in all three seasons than *P vivax* and *P malariae*. Season wise analysis indicates that the prevalence is high in rainy and winter but less in summer and there is round the year transmission. Further it is evident that the prevalence of *P malariae* is more during the rainy season indicates seasonal prevalence.

In Badampahar out of 615 fever cases screened for malaria 60 (9.8 %) were RDK positive and amongst them 33 (5.4 %) were found to be PCR positive and 29 (4.7%) microscopically positive (Table 2). Out of total 33 PCR positive samples 11(18.33%) were found to be *P.falciparum* 17(28.3%) were *P.vivax*. In this area total 5(8.33%) were found have been mixed infections of them 2(3.33%) were of *P, malariae*. The *P.malariae* here were found to be both in rainy and winter season, but not in summer. Microscopically no *P.malariae* could be detected. The prevalence of *P vivax* seems to be equal to the *P falciparum* infection. The *P vivax* infection is more prevalent during the winter season and *P malariae* infection is less than the Ghatgaon CHC. Similar to the situation of Ghatgaon the mixed infections are more common than it was expected. In West Africa and PNG the P.malariae has been characterized to exhibit opposing seasonal fluctuation with *P.falciparum* and *P.malariae* prevalence and / or the parasite densities increasing the dry season.

In our earlier survey carried out during July to October during 2008 we have detected 108 *P. malariae* infections by PCR (>60%) out of 212 positive cases in Mayurbhanj, Sundergarh, Keonjhar, Nayagarh, Rayagada, Kalahandi ,Kandhamal and Angul district. Majority of them were found to be co-infected with *P.falciparum* or *P.vivax*. Since 2009 Odisha has revised its treatment policy by adopting ACT as the first line of treatment in case of *P.falciparum* and CQ +PQ for *P vivax*. Screenings of cases are being done by using RDT. After this the incidence of malaria has come down significantly in both the study areas. In Keonjhar the API has come down from 17.08(2008) to 10.85(2012) and in Mayurbhanj the API has come down to 3.25(2012) from 4.82(2008). Even then during the present survey we have detected the *P.malariae* monoinfections in 11.6% of cases and mixed infections with were 14.2% in Ghatagaon of Keonjhar and 6% (*P.malariae* mixed infections) in Badampahad of Mayurbhanj district. Most importantly these infections are often found as mixed infections as reported in West Africa and PNG. The potential interactions of *P malariae* with *P.falciparum* and *P.vivax* might explain some basic questions of malaria epidemiology and understanding these interactions could have an important influence on the deployment of interventions such as malaria vaccines.

As would appear from Table 3 age wise distribution of species of parasites indicates that the *P.malariae* was more prevalence in older age (>6 years) like *P.vivax* but unlike *P.falciparum* which is present in all age groups in Ghatgaon. Similar to the situation in Ghatagaon the *P.malariae* is more prevalence in older ages in Badampahar here the *P.malariae* is present as mixed infections (Table 4). In West Africa, *P.malariae* prevalence has been reported to peak at ages similar to those of *P.falciparum* (i.e. in children under ten years of age) and in PNG however, *P.malariae* infection is observed predominantly in older children (seven to nine years) like in Odisha.

With respect to asymptomatic cases a total of 241 cases (Ghatgaon: 127 , Badampahar: 114 have been screened. On the other hand total 6 cases (Ghtagaon: 5 and Badampahar: 1) with ARF were admitted to the CHC hospital during this period. Out of 6 cases 4 were with *P falciparum* mono infection and 2 with mixed infection.

Our findings are very important in the current scenario of screen and treat policy of the programme. Under this screen and treat strategy, presence of *P malariae* poses a difficulty if the screening is performed by RDT alone. Further in the present study the species specific RDTs were not able to detect neither the monoinfection nor the mixed infections of *P malariae*. Therefore the molecular method can be used as a tool for surveillance to overcome such problems.

(Table 1 to 4 given as Annexure I separately.)

4. Detection and phylogenetic analysis of chikungunya virus from human cases and vector mosquito species in different endemic regions of Odisha.

Principal Investigator	:	Dr. R. K. Hazra
Co- Investigator	:	Dr. B. Dwibedi
Starting date	:	November 2010
Date of completion	:	October 2013
Duration	:	Three years
Funding	:	Extramural (ICMR)

Objectives

(a) Screening of human cases and selected mosquito species from defined areas of Odisha State for the detection of chikungunya virus infections by serologic and molecular tests.

(b) Nucleotide sequencing of the entire E1 genomic region for phylogenetic analysis.

Progress of the work

Entomological Survey

Mosquitoes both adult and larvae collection was carried out in rural areas of Jagatsinghpur, Kendrapara, Bhadrak, Ganjam, Gajapati,Rayagada districts (Fig 1). The adult Aedes mosquitoes were identified taxonomically based on their distinguishing features like head, thorax, wings, legs, halters, segmentation of body, size of proboscis, sitting posture and habits by following the key developed by Barraud 1934 (The fauna of British India).The larvae and pupae were identified taxonomically based on their morphological features like their size, siphon tube length, "S" shaped movement etc. From larvae and adult collection, five species of adult was confirmed by our team i.e. *Aedes aegypti*, *Aedes albopictus*, *Aedes vitatus*, *Aedes edwardsi*, and *Culex species*.Out of these four *Aedes* species, *Ae.albopic*tus was found to be dominant species in all the above study districts. From the number of positive breeding spot surveyed, the Breteau Index of Aedes albopictus in all the blocks under each district was greater than 100 thereby indicating high vector densities and hence being the main vector responsible for transmission of arbovirus in the affected areas.

Fig 1. Map showing Chikungunya affected areas in Odisha in red colour.

House Index (HI): Percentage of house infested with larvae or pupae.

HI =Number of houses infested / Number of houses inspected X100

Container Index (CI): Percentage of water holding containers infested with larvae or pupae.

CI= Number of positive containers / Number of containers inspected X100

Breateau Index (BI): Number of positive containers per 100 houses inspected

BI=Number of positive containers / Number of houses inspected X100

Multiplex PCR: The multiplex PCR method detected maximum number of *Aedes albopictus* larvae

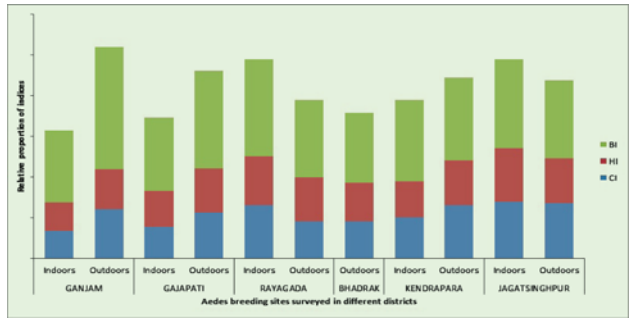


Fig 2: The graph showing the Breteau Index (BI), House Index (HI), Container Index (CI) of the six Chikungunya endemic districts.

and pupae in most breeding spots surveyed which is a prevalent mosquito species of *Aedes* in Odisha. Discarded tires were the most abundant breeding spots of *Aedes* mosquitoes that were obtained from the areas surveyed.

Blood collection:5 ml blood was collected from each suspected case (Patient) showing symptoms of chikungunya in the respective areas which were surveyed. The blood was immediately brought to the laboratory and the serum was separated by centrifugation at 8000 rpm for 10 mins. The serum was then stored at -800C for further study.

ELISA result: IgM captured ELISA was performed to detect CHIKV by using ELISA kit developed by NIV, Pune. All serum samples were tested for the presence of CHIKV specific IgM antibodies using Chikungunya-IgM capture ELISA.176 serum samples from suspected cases of chikungunya collected from affected areas were tested for CHIKV by IgM capture ELISA. Out of 176 samples, 49 (27.8%) shown positive for CHIK ELISA, thereby indicating acute epidemic outbreak in the affected areas (Table 1).

Two step reverse transcriptase polymerase chain reaction (RT-PCR)

Again all serum samples from suspected cases of chikungunya were tested for Reverse Transcriptase PCR (RT-PCR) for detection of virus which was escaped in IgM capture ELISA. So total of 74 numbers of samples were tested positive for RT-PCR (42%). RT-PCR positive samples were tested for viral gene for genomic analysis. RT-PCR detected the chikungunya viral partial E 1 gene specific band at 294 bp from patients serum obtained from different epidemic areas. The E 1 gene band was found in four mosquito pools (10 mosquitoes / pool) collected from Gajapati and Ganjam that were analyzed by RT-PCR. The gel photo is showing the presence of amplified viral E1 region.

Sl. No	Name of the village	Name of the District	Number of samples collected	Samples positive for CHIK V (By ELISA)	Samples positive for RT-PCR
1.	GADADAMDARPALLI	GANJAM	26	9	12
2.	GUDIALI	GANJAM	10	7	6
3.	KAUDIA	GANJAM	23	2	8
4.	AUL	KENDRAPARA	6	0	2
5.	SAHADA	GAJAPATI	2	0	1
6.	POKHARIPADA	JAGATSINGHPUR	5	0	2
7.	BAUDPUR	BHADRAK	6	0	3
8.	GURANDI	GAJAPATI	10	7	8
9.	KUMBHARA SAHI	GAJAPATI	17	8	10
10.	ADARSHA NAGAR	RAYAGADA	2	1	1
11.	ODIA SAHI	RAYAGADA	17	5	7
12.	GOUDA SAHI	GAJAPATI	1	1	1
13.	BISOI SAHI	GAJAPATI	4	0	0
14.	CHRISTIAN SAHI	GAJAPATI	3	1	1
15.	JHOLASAHI	GAJAPATI	4	2	2
16.	KANKRODA	GANJAM	7	0	0
17.	KAUDIA	GANJAM	23	2	6
18.	BOXIPALLI	GANJAM	10	4	4
		TOTAL	176	49 (27.8%)	74 (42%)

Table 1: Number of samples collected and tested positive for CHIKV from 2011 till date.

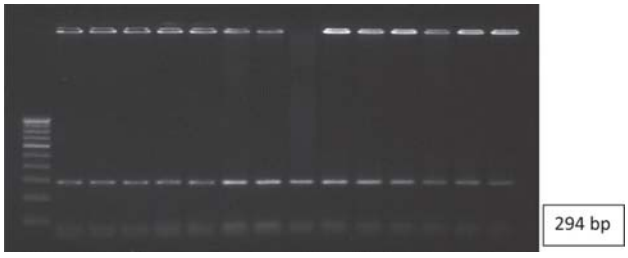


Fig 3: Ethidium bromide stained 1.5 % agarose gel photo showing the amplified E1 region of CHIKV.

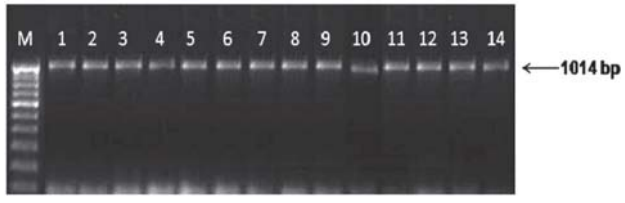


Fig 4: 1.5 % agarose gel photo showing the amplified complete E1 region of CHIKV.

Fig 5: 1.5 % agarose gel photo showing the amplified complete E2 region of CHIKV.

Phylogenetic analysis

From the isolated complete E1 gene, a phylogenetic tree was constructed which is given below. This phylogenetic tree indicates that the sequences from Odisha were grouped along with sequences of CHIKV belonging to IOL (Indian Ocean

Lineage) strain within ECSA (East Central South Asian) genotype, originating probably from the Kenya 2004 strain (predecessor). Hence IOL group, ECSA genotype of chikungunya virus can be attributed to recent outbreaks of chikungunya in Odisha. Further evidence of the ECSA genotype circulation in Odisha was due to the abundance of *Aedes albopictus* vector that efficiently transmits this genotype. This was supported by high larval indices of *Aedes albopictus* in different breeding spots surveyed. The phylogenetic analysis shows more of a temporal pattern rather than a topographical pattern. Many South-East Asian isolates were found to cluster with the isolates under study depicting that a similar genotype has circulated during the recent outbreak in different districts of Odisha.

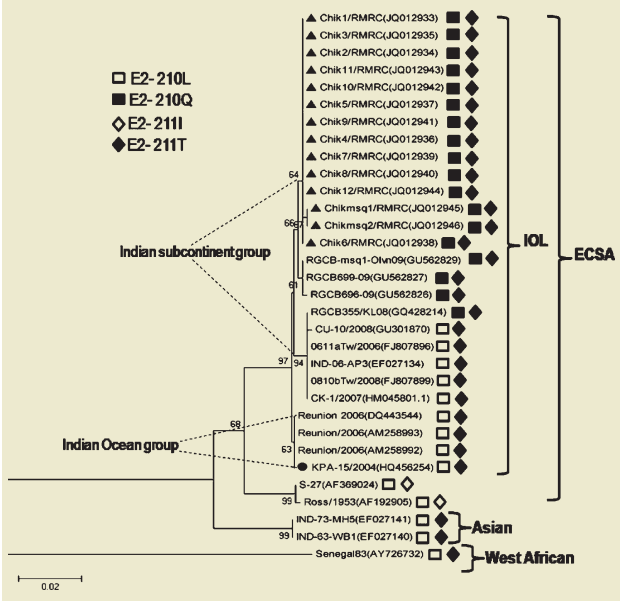


Fig 7: Phylogenetic tree of 768 bp sequence of E 2 gene of different genotypes of chikungunya virus showing all CHIKV sequences from Odisha belong to East Central South African genotype.

Mutation analysis

A226V primary adaptive mutation in E1 gene region along with I211E and E2 L210K in E2 region which acted as second step adaptive mutations were detected in all Odisha isolates (vectors and sera). All the above mutation collectively supported the hypothesis: increase in viral dissemination and

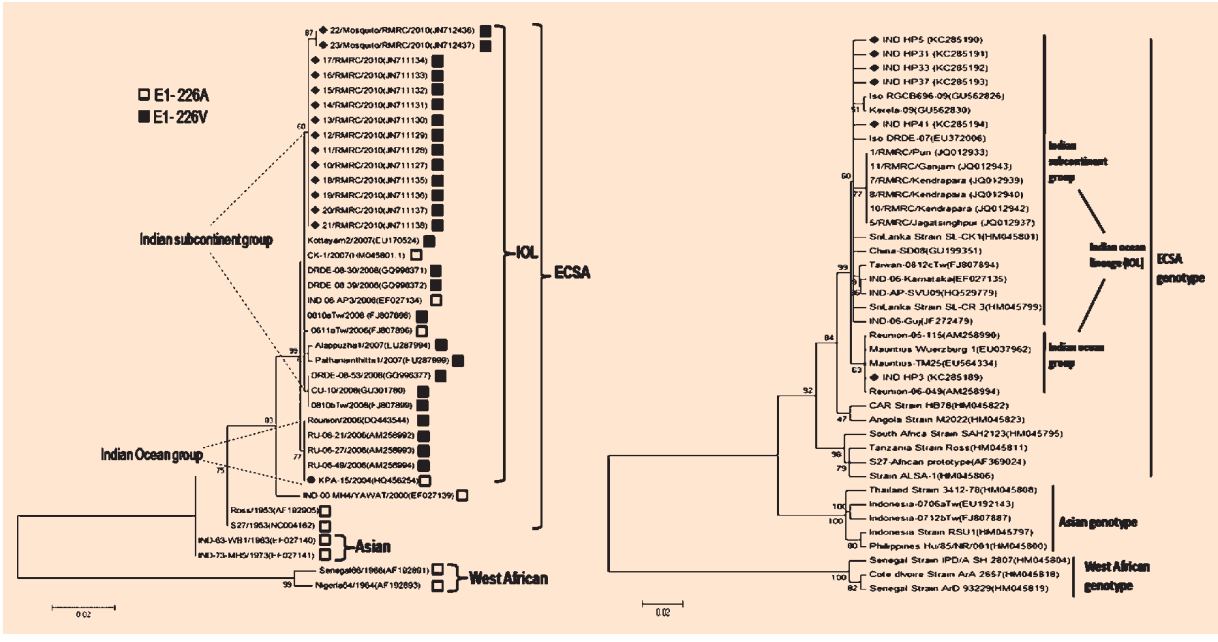


Fig 6: A. Phylogenetic tree of 294 bp sequence of E 1 gene of different genotypes of chikungunya virus showing all CHIKV sequences from Odisha belong to East Central South African genotype.**B**. Phylogenetic tree of E 2 gene of different genotypes of chikungunya virus showing all CHIKV sequences from Odisha belong to East Central South African genotype.

multiplication in *Ae..albopictus* species that finally renders it to be the chief arboviral vector in this region.

Conclusion

From the present results, it can be concluded that the recent outbreaks of chikungunya in Odisha have been caused by viral strains of IOL group of the ECSA genotype with E1-A226V, E2-I211T and E2-L210Q mutations, which in turn has favoured *Ae. albopictus* to be the main arboviral vector in this region. As per our knowledge, this is the first report confirming the association of the IOL strains of ECSA genotype with chikungunya outbreaks in Odisha.

Collection of larvae from breeding spot

Future Plan

- Further screening of more areas during epidemics and intermittent periods needs to be done for entomological survey and vector identification.
- More number of patients' samples and mosquito species needs to be collected for more confirmatory results regarding identification of the chikungunya virus.
- Vectorial capacity of *Aedes albopictus* can be proved further by membrane feeding of the virus isolated from cell culture and development of virus inside the mosquitoes.

5. Etiology of diarrhoea in three tribal districts of Orissa.

Principal Investigator : Dr. B.B. Pal
Co –Investigator : Dr. H.K.khuntia
Collaborator : Dr. Bikash Pattnaik
Starting Date : October 2010
Duration : October 2013
Funding : Funding Extramural (Tribal Task Force, ICMR)

Objectves

1. Phenotypic characterization of common enteric bacteria including the *Vibrio cholerae* from diarrhoea patients from the tribal populations of Orissa.
2. To find out the antibiotic susceptibility test of the diarrhoeagenic *E. coli* (EPEC, ETEC, EHEC, EAggEC), Salmonella, different Shigella spp., Aeromonas spp. and *V. cholerae* isolates.
3. To find out the correlation between clinical isolates of *V. cholerae* by different molecular techniques for the detection of biotype (tcpA-classical/El Tor), serotype(O1/O139), virulence(ctxA) and regulatory genes (toxR) by Quadruplex PCR assay, mismatching amplification for mutation assay (MAMA) PCR for the detection of El Tor variants of *V. cholerae* O1 with ctxB gene of classical strains.
4. The clonality of all serogroups of *V. cholerae* isolates will be done by RAPD PCR, PFGE, sequencing and dendrogram etc. to track their migration from one outbreak area into other.

Progress of work: The project was started in October, 2010 in Kashipur, Dasmantpur, Laxmipur and Mohana blocks with extramural funding. Attempts were done to collect the rectal swabs from diarrhea patients and environmental water samples

from the above project blocks. No rectal swabs were collected from Mohana block of Gajapati District during this period.

A total of 107 rectal swabs were collected from diarrhoea patients from Mohana, Laxmipur, Dasmantpur and Kashipur blocks of Gajapati, Koraput and Rayagada districts from October, 2012 to June, 2013. From total 107 rectal swabs, 81 were culture positive (75.7%) and from which 48 (59.2%) were *E. coli*, 16 (19.8%) were *Vibrio cholerae* O1 Ogawa, 11 (13.6%) were *Shigella* spp. and about 6 (7.4%) were *Aeromonas* spp. respectively (Table 1).

A total of 07 water samples were collected from Mohana, Laxmipur, Dasmantpur and Kashipur blocks of Gajapati, Koraput and Rayagada Districts from October, 2012 to June, 2013. From 07 water samples no V. *cholerae* were isolated.

The distribution of different diarrhoea pathogens in different seasons of Mohana, Kashipur, Laxmipur and Dasmantpur blocks in the year October, 2012 to June, 2013 was given Figure 2. A total of 107 rectal swabs were collected from the above 4 project blocks; out of which 81% were found to be culture positive, 48% were *E. coli*, 16% were *V. cholerae* O1 Ogawa, 11% were *Shigella* spp., and 6% were *Aeromonas* spp. respectively. The cholera organisms were mostly isolated from the adult age groups, *Shigella* spp. and *E. coli* pathogens were isolated from both pediatric and adult age groups, *Aeromonas* spp. were isolated from mostly adult age groups from the all 4 tribal populated blocks. In rainy season, the prevalence of cholera is maximum due to the poor sanitation, lack of knowledge and poor socioeconomic conditions. The distribution of *E. coli* and *Shigella* spp. were found in all blocks as Mohana, Kashipur, Laxmipur and Dasmantpur; but *V. cholerae* was only confined to Mohana block due to the sporadic outbreak of this organisms.

Antibiogram pattern of different pathogens isolated from diarrhoea patients of Mohana, Kashipur, Laxmipur and Dasmantpur blocks:

Table 1. Bacteriological Analysis of Enteropathogens colledted from four tribal blocks from October, 2012 to June, 2013.

	Mohana, Gajapati	Laxmipur, Koraput	Dasmantpur, Koraput	Kashipur, Rayagada	Grand Total (%)
Total samples collected	0	55	21	31	107
Culture positive	0	42 (76.4)	15 (71.4)	24 (77.4)	81 (75.7)
Culture negative	0	13 (23.6)	6 (28.6)	7 (22.6)	26 (24.3)
E. Coli	0	26 (61.9)	4 (26.7)	18 (75.1)	48 (59.2)
V. Cholerae O1 (O)	0	8 (19.1)	6 (40.0)	2 (8.3)	16 (19.8)
Shigella spp.	0	4 (9.5)	5 (33.3)	2 (8.3)	11 (13.6)
Salmonella spp.	0	0	0	0	0
Aeromonas spp.	0	4 (9.5)	0	2 (8.3)	6 (7.4)

Table 2. Analysis of water samples for *V.cholerae* (Mohana, Laxmipur, Dasmantpur and Kashipur blocks of Gajapati, Koraput and Rayagada Districts from October, 2012 to June, 2013).

	Mohana, Gajapati	Laxmipur, Koraput	Dasmantpur, Koraput	Kashipur, Rayagada	Grand Total (%)
Total samples collected	0	7	0	0	7
Culture positive	0	0	0	0	0
Culture negative	0	7	0	0	7 (100)
V. Cholerae O1 (O)	0	0	0	0	0
V. cholerae NAG	0	0	0	0	0
V. Cholerae O1 (I)	0	0	0	0	0
V. Cholerae O139	0	0	0	0	0

The *V. cholerae* isolated from diarrhoea patients of Mohana, Kashipur, Laxmipur and Dasmantpur blocks were uniformly sensitive to Tetracycline, Chloramphenicol, Azithromycin, Neomycin, Gentamicin, Norfloxacin, Ciprofloxacin, Ofloxacin, Doxycycline and resistant to Ampicillin, Nalidixic acid, Furazolidone, Streptomycin, Erythromycin, Co-trimoxazole and Polymixin-B.

The *Shigella* spp. were uniformly sensitive to Tetracycline, Azithromycin, Streptomycin, Neomycin, Gentamicin, Ciprofloxacin, Ofloxacin and resistance to Ampicillin, Chloramphenicol, Nalidixic acid, Furazolidone, Erythromycin, Co-trimoxazole and Norfloxacin.

The *Aeromonas* spp. were uniformly sensitive to Tetracycline, Chloramphenicol, Azithromycin, Streptomycin, Neomycin, Norfloxacin, Ofloxacin and resistance to Ampicillin, Nalidixic acid, Furazolidone, Erythromycin, Co-trimoxazole, Polymixin B, Gentamicin and Ciprofloxacin. respectively.

6. Effectiveness of a bivalent, killed whole-cell based oral cholera vaccine introduced in Satyabadi, Orissa.

Principal Investigator : Dr.Shantanu Kumar Kar
Co-PIs : Dr. A.S. Kerketta, Dr. Hemant K. Khuntia
Collaborators : Department of Health and Family Welfare (HFWDO), Government of Odisha International Vaccine Institute (IVI), South Korea
Starting Date : March 2013
Duration : One year
Funding : Extramural, International Vaccine Institute (IVI), South Korea

Objectives

To evaluate the individual-level protective effectiveness of one or two doses of modified, bivalent, killed, whole cell-based OCV against culture-

confirmed cholera episodes, severe enough to seek a formal health care.

Given the high burden of cholera morbidity, mortality and following policymakers recommendation, state government of Orissa in collaboration with RMRC, Bhubaneswar and IVI, Korea conducted a community-based mass vaccination campaign at Satyabadi block of Puri district, Orissa as a pilot project during May-June 2011. This project aimed at assessment of acceptability, feasibility, and costs of cholera vaccination program delivered under the existing public health infrastructure. A total of ~ 33,000 people living in around 140 villages of the block were vaccinated by June 2011 in two rounds of three days of vaccination. Follow-up and determination of effectiveness of oral cholera vaccination was requested by State government and also advised by 23rd Scientific Advisory Committee of the centre while recommending the project. Understanding the effectiveness of OCV in a public health setting would be important not only for state policy makers and donors, but also for other states in India.

The present study is a matched case-control study conducted in the area included in the mass vaccination with oral cholera vaccine in Satyabadi block. Cases are identified through healthcare facility-based surveillance, which already exists in the project area. Controls are selected among those people who have the same age, gender, and gram panchayat as index cases ("matched controls"), 1 to 4 ratio is used in matching cases and controls. Information on vaccination status and other exposure variables from both cases and controls are obtained.

Cholera Surveillance

Eligible cases are being captured from 5 healthcare facilities that serve the catchment population including the two primary health care centre (s) and Area Hospital in Satyabadi block and the Infectious Disease Hospital & Pediatric ward, District Head quarter hospital. Residents of project area presenting to any health facility with acute, watery diarrhoea were invited to participate in this case-control study. Verbal informed consent was sought followed by collection of requisite information based on a pre-structured questionnaire and rectal swab collection. Rectal swabs were transferred to the RMRC lab in Cary-Blair media within 24 hour period following the collection.

Laboratory case confirmation

The WHO recommended procedures were implemented to identify V. cholera. After confirmation cholera, a follow-up home visit was made on day 7 following the presentation to a health facility by the individual. A pre-structured questionnaire was used to collect additional information and vaccination status. Matched age, sex controls of same gram panchayat and had not sought treatment for diarrhoea in past 3 days from the date of onset of the diarrhoeal illness of their index case was selected. For each index case, 4 matched controls were selected and interviewed. The focal time for cases and their matched controls is the date of onset of the diarrhoeal illness in the index case. First, 8 controls were randomly generated among the individuals who belong to the same sex, age group, and gram panchayat in the baseline census. Each potential control was visited until four controls were recruited based on verbal informed consent through household visit. During the household visit, a pre-structured questionnaire for controls was used to collect information on demographics, risk factors for cholera, and vaccination history to compare with their respective cases.

Besides, in contrast to cholera case, non-cholera diarrhoea cases with non-diarrhoeal controls were selected for bias indicator study. The goal of the bias-indicator study was to assess whether there is an expected absence of vaccine protection against non-cholera diarrhoea. Each week during the main case-

control study, three non-cholera diarrhoea cases were selected, whenever available. The history of receiving one or two doses of OCVC Shanchol during the government-led mass vaccination was ascertained by the review of electronic vaccination registry in addition to the confirmation of vaccination cards during home visits.

Work Progress

Since March to June 2013 a total of 913 rectal swab samples have been collected from the acute diarrhoeal cases attending selected health facilities and subjected to microbiological investigations. The age and sex distribution (Chart:1) indicates that all age group people are affected by acute diarrhoea. In the under five group male are affected more than the females. The cholera organism was isolated from 101(11.1%) rectal swab samples. The month wise cholera incidence shows the increase trend from the month April onwards. The health facilities wise distribution of cholera cases indicates, out of the three facilities within our study area, Sukala PHC reported the maximum numbers of cholera cases.

To assess the prevalence of *V.cholerae* in the environment, water samples from various water

Chart-1: Age & sex distribution of acute diarrhoea cases.

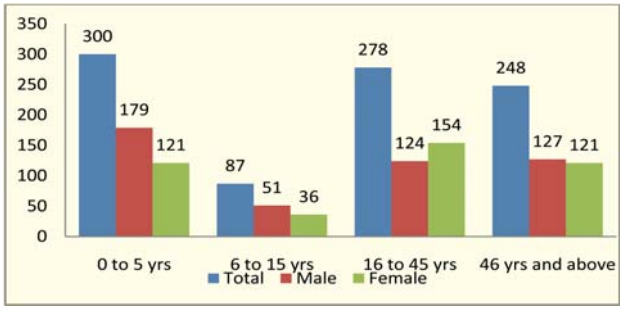


Chart-2 Distribution of various entero - pathogens.

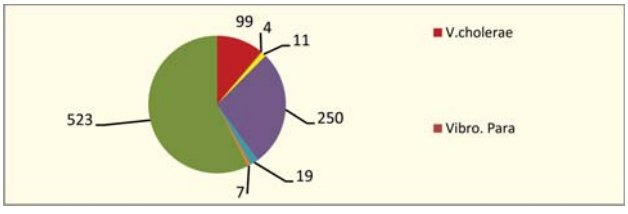


Chart-3: Month wise incidence of cholera.

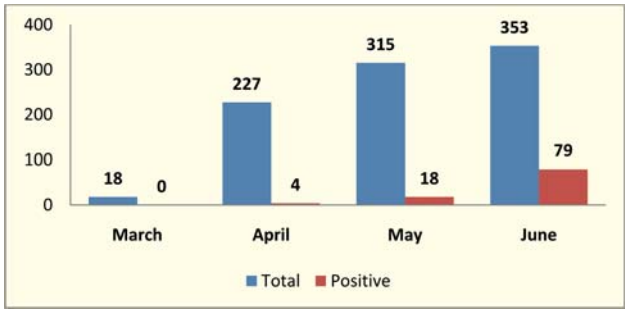


Chart-4: Health facility wise cholera positives.

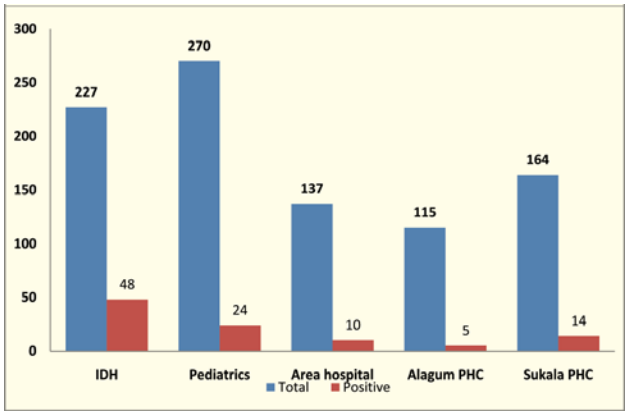


Fig. 1: Cholera Positives among Vaccinated & Non-vaccinated group.

	Total Diarrhoea Cases	Cholera Positives
Vaccinated	263	19(7.3%)
Non vaccinated	170	18(10.5%)
Total	433	37(8.5%)

sources in the study area were collected and tested. So far a total of 115 water samples have been collected. The result shows positivity of *V.cholerae* O1 Ogawa in 1(0.9%) sample (water source-Pond), *V cholerae* non O1 / O139 in 38 (33.0%).

The effectiveness of vaccination was assessed taking cholera incidence amongst the vaccinated and non-vaccinated group. That indicates incidence of cholera among vaccinated 7.2% and among non vaccinated 10.5% (Fig:1). The protective effectiveness of the vaccine at individual as well as community level will be calculated after completion of the total sample size of 48 cases with its matched.

Justification for continuation

As per the sampling a total of 48 Vibrio cholerae cases have to be identified with their matched controls. That needs rigorous screening of the diarrhoea cases. Therefore the study needs to be continued up to complete one year study period.

7. Migration, poverty and access to healthcare: a multi-centric study on people's access and health system's responsiveness in fast-growing Bhubaneswar city of Odisha.

Principal investigator : Dr. Anna S Kerketta
Co Investigators : Dr. G Bulliyya, Dr. D. Das Mrs. G Mallick
Date of Start : July 2011
Duration : Two & half Years
Funding : Extramural (ICMR, National Task Force)

Broad Objective

The broad objective of the study is to assess the migrants' healthcare access in the vulnerability context of migration and livelihood insecurity, and to understand the factors (individual-/community-/system-level) affecting the migrants' access to healthcare services and to identify key points to develop an intervention to improve healthcare access to the socio-economically disadvantaged migrants

Work Progress

The formative research including, both quantitative as well as qualitative methods have been undertaken in 125 slums of Bhubaneswar city. Data pertaining to socio-economic, demographic details, healthcare seeking behavior were collected through pre-tested, interview-administered questionnaire by door to door survey. A total of 3899 households were surveyed covering a total population of 14192.

Qualitative methods like In-depth interviews, focus group discussions and key-informant interviews were used to understand the community (migrant's) perspective (perceived needs, barriers in accessing the healthcare services and their relevance) and in identifying the existing communication channels and their utilization pattern amongst these migrants. These methods were conducted with both community and health providers. As targeted of 72 interviews and discussions were covered that includes 6 Focus Group discussion (3 males and 3 female groups), In-depth interview - 62 (with key persons-19 numbers, with women-20, with CBOs-8, with Medical Officers-4, with high level health personnel-1, with health workers-6 & with NGO personnel-4) and Four case studies (2 each in Bad and Good slums).

Migration History

Out of the households surveyed majority 77% belong to notified slum and 36% have migration duration of 8-10 years. The migration history shows around 91.4% belong to the state of Odisha. The people from the adjacent state like Andhra Pradesh, Bihar and West Bengal also migrated to the city. The outside migrant are mostly from Andhra Pradesh. The reason for migration indicates majority 96% people migrated for better earnings/livelihood. Distribution of households by their caste category shows majority 47.3% of the population belong to other backward class (OBC). Proportion of Scheduled Tribe & Scheduled Caste is 16% and 21% respectively. Majority 96.7% of the residents belong to Hindu religion. The type of house shows, squatter house is predominantly seen among 57.2% followed by semi-pucca in 31% population. Of those migrated since 8-10 years, 74.8% have their own house. Among recent migrant, 28.7% stay in rented house & around 44% have provided free house by the contractor or employer.

Availability of basic amenities shows, the main source of portable water is public tap & hand pump for 69.2% & 21% respectively. Public tap is available in notified slum. Bore well water is used in the migrant camps like construction site. Merely 5.5% people have

own toilet or community toilet and close drain is available in only around 10.4% households in notified slum. The metered electricity connection was found in 35.2% households and 30% use unauthorised electricity supply. Only 3% households are having BPL card. Voter ID is available with 63.7% of the residents. The means of communication channel is TV in 38% households. Majority 85-95% of the residents know the happening in the city through neighbors/co-workers or through announcements.

Existing social networks shows CBOs are located both in the notified & non-notified slums. The CBOs like Self Help Group (SHG), Slum welfare Society, Youth Club, Mahila Mandal exist in these slums.

Morbidity & access to Health services The total morbidity pattern shows 10.4% of the respondents and 22.3% community members reported having various illnesses during past 6 months. The chronic illness reported by 0.3% of the community members. 3.6% participants got hospitalised in last one year and 26.2% had to incur more than Rs.5000/- for their treatment.

Regarding the service of health workers merely 0.6% of the households are aware about the visit of health workers in their locality. Among them, 59.2% informed that she gives ANC services, 90.8% are aware about immunisation services, 32.2% are aware of post partum services, 8.7% said of family planning, 5.3% said IEC, 85.9% are aware of pulse polio vaccination.

About the availability of health facility in their locality, 10.1% have Govt health facility & 7.9% either have private medical college, dispensary and private hospital in their locality. The usual care seeking of people indicated that Govt facilities is used by 45.8%, private hospital by 25.3% and others like quacks, tradition healers, and some other systems also used by the people.

Of the residents who did not avail the Govt. health facility, majority 24.6% said it is not available in their locality, 11.1% long waiting time and 29% opined of poor quality of treatment and diagnostic test, and 2.2% non availability of free medicines. Out of those who never availed the government health facility 73% avail services from private clinic, hospital or nursing home, 26% from nearest medical store.

Obstetric History of women having a child aged less than 2 years, a total of 218 women having child aged less than 2 year of age were interviewed for obstetric issues like MCH care obtained & place of delivery. The result shows 80% had institutional delivery, of which 85% had normal delivery.

Details of antenatal visits: The history on ANC sought indicated around 56% had more than 3 visits. 36% had less than that and 8% did not seek ANC at all. Around 80% ANC sought from Govt. health facilities, 20% from private practicener. The immunisation with injection TT shows 10% did not receive any vaccine where as 90% received more than 2 doses of injection TT. The IFA was received by 78% women where as only 26% consumed entire recommended dose of 100 tablets. When 5.3% did not even consumed single tablets 68% consumed partially of the recommended number of IFA. Number of women received all three components of antenatal care like 3 ANC check up, 2 Injection TT and 100 IFA tablet found to as low as 12.9%. **Regarding birth preparedness,** of the total 8% mothers planned in advance the place of delivery and 50% planned for the accompanying person to the place of delivery. Merely 5% women are aware of availability of free Govt. transport facility thus 65% women used hired auto rickshaw to reach the health facility. Irrespective of duration of migration and slum type almost 80%-100% women had to spent money for delivery. Amount varies from Rs.500/- to maximum Rs.10, 000. Around 19% had to spend more than Rs. 10,000/-. Around 65% received cash benefit at government health facilities.

Awareness on Child immunisation was marked 93% women. Vaccination card was available with 70% women. Majority (around 69%) cards are given by Govt. hospital. Around 80-95% received various routine immunisation however low coverage of measles and vitamin A, of 29% and 26% respectively was found during the survey.

The qualitative survey indicates that in slums of Bhubaneswar city there is internal as well external migration. People have migrated from different districts of state Odisha as well as from other states like A.P, BIHAR, M.P & west Bengal. The important reasons for migration are better livelihood and education of children. Few people migrated due to social dispute or communal riots. The communities are having very good social network and they support one another in need. The success stories of the community shows of getting road and electricity facility and old age pension from the govt. after struggling united for it.

The migrant community usually experience common health problems like common cold &, fever, gastritis, diarrhoea, abdominal pain, vomiting, dysentery, asthma and T.B. The husband or elder person like mother in law / aunt is the decision maker for health issues. The slum area where AWW center exist, the AWW also play vital role in decision making for health service access. Neighbours and relatives are also consulted in case of emergency. People mostly access the private clinics or medical stores for minor illnesses while they access Govt. health facilities for major Illness. For the health need of children, 50% of people access govt. health facility and rest access private clinic. The health workers visit only to the households having pregnant women and children, thus many people are unaware about the health workers availability in their slums. People are aware about the visit of health worker for polio vaccination. The Govt. dispensaries are used by the people who reside close by. The opening time of dispensary is 8.00 AM to 12 noon and 3:00 pm- 5:00 pm which is not convenient for the slum dwellers. As opined by the high level health personnel, the dispensaries are adequately equipped with facilities and basic amenities. It is also opined that, Govt. is having special health policy for migrants. 11 NGOs are working in PPP mode and providing health care services to migrant people of some slums. Each NGO is positioned with 1 doctor, 2 ANMs, 1 pharmacist, 6 link volunteers. These NGOs are funded by NRHM. Each NGO caters 25000 populations through running urban health center. They provide OPD, ANC/PNC, immunization services, health awareness camps for the people resides their catchment area. They also conduct outreach camp for the people residing in the slums located far away from the health centre. Besides these health facilities, there are three private medical colleges, many private clinics and hospital. The families who are financially better off they access private hospital or clinic.

The people get health information through various means like, TV, radio, personal interaction with their neighbours, relatives, and friends. Some AWW and health workers also give the health information particularly on immunization through door to door visit and personal contacts. For obstetric care and delivery women prefer to go to state capital hospital due to less expenditure and to get financial assistance under JSY / MAMATA yojna. Some women prefer private hospital to get better care. It was found that in some tribal slums, due to their cultural practices, home delivery is still being conducted. In the slum where NGOs are working, the link volunteers accompany the pregnant women to the health facilities. It is revealed that the health facilities lack sufficient man power, medicines and test facilities. The community members opined of not receiving proper attention and treatment and majority of them are not satisfied with the services provided for common ailment. The behaviour of the health

personnel is also not acceptable by the poor migrant as they behave rudely.

As perceived by the community the main barriers for not accessing Govt. facilities are working pattern of people, poor socio-economic condition, proximity to the facility, lack of awareness and traditional health practices. The health system related barriers are the non suitable opening time of urban health centers/ dispensaries, lack of awareness of people on existence of urban health centers, insufficient man power, supplies and logistics, non availability of laboratory facilities and lack of proper information of date of outreach camps and wide spread of outreach areas, irregularity in payment of health staff. As most of the medicines are not available in Govt. hospital and people have to purchase it from outside medical store that causes double financial burden (treatment & transport cost) which is out of their affordability. The people who reside away from the heath facility have to bear the transportation cost that creates problem in access of health facility.

The community perceives that engaging more health staffs for primary care and health awareness by government can improve the health services access by the people. They also suggest for doctor's posting with sufficient medicines for common diseases in their slum or provide Mobile Health Unit. In each slum should have health workers health volunteers. They also suggest for health insurance card for all the migrant people.

Justification for continuation: Based on the result of formative research, a community specific of healthcare strategy involving advocacy to various stakeholders, partners & host community, community mobilization and community participation will be developed, implemented and the impact will be evaluated using quasi experimental design. The result of this intervention will be helpful in improving the health care services access by migrants living in slums of fast-growing smaller cities in India.

8. Socio-cultural features and stigma of leprosy for treatment & control in general health services in India: Cultural epidemiological study.

Principal Investigator	:	A. Mahapatra, D.P.Hansdah
Collaborator	:	P.K.B.Patnaik (Government Odisha)
Duration	:	Two years
Starting Date	:	Jan 2012
Status	:	EM (Multi-centric study of the ICMR task force on leprosy)

Background

Notwithstanding accomplishments of the programme, questions remain about the effectiveness of current strategies of leprosy control. Leprosy-related stigma also remains a serious issue, contributing to a frequently overlooked "hidden burden" of this neglected disease beyond the standard epidemiological indicators. Leprosy is no longer considered a public health problem at the national level, since the overall prevalence in India is less than 1 in 10,000 population, even though it remains more problematic in some pockets. Such questions are especially timely with the current integration of leprosy services in primary healthcare, and the importance of maintaining the capacity for diagnosis, access to effective treatment and disability care despite the impact of stigma, both in the general population and among general health services personnel. Research is needed to determine whether and how social and cultural features of leprosy affect access and the quality of clinical services and leprosy control that are required for effective control with integrated services.

The substantial reduction in the prevalence and detection of new cases of leprosy has been documented. Current programme aims are now concerned with motivating people to recognise symptoms and seek appropriate treatment in general

health facilities, but for that to happen, the adverse impact of leprosy-related stigma must be minimised. Despite effective leprosy treatment and massive efforts for public health education to facilitate leprosy control through the general health services, leprosy-related stigma remains a barrier to access to clinical services for diagnosis and treatment. Even though essential features of stigma are changing from enacted to perceived stigma—that is, from overt discrimination to fear among affected persons of what might happen if they were known to have leprosy global and Indian leprosy programmes acknowledge the continuing impact of stigma and the need to reduce it. It had been expected that a reduced burden of leprosy would lead to a lower level of community awareness, more social stigma, atypical skin lesions and late presentation of neurological symptoms. In fact, recent studies in India and Bangladesh do in fact provide evidence for undetected leprosy in communities despite well-established programmes. In addition to problems in identifying cases in integrated programmes, the burden imposed by stigma is not adequately assessed in standard burden of disease estimates, which are typically based on epidemiological measures, disability and functional impairment. Stigma imposes an additional "hidden burden," that is typically omitted from accounts of the burden of illness reported in disability-adjusted life-years (DALYs), which fail to consider the impact of social exclusion and the consequences and concerns about disclosure. Furthermore, an appreciation of how the nature of stigma is affected by social and cultural factors should be considered in formative research to guide policy that recognises the importance of acceptance and social support.

Disease-specific factors may require rethinking of the usual assumption of a direct relationship between the proximity of, and access to, health services. Research has shown that concerns about disclosure of the condition may make nearby health services for leprosy too close for comfort. Nicholls and colleagues state that integrated services may prove effective in reducing delays, but will depend on levels of awareness of leprosy, effective arrangements for referral and sensitivity to the impact of leprosy on those affected. Complementing the issue of whether adequate services are available, both knowledge of the disease and the social impact of the illness substantially affect access and appropriate use of services. Apart from questions of help seeking that affect case finding and treatment, stigma may also affect the quality and effectiveness of primary health services which provide first-line treatment for leprosy. The integration of leprosy into primary health services may result in a low priority for training to recognize and treat a complicated condition, inadequate skills of health workers, weak supervision and monitoring and low motivation of the health workers. In studies conducted in Nepal and Bangladesh, the patients identified priority areas concerning health system issues that included waiting time, privacy during examination, correct diagnosis and prompt treatment, attitude and skills of the health workers and availability of care for preventing disabilities. Even in urban settings, lack of adequate knowledge about leprosy care and persistence of some misconceptions and prejudices about leprosy has been documented among physicians.

Patients emphasize the importance of the quality of health services, and the nature of interactions between patients and providers is also an important factor affecting perceived quality and the motivation initially to seek treatment and continue with it over time. Leprosy patients have identified their own priorities concerning the quality of care and their decisions for seeking care. In the context of integration of services, early detection and adherence to treatment are likely to be even more sensitive to ideas about the disease and perceptions of patients that influence their behaviour. Moreover, very little information is

available, but very much needed, on the quality of care for leprosy patients attending general health facilities, as they seek help not only for leprosy but also for general medical problems other than leprosy. Social and cultural features of leprosy affect not only the quality of services for that condition but also for other conditions for which people with leprosy, like everyone else, seek treatment. Social and family support systems are important for sustained care of all chronic diseases, and stigma affects the quality and effectiveness of that support. Efforts to encourage support and minimize disqualification are important considerations for effective community health services. More information from research examining the attitude and response to leprosy of family members, local leaders, and institutions outside the health sector is needed for effective community health action. In addition to the factors specific to patients, social and family support systems, there is a need to identify the health system specific issues with reference to differential level of integration process in various States and the varied responses of the general health services and vertical staff; A multi-State study documented that all the urban and rural health facilities in Maharashtra were providing MDT and medical officers in all health facilities were diagnosing and treating leprosy cases. The involvement of health sub-centers in treatment delivery was also 100% in Maharashtra. However, in contrast, much lower involvement of the health professionals in recording (10%) and reporting (30%) was noted in Andhra Pradesh. This study emphasized the need to undertake further follow-up studies to address local State specific problems. Since then various other studies have commented on the State and district specific responses and the challenges in integrating leprosy services. A recent study from Bargarh district in western Odisha has also highlighted the need for effective monitoring and evaluation of the integration process. However, all of these studies addressing the integration issues were restricted in scope and descriptive in nature and only to some extent detail out operational and technical constraints.

In this context, this project propose to address important health social science issues concerning patients, health systems and community within a framework of cultural epidemiology.

Aims

- Clarify relevance of socio-cultural features of experience and meaning of leprosy and the current impact of stigma
- Suggest strategies for improving patient-centered leprosy services

Specific objective domains

- ***Patients:***

1. Describe socio-cultural features and stigma among leprosy affected persons
2. Determine effects of socio-cultural features and stigma on preference and utilization of health services by leprosy affected persons

- ***Family/ Community:***

3. Assess the role of family & community members to motivate leprosy affected persons to seek health services.
4. Assess the perception of family & community members on leprosy-related stigma

- **Health system**

5. Assess the level of leprosy-related stigma prevalent among health professionals in general health services
6. Assess the impact of leprosy-related stigma on delivery of services for leprosy affected persons

Expected deliverables

This study will (1) clarify the nature of stigma

and the social burden of leprosy; socio-cultural features of leprosy illness explanatory models; how both of these are understood among patients with leprosy, their family and community; and how these relate to the utilization of primary health services for people with leprosy; and (2) identify strategies to enable health professionals to interact with patients in a manner that clearly responds to patients' concerns.

Progress

Since ICMR fund is released, in june2012, before that, by means of intramural funding preliminary data collection was made from the secondary sources. The data thus is used for calculation of Prevalence Rate (PR) / per 10,000 populations & the Annual Case Detection Rate (ANCDR) / 10,000 populations for all 30 Districts of Odisha from 2001 to 2011. In addition to these the Collaborators of the State Government were apprised of the projects aim and objectives. So far the Annual case detection rate(ANCDR) / 10,000 population is calculated for 30 districts of Odisha and the data reveals that Sonepur District has the highest case detection rate of 5.47 in 2011 and 3.30 in 2011 fig-1.

The finalization of Emic Tool, Pre-testing of the tools, Staff recruitment & training was carried out by NIE till July2012. The Line listing of the Leprosy patients, TB, Malaria were obtained and entered in to the Computer and sent to NIE for sampling, as per the protocol. As soon as the lists arrive the field work started in Aug 2012 onwards.

The other participating Institutes of this were, **NIE-ICMR**, Chennai, Tamil Nadu{co-ordinator}, The Foundation for Medical Research **(FMR)**, Mumbai, Maharashtra, The Maharashtra Association of Anthropological Sciences **(MAAS)**, Pune, Maharashtra, The Regional Medical Research Centre, ICMR, Bhubaneswar **(RMRCB),** Odisha, Regional Medical Research Centre-North East**,** ICMR, Dibrugarh, **(RMRCNE)**, Assam and Rohilkhand Medical College & Hospital **(RMCH)**, Bareilley, Uttar Pradesh.

RMRC Bhubaneswar started the field work of this project from Sept 20th 2012 onwards, after recruitment, training and finalization of Tools at NIE, Chennai. The Three Project Staff (3) were than posted at Sonepur, under supervision of the DLO Sonepur. Every month an advance tour programme was being submitted through the DLO and than last month's progress report is also submitted to the Director, RMRC, Through the DLO and PI of the project along with the monthly attendance sheet duely certified by the DLO Sonepur.

From 10.4.13 on wards one staff was withdrawn from field and engaged in data entry at RMRC, this was also submitted through DLO to Office of RMRC. Subsequently, other two staffs were also withdrawn

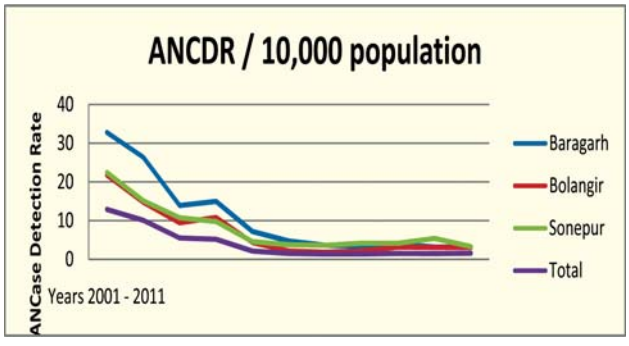


Fig-1 ANCDR / 10,000 Population

Annual Case Detection Rate (ANCDR) /10,000 population.

Slno	district	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
1	Angul	23.50	14.50	7.18	9.42	3.74	3.70	3.14	3.83	3.71	3.32	2.74
2	Balasore	8.20	7.26	2.73	2.56	1.33	0.91	0.85	0.79	1.08	1.08	1.01
3	Baragarh	32.78	26.39	13.92	14.99	7.20	4.74	3.62	3.38	3.95	3.22	3.21
4	Bhadrak	10.24	11.26	4.06	2.72	1.36	1.05	1.01	0.89	0.98	0.80	1.06
5	Bolangir	21.77	14.77	9.44	10.83	4.36	2.14	1.86	2.19	3.18	3.11	3.12
6	Boudh	24.16	17.30	10.78	12.08	4.39	1.78	2.01	1.89	3.18	3.12	2.81
7	Cuttack	7.31	6.85	3.84	3.07	1.43	1.08	1.01	0.96	1.10	0.76	0.88
8	Deogarh	9.87	8.86	5.59	5.01	2.82	2.10	1.95	2.35	2.76	2.46	1.98
9	Dhenkanal	19.41	14.75	8.08	10.08	3.32	2.10	1.79	1.39	1.70	2.54	2.49
10	Gajapati	4.37	4.20	1.78	1.98	0.92	0.61	0.78	0.88	0.80	0.68	0.46
11	Ganjam	12.34	12.14	6.88	5.32	1.60	1.31	1.21	1.12	0.91	0.78	1.19
12	Jagatsinghpur	4.93	3.09	2.23	1.42	0.89	0.87	0.77	0.52	0.75	0.63	0.62
13	Jajpur	7.45	6.58	3.95	3.58	1.14	0.63	1.05	0.99	1.15	1.52	1.66
14	Jharsuguda	31.83	18.78	9.11	10.21	4.27	2.94	2.55	2.75	2.91	2.71	2.51
15	Kalahandi	10.12	8.76	4.61	4.22	1.87	1.39	1.30	1.72	1.55	1.80	2.16
16	Kendrapara	5.37	3.85	2.02	1.74	0.79	0.66	0.70	0.67	0.63	0.49	0.58
17	Keonjhar	7.70	4.72	2.53	1.68	1.10	1.23	1.17	0.99	1.10	1.35	1.14
18	Khurda	13.54	10.36	7.22	5.59	1.83	1.36	1.00	1.10	0.77	0.86	1.03
19	Koraput	6.66	4.62	2.75	2.64	1.46	1.05	1.11	0.94	1.21	1.64	1.78
20	Malkanagiri	4.72	2.98	1.00	1.36	0.60	0.47	0.69	1.10	0.69	0.87	0.66
21	Mayurbhanj	16.98	12.58	5.89	6.50	2.52	1.49	1.41	1.52	1.68	1.85	2.02
22	Nawarangpur	7.92	8.98	3.01	3.96	1.69	0.81	0.72	1.16	1.39	1.62	1.44
23	Nayagarh	9.04	5.74	4.27	4.42	1.95	1.77	1.57	1.67	1.82	1.52	1.74
24	Nuapada	11.48	7.57	2.14	4.76	1.84	1.27	1.41	2.37	3.30	2.02	2.34
25	Phulbani	5.06	2.99	1.81	1.23	0.45	0.37	0.33	0.38	0.54	0.55	0.68
26	Puri	6.70	6.84	5.91	3.62	1.80	1.39	1.21	1.00	1.08	1.01	1.26
27	Rayagada	8.53	6.07	2.97	2.47	1.38	0.51	0.67	0.64	0.47	0.50	0.63
28	Sambalpur	24.91	16.45	12.44	10.90	3.44	2.58	2.33	1.35	1.70	1.67	2.77
29	**Sonepur**	**22.45**	**15.15**	**10.74**	**9.78**	**4.56**	**3.79**	**3.62**	**4.19**	**4.18**	**5.47**	**3.30**
30	Sundargarh	20.02	15.74	6.67	5.32	2.34	1.83	1.42	1.41	1.84	1.98	1.84
	Total	12.91	10.12	5.51	5.19	2.14	1.52	1.38	1.39	1.54	1.54	**1.61**

As on date the Progress report is as follows:

Sl. No	Sample Size of the Study participants type	Target	Number Covered	Due to be Covered
1.	Leprosy –Released from Treatment (RTF)	50	50	
2.	Leprosy – Under Treatment (UT)	50	50	
	LEPROSY Sub TOTAL	100	100	
3.	T.B.	50	50	
4.	Malaria	25	17	08
5.	Skin	25	04	21
6.	Family Members	25	23	01
7.	Community Leader	25	26	
8.	NGO / Pvt. Health Providers	15	11	04
9.	Govt. Health staff	25	16	09
	Other Sub Total		147	
	TOTAL Covered	290	247	43

from the field after being relieved by the DLO, Sonepur from 30.4.13.

Work To be carried out

Another 15- 20% of the Stake holder's interview in their setting is due to be carried out; besides this the FGD – Focus Group Discussion among the different stakeholders is yet to carried out.

The investigators have been in touch through two collaborative workshops, telephone and emails. However, site visits are yet to be conducted. Both quantitative and qualitative data from EMIC questionnaire is yet to be entered in to the computers. The team is currently translating and transcribing qualitative data.

Major Achievements

Sonepur district has the highest number of Leprosy cases in the state of Odisha. Out of the 290 samples we have covered almost 80% of it. In comparison to other centers we are ahead. The data entry is on going for analysis.

9. Hospital Based Sentinel Surveillance for Bacterial Meningitis in India: A Multi centric Study.

Principal Investigator : Dr. S. K. Kar
Co-Investigators : Dr. B. Dwibedi
Prof. Niranjan Mohanty, Head, Department of Pediatrics, SVP Post Graduate Institute of Pediatrics, Cuttack.
Starting date : February 2012
Duration : February 2017
Funding : Extramural (Ministry of H & FW, Govt. of India)

Objectives

Primary Objectives

1. To establish a hospital based sentinel surveillance for bacterial meningitis in children between 1 month and 59 months in six States in India.

2. To determine trends of bacterial meningitis in children 1 month to 59 months of age in these states in India

Secondary Objectives

Determine the etiological profile of bacterial meningitis in children for *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis*.

Application of the research for National Health Policy:

The aim of the project is it to establish a network for sentinel surveillance for bacterial meningitis caused *by H. influenzae, S. pneumoniae and N. meningitidis* in India. Preparations are ongoing by the Government of India for the phased introduction of a Pentavalent vaccine (DPT-Hep.B-Hib) in selected states of the country as part of Universal Immunization Programme. An ongoing surveillance network is critical to facilitate data flow and monitor the changing trends in disease pattern following introduction of potentially lifesaving public health intervention (Pentavalent Vaccine). The study of trends in the pattern of organisms and drug resistance across the country is also being planned as a part of the project.

The surveillance will provide hospital based data on bacterial meningitis specifically those caused by *S. pneumoniae, H. influenzae and N. meningitidis*. Data on drug resistance using MIC will be generated from all the surveillance sites. Generation of this data will help the government not only to observe trends in drug resistance patterns but will ultimately help in formulation of a policy guideline for management of the same.

Progress of Work

Investigators & Staff training / Reorientation: To maintain uniformity in the study methodology and quality of data all the site investigators attended reorientation training on GCP and GLP at CMC, Vellore. Project staffs were trained subsequently on the protocol and procedures involved in the study. The data entry operator engaged in the project was trained on Data entry through Epi info software at NIE Chennai .Technical staff (2 SRFs, 2 research assistants and two technicians) got laboratory training pertaining to identification of isolates using gram stain, blood and chocolate agar culture, biochemical tests during a workshop held at CMC, Vellore.

Standardisation of laboratory procedures at the centre's lab

Laboratory investigation has been undertaken on trial samples (Blood & CSF samples) on the required laboratory procedures including CSF cytology (DC, TLC), CSF biochemistry (glucose, protein) by auto analyzer, culture (blood and CSF) and antibiotic sensitivity. Cultures were run on blood agar, chocolate agar and Mac conkey plates for identification and antibiotic sensitivity.

Quality control

Internal quality check was made on coded samples to see inter observer variations which was negligible. External quality control was inbuilt into the study, where CMC Vellore acted as the reference laboratory. In the process coded isolates were received from CMC, Vellore and relevant laboratory tests were performed to identify the isolates and results communicated to CMC.

Laboratory up gradation at SVPPGIP, Cuttack

The hospital facility selected for the study i.e. SVP Post Graduate Institute of Pediatrics, Cuttack is situated 35 kms away from this Center's laboratory and as per the laboratory protocol the samples need to be put into culture immediately (within 15 – 30 minutes) considering the sensitivity of H *Influenza & S pneumoniae* to external temperature and CO_2 concentration. Hence it was planned to set up a laboratory facility inside the hospital. In this process

laboratory space was identified and necessary civil modification (partitioning and ceiling etc.) undertaken with the help of state R&B Division, necessary equipment have been shifted to the laboratory from RMRC and installed. It has been made functional with help of the project staff who are already trained. Laboratory activity is ongoing to cover 24 hrs. X 7days surveillance activity as desired.

Subject enrolment & Lab Investigation

No. of patients attending the hospital i.e. SVPPGIP, Cuttack, patients suspected of meningitis and no. of hospital admission were recorded during the period of surveillance i.e. March 2012 onwards through 24hr surveillance. After obtaining written consent from parent or guardian accompanying the patient, the subjects were enrolled.

Case report form was filled with the help of resident paediatrician. History of illness and history of immunisation was also recorded. 561 no of suspected meningitis cases were enrolled in the study those who satisfied the inclusion criteria laid down in the protocol. The children enrolled were between the age group of 1 to 59 months as per the protocol. The major presenting illness was fever with convulsion (51%). Other associated features were bulging fontanelle (21%), neck rigidity (14%), and altered sensorium (12%).Around 35% of patient reported to the above hospital within 24hrs of onset of fever. History of use of antibiotics before admission was observed in 61% of cases.

There was no recorded history of immunisation against Hib and *Streptococcus pneumoniae* in all cases that were enrolled. CSF and blood samples were collected following standard practise and procedure in the hospital for investigation. 463 CSF samples and 179 blood samples was collected for investigation. In all cases samples were processed immediately and put into culture (within 15-30 mins). Latex agglutination test was done in 379 samples. Previously latex test was done only in probable cases but now latex test is being carried out in all suspected cases of meningitis. Out of 379 samples subjected to latex test, 13 samples were latex positive (five for Hib, seven for *S.pneumoniae* and one for group B Streptococcus). CSF cell count varied from 0 to 16500. About 36% of CSF samples presented with cell count more than 10, while only 9% of CSF samples had WBC count more than 100.

Of the total CSF samples subjected to culture, 3 were culture positive for *Staphylococcus aureus* and 1 was positive for *Salmonella typhi*. The culture positive cases were subjected to antibiotic sensitivity testing and *Staphylococcus aureus* was found to be sensitive to Vancomycin, Ampicillin, Erythromycin, Chloramphenicol, Cefotaxime, Gentamicin and resistant to Ceftazidime. *Salmonella typhi* showed susceptibility towards Gentamicin, Cotrimaxozole, Chloramphenicol, Ceftriaxone and was resistant to Ampicillin, Cefotaxime, Ceftazidime.

Out of 179 blood samples processed for culture, 2 were culture positive for *Klebseilla pneumoniae* and 4 were positive for *Pseudomonas aeruginosa*. Antibiotic susceptibility done against *Klebseilla pneumonia* revealed that it was sensitive to Norfloxacin, Cephotaxime, Azithromycin, Chloramphenicol, Ciprofloxacin, Gentamicin, Ceftazidime and resistant to Ampicillin and Neomycin. *Pseudomonas aeruginosa* was found to sensitive to Cefotaxime, Gentamicin, Ciprofloxacin and resistant to Ampicillin, Ceftazidime and Penicillin.

35 no of CSF samples were sent to CMC Vellore in first batch for real time PCR analysis, out of which 12 were positive for *S.pneumoniae*. At RMRC Laboratory we analysed 100 samples by real time PCR, out of which 21 were found to be positive. (*S.pneumoniae* 17 and *H.influenzae* type b 4).

Future Plan

Enrolment of the subjects into the project will be carried out as per protocol. Latex test will be carried out in all cases from now onwards.

10. New borne screening for sickle cell diseases in tribals of Odisha and association of disease morbidity with genetic and nutrition modifiers of HbF levels.

Principal Investigators : Dr. S K Kar, Director, RMRC Bhubaneswar
Co-Investigator : Dr. Sapna Negi, Scientist D, Director, RMRC, Bbsr
Dr. AS Kerketta, Scientist D, Director, RMRC Bbsr
Funding: Extramural

Duration of the Project : 5 year

Project has been approved by 26th SAC and submitted to ICMR for extramural funding. In the meantime research activity on the project has been started from March 2013 at Kalahandi field unit with intramural support with the **objectives**:

- To standardize and establish a protocol for collection of cord blood samples for screening of neonates for Sickle Cell Disease (SCD) and followup of positive subjects
- To get data on incidence of Sickle cell disease among Institutional deliveries of Kalahandi district hospital

Background information

Sickle cell disease is a major health problem in the state of odisha. As per the State Health Department 2008-2012 report – Odisha has 5.35 lakh of population affected by the Sickle Cell disease, of which nearly 94% per cent live in 13 western Odisha districts including Kalahandi. Although, no systematic data is available from Kalahandi district, data collected from District Hospital shows that a total of 602 units of blood (one unit per hospital admission) had been transfused to patients with SCD in Kalahandi District hospital during the year 2011-2012. A newborn screening in this region will allow us to get data on incidence of SCD in the district and education of parents of the diseased about signs and symptoms of the SCD crisis which might help in reducing mortality and morbidity among those affected.

Progress of Work Done

Standardization of cord blood collection procedure was carried out at Gynecology & Obstetric Department, Capital Hospital which is close to our centre. The standardized procedure was then applied at Gynecology & Obstetric Department, Kalahandi District hospital in collaboration with State Government. Nurses of the Department were trained in collecting cord blood sample under sterile conditions. In order to get the systematic data, a preliminary newborn screening activity in this district was started intramurally from March to July 2013 and 761 newborns cord blood samples were screened for SCD using Hb variant HPLC analysis. The report of our screening has been given to the HOD Gynecology & Obstetric Department, District Hospital Kalahandi. A three monthly door-to-door followup of 11 families with 2 homozygous and 9 heterozygous SCD neonates was carried out for the confirmation of parental SCD status and to know any health problems encountered by the SCD babies and their parents using a predesigned morbidity assessment questionnaire.

Results

Of the total cord blood samples tested, 125 (16%) were found to be SCD positive of which 13 (1.7%) were homozygous and 112 (14%) were heterozygous for the disease. The demographic data of the studied subjects indicates approximately equal proportion of males and females born at Kalahandi district hospital as well as those born with the disease (**Table 1)**. The percentage of SCD homozygous cases was found to be highest in M. Rampur block (10%) of Kalahandi district (**Table 2**). The result is obtained on a small number of deliveries therefore needs to be confirmed on large dataset. HPLC analysis of the cord blood samples of neonates demonstrated an overall higher average HbF levels among SCD homozygous than in heterozygous and healthy control neonates (**Fig 1**).

A followup of two newborns with Sickle cell disease and 8 SCD carriers was carried out at 3 months of age and blood samples of the parents were collected

for confirmation. No SCD related morbidity was detected in the newborns except for one SCD homozygous baby who was found to be having problem of stomach distention. HPLC analysis of the parent's blood confirmed SCD condition of the newborns.

From the door-to-door followup it was experienced that 1) the villages visited at Kalahandi district are far from each other and their sizes are very small with an average of 32 households per village, 2) the frequency of SCD per village was ~1, 3) confidentiality of the health status of the baby and his / her family members is at risk due to the frequent visits of a clinician / researcher to the affected households. Therefore, an alternative strategy like involving ASHAs of the village for informing family of the patient and asking them to report to RMRC field unit at Kalahandi District hospital for confirmation of result and for subsequent follow-ups should be implemented. This approach will also help us in guiding them for a regular checkup by a pediatrician at the District hospital.

Table 1: Demographic data of the subjects studied.

SI NO.	AREA	TOTAL NUMBER (n=761)	NORMAL (n=635) (%)	SICKLE CELL TRAIT (n=112) (%)	SICKLE CELL DISEASE (n=13) (%)
1	BISWANATHPUR	6	5(83.33)	1(16.67)	
2	BHAWANIPATNA	292	243(83.21)	44(15.07)	5(1.71)
3	DHARMAGARH	20	18(90)	2(10.00)	
4	GOLMUNDA	11	9(81.810)	2(18.18)	
5	JAYPATNA	42	37(88.09)	5(11.90)	
6	JHUNAGARH	175	152(86.85)	20(11.43)	3(1.71)
7	KEOGAON	29	20(68.96)	9(31.03	
8	KESINGA	36	31(86.11)	5(13.89)	
9	KOKSARA	26	23(88.46)	3(11.54)	
10	M.RAMPUR	19	13(68.42)	3(15.79)	2(10.52)
11	NARLA	27	23(85.18)	4(14.81)	
12	OTHER	71	55(77.46)	13(18.31)	3(4.22)
13	TH.RAMPUR	7	6(85.71)	1(14.29)	

Figure-1

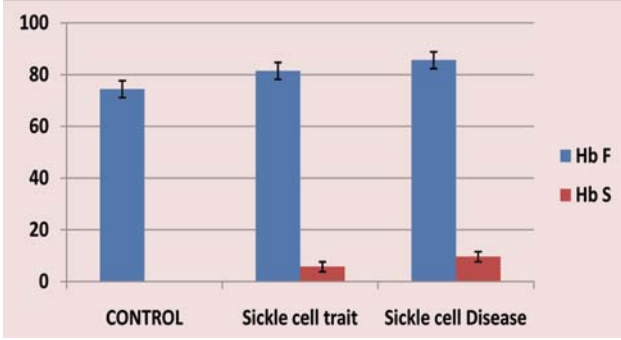


Table 2: Area Wise Distribution Of Sickle cell Disease in Kalahandi.

DISEASE STATUS	TOTAL (n=761)(%)	MALE (n=383)		FEMALE (n=375)	
		NO.OF MALE S	MEAN WT±SD	NO.OF FEMALES	MEAN WT±SD
NORMAL	635 (83%)	320 (84%)	2.86±1.19	312 (83%)	2.72±1.20
SICKLE CELL TRAIT	112 (15%)	53 (14%)	2.68±0.86	59 (16%)	2.61±0.86
SICKLE CELL PATIENTS	13 (1.7%)	9 (2.3%)	2.8±0.46	4 (1.1%)	2.42±1.10
Hb D trait	1(0.13%)	1 (0.3%)	3.2		

11. Characterization of clinical features of Diabetes mellitus specific to low BMI individuals.

Principal Investigator : Dr. Sapna Negi
Co-Investigators : Dr. S.K Kar,
Dr. Nilam M. Somalkar
Collaborators : Dr. CBK Mohanty,
Prof. Medicine, SCB Medical College, Cuttack
Dr. Abhay Sahoo,
Endocrinologist, Kalinga Hospital, Bhubaneswar
Funding : Extramural

Duration of Research Project: 3 years

Objectives

- To classify subjects into low BMI and high BMI diabetics and age and gender match healthy controls
- To identify subjects with low levels of insulin and those with insulin resistance

- To study pancreatic autoimmunity among the group with inadequate insulin secretion
- To study the subjects for vitamin insufficiency mainly Vitamin D and Vitamin B12
- To perform association studies on data obtained from anthropometric and clinical analysis.

Background information: Diabetes has been extensively investigated, but so far these investigations has not led to any major insight into its etiology, mainly due to the clinical heterogeneity among diabetes patients. The major form of late onset diabetes, Type II diabetes, is usually linked to high BMI, but in many cases is also observed in low BMI individuals. Cause of adolescent diabetes in low BMI individuals is not well defined. In the present study we will specifically look into the characteristics of diabetes in low BMI individuals and compare them with high BMI diabetics. The characterization will be based on insulin secretion and sensitivity, levels of triglycerides, presence of pancreatic auto-antibodies and vitamin D and B12 insufficiency among newly diagnosed diabetics. The study will provide insight for developing strategies for proper management of low BMI diabetes.

Progress of Work Done

The project has been approved by 26th SAC and submitted to ICMR for extramural funding.

12. Assessment of adolescent reproductive and sexual health programme in Orissa: Advocacy for intervention strategies.

Investigator	:	Dr. G. Bulliyya
Co-Investigators	:	Dr. A. S. Kerketta
Starting date	:	June 2011
Closing date	:	May 2014
Status	:	Extramural (ICMR Adhoc project)

Adolescents (10-19 years of age) comprise 22.8% (225 million) of the Indian population. It is a heterogeneous group, marked with physical, physiological, sexual and behaviour changes and their situation varies by age, sex, marital status, class, region and cultural context. A large proportion are out of school, malnourished, get married early, work in vulnerable situations, sexually active, and exposed to peer pressures. Adolescence perceived to be healthy period of life because mortality is relatively low in this age group. They possess a distinct array of health challenges including teenage pregnancy, unsafe abortions, excess risk of maternal and infant mortality, high-risk behavior, and lack of awareness about contraception and reproductive tract infections and rapidly rising incidence of HIV / AIDS. Yet they face many challenges in their life, which are related to their health and inadequate access to health care. Recognizing the adolescent health needs, Adolescent Reproductive and Sexual Health (ARSH) program launched as a part of Reproductive and Child Health (RCH-II) under National Rural Health Mission (NRHM 2005) and Programme Implementation Plan (PIP 2006) in anticipation of positive influence maternal and infant deaths, delay in age of marriage, reduce incidence of teenage pregnancy, meet unmet contraceptive needs, sexually transmitted diseases and proportion of HIV positive cases. To achieve the goals, Adolescent Friendly Health Clinics (AFHC) established at institutional levels to improve quality of healthcare, counseling services and to build a supportive environment.

Objectives

The general objective is to evaluate the adolescent reproductive and sexual health (ARSH) program and adolescent friendly health clinic (AFHC) services through developing advocacy-based intervention in Orissa.

Specific

- To assess the knowledge, attitude and behavior on reproductive health problems of adolescents
- To assess the quality of care at Adolescent Friendly Health Clinics;

- To assess the accessibility and utilization of health care services by adolescents; and
- To devise plausible ways and intervene with package of services to explore opportunities for improving utilization of adolescent health services.

Methodology

The study is being conducted in two phases:

Phase-I comprised formative research on evaluation of baseline process indicators of ARSH program and quality of care at AFHC. At the community level, health needs and accessibility of adolescent health services and stakeholder was assessed. Further situation analysis was made as the first step for systematic scaling up that involved analysis of data and identifying factors for promoting optimal accessibility and coverage of ARSH services.

Phase II: The findings of phase-I are being used for strategy implementation based on indentifying gaps from baseline evaluation to improve ARSH services towards achieving the envisaged goals.

Study area

The study is being carried out in two districts viz Kalahandi and Rayagada, where 29% and 35% of women married before 18 years (DLHS-3, 2010). Multistage stratified random sampling adopted for selection of two identical blocks, sub-centres and villages. In Kalahandi district, Junagarh and Dharmagarh blocks while Rayagada rural (Jamdehipenta) and Gunupur blocks in Rayagada district were selected. In each study block, one sector covering two Sub-Centres having maximum SC/ST population was included in the study. Data was collected through pre-tested questionnaires separately for household survey, adolescents, and married adolescent groups in the community. Nutritional status was assessed by anthropometric measurements and anaemia by hemoglobin estimation. Similarly stakeholder's questionnaires used for Anganwadi Workers (AWW), Accredited Social Health Activist (ASHA), Panchayat Raj Institutes (PRI) and teachers. Further independent structured questionnaires were used for facility-based survey and healthcare providers such as Medical Officers, ICTC Counselors, Lady Health Visitors (MPHS-Female), MPHS-Male, ANM (MPHW-Female), MPHW-Male.

Progress

The study included 1615 households (HHs), 872 in Kalahandi and 743 in Rayagada districts. A total 2087 adolescents were covered from four blocks in Kalahandi (1132, Junagarh-672 and Dharmagarh-460) and Rayagada (955, Rayagada-531 and Gunupur-424). Adolescent (early) marriages found to be 7.9% (male 4.1%, female 10%) in Kalahandi and 9.1% (male 3.7%, female 13.6%) in Rayagada districts In Kalahandi, 16.4% of married adolescents are pregnant and 34.3% are lactating, while 10.8% and 8.1% are pregnant and lactating mothers in Raygada (**Table-1**). Majority of adolescents are educated in both the districts and female adolescents are at par with their male counterparts. Drug abuse is confined to male adolescents in terms of chewing tobacco followed by smoking and alcohol intake.

Age at menarche was 12.3 years in both the study districts. The level of awareness on pubertal changes vary (24-34%) and (77-25%) in Kalahandi and Rayagada districts. Knowledge on legal age of marriage is 24-34% and consequences of teenage marriage known to 36–39% adolescents. Knowledge about menarche prior to attaining menarche is higher in Kalahandi (38%) than in Rayagada (21%). Adolescent females experienced premenstrual syndrome is more in Rayagada than in Kalahandi district, while 21.3-31.8% of girls had problem during menstrual periods. About 50% of girls are maintaining

menstrual hygiene practices and only 4-12.7% of using sanitary pads.

Adolescent's knowledge about RTI / STI and HIV / AIDS reveals that 23.1% and 6.5% in Junagargh and Dharmagargh, and 33.3% and 18.8% in Rayagada and Gunupur blocks having knowledge on RTI / STD. However, majority of them are unaware of the symptoms and ways of acquiring RTIs / STD. Similarly, most of adolescents are aware about HIV / AIDS, 19-29% have knowledge on symptoms of HIV / AIDs, 50% are aware about the routes of transmission and preventive methods. Sources of information about HIV / AIDS and RTI / STDs include TV / radio, followed by Poster / Wall paint, healthcare workers, newspaper / magazine, exhibition, school teachers, friends / peer and husband / parents (Table 2). About 36-42% of adolescents have knowledge on various methods of family planning getting information through public channels. Tubectomy is the commonly known method of family planning and awareness about contraceptive methods include emergency pills followed by condoms, oral pills, injections, IUD and spermicidal. The level of awareness about contraception and family planning methods are greater in Kalahandi as compared to Rayagada district.

Knowledge about programs related to adolescent health are in order of Kishori Shakti Yojana (KSY), Balika Samridhi Yojana, Village Health & Nutrition Day (VHND), Gaon Kalyan Samiti (GKS), SABLA, and Adolescent Education under NACO in both the sexes and in the study districts. However, participation in these programs is very low (4-9%). Adolescents received health services included iron-folic acid (IFA), followed by tetanus injections, deworming tablets and health check-ups. Quite a few adolescent girls attended VHND / GSK sessions (5-9%) and received knowledge about their health issues. Awareness about the ARSH programme is far from expectations (<3%) in both the districts. Similar is the case for those aware and access the adolescent-specific health facility namely Adolescent Friendly Health Clinics in Kalahandi and Rayagada districts (Table 2).

Nutritional status reveals that 16.5% and 21.4% of adolescents respectively in Kalahandi and Rayagada districts are underweight (BMI <-2SD of WHO reference) and stunting (z-scores height-for-age <-2SD of median of WHO reference) or shortness-for age is seen 34.9% and 45.3% respectively (Table 3). Hemoglobin estimated from a sample of 871 prick blood samples show that 70% of adolescents are suffering from different grades of anaemia in both the study districts, Kalahandi (Junagarh 60%, Dharmagargh 88%) and Rayagada (Rayagada 76%, Gunupur 78%). Severity of anaemia in terms of mild (16.5%) moderate (30.1%) and severe (20.6%) forms in Kalahandi and it is 36.4%, 28.1% and 11.2% respectively in Rayagada districts. Anemia prevalence is more common among adolescents in Kalahandi district as compared to Rayagada district (Table 4).

Mean age at marriage for male and female adolescents is 18.3y for both Kalahandi (Junagarh 18.5y, Dharmagarh 18y) and Rayagada (Rayagada 18.9y, Gunupur 17.7y) districts. Similarly, mean age at first conception for female adolescents is 19.6y in Kalahandi whereas it is 19.2% in Rayagada. Awareness about ideal age of marriage (>18 y) is more in Kalahandi (40%) as compared to that in Rayagada (19%) districts. Of total married adolescent girls, 62-82% of them had a pregnancy and 7-12% of them had an abortion. While, knowledge on ideal birth spacing (>2 y) and consequences of early marriages is known to 20-41%, only 10% of them are currently using various birth spacing methods. A majority of married adolescents exposed to family planning messages (32% in Kalahandi and 49% in Rayagada) through various IEC media.

A majority 88% of ever-married adolescent females registered for ANC in Kalahandi and 63% in Rayagada districts. Most of them registered with local health workers (ANM / ASHA / AWW) followed by Govt. hospital and private clinics. More than 83% and 74% of adolescent women in Kalahandi and Rayagada districts respectively received 2 or more antenatal check-up and 80% of married women had ANC tests such as weight / height, blood pressure, blood test, urine test, examination of abdomen / breast. In Kalahandi and Rayagada districts 97.4% and 87.5% respectively had received IFA tablets during

Table 1. Study characteristics of adolescents by age and sex in Kalahandi and Rayagada districts.

Study character	Kalahandi (1132)			Rayagada (955)		
	Junagarh	Dharmagarh	Total	Rayagada	Gunupur	Total
Number	59.4 (672)	40.6 (460)	100.0 (1132)	55.6 (531)	44.4 (424)	100.0 (955)
Sex						
Boys	34.0	38.3	35.7	38.9	47.8	42.9
Girls	66.0	61.7	64.3	61.1	52.2	57.1
Age group						
10-14 yr	60.0	58.6	58.5	57.1	53.1	55.1
15-19 yr	40.0	41.4	41.5	42.9	46.9	44.9
Marital status						
Unmarried	90.8	94.1	96.0	91.8	90.6	93.7
Married	9.2	5.9	7.9	8.9	9.4	9.1
Male	7.0	2.0	4.5	4.3	3.0	3.7
Female	10.4	9.5	10.0	11.7	15.3	13.6
Physiologic status						
Pregnant adolescents	15.2	18.5	16.4	10.5	11.1	10.8
Lactating adolescents	15.2	66.7	34.3	8.0	8.3	8.1
Non-pregnant/Non-lactating	69.6	14.8	49.3	81.5	80.6	85.1
Schooling/Occupation						
Student	90.5	78.9	50.1	84.5	94.9	89.1
HH work	7.4	17.0	11.3	10.2	3.0	7.0
Earning member	2.1	4.1	2.9	5.2	2.1	3.9
Literacy						
Primary (<5th std)	42.4	44.7	39.6	53.8	42.3	48.7
Secondary (5-10th std)	43.5	34.7	43.5	33.2	37.6	35.1
Higher Sec. (>10th std)	5.5	8.2	6.9	4.8	15.8	9.7
No education	8.6	12.4	10.1	8.2	4.3	6.5
Drug abuse						
Chewing	7.3	10.3	7.0	17.3	8.9	13.6
Smoking	1.4	1.2	1.1	2.5	0.6	1.8
Alcohol	1.0	0.2	1.0	6.8	0.7	4.2

Table 2. Adolescent's knowledge about RTI / STD and HIV / AIDS in Kalahandi and Rayagada districts.

Knowledge on RTI/STD	Kalahandi			Rayagada		
	Junagarh	Dharmagarh	Total	Rayagada	Gunupur	Total
N	672	460	1132	531	424	955
Heard/Knowledge about RTI/STD	23.1	6.5	16.8	33.3	18.8	26.9
Symptoms of RTI/STD	1.9	0.9	1.4	14.1	9.5	12.1
Sources of acquiring RTI/STDs	1.2	4.6	2.6	20.9	14.6	18.1

Complication of RTI/STDs	1.6	2.8	2.1	9.7	6.8	8.5
Knowledge on HIV/AIDS						
Symptoms of HIV/AIDS	6.3	37.2	18.8	34.5	23.5	29.6
Know at least 3 routes of transmission	57.7	43.0	51.8	39.7	30.4	35.6
Prevention of HIV/AIDS	51.8	45.7	49.3	33.3	28.3	31.1
Knowledge on family planning	39.9	31.1	36.3	52.6	28.3	42.8
Methods of family Planning						
Vasectomy	18.7	15.4	2.38	7.9	18.9	11.3
Tubectomy	56.0	31.5	17.2	35.5	18.9	33.1
Both	0.4	25.2	3.3	41.9	61.1	47.6
	41.8	27.9	13.4	14.7	1.1	10.5
Heard about contraception	44.9	39.8	42.8	41.7	21.8	30.8
Aware about contraception						
Condom	11.9	16.9	13.8	0.6	15.1	3.5
Oral Pill	15.2	41.5	25.2	41.7	20.5	35.5
Emergency Pills	0.0	0.5	0.2	1.7	4.1	2.6
Injection	4.6	1.1	3.3	0.6	1.4	0.6
I.U.D.	0.7	1.1	0.8	5.7	1.4	4.5
Spermicidal	0.0	0.0	0.0	0.6	0.0	0.3
Others (traditional)	1.1	0.5	0.8	0.0	1.4	0.3
Don't know	65.1	34.9	53.8	24.0	27.4	24.9
Knowledge on ARSH Program						
Aware programs at least more than one	1.8	6.7	3.7	24.3	16.0	20.6
Ever participated in VHND	3.1	6.5	4.5	15.6	0.2	8.8
Ever participated Kishori Swasthya Mela	2.5	3.5	2.9	20.0	0.2	11.2
Aware about ARSH program	3.3	3.0	3.1	0.8	0.7	0.7
Aware of Adolescent Friendly Health Clinic	3.0	0.2	1.8	0.0	0.2	0.1

Table3. Nutritional status of adolescent population in Kalahandi and Rayagada districts, Odisha.

Block/District	Thinness (BMI-for-age z-score <-2SD)			Stunting (Height-for-age z-score <-2SD)		
	Male	Female	Total	Male	Female	Total
Kalahandi	N=255	N=616	N=871	N=255	N=614	N=869
Junagargh	4.7	6.5	6.0	5.5	21.2	16.6
Dharmagargh	18.0	7.5	10.6	23.5	16.3	18.4
Total	22.7	14.0	16.5	29.0	37.1	34.9
Rayagada	N=212	N=260	N=472	N=212	N=260	N=472
Rayagada	11.3	9.6	10.4	22.2	33.5	28.4
Gunupur	17.5	5.8	11.0	28.8	7.3	16.9
Total	28.8	15.4	21.4	50.9	40.8	45.3

Table 4. Prevalence of anaemia among adolescent by sex in Kalahandi and Rayagada districts, Odisha.

Anaemia grade (Hb g/dl)	Kalahandi district			Rayagada district		
	Junagarh (158)	Dharmagarh (241)	Total (399)	Rayagada (360)	Gunupur (112)	Total (472)
Normal (>12.0)	39.9	22.0	29.1	23.6	21.5	23.0
Mild (10-12)	8.2	28.2	16.5	37.8	32.1	36.4
Moderate (7-10)	22.2	35.3	30.1	27.2	9.7	28.1
Severe (<7 g/dl)	29.7	14.5	20.6	10.0	15.1	11.2

Table 5: Awareness of health care providers at Adolescent Friendly Health ClinicsField Activities.

Health care providers Awareness	Kalahandi district			Rayagada district		
	Junagarh (27)	Dharmagarh (18)	Total (45)	Rayagada (19)	Gunupur (24)	Total (43)
Aware about Promotive services of ARSH	**63.0**	**44.4**	**55.6**	**84.2**	**72.2**	**81.4**
Focused care during antenatal period	18.5	5.5	13.3	5.3	4.2	4.6
Counseling & provision for emergency contraceptive pills	3.7	5.5	4.4	5.3	4.2	4.6
Counseling/ provision of reversible contraceptives	0.0	0.0	0.0	0	0	0.0
Information & advice on ARSH issues	3.7	11.1	6.7	57.9	33.3	44.2
All the above	40.7	22.2	33.3	21.0	29.2	25.6
Don't know	33.3	90	42.2	10.5	29.2	4.7
Aware about Preventive services of ARSH	**59.3**	**38.9**	**51.1**	**68.4**	**62.5**	**65.1**
Services for tetanus (TT) immunization	7.4	16.7	11.1	5.3	33.3	14.0
Services for prophylaxis against nutritional anaemia (IFA tablets)	3.7	5.5	4.4	5.3	4.2	4.7
Early/safe termination of pregnancy & management of post abortion complication	0.0	5.5	2.2	15.9	4.2	9.3
Counseling on abuse & dependence on alcohol, drug, smoking, tobacco etc	3.7	0	2.2	21.1	8.3	14.0
Dietary/nutrition counseling	14.8	0	8.9	5.3	8.3	7.0
All the above	25.9	11.1	20.0	26.3	20.8	23.3
Don't know	44.5	61.1	51.1	21.1	33.3	27.9
Aware about curative services of ARSH	**51.9**	**50.0**	**51.1**	**57.9**	**58.3**	**58.1**
Treatment for common RTI/STI	18.5	16.7	17.8	10.5	4.2	7.0
Treatment & counseling for menstrual	11.1	5.5	8.9	5.3	4.2	4.7

disorders						
Treatment & counseling for sexual concerns of male/female adolescents	7.4	5.5	6.7	15.9	4.2	9.3
Management of sexual abuse among girls	0.0	0	0.0	5.3	0	2.3
All the above	22.2	22.2	8.9	21.1	50.0	37.2
Don't know	40.7	50.0	44.4	42.1	41.7	41.9
Aware about referral services of ARSH	**48.1**	**50.0**	**48.9**	**89.5**	**83.3**	**86.0**
Referral for screening of sickle cell anemia	7.4	11.1	8.9	5.3	0	2.3
Counseling of HIV/AIDS RTI/STI through VCCTC at ICTC	11.1	5.5	8.9	36.8	16.7	27.9
Prevention of parent to child transmission (PPTCT)	3.7	5.5	4.4	0.0	4.2	2.3
Critical cases referred to the district/state health institution	11.1	0.0	6.6	21.1	20.8	20.9
All the above	14.8	22.2	17.8	26.3	41.7	34.9
Don't know	59.3	55.6	57.8	10.5	16.7	14.0
Aware about outreach services of ARSH	**74.0**	**66.7**	**73.3**	**73.7**	**70.8**	**72.1**
Periodic health check-ups & community camps	22.2	33.3	26.7	31.6	25.0	27.9
Periodic health education activities	14.8	22.2	17.8	21.1	16.7	18.6
Co-curricular activities (adolescent meet at block/district)	0.0	5.5	2.2	5.3	0.0	2.3
All the above	25.9	16.7	22.2	21.1	29.2	37.2
Don't know	37.0	22.2	31.1	21.1	29.2	14.0
Are you satisfied with the services delivered under the ARSH program	**70.0**	**42.3**	**60.0**	**84.2**	**83.3**	**83.7**

pregnancy, 92.3% and 80.0% of them had consumed total 100 tablets. Majority received tetanus (TT) injection and ICDS supplementary food in both the districts and exposed to IEC advices on breastfeeding, keeping baby warm, institutional delivery, cleanliness at delivery, nutrition counseling and family planning. A considerable adolescent deliveries taking place at facility level both in Kalahandi and Rayagada districts that are motivated by health workers ASHA / ANM / AWW, however, in case of home delivery, reasons attributed are cost too much, too far / no transport and better care at home. Majority of deliveries were conducted by doctor or TBA.

A checklist of process indicators used to evaluate at each AFHC as per ARSH guidelines in selected blocks Kalahandi (Bhawanipatna and Junagarh) and in Rayagada (Rayagada and Gunupur) districts. These AFHC are scored as per quality services available in the Guidelines. A total 88 healthcare providers were interviewed on process indicators that include Medical Officers (6), ICTC Counsellors (5) Lady Health Visitors (MPHS-Female 10), MPHS-Male (13), ANM (MPHW-Female 41), MPHW-Male (13) in Kalahandi (45) and Rayagada (43). Healthcare providers aware about all 5 service components 55-82%, whereas majority aware on independent components rather than combinations of five (Table 5). Promotive services (focused care during antenatal period, counseling / provision for emergency contraceptive pills, provision of reversible contraceptives, information-advice on ARSH issues) are known to 22-40.7.%, preventive services (TT, IFA, Early / safe termination of pregnancy & management of post abortion complication, counseling on abuse & dependence on alcohol, drug, smoking, tobacco etc) 51-65.1%, curative services (treatment for common RTI / STI, treatment & counseling for menstrual disorders & sexual concerns, management of sexual abuse among girls) 50-58.1%, referral services (screening of sickle cell anemia, counseling of HIV / AIDS RTI / STI through VCCTC at ICTC, prevention of parent to child transmission, critical cases referred

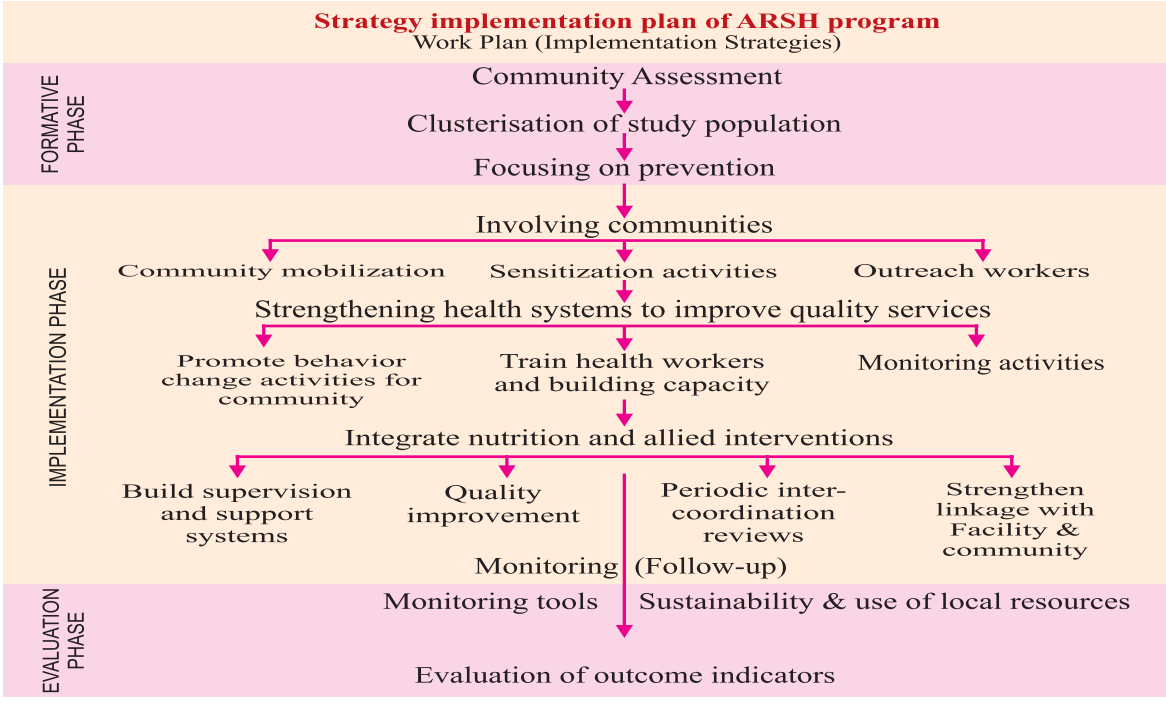
to the district / state health institution) 49-86%, and outreach services (periodic health check-ups, community camps, health education activities, Co-curricular activities) 72-74% (Table-5).

The quality of services available at Adolescent Friendly Health Clinics is adequate in Kalahandi and Rayagada districts in terms of displaying name plate (local language 'Shraddha' or 10-19 Barsa ra Kisora / kisorinka Pain and timings (every Saturday afternoon 3-5 PM). Medical Officer and ANM or staff nurse deputed for extending ARSH services and maintained records and registers in confidentiality as per policy guidelines. Equipments, measuring tools (weight scale), supplies such as oral contraceptive pills (OCP), emergency contraceptive pills (ECP) and condoms are available, however, vaccination (TT), pregnancy test strips, rapid plasma reagent kits for syphilis remain to be extended. However, accessibility of ARSH services by adolescents is very poor in each AFHC (monthly <10) which is far from achieving the health objectives.

Strategy implementation is on using various advocacy tools selecting one block in each district by random selection as study block covering two sub-centers (Study Plan). The intervention blocks include Junagarh (Pilikia, Habaspur) in Kalahandi and Jamdehipento or Rayagada rural (Pitamahal, Kereda) in Rayagada district. Interventions strategies include orientation on ARSH components to community health workers and other stakeholders in the community. At facility-level, orientation workshops conducted for health care providers about AFHC process indicators of delivery for effectiveness of the programme and study population assessing the quality and satisfaction level of services. Community-level workshops conducted at sub-centre-level covering target adolescents of school-going, non-school going and married adolescents including their parents and family members.

Future plan

The ongoing intervention strategy is being monitored using process indicators of the programme and advocacy tools such as IEC materials (posters, pamphlets, banners) targeting adolescents (school going, non-school going and married), health workers, stake holders in the community for knowledge generation on ARSH, preventing methods and curative services accessibility at specific health



Field Activities of RMRC

facilities creating confidence and confidentiality. The effectiveness of the strategy will be assessed in terms of promotive, preventive, curative, referral and outreach services at facility level and it will be used to reduce the incidence of early marriage, teenage pregnancy, meeting unmet needs of contraception, and sexually transmitted diseases and substance abuse in the community.

13. Distribution and bionomics of *Culex 'vishnui'* group of mosquitoes with reference to Japanese Encephalitis transmission in Odisha.

Principal Investigator	:	Dr.N.Mahapatra
Co-Investigator	:	Dr.R.K.Hazra
Duration	:	3 years
Funding	:	Intramural (ICMR)
Started	:	April 2013

During the month of July and August,2012 suspected cases of encephalitis were admitted to tertiary care hospital of Cuttack and Bhubaneswar was conformed in the virology laboratory of the centre. It has identified eight JE sporadic cases during 2012.The cases were reported from five coastal districts of the state (Puri, Balasore, Jajpur, Kendrapara and Jagatsingpur).

Mean while, during16.09.2012 to 14.11 2012, a sudden deaths of children suspected of encephalitis were reported from tribal areas of Malkangiri district**.** There was a report of 32 deaths, 21 in Potrel ,Ushakapli and Badili villages of Korkunda Community Health Centre(CHC) ,two in Namkonda village of Kalimela CHC, three in Charkiguda of Malkangiri NAC, and six in Mathili CHC, Malkangiri district. Investigation conducted by RMRC during that period conformed 11 cases of JEV infection out of 86 cases by serological tests, which was cross checked at NIV laboratory.

Following that, this year an entomological survey was conducted to find out the possibility of JE transmission, For this purpose, a survey was conducted from 9.6.13 to 13.6.13 by a team of entomologist and two technician in Malkangiri district. The team went to four affected villages and Blood samples were collected from inhabitants of affected house as well as from their neighbours. Indoor(human dwelling and cattle shed) and outdoor day time resting mosquito collection were done in the villages.

Entomological investigation in Potrel village

The village is situated 4 km away from the CHC HQ. Human dwelling are scattered not in a row. The houses are thatched with terracotta tiles or asbestos. Maximum holdings in the village were electrified.

Cattle and goat sheds were away from the human dwelling .In some cases, mixed dwelling were also seen. All the pig sheds were found within 5 to 50 ft away from the human dwelling. The village had two pits, one pond (a perennial water source) and one well for their drinking purpose. Paddy fields are all around the village. When the team visited the village, almost all paddy field were found dried.

Mosquito species like *Culex vishnu i*(the known JE vector),*Cx.quinquefaciatus* ,*An.vagus, An.culicifacies Anopheles subpictus* were collected(table 1). Due to summer season mosquito populations were low.

Investigation in Ushakapli village

The Uskapali village is situated 10km away from the CHC HQs. Position of human dwellings, cattle and goat sheds and pig sheds was almost similar to the other village. There were four tube wells in the village and all were used by the villages for drinking. In addition, there were two wells , two pits and one pond inside the village and small stream at 750 m away from the village. Dry paddy fields were present all around the village.

Only the stream, pond and pits were the breeding habitats for mosquitoes.

Five mosquito species *Culex.vishnui, Cx.quinquefaciatus, An.subpictus An.vagus and An.culicifacies* were recorded. The per man hour density (PMHD) is given below (Tab-2)

Investigation in Pradhaniguda village

The Pradhaniguda village is situated 5km away from the CHC HQs. Position of human dwellings,

Tab-1. Man Hour density of mosquitoes in Potrel village.

Sl.No.	Malkangiri NAC	Village name	Mosquito species	PMHD
1			*Cx.quinquefasciatus*	2.0
			Cx.tritaeniorhynchus	1.0
2			*An.culicifacies*	4.5
3			*An.subpictus*	20.5

Tab-2: The per man hour density (PMHD) of mosquitoes of Ushakapali village.

Sl.No.	Sub centre	Village name	Mosquito species	PMHD
1	MV-34		*Cx.quinquefasciatus*	2.0
2			*Cx.vishni*	0.5
3			*An.vagus*	0.5
4			*An.culicifacies*	1.5
5			*An.subpictus*	7.0

Table 3: The per man hour density (PMHD) of mosquitoes of Pradhaniguda village.

Sl.No.	Malkangiri NAC	Village name	Mosquito species	PMHD
1			*Cx.quinquefasciatus*	2.0
			Cx.tritaeniorhynchus	1.0
2			*An.culicifacies*	4.5
3			*An.subpictus*	20.5

cattle and goat sheds and pig sites was almost similar to the other village. There were two wells and small irrigation canal 150-200 meter from the village . Mosquito and blood samples were collected from the different household.

Observation from the entomological investigation:

1. Paddy field, the major mosquito breeding habitat in the villages, were dry during the time of survey.
2. Outdoor sites for mosquito resting, such as bushes, were plenty around the village
3. Vector density was very low in this season
4. During the time of survey, there was no report of fever or other related illness.

Blood samples collection from human

A total of 45 blood samples were collected from the members of the affected household (contacts) as well as neighbors in the presence of the PHC doctor. Five samples were also collected form the hospital who had fever and suspected to have malaria. The samples were brought to the laboratory to detect IgM and IgG. All the samples were negative for IgM.

Collection of Larvae

Team Collecting information from household

Pig population near the Household

Pig shed near to utensil cleaning site

Collection of blood sample from the cattle

Pig population and their shed

14. Performance of Light Emitting Diode microscope in different settings for TB diagnosis: a multi-centric study.

Principal Investigator : Dr Dasarathi Das
Starting Date : September 2013
Period : One year
Funding : ICMR

Development

Preliminary work has been initiated with fluorescence microscopy. Since the centre is not having LED Fluorescence microscope the budget for the same was made in the project proposal. For standardization of fluorescence staining and microscopy,318 slides were examined with fluorescence microscope and compared with ZN microscopy.

15. A Prospective Study to determine the Incidence of Tuberculosis among Patients with Type 2 Diabetes Mellitus (Multi-centric).

Principal Investigator : Dr. T. Hussain,
Co Investigators : Dr.M. Makeshkumar & Dr.Soumya Swaminathan (NIRT, Chennai)
: Dr. S.K.Kar, Dr. D. Das, & Dr.P.K.Sahoo (RMRC, BBSR)
Collaborators : Dr. Abhay Sahoo, SUM Hospital, Bhubaneswar
Starting Date : Aug. 2013
Duration : 3 Years

Work Progress

Preliminary work has been initiated with intra-mural funds. Some of the works are reflected below:

(i) Prevalence & correlates of Metabolic Syndrome, Pre-Diabetes and Type 2 Diabetes mellitus among adults in an urban area of Bhubaneswar - a hospital based study

Indians are more prone for Metabolic syndrome (MS), Pre-Diabetes and Type 2 Diabetes mellitus (T2DM) than almost any other population in the world. MS, Pre-Diabetes and T2DM are major health problems associated with significant mortality and morbidity but can be prevented or delayed through lifestyle interventions. This study was carried out to determine the prevalence and correlates of MS, pre-diabetes and T2DM among adults in an urban area of Bhubaneswar. 105 adults were enrolled, after obtaining pre-informed consent. The plasma and sera samples were used for various investigations namely random blood sugar, Liver function tests, blood urea, serum creatinine and lipid profile. The socio-demographic and anthropometric profile, reasons for stress, complications at the time of testing, habits, etc. were correlated with Blood pressure, fasting blood sugar levels and lipid profile at the time testing. Out of 105, 45 were having MS, 10 were pre-Diabetic and 60 were Diabetic. Further, 71 were males whereas 34 were females. About 32% people in the age range of 41-50 years are pre-disposed to develop MS, pre-diabetes and T2DM. Adults with regular job were prone to MS, pre-Diabetes and T2DM. With regard to lifestyle, 22% of adults with MS, 30% with pre-Diabetes and 52% with T2DM were sedentary. Most of them were having a genetic pre-disposition for developing T2DM as they had at least 1 of their family member with the similar condition. Stress appeared to be higher among the adults with MS and pre-diabetes. 20% adults with pre-diabetes had the habit of smoking whereas 44% with MS and 30% with pre-diabetes indulged in binge drinking. 89% of the adults with MS were overweight 36% of adults with MS, 30% with pre-Diabetes and 48% of the T2DM were having hypertension. Majority of adults with MS were having high cholesterol, high triglycerides and high BP (blood pressure). Random blood sugar levels were high among 56% of adults with MS, 30% with pre-Diabetes

and 62% with T2DM. 4 were having extra-pulmonary TB (TB Spine) and were undergoing treatment for the same. Only 1 was having arthritis and spondylitis. Further, about 30*% adults were not aware that they were having anyone of the conditions, viz., MS, pre-diabetes and T2DM*. Those diagnosed with MS, pre-diabetes and T2DM were referred to the local Diabetes Clinic for further treatment, care and management. The increased risk of the 3 entities among adults warrants specific preventive action. All of them were counselled for adopting healthy life style changes, in terms of diet restriction, regular exercise and adherence to treatment. An early diagnosis, appropriate treatment and adequate care form the basis for control of Diabetes, Stroke and heart disease which would otherwise lead to various complications like neuropathy, nephropathy, CVD, blurred vision, etc. We, therefore, feel that monitoring adults above 40 years of age, irrespective of their complaints and symptoms, at regular intervals, for Blood pressure, Blood sugar and Lipid profile would go a long way in early detection of the MS, pre-diabetes and T2DM conditions. This is the first report of MS, pre-Diabetes and T2DM among adults in this region of the country.

(ii) Prevalence of Type 2 Diabetes mellitus among patients with active Tuberculosis disease

This has been initiated. About 38 patients with active TB disease attending an OPD of a primary care hospital in Bhubaneswar were screened for random blood sugar levels by finger prick method using Glucometer. Out of these, 28 were males and 10 were females. 36 were having pulmonary TB and 1 was having extra-pulmonary TB. Out of 38, 20 were having Diabetes, i.e., blood sugar levels more than 200 mg / dl. 28 were sputum positive and 10 were sputum negative. 15 were cat-I , 10 were Cat-II. 2 out of 38, were HIV-positive. Study is going on.

16. Virology Network Laboratory. (Grade-I)

Principal Investigator	:	Dr.B.Dwibedi
Co-Investigators	:	Dr.R.K.Hazra, Miss S.Dixit
Co-ordinator	:	Dr.S.K. Kar
Starting date	:	March 2010
Closing date	:	March 2015
Funding	:	Extramural (ICMR)

Background

It was aimed at creating regional facilities for laboratory diagnosis, surveillance and research in viral diseases of importance.

The proposal involves construction of the laboratory, procurement of equipments, training of involved staff, establishment of laboratory techniques like serology, molecular diagnosis, sequence analysis, cell culture and isolation etc. in phased manner. Outbreak investigation, surveillance during epidemic and inter epidemic period and sporadic disease diagnosis of important viral diseases of the region and emerging infections would be carried out which will be strengthened by research subsequently.

Objective

To establish a grade I diagnostic virology laboratory for investigation of viral diseases of regional and national importance including but not limited to

1. **Viruses transmitted by respiratory route**: Measles, Rubella, Mumps, Influenza viruses (A, B and C), Parainfluenza virus, Adenoviruses, Respiratory Syncytial Virus, Rhinoviruses, Coronaviruses.
2. **Viruses transmitted by intestinal route**: Poliovirus, Hepatitis A & E viruses, Rotavirus, Astroviruses, Calciviruses, Norwalk viruses, Enteroviruses.
3. **Vector Borne Disease Viruses**: Dengue, Chikungunya, Japanese encephalitis, West Nile, Kyasanur Forest Disease, Chandipura viruses.
4. **Zoonotic viruses**: Rabies virus, Nipah virus, Hanta virus

5. **Viruses transmitted by body fluids**: HIV, Hepatitis B and C viruses.

Progress of work

Current strength of laboratory diagnosis

Laboratory investigation of different viruses coming under broad divisions like respiratory viruses, enteric viruses and Arbo viruses were standardized and undertaken with quality control. Different viruses diagnosed following different sero molecular test are presented in the table below.

Man power training

Project staff was given training on molecular techniques and epidemiology during the period. One Scientist (non medical) undergone short training on Rota virus molecular typing at CMC, Vellore. Two scientists(Medical) were trained on GCP methodology of outbreak investigation and epidemiology of viral disease at NIE , Chennai.

Networking for obtaining information, Sample receipt, Investigation and reporting

Network with the State Health Department,

Table 1: Laboratory investigation established for different group of viruses.

Group/Test	Serology	PCR/ RT PCR	Realtime PCR	Sequencing & Genotyping	Cell Culture	IFA
Respiratory viruses	Measles, Rubella, Mumps Varicella	Parainfluenza 1,2,3, HMPV, Measles, Rubella & Varicella Zoster	Influenza A, H1N1 & B, Para influenza, Corona, Rhino, RSV A &B, Adeno, Entero, Parecho, Boca & Measles,	H1N1 & Flu-A	Measles Varicella	Varicella
Enteric viruses	Rota, Adeno, Astro, Noro, Entero, Coxackie, HAV &HEV	Rota, Entero	Rota, Adeno, Astro, Noro, Entero, Coxackie	Rota Entero	Rota	
Vector Borne Disease Viruses	Dengue, JE, Westnile & Chikunguniya	Dengue, JE, Westnile, Chikunguniya & Chandipura	Dengue	Dengue, JE, & Chikunguniya	Dengue JE	Dengue
Viruses transmitted by body fluids & others	Hepatitis B,C,D CMV, EBV HPV Adeno	HBV, HCV, Parvo B19	CMV, EBV HHV 6 and 7 and Parvo B 19	HBV HCV		
Neurotropic viruses	HSV-1,HSV-II, Entero	HSV-1,HSV-II, Entero		HSV	HSV	HSV

Medical Colleges and Hospitals of the region for referral investigation of sporadic cases and outbreak investigations was further strengthened through frequent interaction. Outbreak investigations are being undertaken along with the state health team upon getting information through media or health system. Immediate report is being communicated to the concerned hospital within 3 days of sample receipt.

I. Sample collection

A. sporadic/ referred cases

Sporadic / referral cases were received by the centre from different hospitals from different districts. From Jan to June 2013, 2751 number of samples were received by lab from different Govt. and Private hospitals from Odisha. The details of sample receipt from hospitals has been given in below mentioned tables (1 and 2).

Table 1: Sample Receipt from different hospitals and Medical colleges (Till June 2013)

Source Hospital/Centre	No. of subjects enrolled
Capital Hospital, BBSR	562
SCBMH, Cuttack	314
SVPPGIP, Cuttack	289
SuM Hospital, BBSR	816
Outbreak investigation	183
Other hospitals and PHC	587
Total	2751

Table 2: Suspected viral diseases investigated.

Sl. No.	Suspected Diseases under investigation	No. of samples for the respective disease
1	Chikungunya	0
2	Dengue	103
3	Respiratory infection	97
5	Measles	78
6	Chickenpox	72
7	Mumps	6
8	Hepatitis	919
9	Encephalitis	442
10	Viral diarrhea	546
11	Rubella	444
12	Human Papilloma Virus	17
13	CMV	11
14	EBV	1
15	Fever and rash (HFMD, Parvo)	4

B. Outbreak investigations

Outbreaks were reported from different parts of the state and investigation was done in collaboration with the state health department. The team collected the samples both by direct investigation and through collection by the primary health centre / district hospital of the concerned area. Outbreaks of Measles, Rubella, Varicella and Hepatitis virus infection has been investigated with immediate reporting to State Health Department with recommendations for timely prevention. The outbreaks included 1 outbreaks of jaundice, 4 chikenpox ,4 measles, 2 Rubella and 1 Encephalitis (Follow up) covering 11 districts. The outbreak investigations conducted along with State Health Departments during this period were summarized below. (Table 3)**.**

The major outbreaks investigated are summarized below

- **Rubella Outbreak in kendrapara district (22nd & 23rd April 2013)**
- A research team from virology laboratory comprising of clinician, research assistant and technician had visited Parabari village PHC II of Derabis Block on 22nd April and Sailendra Narayanpur village under Talachua CHC of Rajnagar Block of Kendrapada district on 23rd April 2013 to investigate the reported illness manifesting as fever and rash. 55 cases of fever and rash were reported from 2nd April to 15th April 2013 in two villages affecting 4-33 years of age group.

20 cases were reported from village Parabari having the population of 460 in 80 households. 23 samples were collected Out of which, 19 were asymptomatic and 4 samples were symptomatic suspected for either Measles or Rubella. Three samples were collected from pregnant ladies in that village.

The village Sailendra Narayanpur is a remote and inaccessible village which is approximately 110 kilometers away from the district headquarter. 37 samples were collected from that village having a total population of 975 in 104 households. Out of 37 samples, 21 were from asymptomatic and 16 samples were from symptomatic individuals suspected for either measles or rubella. Also there were four pregnant ladies in that village and their samples had been taken. Death was not reported in that village.

Laboratory Investigation has revealed 13 out of the 40 samples were positive for Rubella IgM and 11 out of 12 samples were positive for Rubella IgG antibody through ELISA. Samples tested for Measles IgM antibody (n=10) were found to be negative

- A follow up study for JE was conducted during 9th to 13th June, 2013 in Potrel, Uskapali and Charkiguda village of Malkangiri district to find out the persistence of JE virus in animal reservoirs and to investigate the clinical outcome and neurological sequelae if any in JE infected individuals.

II. Laboratory Investigation results

The lab has undertaken 4504 sero-molecular tests for diagnosis of around 50 Viruses covering all the major viral diseases of importance of the region. The results of laboratory investigation is summarized below for reference.

Table 3: Outbreak investigation report :

Time period	Disease	District	No of village	Samples collected/Recieved	Lab Test	Result
April 2013	Chikenpox	Bolangir	1	12	Varicella IgM	11
	Jaundice	Cuttack	1	5	HAV/HEV	0/5
		Kalahandi	1	5	HAV/HEV	5/1
	Chikenpox	Raygada	1	5	Varicella IgM	5
	Measles	Balesore	1	5	Measles IgM	0
	Measles/Rubella	Kendrapara	2	6	Measles IgM	0
					Rubella IgM	5
	Measles	Deogarh	2	5	Measles IgM	2
	Chikenpox	Raygada	1	5	Varicella IgM	4
	Rubella	Kendrapara	1	60	Rubella IgM	13
May 2013	Measles	Balasore	1	5	Measles IgM	1
		Mayurbhanj	1	10	Measles IgM	0
		Jajpur	1	5	Measles IgM	2
June 2013	JE	Malkangir	4	51	JE IgM	0
	Chikenpox	Anugul	1	4	Varicella IgM	4
TOTAL	**14 outbreaks**	**11 districts**	**19**	**183**		**58**

Suspected disease, affected area and lab investigation result.

A. Investigation on hospital based recruited cases

Result of investigation as summarized in the following Table.

Summary of observations on the major viral diseases investigated during the period.

Arboviral diseases

The suspected viral diseases included Dengue, JE virus collected from outbreaks or sporadic hospital admissions. A total of 62 number of samples were received from different hospitals. Out of the total samples tested for Dengue IgM 16(15%) were positive where as in 2(2.6%) cases NS1 antigen was detected. Dengue serotypes III was detected in these 2 NS1 antigen positive cases. During 2010 serotype II was prevalent where as in 2012-13 all four serotypes were reported with serotype II as the dominant type. In the northern region serotypes I, II and III where as in sourthern regions serotype II and IV were reported.

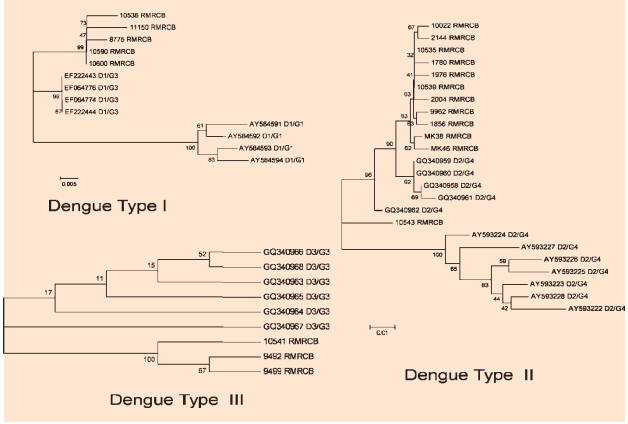


Fig 1: Phylogenetic tree of Cpr M nucleotide sequences of Dengue strains and other strains with their Genebank ccession number.

20 samples from three serotypes (I,II,III) of Dengue virus has been sequenced and the blast search has revealed that Serotype I has the maximum similarity(93%) with Delhi strains, 92% similarity with Thailand and 91% similarity with Mianmar strains. Dengue serotype II has the maximum similarity (98%) with southern and northern strains. Dengue serotype III has the maximum similarity (97%) with Kerala strains. Genotypes identified from serotype I, II, III were III, IV and III respectively.

Table 4: Result of investigation on hospital based cases.

Sl No.	Virus	IgM +ve (%)	Antigen +ve (%)	PCR +ve (%)	Real time PCR +ve (%)	Genotype/ Serotype
1.	HSV I	88/9(10.2)		152/28(18.		
2.	HSV II	92/8(8.7)		4)		
3.	JE Virus	62/0(0)				
4.	Dengue	106/16(15)	86/2(2.3)	2/1(50)	4/1(25)	D3
5.	CHIK	2/0(0)				
6.	Rota		549/145(26.4)	145/45(31)		G1,G9,G10,G12 P8,P10
7.	Astro		227/0(0)			
8.	Adeno(Enteric)		228/6(2.6)			
9.	Noro G1		227/0(0)			

10.	Noro G2					
11.	Coxackie					
12.	Measles	95/36(37.8)		12/0(0)		
13.	Varicella	76/53(70)		12/0 (0)		
14.	Mumps	5/1(20)				
15.	Rubella	355/27(7.6), IgG- 26/23(88.4)		62/0(0)		
16.	Entero	31/2(6.4)		2/0(0)		
17.	HAV	186/70(37.6)		15/3(20)		
18.	HEV	165/44(26.6)				
19.	HBV		855/140(16.3)	80/46(57.5)		10(7Genotype D,2 Genotype C, 1 Genotype A)
20.	HCV	764/12(1.5)		10/1(10)		1(genotype1b)
21.	HDV	65/0(0)				
22.	HPV	15/2(13.3)				
23.	EBV	3/2(66.6)				
24.	CMV	12/1(8.3)				
25.	Adeno				20/90(22 .2)	
26.	Influenza A(FluA)				10/90(11)	
27.	FluA(H1N1)				1/….()	
28.	Flu B				0(0)	
29.	HMPV A/B				4(4.4)	
30.	Rhino				13(14.4)	
31.	Para influenza 1				1(1.1)	
32.	Para influenza 2				0(0)	
33.	Para influenza 3				4(4.4)	
34.	Para influenza 4				0()	
35.	RSV A/B				0()	
36.	Corona viruses (Cor63,Cor2 29,Cor43, HKU1)				6(6.6)	
37.	Parecho virus				0(0)	
38.	Boca Virus(HBoV)				14(15.5)	
39.	EV				3(3.3)	

Encephalitis viruses

Viruses that cause encephalitis were also investigated. 442 subjects were enrolled with symptoms of encephalitis during this period. Blood and CSF samples were collected and tested for JE virus, HSV I, HSV II, Varicella, Measles, Entero virus and WNV. Antibody to Herpes simplex virus I was detected in 10.2 % (n=88), Herpese Virus II in 8.7% (n= 92) cases. PCR amplification was done to detect

Entero virus, HSV 1, HSV 2, JEV and WNV from CSF collected from cases of encephalitis. PCR positivity for HSV was noted in 18.4% of cases (n=152). In no cases Entero or WNV was detected.

Case suspected for encephalitis(n=3) were tested for Coxackie IgM Antibody and one case was found positive with clinical history of diarrohea.

Out of the total samples tested for different viruses HSV 1 was found to be the prevalent causative agent in most of the encephalitis cases.

Enteric viruses

546 patients with diarrhoeal disorders were investigated for enteric viruses, those were enrolled during this period. The subjects were mostly children below 5 years who presented with moderate to severe diarrhea. Rota antigen was detected in 26.4% (n=549) and Adeno in 2.6% (n=228) of cases where as Astro and Noro antigen test was negative for all cases. The most common viral agent was Rota. Specimens were subjected to PCR using type specific primer for genotyping of Rota virus. Majority of G(G1, G9, G10, G12) types and P (P8, P10) types were detected. 7m to 2 yr age group was the most prevalent group where 84% of the children were affected with rota virus infection. In all the groups G1 was common among all G types and P8 and P10 were common among all P types.

Hepatitis Viruses

Among the cases of jaundice screened for hepatitis virus infection, HBV and HCV were detected serologically in 140(16.3) and 12(1.5) of cases respectively. PCR was positive in 46(57.5) and 1(10) of cases respectively for HBV & HCV. Out of 10 samples sequenced, genotype D,(n=7), C (n=2), A(n=1) and HCV genotype 1b(n=2) were identified as the genotypes circulating in this region. Among HBsAg

Table- 5: Distribution of viral infections causing ARI during Jan-June 2013.

VIRUS	PCR POSITIVITY(%)
Adeno	**22.2**
Influenza A(FluA)	11
FluA(H1N1)	**4.5**
Flu B	0
HMPV A/B	4.4
Rhino	**14.4**
Para influenza 1	1.1
Para influenza 2	0
Para influenza 3	4.4
Para influenza 4	0
RSV A/B	0
Corona viruses (Cor63,Cor229,Cor43, HKU1)	6.6
Parecho virus	0
Boca Virus(HBoV)	**15.5**
EV	3.3

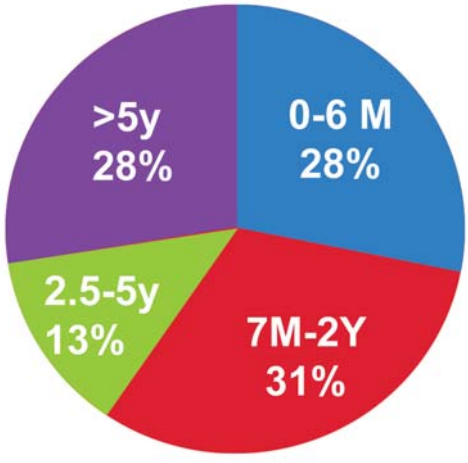
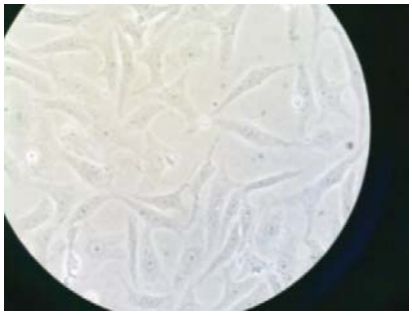
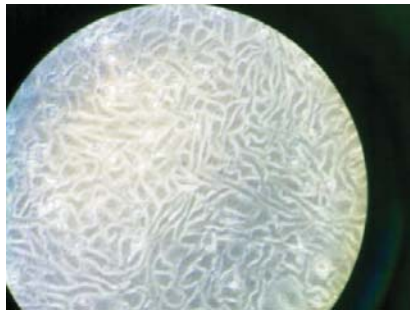


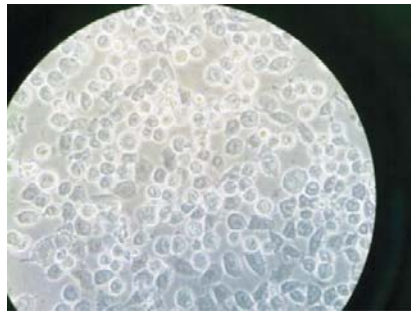
Fig 2: Age wise distribution of RTI Cases.



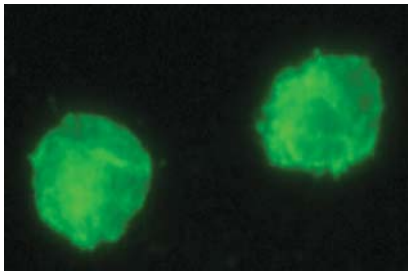
a. Normal Vero Cell Line.



b. HSV Infected Cell Line after 1 day



c. Cytopathic effect after 2 days Detachment and rounding



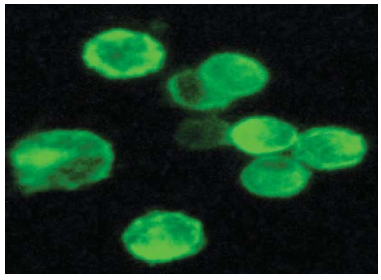


Fig.3.

d. HSV culture in Vero Cell Line and detection by IFA

Detection by IFA

(140) positive 19% (8 / 42) were HBeAg positive where as 50 %(24 / 48) were antiHBe positive.

Antibody positivity for Hepatitis E virus infection was 44 / 165(26.6%). Hepatitis A virus specific IgM was positive in 70 / 186 (37.6%) of the subjects.

Age distribution of cases indicated that HAV infection to be more in 6-15 years of age group (42.8%) showing decline with increasing age. HEV infection was high in adult population (56.8%) in comparison to children.

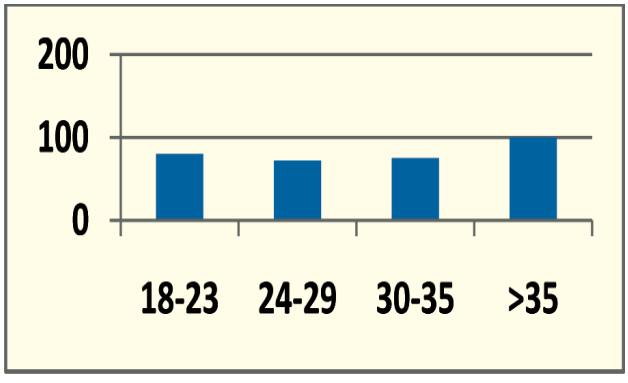


Fig 4: *Rubella IgG Positive (%) in different age groups.*

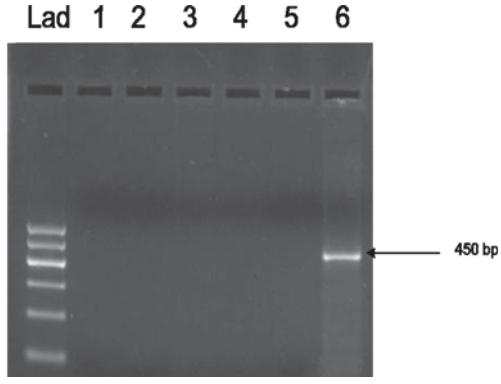


Fig. 5: *Amplification of L1 gene for HPV.*

Respiratory virus infection

Viral respiratory infection was another important disease mostly affecting children which was important for laboratory diagnosis. Using Real Time PCR assay, many respiratory viruses were identified including some emerging viruses. During 2013, 90 number of cases suspected with Acute Respiratory Tract Infection were tested by Real Time PCR, out of which 55(62%) patients were diagnosed with any one viral infection from the respiratory panel. Out of all tested samples Adeno Virus was detected in 22.2% of cases followed by Human Boca, Rhino and Corona Virus. One case positive for H1N1 was detected in the month of February out of 22 no. of cases received for suspected swine flu during this period.

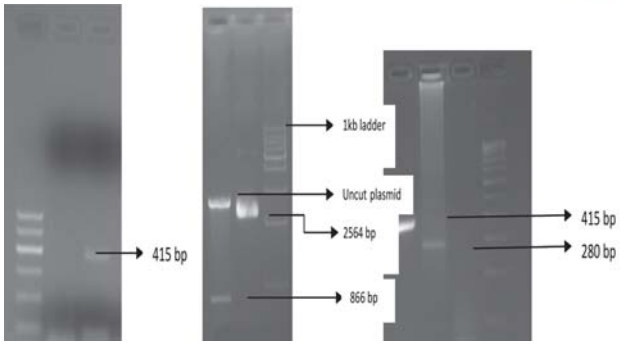
Among all cases studied for ARI 7m to 2 yr Age group was found to be the most common age (Fig. 2)

Exanthematous illness

Among viral infections causing fever with rash. Measles, Herpes simplex, Entero (HFMD) and Chiken pox were investigated.

78 No of cases were investigated with suspected Measles infection out of which 36 no of cases were from outbreak sources and 42 no of cases were collected from sporadic hospitalized cases. IgM antibody was detected in 37.8% of the cases. Twelve samples were subjected to PCR but in no case the viral RNA was detected. During this period 72 cases suspected with Chickenpox were enrolled and in 70% of cases the infection was confirmed by detection of IgM antibody.

Samples from 3 cases with skin lesions for suspected HSV infection was collected and infected with vero cell line and after 3 days characteristic cytopathic effect was observed in three cases. This was further confirmed by Immunoflourescent assay by using fluorescent labeled antibody specific to HSV virus.



Purified 1st PCR product (415 bp) for HSV (glyco protein D gene)

PVuII restriction enzyme, Digestion product of HSV clone.

Colony PCR for screening of HSV clones.

Fig. 6: Cloning of HSV glyco protein D gene.

Rubella infection in pregnant women

Rubella infection and susceptibility was looked for in woman attending antenatal clinics. Out of the total 355 cases enrolled 30% of cases were confirmed with a bad obstetric history. 7.6% (n=15) were positive for IgM antibody and IgG was detected in 88.4% of cases. The results indicated that still 10% are susceptible to Rubella who may need vaccination. All women were protected beyond 35 years. (Fig. 4)

HPV infection in Women

17 no. of cases with chronic cervicites or suspected cervical discharge were enrolled during this period. Cases were within the age range of 26-65 Yrs. and in 7.6% of cases of cervical swabs PCR positivity was obtained(Fig.5).

Cloning

In 1 sample positive for HSV DNA, glycol protein D gene was cloned in TA vector(p^{GEMT} Easy vector, Promega) and sequenced. The cloned product is preserved for future use like for standardization of quantitative PCR & library preparation for whole genome sequencing.

17. Development of integrated vector management for demonstrating control of co-exiting mosquito borne diseases such as malaria, filariasis and chikungunya in Nayagarh District of Odisha.

Principal Investigator	:	Dr. N. Mahapatra
Co-Investigator	:	Dr R K Hazra Mr. N. S. Marai
Starting Date	:	April 2012
Closing date	:	March 2015
Funding	:	Started intramurally and Applied for Extramural funds of ICMR Vector Task Force

Objectives

1. To study the bionomics of the vector of co-exiting mosquito borne diseases such as malaria, filariasis and Chickunguya.
2. To develop evidence based, location specific and technically sound vector control strategies to reduce prevalence of co-exiting mosquito borne diseases.

Rationale

Several vector control programme targeting specific vector borne diseases like filariasis, malaria and Chikungunya are being operated in the country, while the vectors that transmit these diseases are prevalent in and around the households where population resides. There are lots of similarities in those causative vectors bionomics and habitats. Hence comprehensive strategy will help in controlling the vector population and transmission of the above three diseases which will be feasible, less laborious, and cost effective.

Thus the study is planned to be carried out in Nayagarh district where Malaria, Filariasis and Chikungunya are prevalent with the following.

Therefore a study is being initiated in Odogaon PHC of Nayagarh district for implementing comprehensive vector control strategy.

Fig.1: Odisha map showing the study area.

Progress

Study site selection

The initial vector survey was intensely carried out to study their bionomics and transmission indicator which will be compared after intervention

Kural village of the Odagaon PHC of Nayagarh district (Fig.1) was selected as study site which showed co-prevalence of malaria, filariasis, chikungunya as per the Govt. data. Mashabari village was taken as control.

The initial vector survey was intensely carried out in the selected population reporting all the three diseases to assess their density and bionomics to generate data on transmission indicator which will be compared after intervention.

Prevalence of diseases:

Epidemiological data collected from the District Health Services, showed prevalence of Malaria: Slide Positivity Rate-7.8%, Filariasis: microfilaria rate- 6.8%, Chikungunya: attack rate-10.5% in Kural village.

Seasonal Prevalence of the vectors

***Cx.quinquefasciatus, An.annularis, Ae.aegypti* showed highest density in winter**

Density of *An.culicifacies and Ae.albopictus* were highest during Rainy season. Significant variation in the density of *Cx.quinquefasciatus ,An.culicifacies and An.annularis* were seen in between summer and rainy season where as for *Cx.quinquefasciatus* significant variation was seen between summer and winter and between rainy and winter variation was seen in *An.culicifacies.*

In control village Mashabari , Rise in all the above vector density were observed during Rainy season.

Abdomination condition of vectors

All the vectors showed high proportion of seeking stage (UF+FF) than resting stage (HG+G) condition. High un fed & freshly fed population indicates the vector preference to indoor resting.

Accordingly it suggests a to intensify the Indoor Residual Spray (IRS)

High Transmission of filariasis (Infectivity rate 1.97 %) and malaria was observed with sporozoite rate of 1.2 % in *An.culicifacies* and 0.31% in *An.annularis.*

Sibling Identification.

An.culicifacies A,B & C were found to be prevalent and presence of *An.culicifacies*- C indicates for IRS as it bit during evening.

Insecticide Susceptibility

All the vectors were susceptible to Deltamethrin

Larval density- Total 1235 numbers of breeding places were surveyed. Out of which 845 (68.42%) spots were positive for *Cx.quinquefasciatus*. Larval density was found to be 65 / dip.

3. Breeding Sites

- The pits meant for keeping cow dung were the major breeding spot in rainy season and drain was the major breeding spot for *Cx.quinquefasciatus* during winter and summer season.
- Rice field contributes 57% of the breeding of *An.culicifacies* and *An.annularis* during rainy season where as during winter and summer ponds and pools the major breeding source.
- Earthen pot was found to be the major breeding spot for Aedes in domestic and peridomestic areas under the tree clad.
- Amongst all different breeding sites 30% are common to all the three disease vectors.
- The HI, BI and CI of both Aedes Species are higher than the recommended value. larval Indices recommended by NVBDCP : House Index<5 , Container Index<10 , Bretaue Index<20

Future Plan

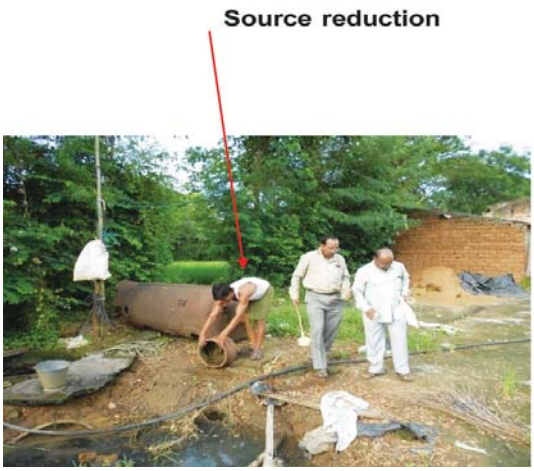
- Basing on the information on vector bionomics and association of more than one vector sharing common breeding and resting place, the control strategy is being developed in consultation with State entomology. The intervention will be done with one or more in combination of the following control methods.
- 1. Chemical control - by impregnation of bed nets.
- 2.Environmental management and source reduction
- Biological control - Larvivorous fish.

Senstization & Capacity Building

Meeting held with the Sarpanch and local leader of the village, ASHA, PHCs Doctor, MPHW, GKS members, School teacher, students Primary school curriculum for CVC Source reduction.

Educating the school children about larval and adult stages of mosquitoes

Children are seeing the life adult of mosquitoes through lens



18. Transfer of a molecular technology from laboratory based study to field for mapping of malaria vectors and their vectorial attributes.

Principal Investigator	:	Dr. R. K. Hazra
Co-Investigator	:	Dr. N. Mahapatra (Collaborator from VCRC, Pondicherry NIMR, Rourkela and Director of Health Services, Govt. Of Odisha)
Starting date	:	20.03.2012
Closing Date	:	20.03.2015
Funding	:	Extramural (ICMR, Translational Research)

Objectives

1. To standardize methodologies for different parameters for vector mapping.
2. To test the standardized methodologies from Phase-1 (Phase-1 objectives are given below)
3. To map the vectors at PHC level and identify operational issues.
4. To prepare a vector map at district level.
5. Transfer the laboratory based technology to field.

In Orissa there is lack of trained entomologist for control programme. Molecular methods for species identification have received great attention in recent years. Recently we developed a molecular tool for identification of main malaria vector of Orissa. The method was also developed for simultaneous detection of species complex, their human blood indices and presence of sporozoites from single mosquito. Basing on this technique developed by our center the total screening of Anopheline vectors can be undertaken in Orissa. Therefore the present study will be undertaken to screen the malaria vectors from different parts of Orissa and their vectorial attributes.

PHASE 1: (Duration: 12 months)

I. Technical Workshop on the methodologies to be used in Phase I with the participation of all the investigators.

II. Establishment of PCR assays in collaborating institutes.

1. Mosquito samples will be collected from plain, riverine, foot hill and hill top (one village from each ecotype) areas in three districts, Keonjhar (RMRC), Sundargarh (NIMR) and Koraput (VCRC). All ecotypes will be included in all the three districts.
2. Habitat of collection: indoors (human dwelling / cattle shed) and outdoors (pit, box shelters, tree hole / hollows).
3. Standardization of collecting devices (hand catch and light traps) for both adult and immature stages (as and when required).
4. Frequency of collection: Collection will be done during July and August for *An. culicifacies* and during cold season (October to December) for other anophelines.
5. Anophelines belonging to different sibling species (after identification by PCR) will be pooled as groups and distributed to the three institutes for standardization.
6. Preservation of samples

(a) Kits for sample preservation: filter paper and mosquito drying stand.

(b) Dried mosquito samples will be collected in individual microtubes.

7. Vector species composition: The samples collected will be identified to species morphologically and coded. These samples will be subjected to the PCR assay developed by RMRC for molecular identification of species and sibling species. Molecular identification of species will be

compared with morphological identification for sensitivity and specificity.

8. PCR assay for blood meal analysis, vector incrimination*Plasmodium falciparum* (*Pf*) and detection of *kdr*.

9. Optimization of pool size for PCR assays (species composition, human blood, vector infection).

10. PCR assay for detection of *Plasmodium vivax*.

III. Standardization of multiplex PCR for *P. vivax*and*kdr*.

IV. Data analysis and report preparation

Progress of work

Standardizations:

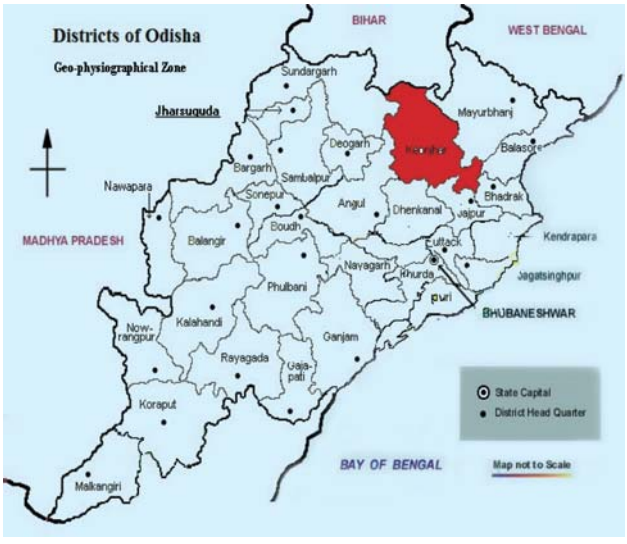


Fig.1 Map showing Keonjhar district of Odisha in red colour.

Selection of study site

The studies were conducted for one year in four different ecotypes like plain, riverine, foot hill and hill top of Keonjhar district of state Odisha. Jhumpura one of the highly endemic PHC for malaria of Keonjhar district was selected for the study. One village from each ecotype was selected from the PHC.

Place of collection

Resting adult mosquitoes were collected from each village in every month during dawn and dusk. Collection was made from both indoor and outdoor. In the Indoor, mosquitoes were mainly collected from human dwelling and cattle shade. Since most of the houses in every village have a cattle shade nearer to their houses. Collections were also carried from mixed dwelling. Likely in the outdoor mosquitoes were collected from tree holes and shelters.

Frequency of collection and standardization of collection device

Mosquitoes were collected by trained Insect collectors, technician and Research Assistant for 4-5 days every month during the study period. Collection both by CDC light trap and hand catch by oral aspirator were done. Seven time collection were carried out in the study site, 264 man hours of collection in the hand catch and 21 traps night collections were made from all the villages. In each village the location of the trap remained the same throughout the study period.

Morphological identification

The adult *Anopheles* mosquitoes were identified taxonomically based on their distinguishing features like head, thorax, wings, legs, halters, segmentation of body, size of proboscis, sitting posture and habits by following the key developed by Barraud 1934 (The fauna of British India). A key for morphological identification of *Anopheles* species was developed by Entomological division of RMRC and followed during the study .The adult mosquitoes were also identified using molecular methods.

Mosquito Preservation and processing

After identification each individual specimen was dissected in two parts, the head thoracic part was kept in one micro centrifuge tube and rest of the body in another. These different parts were subjected for DNA isolation. The head thoracic part was processed further for sporozoite detection and abdomen part was

processed for blood meal detection and for species identification. Twomethods of preservation of mosquito samples were adopted.In the first method adult mosquitoes were kept in isopropanol and transported to the laboratory and in the second way simply they were dried in a mosquito drying chamber maintaining a temperature of 37 º C.

Molecular study

DNA Isolation: New Protocol

DNA was isolated from morphologically identified *Anopheles* species by modified phenol chloroform method (Coen et al., 1982) and following Insect DNA purification kit (Hi-media). A new protocol was developed in our laboratory for isolation of DNA in which genomic DNA extraction from insects takes 4.30hours only. This method is also implemented in our study. The detail of the protocol is given below. The preserved mosquitoes in isopropanol were washed in sterile distilled water or phosphate buffer saline (PBS) to remove excess alcohol. Fresh mosquitoes can be grinded directly. Mosquitoes were grinded in 1.5 ml eppendorf tube with micropestle taking 50-100 µl 1X STE buffer (50mM Nacl,50mM Tris- HCL,100mM EDTA, PH 8.0) along with 100mM sucrose. 1X STE buffer was added to a total volume of 300-500 µl for a single mosquito and 1 ml for mosquito pool like 4, 6,8,10 numbers. Then 1% SDS, 1% Triton -X, 10 µl / ml RNase A (20mg / ml) and 20 µl / ml Proteinase K (20mg / ml) were added and mixed properly. The tubes were incubated for 1 hour 30 minutes at 37ºin dry bath with intermittently mixing in every 15 minutes. Then the tubes were subjected to centrifuge at 12,000g for 10 minutes at 4º C and the supernatant were transferred to a fresh tube. Equal volume of phenol: chloroform (1:1) was added and the tubes were shaken well for 5 minutes then centrifuged at 12,000g for 10 minutes at 4º C. The above step was repeated again. Then chloroform: isoamylalcohol (24:1) was added to it and centrifuged at 12,000g for 10 minutes at 4º C. The supernatant were transferred to a fresh tube. Then twice the volume of cold isopropanol were added to it and kept for 1 hour in -20º C. After the incubation is over the samples were centrifuged at 12,000g for 30 minutes at 4º C and the supernatant were removed. The pellet were washed with 70% ethanol and kept at 37 º C for 10 minutes. At last the dry pellet was dissolved in nuclease free water or TE buffer (PH 8.0) andstored at 4 º C or -20 ºC for future use.

Optimization of pool size for PCR assay and Identification of mosquitoes, blood meal analysis and vector incrimination *(pf)* by multiplex PCR assay

For rapid evaluation and monitoring of major vector species of a particular region, we adopted a multiplex PCR diagnostic assay for a pool of mosquito. Pooling of mosquitoes was carried out in two ways i.e. DNA from pool mosquitoes and individual mosquito DNA pooled. Mosquitoes collected from field were grouped in to different pool size up to ten numbers irrespective of their species composition. After DNA isolations they were subjected to PCR assay. A total of 3670 numbers of *Anopheles species* were screened from the four ecotypes. Initially we incorporated the species specific primers for *An.fluviatilis, An. culicifacies, An.annularis*and *An.varuna*. Along with the vectors, one non-vector*An.pallidus*was also included. Simultaneously, the mosquito samples were screened for blood meal analysis and vector incrimination *(pf)* by using species specific primers for *Pf* and human blood. The primers designed for A*nopheles*species, human blood and *Plasmodium falciparum* were taken from Swain et al (2009 and 2010) and used for identification of collected mosquitoes.

PCR Standardization

For the standardization of the human and *P.falciparum*, specificprimers, DNA isolated from the blood of normal and *P.falciparum* infected people was used respectively. All primers were used to develop a one-step reaction.In a final volume of 25µl the PCR reaction mix contained 1.5 XPCR buffer (Containing 1.5mM $MgCl_2$), 400µM dNTP mix, 1 unit of Taq polymerase, 3µl of DNA template, 0.8µM of each

forward and reverse universal and 0.6µM of each species specific primer of five *Anopheles* along with 0.8µM of forward and reverse primers for human blood and *Plasmodium falciparum*respectively.After an initial denaturation carried out at 96 º C for 6 minutes, 30 cycles were programmed as follows: 96 º C for 30 s.50 º C 25 s,72 º C for 1 minute and final extension at 72 º C for 8 minutes.The PCR product was run in 1.8% agarose gel using TBE buffer.

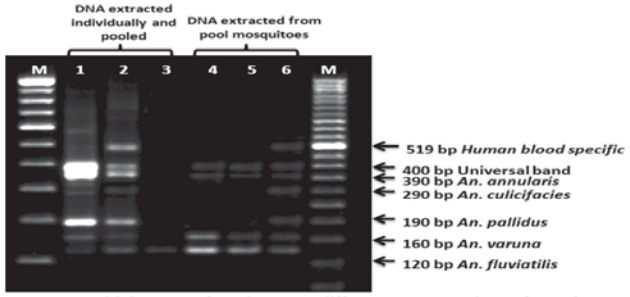
Primer designing for inclusion of non-vectors in multiplex PCR is under progress. Primer designing for inclusion of *Plasmodium vivax* in multiplex PCR is under progress.

Validation of the assay

The establishment of Laboratory and validation is in progress in other Institutes like in NIMR Rourkela. Validation completed in own Institute in other laboratories.

Workshop

A workshop on **"Transfer of a molecular technique from lab based study to field for mapping of malaria vectors and their vectorial attributes"** was held from 4th to 8th February 2013 in Regional Medical Research Centre, Bhubaneswar. Delegates from different NIMR and field stations like New Delhi, Raipur, Rourkela, Goa, Dwarka, Hardwar and representatives of the state NVBDCP were



Lane 1-3 Multiplex PCR of pool DNA at different concentrations of reaction mixture. DNA extracted individually and pooled. Lane 4-6 Multiplex PCR of DNA extracted from pool mosquitoes at different concentrations of reaction mixture.(pool size 10)

participated in the above work shop .The workshop was continued for five days where they were taught different concept regarding the use of different molecular technique and its practical aspects that would be useful in rapid monitoring and evaluation of malaria of anarea.

Entomological collection

A total of 6556 of Anopheles mosquitoes were collected during 264 human hours and 21 night trap collections. *Anopheles* mosquitoes belonging to different species like***An.annularis, An.culicifacies, An.hyrcanus, An.pallidus, An.subpictus, An.vagus, An.aconitus,An.barbirostris, An.fluviatilis, An.jeyporiensis, An.karwari, An.phillipinensis, An.tassellatus, An.minimus, An.sergenti, An.splendidus, An.stephensi, An.varuna*and*An. maculates***were collected during the survey.

Table 1: Primers list for multiplex PCR

Species	Primer Name	Sequence (5'-3')	Bases	Tm (^{0}C)
Universal forward primer	ANF	ACCAAGAAGTCTATCTTGCGCG	22	54.8
Universal reverse Primer	ANR	TTTGCACGTCAGAATTGCTT	20	47.7
An. annularis	ANN	CGTGGTACCGGCGTGTACTGCGCC	24	65.9
An. culicifacies	CUL	CCCACAGGCGAAGACAACTCGA	22	58.6
An. fluviatilis	FLU	GTTGAAGTCAGGGGAAACCCT	21	54.4
An. pallidus	PAL	CGTGAAGAGCGAATACAGCCCATG	24	59.1
An. varuna	VAR	GCTTGCCGTCCAGCAAACTG	20	55.9
P.falciparum	Pf F	ACGTTTAGGGGCGAAAGACT	20	51.8
P. falciparum	Pf R	TTAGCCCATCCATTTTCAGG	20	49.7
H. sapiens	HUM1	CGAGAGTTCTCTGGAAGAATTGA	23	63.5
H. sapiens	HUM2	TGATAGCCTGGAAGTGACAAAAT	23	63.5

Species	UV TRAP	%	HAND CATCH	%	Total
An.annularis	180	20.27	1450	25.58	**1630**
An.culicifacies	62	6.98	285	5.03	**347**
An.hyrcanus	194	21.85	647	11.41	**841**
An.pallidus	183	20.61	1469	25.92	**1652**
An.subpictus	89	10.02	547	9.65	**636**
An.vagus	41	4.62	99	1.75	**140**
An. aconitus	0	0.00	4	0.07	**4**
An.barbirostris	34	3.83	126	2.22	**160**
An.fluviatilis	41	4.62	599	10.57	**640**
An.jeyporiensis	3	0.34	3	0.05	**6**
An. karwari	0	0.00	3	0.05	**3**
An.phillipinensis	1	0.11	1	0.02	**2**
An.tassellatus	7	0.79	15	0.26	**22**
An.minimus	1	0.11	1	0.02	**2**
An.sergenti	0	0.00	5	0.09	**5**
An. splendidus	16	1.80	35	0.62	**51**
An.stephensi	6	0.68	6	0.11	**12**
An.varuna	30	3.38	372	6.56	**402**
An.maculatus	0	0.00	1	0.02	**1**
TOTAL	888 (13.54)		5668 (86.46)		6556

Table: 2 Comparison of collection methodologies.

Out of the total number of collection **888 (13.54%)** were collected by **CDC light trap** and **5668(86.46%)** were collected by **hand catch using oral aspirator**. So**hand catch using oral aspirator is preferred over CDC light trap** where the collection densities of each species are always more than CDC light trap. Some species that never found in CDC trap were collected from Hand catch. In the light trap collection indoor collection was also found to be having more number of *Anophelines* than that of the outdoor collection.**Indoor**collection comprised **59%** while **outdoor**collection comprised **41%** of the total light trap collection respectively.

Season wise analysis of the mosquito's collection indicated that **highest (55.22%)** density was observed in **post monsoon** while lowest during **monsoon (11.42%)**. The winter contributes **33.36%** of the total collection.

Internal validation was conducted in two laboratories of RMRC (Molecular Biology and Microbiology Division). External validation by state govt. entomology unit is under progress.

Among the four different ecotype selected the density of

	INDOOR						OUTDOOR				
Species	CS	%	HD	%	MD	%	Shelters	%	Tree holes	%	Total
A.annularis	1152	25.62	56	29.17	228	26.45	5	10.00	9	13.24	**1450**
An.culicifacies	216	4.80	22	11.46	24	2.78	8	16.00	15	22.06	**285**
An.hyrcanus	519	11.54	11	5.73	96	11.14	2	4.00	19	27.94	**647**
An.pallidus	1179	26.22	54	28.13	197	22.85	18	36.00	21	30.88	**1469**
An.subpictus	447	9.94	25	13.02	69	8.00	5	10.00	1	1.47	**547**
An.vagus	80	1.78	3	1.56	12	1.39	1	2.00	3	4.41	**99**
An.aconitus	4	0.09	0	0.00	0	0.00	0	0.00	0	0.00	**4**
An.barbirostris	117	2.60	0	0.00	7	0.81	2	4.00	0	0.00	**126**
An.fluviatilis	441	9.81	8	4.17	145	16.82	5	10.00	0	0.00	**599**
An.jeyporiensis	3	0.07	0	0.00	0	0.00	0	0.00	0	0.00	**3**
An. karwari	3	0.07	0	0.00	0	0.00	0	0.00	0	0.00	**3**
An.phillipinensis	1	0.02	0	0.00	0	0.00	0	0.00	0	0.00	**1**
An.tassellatus	9	0.20	0	0.00	6	0.70	0	0.00	0	0.00	**15**
An.minimus	1	0.02	0	0.00	0	0.00	0	0.00	0	0.00	**1**
An.sergenti	4	0.09	0	0.00	1	0.12	0	0.00	0	0.00	**5**
An. splendidus	25	0.56	5	2.60	5	0.58	0	0.00	0	0.00	**35**
An. stephensi	5	0.11	1	0.52	0	0.00	0	0.00	0	0.00	**6**
An.varuna	289	6.43	7	3.65	72	8.35	4	8.00	0	0.00	**372**
An.maculatus	1	0.02	0	0.00	0	0.00	0	0.00	0	0.00	**1**
			192		**862**						
Total	**4496 (79.32%)**		**(3.39%)**		**(15.21%)**		**50 (0.88%)**		**68 (1.20%)**		**5668**

Table 3: Hand catch collections of Anophelines from indoor and outdoor habitats.

From the total hand catch, **Indoor** collection contribute **5550 (97.9%)** of the collection and very few mosquitoes **83 (2.01%)** were collected from the outdoor. From the indoor collection **cattle shade** contributes **highest number (79.32%)** of collection followed by mixed dwelling. Human dwelling gives least number of mosquitoes.

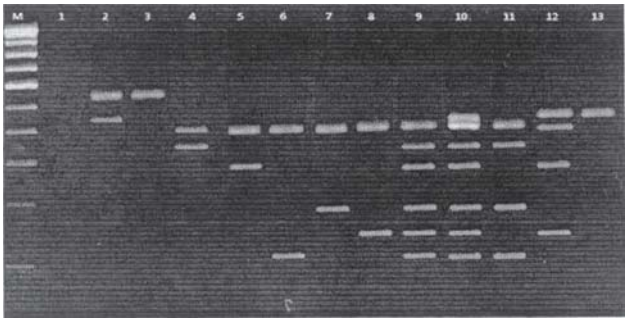
Table 4: Percent composition of Anopheles mosquitoes in four ecotypes of Jhumpura PHC:

Species	Plain		Riverine		Foot hill		Hill top		Total	
	No	%	No	%	No	%	No	%	No	%
An.annularis	188	9.09	173	11.80	1022	56.03	247	20.62	1630	24.86
An. aconitus	1	0.05	1	0.07	2	0.11	0	0.00	4	0.06
An. barbirostris	91	4.40	30	2.05	12	0.66	27	2.25	160	2.44
An. culicifacies	23	1.11	240	16.37	45	2.47	39	3.26	347	5.29
An. fluviatilis	8	0.39	14	0.95	76	4.17	542	45.24	640	9.76
An. jeyporiensis	1	0.05	2	0.14	0	0.00	3	0.25	6	0.09
An. karwari	0	0.00	0	0.00	1	0.05	2	0.17	3	0.05
An. pallidus	721	34.86	591	40.31	254	13.93	86	7.18	1652	25.20
An.phillipinensis	0	0.00	1	0.07	1	0.05	0	0.00	2	0.03
An. subpictus	417	20.16	153	10.44	53	2.91	13	1.09	636	9.70
An. vagus	65	3.14	15	1.02	51	2.80	9	0.75	140	2.14
An.hyrcanus	527	25.48	172	11.73	88	4.82	54	4.51	841	12.83
An.tassellatus	6	0.29	0	0.00	0	0.00	16	1.34	22	0.34
An.minimus	0	0.00	0	0.00	1	0.05	1	0.08	2	0.03
An.sergenti	0	0.00	2	0.14	1	0.05	2	0.17	5	0.08
An.slpendidus	3	0.15	3	0.20	27	1.48	18	1.50	51	0.78
An.stephensi	1	0.05	5	0.34	6	0.33	0	0.00	12	0.18
An.varuna	16	0.77	64	4.37	183	10.03	139	11.60	402	6.13
An.maculatus	0	0.00	0	0.00	1	0.05	0	0.00	1	0.02
Total	**2068(31.54%)**		**1466(22.36%)**		**1824(32.35%)**		**1198(18.27%)**		**6556**	

anopheles are **high (31.54%) in plain**, **22.36% in foot hill,(22.36 %)**in **riverine** and **(18.27%) in hill top**. Though the density of anopheles is highest in plain ecotype but the prevalence of vector species is very low whereas in three other ecotype the vector species are distributed in comparatively higher density than plain. Some specific vectors were found in high density at a particular ecotype. ***An. culicifacies***was always found in **high density in riverine** whereas ***An.fluviatilis* in hill top and foot hill** and ***An.annularis* in foot hill.**

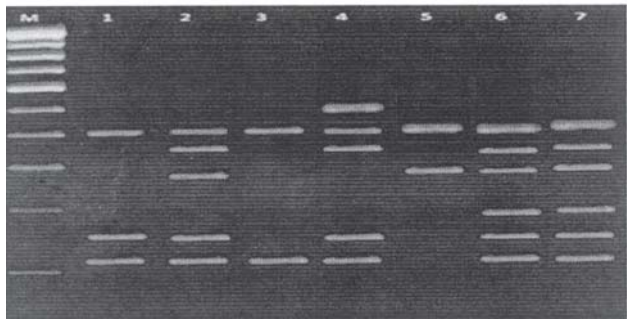
Report by Division of Microbiology

Control Result



Species	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13
Universal				+	+	+	+	+	+	+	+	+	
An. annularis				+					+	+	+		
An. culicifacies					+				+	+		+	
An. fluviatilis						+			+	+	+		
An. pallidus							+		+	+	+		
An. varuna								+	+	+		+	
Plasmodium falciparum		+								+		+	+
Human blood presence		+	+										

Validation Result

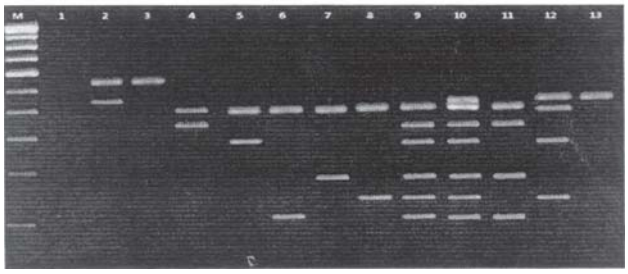


Species	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13
Universal	+	+	+	+	+	+	+						
An. annularis		+		+		+	+						
An. culicifacies		+			+	+	+						
An. fluviatilis	+	+	+	+		+	+						
An. pallidus						+	+						
An. varuna	+	+		+		+	+						
Plasmodium falciparum													
Human blood presence				+									

Signature and Designation of Validating Authority

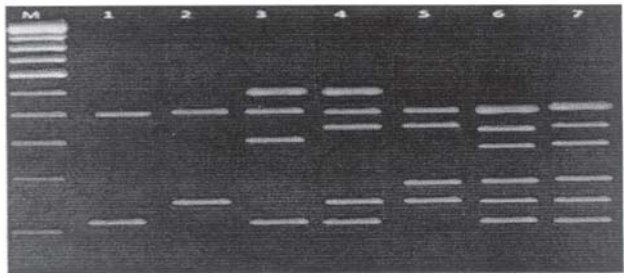
By Division of Molecular Biology

Control Result



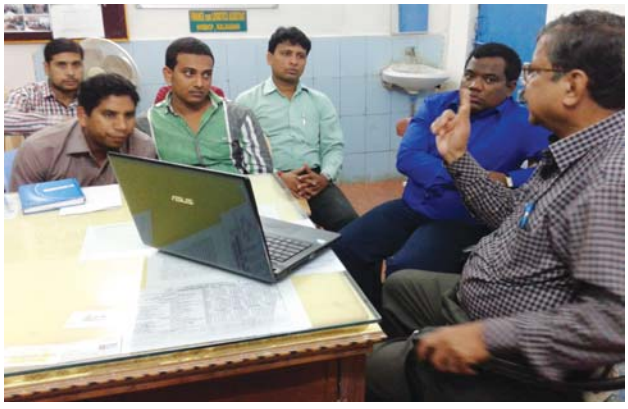
Species	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13
Universal				+	+	+	+	+	+	+	+	+	
An. annularis				+					+	+	+		
An. culicifacies					+				+	+		+	
An. fluviatilis						+			+	+	+		
An. pallidus							+		+	+	+		
An. varuna								+	+	+		+	
Plasmodium falciparum		+								+		+	+
Human blood presence		+	+										

Validation Result



Species	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13
Universal	+	+	+	+	+	+	+						
An. annularis				+	+	+	+						
An. culicifacies			+			+	+						
An. fluviatilis	+		+	+		+	+						
An. pallidus					+	+	+						
An. varuna		+		+	+	+	+						
Plasmodium falciparum													
Human blood presence			+	+									

Ramakanta Rana SPO
Signature and Designation of Validating Authority



Training of MTS workers in Kalahandi field unit.

Major Achievements

1. A simple, rapid and very efficient protocol for DNA isolation from mosquito specie was established in our laboratory.

2 A change in behavioral pattern of Anopheles fluviatilis was observed in Keonjhar district during our study period i.e. July 2012-March 2013. Their densities was found to be maximum during post monsoon period and were collected mostly from mixed dwelling followed by cattle shed and very few numbers from human dwelling. Sibling species analyses revealed that *An. fluviatilis* T were found to be in higher density than other siblings. An. fluviatilis was found to be more Anthropophilic (55%) than zoophilic which contradicted with earlier report of being zoophilic only.

3. A workshop was organized for five days where different concept regarding the use of different molecular technique and transfer these technique to field was taught. These aspects would be useful in rapid monitoring and evaluation of malaria of an area. The participants were from entomologists of health department Govt.of Odisha and scientists and research staffs from five field unit of NIMR.

Future Plan

PHASE 2:

Testing in small scale (One / Two PHC, Duration: 12 months)

Work plan

(i) Selection of PHCs (all eco-types, drug and insecticide resistance).

(ii) Strata: 5 villages x 4 eco-types x drug and insecticide resistance

(iii) Sample collection by
   (a) Investigators
   (b) Male MTS / male health workers (workers from state Govt.).

(iv) Hand catch by MTS / male health worker: randomly selected 3 human dwellings (HD) and 3 cattle sheds (CS) in each village.

(v) Hand catch by investigators: randomly selected 3 HD and 3 CS in the same village for verification initially of the collection by the workers.

(vi) Four traps per village (two each in HD and CS) Frequency of mosquito sampling (three seasons, one collection per season).

(vii) PCR assay for vector species composition, Human blood meal identification, sporozoites detection, drug resistance and insecticide resistance.

Workshop on Translational Research at RMRC

(viii) Screening of all fever cases (by ASHA and ANM) in the selected villages and for Pfcrt and Pfmdr1 from Pf +ve cases (by investigators): Comparison of human with mosquitoes for drug resistance.

19. Quadruplex PCR for diagnosis of V. cholerae O1 and/or O139 Serogroup causing Cholera.

Principal Investigator : Dr. H. K. Khuntia

Objectives

1. To optimize, inter and intra observer variations of the Qudruplex PCR will be checked for detection of *V. cholerae*
2. To map out the *V. cholerae* strains found in Orissa by Quadruplex PCR by examining both hospital and outbreak samples
3. Transfer of the Quadruplex PCR technology from laboratory to the field.

Progress of Work

1. Intra observer variation of the Qudruplex PCR:

To study the intra observer variation of the PCR technique, an in-house validation was conducted with coded *V. cholerae* O1 and O139 strains. A total of five PCR expert Research Scholars of RMRC, Bhubaneswar were assigned each with five coded *V. choleare strains* and three control strains (Positive control: *V. cholerae* O1 and O139 and Negative control: Salmonella spp). Each student was taught in detail about methodology and a protocol of Quadruplex PCR assay was given

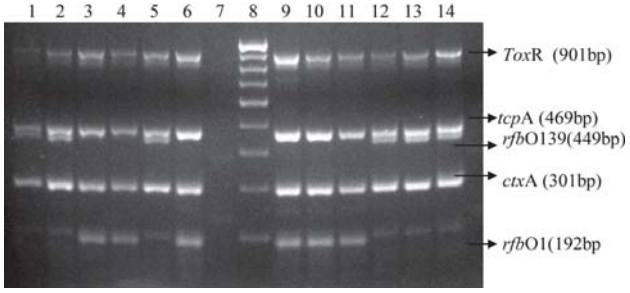


Fig 1. shows results of in-house validation of Quadruplex PCR assay of *V. cholerae* O1, O139, positive control (*V. cholerae* O1 and O139) and negative control (Salmonella spp.)

before the experiment. The results of Quadruplex PCR assay of all 25 coded *V. cholerae* strains confirmed genetically their serogroup encoding *rfb* O1 / *rfb* O139 sreogroup that matched with their respective actual serogroups showing positive for other genes *ctx*A, *tcp*A, and *Tox*R (Fig1).

Response to the recommendation of Pre-SAC members, the terminology " Novel Technique" in the title of the project has been dropped. Secondly, the results of culture +ve samples and their corresponding PCR has been prepared.

2. Mapping of *V. cholerae* strains found in Odisha by Quadruplex PCR assay by examining both hospital and outbreak strains.

A total of 332 rectal swabs sample in Carry Blair transport (CBT) medium were collected / referred from hospitalized diarrhoea patients. Rectal swab sample were inoculated on TCBS plate and incubated at 37^{0}C for 18 hour. DNA was extracted from the colonies resembling the *V. cholerae* strains and subjected for Quadruplex PCR assay for genetic confirmation of serogroup and other virulent genes. Of the 332 rectal swabs, 23 *V. cholerae* were confirmed by PCR assay with the detection of *rfb* O1 gene encoding surface antigen that matched with the conventional method of sero-diagnosis. All the *V. cholerae* strains showed positive for *ctx*A, *tcp*A (El Tor) and *Tox*R genes.

Future Work

- Attempt will be made to transfer the Quadruplex PCR technology from laboratory to field very soon for early diagnosis of cholera.

Annual Report 2013

Completed Studies

Contents

Field activities of RMRC, BBSR

1. Effect of maternal infection on neonatal immune responses in bancroftian filariasis.

Principal Investigator : Dr A.K. Satapathy
Co-Investigators : Dr M. S. Bal
Dr N.N. Mandal,
Dr S. K. Kar
Duration : Three years
Starting date : Dec 2009- Dec 2012
Status : Extramural (Immunology Task Force, ICMR)

Objectives

1. To study the B cell response (antibody isotypes) to filarial antigens in cord blood samples of offspring and in their corresponding mothers
2. To evaluate the influence of maternal filarial infection on mitogen and filarial antigen induced cellular responsiveness and cytokine production in cord blood of neonates
3. To compare the expression profile of T regulatory cells in cord blood of infected and uninfected mothers

Background

Filarial infection acquired through maternal origin has been considered a risk factor for increased susceptibility. A number of studies have shown that children of microfilaraemics mothers were more likely to be microfilaraemics than those of amicrofilaraemics mothers. In general, helminthes induced Th2–type of response that confers host protection and expulsion of intestinal nematodes in experimental models. Similarly filarial parasites selectively induce Th2 type response. Children borne of filarial infected mothers have been shown to impair filarial Ag-specific T cell responses. Women commonly harbor filarial infections during their childbearing years, raising the possibility that the developing fetus may be exposed to filarial antigens in utero and thereby have altered immunity and susceptibility to infection during early childhood.It is not known how maternally conferred immunity affects the evolution of parasite specific T cell immunity and susceptibility to infection during childhood.

Results

The prevalence of microfilaraemia and antigenemia in paired maternal and cord samplewas determined.Interestingly, 39 of 105 amicrofilaraemic mothers were also found positive for CFA indicating the presence of adult worm within them. An overall CFA positivity among mothers was noted to be 44.5% (53 / 119). None of the cord samples from CFA negative mothers were CFA positive whereas 24.5% of neonates from CFA positive mothers were tested for CFA positive suggesting placental transfer of CFA from mother. An overall prevalence of antigenemia among all the cord samples was observed to be 10.9 % (13 / 119). Out of 13 mothers who have transferred CFA to their respective cord, 11 were microfilaraemics and two were amicrofilaraemics.

Antibody isotypes to filarial antigen was determined in paired maternal and cord blood

Table-1 Antibody isotypes (IgG, IgM, IgE) in maternal and cord samples.

Maternal infection status	N	IgG Maternal sera	Cord sera	IgM Maternal sera	Cord sera	IgE Maternal sera	Cord sera
		N %	N %	N %	N %	N %	N %
CFA Positive	53	32 (60.4)	8 (15.1)	34(64.2)	6 (11.3)	29(54.7)	13 (24.5)
CFA Negative	66	40 (60.6)	18 (27.3)	40 (60.6)	0 (0)	47 (71.2)	2 (3.03)

samples as shown in Table- 1. No significant difference was observed in prevalence of IgG, IgM&IgE antibodies in CFA positive and negative mothers. Anti filarialIgG, IgM and IgE positivity did not vary with the antigen status of the mothers. IgG positivity was determined in 21.8% (26/119) of cord samples. IgM only could be detected in infants, who were CFA negative, but born from CFA positive mothers. IgE antibody was detected in 15 cord blood samples of which 13 were from CFA positive mothers.

The prevalence of anti-sheath antibodies in mothers and their respective cord bloods was found to be similar i.e. 48.3% (29/60). However, 38.3% (23/60) of cord blood samples were negative for both CFA and anti-sheath antibodies, and only 3.3 % (2/60) of cord blood samples were positive for both parameters (Table-2). Interestingly all the cord blood samples from anti-sheath antibodies-positive mothers (69.45%) were positive for anti-sheath antibodies indicating transpalcental transfer of anti-sheath antibodies.

The plasma level of cytokine for IL-10 and IFN-? were measured in the mother and their respective cord blood samples and the results are shown in Fig. 1&2.As shown in Fig.1cord blood samples of infected mothers had significantly higher IL-10 than cord bloods of uninfected mothers (p= 0.007). We analysed further the association of anti-sheath antibodies and levels of cytokines in mother and their respective cord blood samples. Elevated levels of IL-10 were observed in anti-sheath antibodies negative cord bloods compared

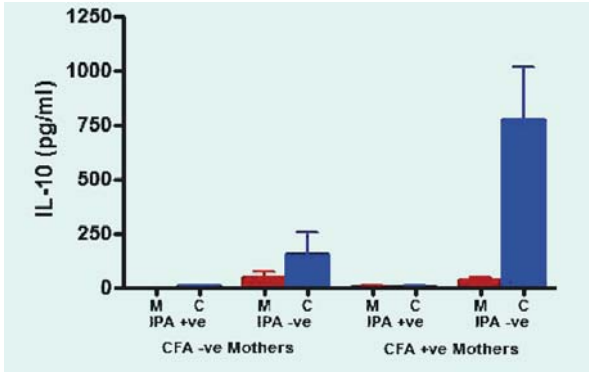


Fig.-1: Plasma level of IL-10 in mother and their respective cord blood samples obtained from newborns of women with (CFA +ve) or without (CFA –ve) filarial infection. Groups were further stratified according to anti-sheath antibodies positive (IPA +ve) and negative (IPA -ve) status. Bars represent geometric mean ± SE values. M – mother, C- cord blood.

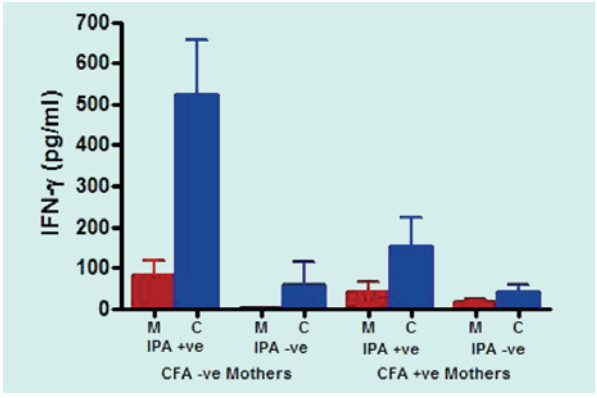


Fig.-2: Plasma level of IFN-ã in mother and their respective cord blood samples obtained from newborns of women with (CFA +ve) or without (CFA –ve) filarial infection. The groups were further stratified according to anti-sheath antibodies positive (IPA +ve) and negative (IPA-ve) status. Bars represent geometric mean ± SE values. M–mother, C-cord blood.

Table2. Prevalence of anti-sheath antibodies and circulating filarial antigen (CFA) in mother and their respective cord blood.

Infection Status	Maternal N	Anti-sheath antibodies +ve	Anti-sheath antibodies -ve	Cord blood N	Anti-sheath antibodies +ve	Anti-sheath antibodies -ve
CFA +ve	24	4(16.6%)	20 (83.4%)	10	2 (20%)	8 (80%)
CFA –ve	36	25 (69.4%)	11(30.5%)	50	27(54%)	23(46%)

to anti-sheath antibodies positive cord bloods irrespective of the infection status of mother. Cord blood samples from infected mother that were anti-sheath antibody negative had significantly higher levels of IL-10 than cord blood samples that were anti-sheath antibodies positive. In contrast the levels of IFN-? were found to be significantly high in CFA-ve cord blood than that of CFA +ve cord blood samples ($p<0.02$) as shown in Fig.2. Elevated levels of IFN-? were observed in anti-sheath antibodies positive cord blood samples compared to anti-sheath antibodies negative cord blood samples irrespective of the infection status of mother.

Purified CBMC of infected mothers displayed weak T-cell proliferative response to filarial parasite antigens in comparison to CBMC of uninfected mothers. CBMCs from uninfected mothers have shown significantly high proliferative response to filarial antigens compared to CBMCs from infected mothers. However the extent of proliferation, stimulated with mitogen (PHA) was found to be similar in CBMCs of both uninfected and infected mothers. The proliferative response to purified

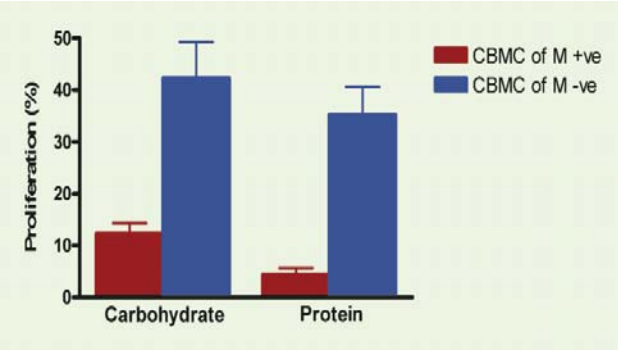


Fig.-3: Proliferative responses of CBMC induced with Purified antigens of filarial parasite.

carbohydrate antigen was significantly high in CBMCs of uninfected mother as shown in Fig.6. This indicates that transfer of filarial antigens influences the cellular proliferative response of the newborns.

Conclusions

Maternal infection has been considered to be a risk factor for filarial infection in offsprings.To examine the influence of maternal infection in neonates, we have examined the prevalence of circulating filarial antigen (CFA) and anti-filarial antibodies in maternal and corresponding cord blood samples collected from an area endemic for bancroftian filariasis. Our study demonstrateed transplacental transfer of circulating filarial antigen from mother to cord. Filaria-specific IgM and IgE antibodies were higher in cord blood from infected mothers than from non-infected mothers. The findings of the study provide additional circumstantial evidence for pre-natal sensitization to filarial antigens developed in utero.

Since a protective role for anti-sheath antibodies has been considered in a broader perspective for anti-filarial immunity we also assessed the impact of maternal infection on the anti-sheath antibodies and cytokine production in neonates born from infected and uninfected mother. About 69.4% of non-infected mothers and their cord bloods showed presence of anti-sheath antibodies, while only 16.6 % of the cord bloods from infected mothers were positive for anti sheath antibodies. IL-10 level was observed to be remarkably high in cord bloods of both infected and non-infected mothers negative for anti-sheath antibody. In contrast, IFN-ã level was significantly high in cord bloods of non-infected mothers compared to infected mothers. The study reveals that presence or absence of anti-sheath antibodies in association with cytokines skews the filarial specific immunity to either Th1 or Th2 responses in neonates.

An increased level of IL-10 (Th-2) and down regulation of IFN-γ (Th-1) has been detected in cord blood of children born to filarial infected mothers. High level of T- Regulatory cells and increased production of IL-10 in cord blood from infected mothers indicate that increased T-regulatory cells could down regulate inflammatory responses and may be associated with parasite survival.

2. Study on drug resistance among sputum positive tuberculosis patients in Rayagada district, Orissa.

Principal Investigator : Dr Dasarathi Das
Co- Investigator : Dr B Dwibedi
Starting Date : June 2011
Period : Two years
Funding : ICMR

Objective

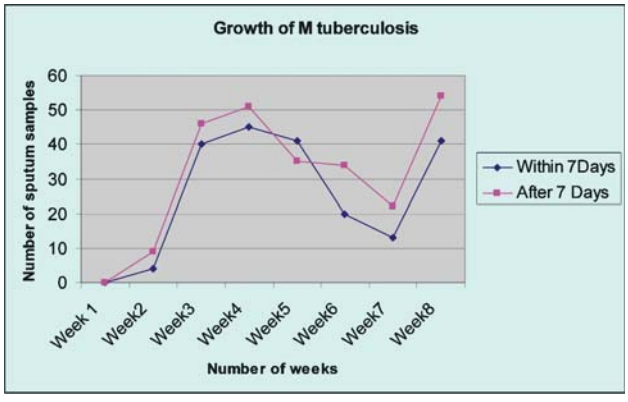
To estimate the prevalence of drug resistance among sputum positive tuberculosis patients in Rayagada district, Odisha.

Methodology

The sputum samples were collected from 18 DMCs of Rayagada district of Odisha state and direct smears were made and stained by Ziehl-Neelsen (ZN) staining method for Acid Fast Bacilli (AFB) by the RNTCP technicians. Slide preparation, ZN staining, microscopic examination were made as per RNTCP guidelines. Smears were graded as follows: negative when no AFB was observed after examining at least 100 oil immersion fields; scanty positive if there were 1–9 bacilli per 100 fi elds; 1+ for 10–100 bacilli / 100 fields, 2+ for 1–10 bacilli / field in at least 50 fields; and 3+ for >10 bacilli / field in at least 20 fields. The DMCs were supplied with freshly prepared CPC-NaCl working solutions which was added to the sputum samples in equal volumes and tightly capped, sealed, labeled and kept in DMCs. The CPC containing sputum samples, specimen request forms, consent forms were collected by person and transported to Rayagada district hospital from where sputum samples transported RMRC laboratory for processing. For culture the specimens were centrifuged at 3000 x g for 15 minute, sediment was re-suspended again in almost 40-45 ml sterile distilled water and centrifuged at 3000 x g for 15 minute. Sediment was resuspended in 1-2 ml of sterile distilled water and a loopful was inoculated onto two Lowenstein- Jensen (LJ) and one LJ containing Para Nitro Benzoic Acid (PNB) slopes. The medium bottles were incubated at 37°C in walk-in- incubator and were examined for growth of *M. tuberculosis* complex once a week for 8 weeks. Growth of *M. tuberculosis* complex was graded as follows: actual number of colonies if between 1 and 19 colonies; 1+ for growth of >20 but <100 colonies; 2+ for growth of >100 colonies; and 3+ for confluent growth. Growth on LJ was identified by colony morphology and biochmecal tests including niacin production, catalase activity at 68^0C and susceptibility to p-nitrobenzoic acid. The drug susceptibility testing of the first line anti TB drugs was carried out by Proportion Sensitivity Test (PST) method of RNTCP. The drug concentration used was Isoniazid 0.2 ìg / ml, Ethambutol 2 ìg / ml, Streptomycin (dihydrostreptomycin sulfate) 4 ìg / ml, Rifampicin 40 ìg / ml.

Result

Out of the 634 patients 577 were newly diagnosed and 57 were previously treated cases. The majority, 77.8% of sputum positive patients were of males in the productive age group of 21-50 years. Of the 634 sputum samples collected in CPC, 539(85.0%) yielded *M tuberculosis*, 74(11.7%) yielded no growth, 18(2.8%) grew contaminants and 3(0.5%) were NTM. About 56% sputum samples could not be processed within a week's time as transportation took longer time to reach



the laboratory. In both the intervals of transportation, the highest mycobacterial growth was observed at 4th weeks of incubation.(Fig-1)

Out of 539 tuberculosis patients found positive by culture in LJ medium, Drug susceptibility testing was successful in 532 cases out of which 489 were new and 43 were previously treated cases. It was observed that 6.3% new and 23.3% previously treated patients showed some form of resistance to first line anti TB drugs. The level of mono resistance observed among newly diagnosed patients were 3.7% to streptomycin and 2.5% to isoniazid. While mono resistance observed among previously treated TB patients were 7.0% to streptomycin and 4.7% to isoniazid. The level of MDR TB was 0% and 7.0% among new and previously treated patients.

Conclusion

This study reported a very low level of MDR TB prevalence in the Rayagada district and suggests that more attention to early detection and treatment through DOTS can sustain this low level of drug resistance to anti-TB drugs for a longer period.

Project Investigator with DTO and STS verifying Lab records at a DMC of Rayagada district of Odisha

3. Development of a LAMP assay for diagnosis of human malaria.

Principal Investigator	:	Dr M R Ranjit
Co-Investigator	:	Dr S K Kar
Starting Date	:	2 / 7 /2010
Closing Date	:	2 / 7 /2012
Funding	:	EM: DBT (BT/PR12536/ MED/29/02/2009)

Objectives

(i) To design species specific loop primers for detection of human malaria parasites

(ii) To optimize the reaction conditions for easy detection of the LAMP derived products

(iii) To find out the efficacy of the test compared to nested PCR and light microscopy

Background

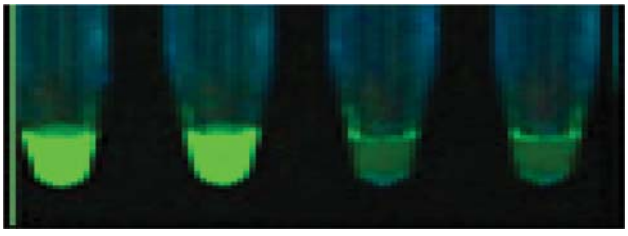
Microscopy is the gold standard for diagnosis of malaria even though various rapid and simple tests have been developed in recent years. But loop-mediated isothermal amplification (LAMP) of nucleic acids seems to be a promising new technique, which enables to detect malaria parasites in a setting with limited resources. However, LAMP assay in its current form lacks sufficient accuracy in detection of the end product. Therefore, optimization of the current method for visualization of LAMP end products is important. The proposed project will help to develop a suitable method for detection of end product.

Progress of Work

With continuous effort we have been able to optimize the reaction conditions using Loop Amp DNA Kit of Eiken Company, Japan. To prevent contamination different sets of pipettes and different work areas were designated for DNA template preparation, reaction mixture preparation and DNA amplification.

For every 25 µl reaction mixture the optimum primer mix was in the proportion of 10(FIP & BIP) :5 (FLP &BLP) :1 (FOP &BOP) and the concentrations of

FIP& BIP , FLP &BLP and FOP & BOP were 50, 25 and 5 picomoles respectively. The optimum temperature for reaction was 63 ºC and duration was 60 minutes for *Plasmodium falciparum* and 45 minutes for *Plasmodium vivax* and *P malariae*. The LAMP positive tubes can be clearly distinguished from negative tubes by naked eye as well as under UV. The reaction mixtures with amplicons will show stronger luminescence under UV compared to reaction mixtures without amplicon (Fig 1).



+ve +ve -ve -ve

Fig 1: Lamp Products Under UV Transilluminator.

Our selected LAMP primers did not show any cross reactions with each other as would evident from Table 1.

Table 1: Test cross specificity of different species specific primers**.**

Sample primers	DNA *P. falciparum* (n=5)	DNA *P. vivax* (n=5)	DNA *P. malariae* (n=5)
P. falciparum	+	-	-
P. vivax	-	+	-
P. malariae	-	-	+

When we tested the repeatability (5 samples repeated for 10 times) of the selected set of primers , it was found that the LAMP primers selected for *P falciparum* can give the same amplification in 100% of time, while *P vivax* and *P malariae* in 100% of time (Fig2)

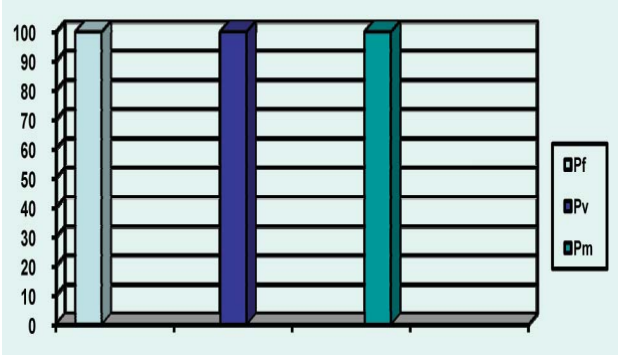


Fig 2: Repeatability of the test with same set of primers.

The detection limit of parasites for *P falciparum* was found to be 2 per µl of blood and in case of P *vivax* and *P malariae* it was 5 per µl of blood and can be compared to PCR. (Table 2)

Conclusion

During the project period the primers have been designed and the reaction conditions have been standardized and the efficacy of the test has been compared to PCR. The results are promising. The test now needs to be validated in the field condition and also inter and intra observer variations are to be investigated. To reduce the cost of the tests attempts has to be made to prepare the in house reagents.

Table 2: Detection limit of parasites in LAMP and PCR techniques.

Plasmodium falciparum			***Plasmodium vivax***			***Plasmodium malariae***		
Parasite/µl	PCR	LAMP	Parasite/µl	PCR	LAMP	Parasite/µl	PCR	LAMP
200	+	+	100	+	+	20	+	+
100	+	+	50	+	+	10	+	+
20	+	+	20	+	+	5	+	+
10	+	+	10	+	+	2	+	-
5	+	+	5	+	+	-	-	-
2	-	+	2	-	-	-	-	-

4. Multicentric evaluation of L3 stage specific RT-PCR Assay for the detection of infective stage (L3) *W.bancrofti* in vector.

Principal Investigator : Dr. N. Mahapatra
Co-Investigator : Mr. N. S. Marai
Starting Date : Jan 2012
Closing date : April.2013
Funding : ICMR Task Force

Objective

To assess the sensitivity and specificity of the infective stage specific RT-PCR assay in detecting infectivity in vector and evaluate its usefulness in control programmes at various Natinal Research Centres.

Rational

- Dissection of vector mosquitoes for L3 stage larvae is presently the only proven method to estimate transmission in an LF endemic community.
- Effort are underway globally to develop PCR based method for the detection of infective (L3) stage larvae of *Wuchereria bancrofti.*
- At the VCRC, infective stage specific RT-PCR has been developed and validated in the laboratory.
- The success of this assay in specific identifying the infective
- (L3) stage of *W.bancrofti* will be of great value in assessing the transmission of filaria infection and hence in decision making related to elimination programme.
- Therefore, the assay needs to be incorporated in operational programmes for large scale surveillance and monitoring.

Objective

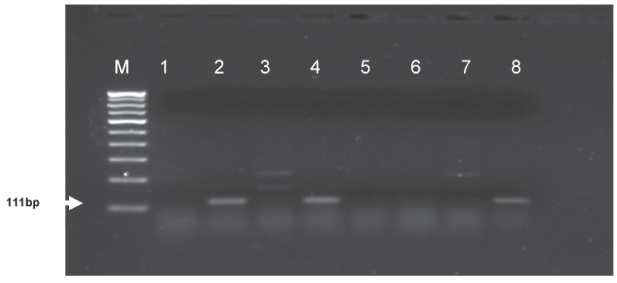
To assess the sensitivity and specificity of the infective stage specific RT-PCR assay in detecting infectivity in vector and evaluate its usefulness in control programme at various National Research Centers.

Result

Detection of infective stage (L3) larva of filaria parasite, *Wuchereria bancrofti in* mosquito vector is done by stage specific RT-PCR assay. Mosquito samples were homogenized with denaturing agent ,from which RNA is extracted using organic solvent .First strand cDNA is synthesized by reverse transcription from extracted RNA ,by using oligo-dT primer and reverse transcriptase enzyme. Stage specific primer (Forward and reverse) are used to amplify the first strand cDNA to second cDNA, which is run on agarose gel, stained with ethidium bromide, observed for a band of 111 bp on a UV transilluminator. The detail procedure was followed as per the protocol provided by VCRC Pondicherry.

Out of the 12 known coded sample, result of nine sample tallied with the known coding of VCRC, however code no 8 and 12,which were negative samples showed positive results and code no 9 which was a +ve sample showed negative results .Fifty coded samples were processed and 25 samples were found to be positive. The results were communicated to VCRC.

Gel photo map showing positive bands of L3 larvae.



Lane M ,100 bp marker. Lane 1 to 8 (Unknown coded sample of no 22 to no 29)

Phase III

The numbers of adult *Culex quinquefasciatus* collected from the three endemic areas are 578 (Cuttack) 308 (Khurdha) and 664 (Nayagarh). All the mosquitoes were dissected and THE presence of infective larvae recorded. The number of mosquitoes with infective larvae (L3) was 8, 7 and 14 in Cuttack, Khurdha and Nayagarh respectively. The infective larvae were detected in all the three villages with infectivity rate ranging from 1.3% to 2.27%.

Out of 40 pools processed 2 pools were found positive for L3. As these areas are covered under MDA, the infectivity rates were found to be low. However, pool positivity was found to be higher (5%). Pool positivity was higher in Cuttack and Khurdha (fig. 4) when compared to the observed infectivity rate.

Discussion

The twelve known samples were provided by the VCRC for standardizing the procedure in RMRC, Bhubaneswar. Out of twelve samples 9 samples were found positive and matched with the result of VCRC and 3 samples did not. The sensitivity and specificity of the assay was found to be 60%.

The results show that the procedure of RT-PCR assay has been well standardized in the RMRC, Bhubaneswar. Dissection of mosquitoes collected from the three endemic areas showed infectivity rate ranging from 1.38 to 2.11 after 7 rounds of MDA. Now the mf rates of these areas vary from 2 to 3 %.

Out of 40 mosquito pools (Khurdha 20, Cuttack 15 and Nayagarh 5) two pools were found positive, one in Khurdha and another in Cuttack respectively. Pool screen data showed infectivity rate varying from 0.0 to 0.28%. This difference may be due to individual mosquito dissection or in the pool even if more than one mosquito would have been positive still it will show as one positive. However, pool positivity rates, found to be higher than the infectivity rate, which shows the assay to be very sensitive and stage specific.

Conclusion

The above data indicates that the RT-PCR assay is as sensitive and stage specific as the traditional dissection techniques for monitoring transmission intensity. The assay can be used as a monitoring tool to assess the filariasis elimination programme. Furthermore, screening of mosquitoes in pools speeds up the processing of large number of specimens and thereby maximizing the efficiency of monitoring.

5. Assessment of treatment seeking behavior, LLIN use and IRS acceptance by the tribal community of Odisha.

Principal Investigator : Dr N.Mahapatra
Co-Investigator : Dr R.K.Hazra
Starting Date : October 2012
Closing Date : February 2013
Funding : EM

Objectives

To build up capacity for scaling –up implementation of malaria control through COMBI approach.

Specific

- To asses the malaria treatment seeking behaviour of the people.
- To asses community acceptability and usage pattern of bed nets
- To asses community acceptance of IRS for malaria control.

It was seen from the study that though, in the studied villages, IRS was done and 90.3% respondent allowed their house to spray and they know the benefit of spraying and they get the prior intimation before

Focus Group discussion

Discussion with Key informant
(Teacher, Sarpanch, Health worker)

spraying, still 90 (60%) respondents revealed that, after spraying mosquitoes' nuisance did not came down. But in LLIN area, the acceptance to LLIN were more and they have the knowledge that LLIN nets were treated with insecticides at the factory and 92.6%(139) respondents were willing to buy ITN / LLIN net for their family by paying low prices / Govt. prices (Rs 10 / -).However in spite of IRS / LLIN distribution, all the studied villages showed high API (more then 14) in both the block.

Conclusion: The study showed acceptance of bednet is higher than IRS. After implementing both the strategy API was still high.Therefore , potency of DDT / ITN / LLIN is highly essential along with continuos monitoring .Awareness campaign should be conducted more frequently.

Out break of Dengue in Ganjam District of Odisha.

An outbreak of Dengue fever occurred in Ganjam district of Odisha during mid of September 2012. On request of the state Govt, a team consisting of entomologist, clinician conducted a survey from 25.09. 2012 to 28.09.2012

Collection of Blood samples and mosquitoes larvae from village KUKUDAHANDI

Awareness development in Ayurvedic College Kukudahandi

Objective

1. To find out the vector prevalence and transmission in the affected villages of Ganjam district.

Meeting with ADMO

At first, CDMO, of Ganjam district was contacted on 25.09.2012, and the problem was discussed and the affected villages were identified along with VBD consultant.

Visit to the study village

The team went to the affected village KUKUDAHANDI, **HINJILIKATO Langipali, Gopalpur** and, **Galiri** contacted the Sarpanch of the villages. The objectives of the project were explained to the Sarpanch along with local leaders in order to report and co-operate the team during various entomological, epidemiological and demographical survey.

A total of 53 adult mosquitoes were collected to see the mosquitogenic condition. *Cx.quinquefasciatus, An.subpictus, An.annularis, An.vagus and Ae.aegypti* were collected. Larval breeding places were searched for presence of Aedes larvae. Out of 65 breeding places, 16 were found positive for Aedes larvae. The larvae and adult were brought to the laboratory for further processing .

The intra venous blood were collected from the patient, their contacts and neighbors, and suspected cases.

Public support for adult mosquito collection

A meeting was held with the principal of Ayrbedic college, a awareness demonstration was given by audio visual to the students and staff of the college.

Conclusion

The presence of both the vector Aedes aegypti and albopictus and detection of dengue 2serotype in vector gave enough evidence of transmission dengue in the affected area. The information was immediately communicated to the VBD consultant / entomologist of state health Deparment and immediately fogging and source redction were done to control dengue transmission.

6. Role of CD5+ B-lymphocytes in human lymphatic filariasis.

Principal Investigators : Dr A.K. Satapathy
Co-Investigators : Dr B. Dwibedi, Dr P.K.Sahoo, Dr S.K.Kar
Duration : Three years
Starting date : April 2010
Closing Date : Dec 2012
Status : Extramural (DST)

Objectives

1. To study the profile of B1 cell populations and its association with poly reactive antibodies in filarial infected human population.
2. To study the role of B1 cells in cytokine responses by filarial proteins and carbohydrates antigens in filarial infected human cells.

Background

The role of B cells in host protection against filariasis remains unclear. There are two major subsets of B-lymphocytes, B-1 and B-2 cells. Several studies have shown that B1 subset of B cells play an important role in the outcome of infection in schistosomiasis, S. pneumoniae and experimental filariasis. However, the biological role played by B1 lymphocytes to provide host protection against filarial infection is largely unknown. In our previous year study we monitored the levels of B-1 cells (CD5+ and CD19+) in the clinical spectrum of lymphatic filariasis. B-1 cells were found to be low in microfilariamic patients. In normal humans and mice, B-1 cells produce antibodies that are mostly polyreactive nature and have low affinity. Most B-1 cells produce IgM, which bind to a variety of self-antigens. It has been shown that parasite carbohydrates polyclonally stimulate CD5+ B-lymphocytes to produce IL-10 which down regulate protective Th1 type of immune responses in schistosomiasis. We investigated the role of filarial carbohydrates in mediating immune deviation and prolonged survival of parasites in infected hosts. An attempt has also been made to investigate induction of T cell apoptosis during filarial infection.

Results

An increased level of IgM and IgG2 antibodies to filarial carbohydrate is associated with absence of active filarial infection. As per the 2nd objective we measured the antibodies responses to fil protein antigens in the clinical spectrum of filariasis. A filarial protein antigen devoid of carbohydrates was prepared. We analyzed the antibodies response in clinical spectrum of human lymphatic filariasis using the purified proteins from filarial worms. IgG antibodies to fil protein in human filariasis and shown

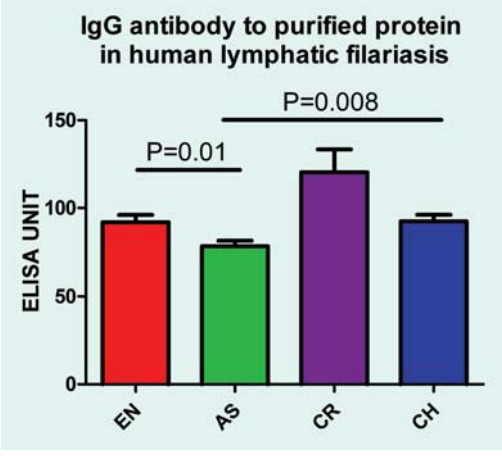


Fig-1

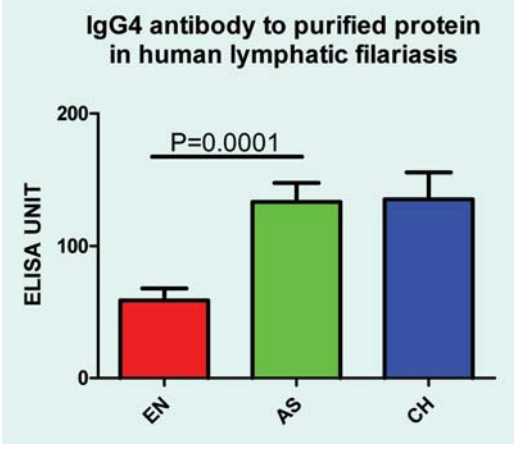


Fig-1

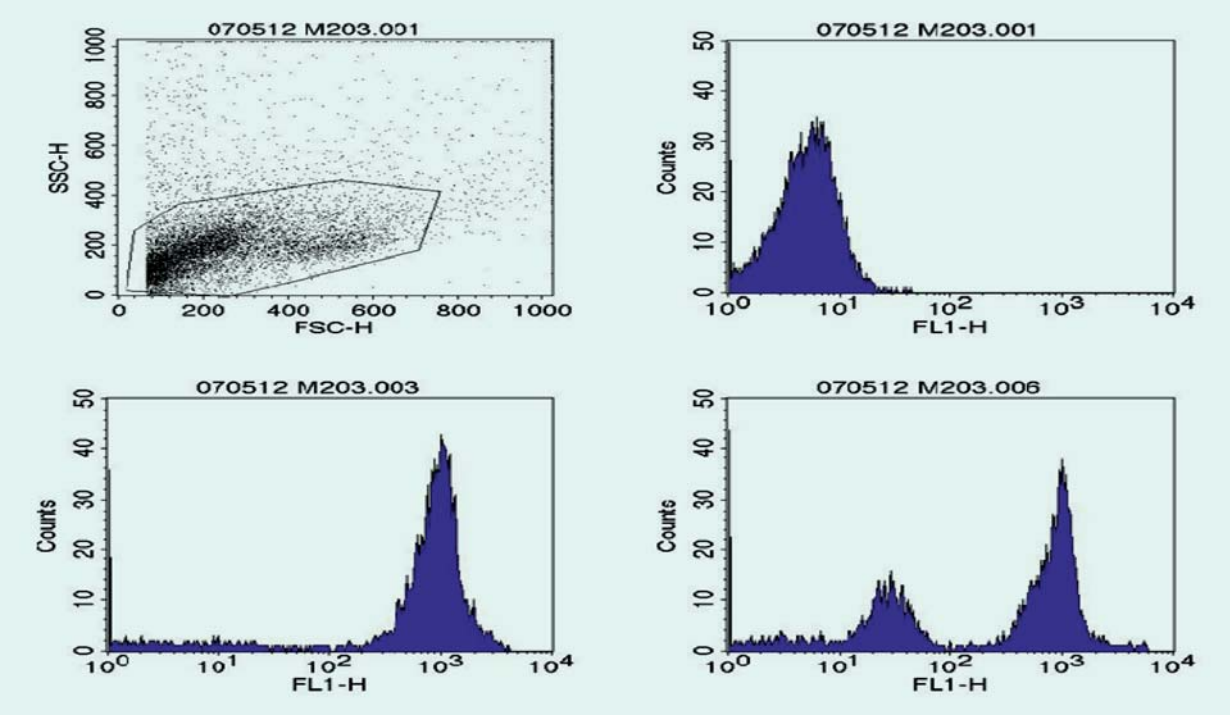


Fig-3

in Fig-1. IgG antibodies to fil protein antigen was found to be significantly low in case of microfilaremic carriers in comparison to other groups. Ig G subclass response to fil protein antigen was also analyzed. We have found that microfilaremic individuals had significantly high IgG4 antibodies to protein antigens compared to endemic normal Fig-2. An increased level of IgG4 to filarial protein is associated with presence of active filarial infection.

An attempt was made to study the cellular proliferative responses to filarial carbohydrates antigens in infected and uninfected individuals. Peripheral blood mononuclear cells (PBMC) of were purified, statined with CFSE before culture and stimulated with different filarial antigens (Crude, Purified carbohydrate and purified protein). The cells were cultured for 72 hours under stimulation with different filarial antigens. Then the cultured cells were stained with Propidium Iodide to exclude the dead cells and proliferation was measured by flowcytometer as shown in Fig-3.

The proliferative responses of T cells to filarial antigens (crude, purified carbohydrate antigen and purified protein) in endemic normals and microfilariaemic individuals are shown in Fig-4. Purified PBMC of infected individuals displayed weak T-cell proliferative response to filarial parasite antigens in comparison to PBMC of uninfected individuals. The proliferative response to purified carbohydrate

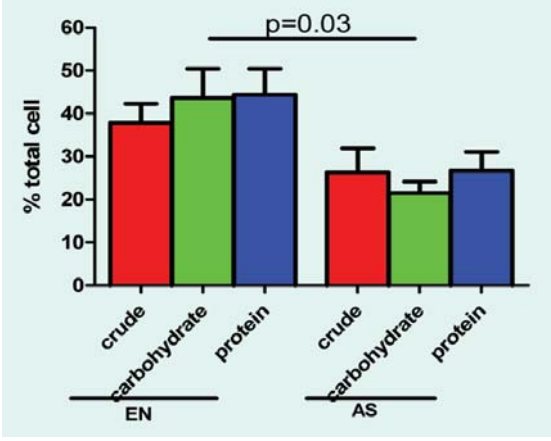


Fig-4

antigen was significantly low in PBMCs of infected individuals compared with endemic normal. This indicates that cellular response to filarial carbohydrates antigens suppressed in microfilariaemic individuals.

Cellular hyporesponsiveness observed during helminth infections are attributed by factors like APC dysfunction, increased IL-10, regulatory T cells and induction of $CD4^+$ T cell apoptosis. The profile of apoptotic $CD4^+$ T cell in filariasis remain unexplored. We scored apoptosis of peripheral T cells in filarial infected individuals. Peripheral blood was stained with CD4 and annexin-v and analysed by flow cytometry. It was observed that population of annexin-v positive helper T cells in filarial infected individuals were significantly more than endemic controls whereas population of annexin-v positive helper T cells between asymptomatic carriers and chronic patients were comparable (Fig-5).

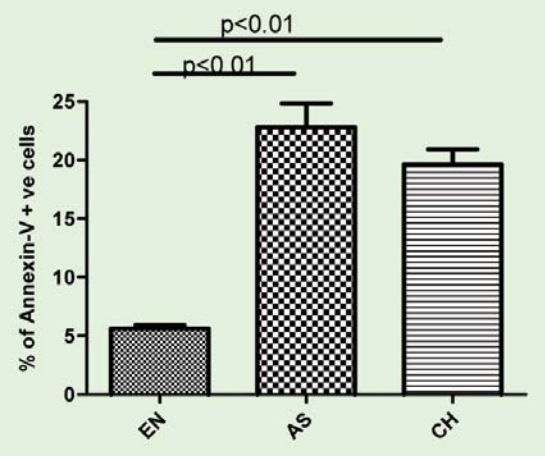


Fig-5

Fas ligand expression on the surface of B1 cells induces apoptosis of T helper cells. We scored the Fas ligand expression on the surface of B1 cells in different clinical categories of filariasis. Peripheral blood was stained with CD5 and CD19 to score B1 cells. The population of B1 cells were analysed for expression of Fas ligand with appropriate isotype control. Fas ligand expression on the surface of B1 cells of filarial patients (AS, CH) was significantly more than endemic controls (Fig-6). Membrane bound Fas ligand expression is responsible for induction of apoptosis in different cells. Correlation between Fas ligand expression of B1 cells and apoptotic T cells was analysed. The results are shown in Fig-7. A positive correlation was found between Fas ligand expression of B1 cells and peripheral $CD\ 4^+$ T cell apoptosis ($p=0.571$). Enhanced apoptotic T cell population in individuals with active filarial infection as compared to endemic controls may be responsible for immunohypo responsiveness.

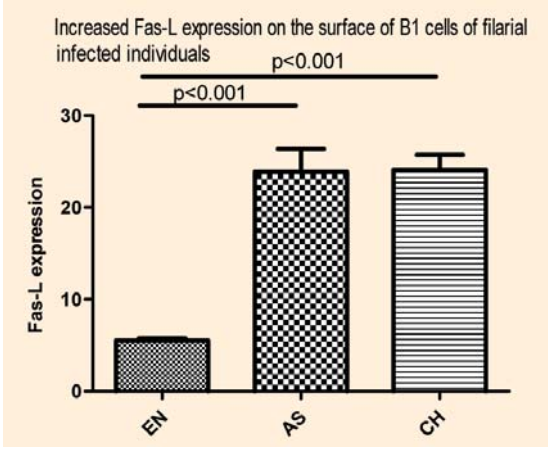


Fig-6

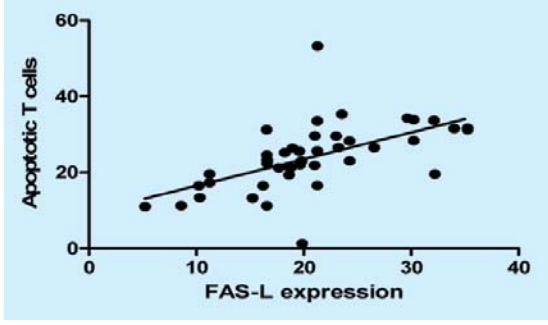


Fig-7

Conclusions

B1 subset of B cells have immense importance in the outcome of infection in Schistosomiasis, Pneumonie and experimental filariasis. However, no information regarding status of B-1 cells population in clinical manifestation of human bancroftian

filariasis exist. We investigated levels of B-1 cells from the total B cells by flow cytometer. Significantly low level of B-1 cells and IgM antibodies against a wide variety of auto-antigens (mostly produced by B–1 lymphocytes) were detected in microfilariaemia carriers compared to other clinical groups. A positive correlation was also found between IgM antibodies to actin and ss-DNA. Absorption of plasma with soluble actin, myosin and lipopolysacharides (LPS) resulted in significant removal of filarial binding antibodies. Affinity purified anti-ss-DNA antibodies were found to be reactive to filarial antigens, actin, myosin, tubulin and ss-DNA. Further, a positive correlation was found between polyreactive antibodies and B-1 cells in filarial infected human population attributing a role for $CD5^+$ B cells producing polyreactive naturally occurring antibodies.

Cellular hyporesponsiveness observed during helminth infections are attributed by factors like APC dysfunction, increased IL-10, regulatory T cells and induction of CD4+ T cell apoptosis. The profile of apoptotic CD4+ T cell in filariasis remain unexplored. Peripheral apoptotic T helper cells were significantly increased in filarial patients compared to endemic controls. Expression of Fas-ligand on the surface of peripheral B-1 cells increased in filarial patients and positively correlated with peripheral apoptotic T helper cell indicating FasL expressing B-1 cells are important mediators of CD4+ T-cell apoptosis.

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

Principal Investigators	:	Dr. S. L. Hoti, VCRC, Pondicherry
Co-Investigators	:	Dr A.K. Satapathy, RMRC, Bhubaneswar
Duration	:	18 months
Starting Date	:	Jan 2012
Closing Date	:	
Status	:	Extramural (Task Force Project, ICMR)

Objectives

1. To establish a national network of researchers and programme managers interested in genotyping of *W.bancrofti* and *B.malayi* prevailing in different endemic areas.
2. To determine the frequency of alleles of different loci in different genes (â tubulin, ALT-2, ITS region of r-DNA) among *W.bancrofti* parasite populations in different parts of the country.

Background

Elimination of human lymphatic filariasis has been launched on a global scale, basing its strength on a single chemotherapeutic (Mass Annual Single dose of DEC with Albendazole) strategy. Long term chemotherapy is likely to undergo changes to the extent of restructuring its own populations leading to drug resistance. It is unrealistic to adopt a single strategy because variability is the hallmark of biological systems and in reality the programme will be dealing with several variants of parasite, some exhibiting polymorphism of genes important for pathogenesis, drug sensitivity and transmission, rather than one single variety. Therefore, it is essential to fingerprint the genome of the lymphatic filarial parasite, *W. bancrofti*, the major filarial parasite in the country .

Results

Morphometry

Three villages (palaspur, padanpur and badatota) in the district of were surveyed. We have identified and isolated microfilariae from the 18 infected people and were chosen for the morphometry analysis and the microfilariae from their blood slides were taken for the DNA extractions. Microfilariae from Giemsa stained blood smears were examined for parameters such as the body length, width, cephalic space, distance from head end to nerve ring and caudal nuclei using LAS soft-ware and digital image of the parasites were stored in a data bank.

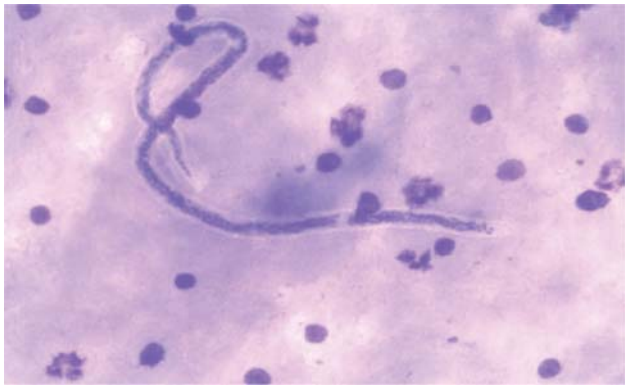


Fig-1. showing *Wuchereria bancrofti* microfilariae from Odisha Region.

Polymorphism in tubulin gene of *W. bancrofti*

Replacement of phenylalanine with tyrosine at amino acid position 200 of ?-tubulin isotype 1 conferred BZ-resistance in nematode parasites of farm animals has been established. Using this information we examined the polymorphism in the codon of this residue in *W.bancrofti* populations representing filarial endemic areas of Odisha.

Real time PCR was employed to analyze the single nucleotide polymorphism at 200 position of beta tubulin gene of *W. bancrofti*. The 200 position is associated with albendazole sensitivity. A change in single nucleiotide replace Phe (TTC) with tyrosine(TAC) at position 200 of beta tubulin resulting to Benzimidazole resistance in *W.bancrofti*.

Individual mfs were picked up from 10 patients (10x10=100) and were transferred into .2 ml PCR tubes containing TE. Mf containing pcr tubes were incubated in eppendorf thermal cycler for denaturation at 95 degree C for 10 min and immediately placed in to the ice for 5 minutes. The tubes were given a short spin for 30 seconds and are stored at -20 degree C. 10 µl reaction mixture was prepared with 2x Taq man master mix, 40x probe / primer mix, DNA and ionized water. The samples were then transferred into 96 well optical reaction plate and then short spin briefly. The plated were covered with adhesive cover and sealed with adhesive seal applicator. The plates were then loaded into ABI prism 7000 real time PCR machine.

Allele discrimination and absolute quantification program sheets were prepared using the ABi7000 software and the pre read for allele discrimination. Then the amplification carried out for absolute quantification and finally post read for 10 min.

A total of 93 mf collected from 10 different patients were analyzed for beta tubulin assay. All the samples were analysed for the single nucleotide polymorphism at aminoacid 200 position of beta tubulin. Polymorphism at 200AA position of beta

TABLE-1: MORPHOMETRIC DATA OF MF COLLECTED FROM DIFFERENT VILLAGES OF KHURDA DISTRICT OF ODISHA.

MORPHOLOGICAL PARAMETRE	MEAN± SD (N=94)
BODY LENGTH(MEAN±SD)	417.39± 60.22
CEPHALIC LENGTH(MEAN±SD	9.33± 3.64
CEPHALIC WIDTH(MEAN±SD	7.81± 2.51
CEPHALIC RATIO(MEAN±SD)	1.2± 0.38
DISTANCE TO THE END OF NERVE COLUMN(MEAN±SD)	408.05± 59.30
WIDTH AT NERVE RING(MEAN±SD)	10.76± 2.16
DISTANCE AT NERVE RING	78.30± 32.55
PRESENCE OF SHEATH	PRESENT
PRESENCE OF CAUDAL NUCLEI	ABSENT
NO OF NUCLEI IN SINGLE NUCLEAR COLUMN	4-12

Table-2 Polymorphism in ? *tubulin* gene in 93 microfilariae of *W. bancrofti.*

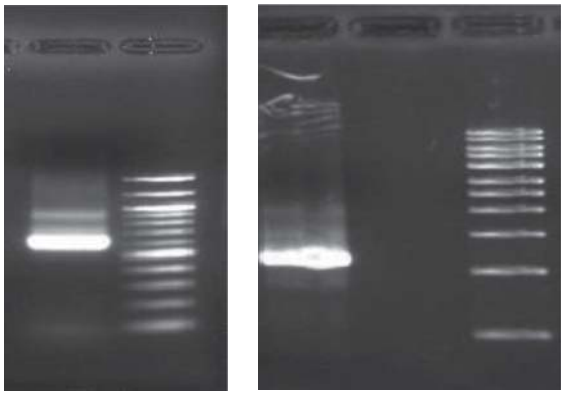
Sl no	MF code	1	2	3	4	5	6	7	8	9	10
1	PA-12	AA	AA	AT	AT	AA	AA	AT	AT	AA	AA
2	PP-9	AA	AA	AA	AA	AT	AA				
3	PA-102	AT	AT	AT	AT	AT	AT	AT	AT	AT	AA
4	PP-2	AA	AA	AT	AT	AT	AA	AA	AT		
5	PP-116	AA	AT	AT	AA	AA	AA	AA	AA	AA	AA
6	PP-5	AA	AA	AT	AT	AA	AA	AA	AT	AA	
7	PP-96	AA	AT	AT	AT	AT	AT	AT	AT	AT	AT
8	PP-24	AA	AA	AA	AA	AA	AA	AA	AA	AT	AT
9	PP-57	AT	AT	AT	AA	AT	AT	AT	AT	AT	AT
10	PP-14	AT	AA	AT	AT	AA	AA	AT	AA	AA	AA

Tubulin gene in 93 microfilariae is shown in Table-1. As per the experimental design we consider AA as dominant homozygous for sensitive allele, TT is the recessive homozygous which is for resistant and AT is the heterozygous allele. Out of 96 mf, 46 showed signal for AA alleles, and 47 for AT alleles. There was no signal indicating the presence of TT alleles.

Cloning and sequencing of Alt2 and ITS genes

100 mf were pooled from 10 purified mf sample (10 mf from each sample: 10 x10=100) and centrifuged at 10,000 rpm for 10 min to collect the mf as pellet. Genomic DNA was isolated by using Qiagen Micro Amp Kit. This DNA was used to amplify the target genes. Alt-2 gene was amplified from the microfilarial genomic DNA using primers (forward 5'- TGT ATC GCT GCA ATC TTT CAT T-3' and reverse 5'-GGT GCA CAG TAC GAA TAC TCC A-3' (Fig 2A). The amplified product was then cloned into TOPO. ITS-1 gene was amplified from the microfilarial genomic DNA using the primers, forward 5'- TTC CGT AGG TGA ACC TGC-3' and reverse 5'- CAT TTA TTA CTT ATC AGG GGA TTT TTG-3' (Fig-2B).

Fig-2



A **B**

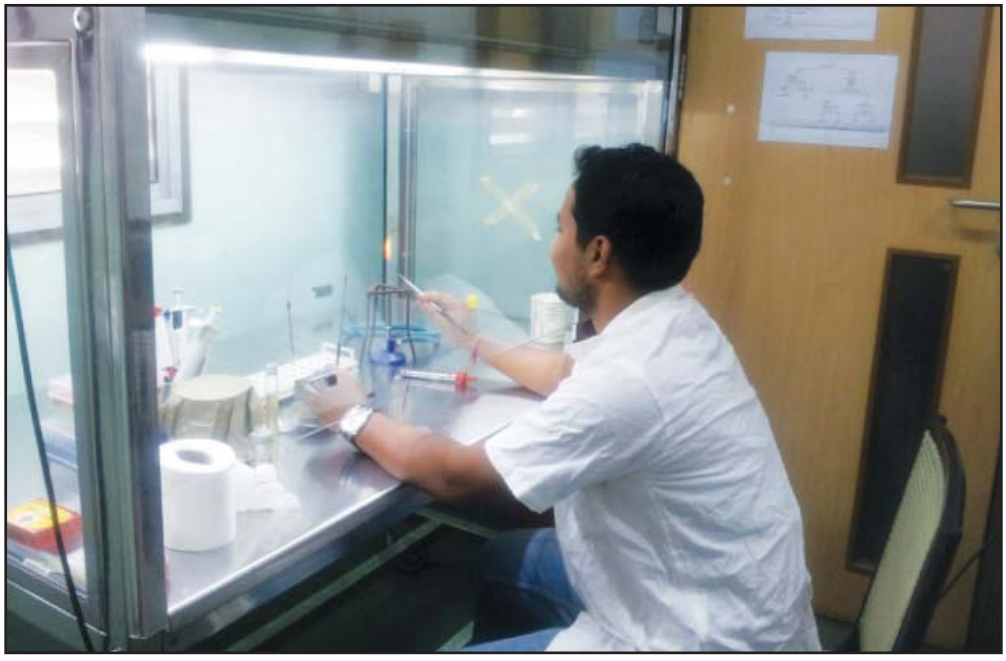
Amplification of Alt-2 (A) and ITS (B) genes from *Wuchereria bancrofti.*

Conclusion

Morphological parameters such as the body length, width, cephalic space, distance from head end to nerve ring and caudal nuclei of microfilariae of this region was examined by using LAS soft-ware. Each mf was photographed and stored in data bank. This data will be compared with other collaborating laboratories. We analyzed the single nucleotide polymorphism at 200 position of beta tubulin gene of *W. bancrofti.* A change in single nucleiotide replaces Phe (TTC) with tyrosine (TAC) at position 200 of beta tubulin resulting to Benzimidazole resistance in *W.bancrofti.* Out of 93 mf, 46 showed signal for AA alleles, and 47 for AT alleles. There was no signal indicating the presence of (Resistance) TT alleles.

Other Scientific Activities

Contents



Technology transfer to District Hospital staff of Rayagada

Demonstration of importance of hand washing
practices in school in tribal area of Rayagada District

1. Activities in RMRC Field Station at Raygada

The laboratory space provided by the state health department in Rayagada district headquarters hospital (DHQ) premises has been renovated from the budget sanctioned by ICMR during 2013. The laboratory facility comprised of separate rooms for (i) bacteriology (culture and drugs sensitivity testing) (ii) serology (iii) DNA / RNA extraction, PCR and gel documentation (iv) nutrition (v) data entry, reporting and sample receiving room and (vi) transit room. The minimum necessary equipments for above facilities purchased from ICMR grant have been installed. During the period under report the following activities have been undertaken.

(a) Diarrhoea including cholera

A continuous surveillance is being undertaken in the district to help the local health authorities and district administration to prevent epidemic. A strong networking has been established to collect, preserve and transport the rectal / stool samples from diarrheal patients and water samples from the sources used by the communities for drinking and domestic purposes to the Rayagada field unit laboratory. During March 2013 to Feb 2014 total 629 samples (rectal swabs: 422, water samples: 207) have been tested for diagnosis of diarrheal organisms. Out of the 422 rectal swabs, 243 samples were found to be positive for enterobacteriaceae. Of them 12.3 % (n=30) were *Aeromonas spp*, 47.3 %(n=115) for *V cholerae*, 33.3% (n=81) for *E coli*, 0.82 % (n=2) for *Salmonella* and 6.2 % (n=15) for *Shigella.* Similarly out of 207 water samples collected from sources like stream, nala and house hold water 1.4% (n=3) were positive for *V cholerae* . Timely identification and reporting has helped the local health authorities to check the outbreak of epidemic by implementing adequate control measures.

(b) Fever Survey

A total of 5541 individuals belonging to all age and sex groups from 22 villages of 3 sub-centres of Jemadeipentha PHC were included in the study. The age group composition showed 10.18% in under-five, 23.48% in 5-15 years and 66.34% above 15 years of age. Among total study subjects, 46.60% were males and 53.40% were females. Sex ratio of study population was 1146 per 1000 male.The survey indicated an overall morbidity prevalence of 14.98%. Morbidity prevalence was higher in female (16.94%) than male (12.86%) ($P < 0.001$). Febrile illness shown to have the highest prevalence of 7.69%, which was 6.85% in male and 8.47% in female ($p=0.033$). It was also higher in both extremes of age. Second common morbidity was musculoskeletal disorder found in 3.82%; of which 2.55% in males and 4.96% in females ($p=<0.0001$). Age wise distribution of morbidities indicated that, fever and diarrheal diseases were more prevalent in extreme age groups i.e. 0 to 5 years and > 60 year age while musculoskeletal disorder; eye infection and pulmonary tuberculosis (PTB) were more in other age groups.

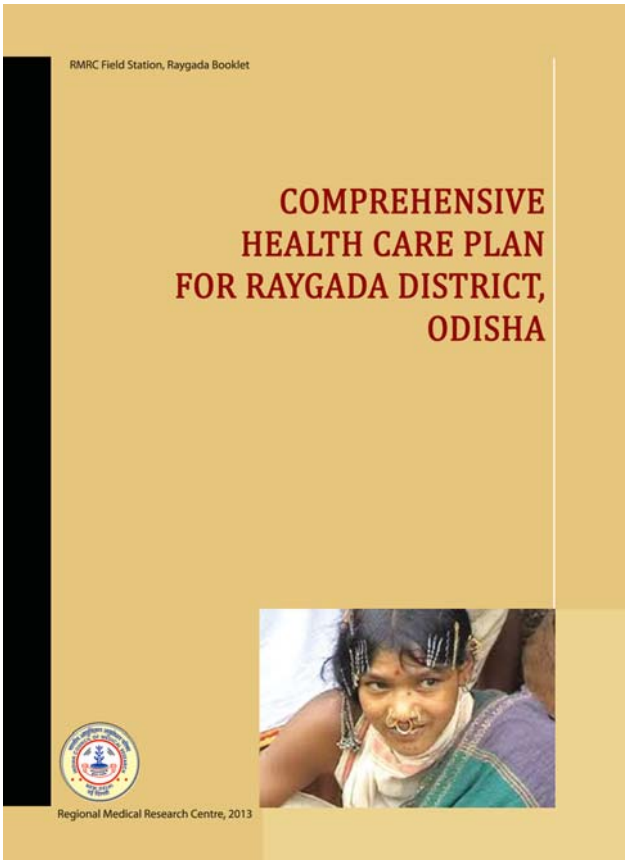
Out of total fever cases, flu like illness commonly called as viral fever contributed the most (47.22%) followed by acute respiratory tract infection (ARI) (19.22%) and malaria (15.73%). Fever was also found to be associated with acute gastroenteritis (6.57%), skin infection like scabies with secondary infection, chicken pox, measles, cellulitis (6.1%), ENT infections like acute and chronic suppurative otitis media (CSOM) and eye infections like conjunctivitis (5.16%) of individuals. Etiological agent responsible for acute respiratory tract infections isolated from throat swab were bacteria like *H influenzae*, *S. pneumoniae* in 19.6% cases and viruses like *Corona virus, Para influenza, Rhino virus* in 10.3% cases. Mixed infection was found in 08.2% cases. E coli infection observed in 30% of stool sample collected from diarrhoea cases.

The treatment seeking for various morbidities indicated that, only 21.1% individuals sought treatment from different sources for various illnesses, of which 55.5% received treatment from different

government health providers, 40.0% from trained and untrained practitioner and 4.5% from traditional healer. Average duration of seeking treatment from any health care source was 2 days. Perception of ASHA, AWW, GKS members and the general public towards the problem is being collected in pre tested format as part of the strategy development. The study is underway to develop a strategy to prevent the morbidity.

(c) Improving Health of Under 5 Children

A proposal to improve the health parameters of under 5 children with special reference to reduction of morbidity and mortality (prenatal, perinatal, childhood mortality and MMR) through health system strengthening using innovative approaches have been developed, discussed in the SAC and core committee of the Translational Research Committee of ICMR, Ethical committee of the institute and finally has been approved by ICMR. The main target of the proposed activity is to reduce the infant mortality rate (Raygada: 65/ Odisha: 62) and under-five mortality rate (Rayagada: 105/ Odisha 82) of the district to the level of state average. The project period is for three years (2014-2017).The fund is awaited to initiate the activity.



Books Published by RMRC on Raygada Field Station

2. Activities RMRC Field Station at Kalahandi.

The laboratory infrastructure was developed by remodeling one wing of the existing district headquarters hospital provided by District Health Authority. Civil and Electrical up-gradation was done with fund from ICMR. The laboratory facility comprised of separate rooms for:

A. Serology and Hematology

B. Bacteriology (Culture and Drugs sensitivity testing)

C. Entomology (Insect rearing and dissection)

D. RNA / DNA extraction, PCR and documentation

E. Data Entry, Reporting and Rest room

Equipments, we installed for above facilities with ICMR grant.

One research project has just been initiated for hypertension control through Diet & Life Style Modification in tribal population of Kalahandi district. Another proposal of operational research to reduce under five morbidity and mortality has been approved by ICMR as a translational project which is to be initiated soon.

Transfer of technology to District Health staff has been undertaken by providing hands on training to four paramedical personnel and two project assistants (MSc Biotech) identified by District Health Authority. They were trained on Bacterial Culture and Isolation

Books Published by RMRC on Kalahandi Field Station

of enteropathogen including Vibrio cholerae. ELISA based diagnosis of Dengue (NS 1, IgM), Chik (IgM), HBV (HbsAg), Measles (IgM) and PCR diagnosis of HPV and Malaria. The acquired skill was also evaluated by RMRC team. 15 Malaria technical supervisors of the district were trained regarding vector collection, processing and Identification. The laboratory is providing regular service to referred samples on hemoglobinopathy, diarrhoeal disorder, dengue and measles cases. Malaria control activities were also strengthened with above inputs.

Further strengthening of activities is planned with networking of all the PHCs under supervision of public health officials of the district.

3. Genetic characterization of E2 region of Chikungunya virus circulating in Odisha.

The work was carried out by one of the student of Dr. R. K. Hazra who was awarded with Lady Tata Memorial Trust and she has done the work with our field collected samples. The results are as follows

The CHIKV E2 peptide 57KTDDSHD63 (Fig 1) depicted the maximum score using the Immune Epitope Database and Analysis Resource and NetCTLpan 1.1 T-cell epitope prediction server in all the CHIKV isolates of Odisha. It bound to HLA alleles with high affinity and hence predicted to be the most probable T-cell epitope, thus suggesting it to be a highly regarded region for the formulation of synthetic peptide vaccines.

The CHIKV E2 gene consisted of three domains- A, B and C. Target-template identities ranged from 68% to 82% for the CHIKV E2 domains A, B and C. Adaptive mutations like E2-L210Q, E2-I211T and E2-V229I were mapped on the domain B thus making it

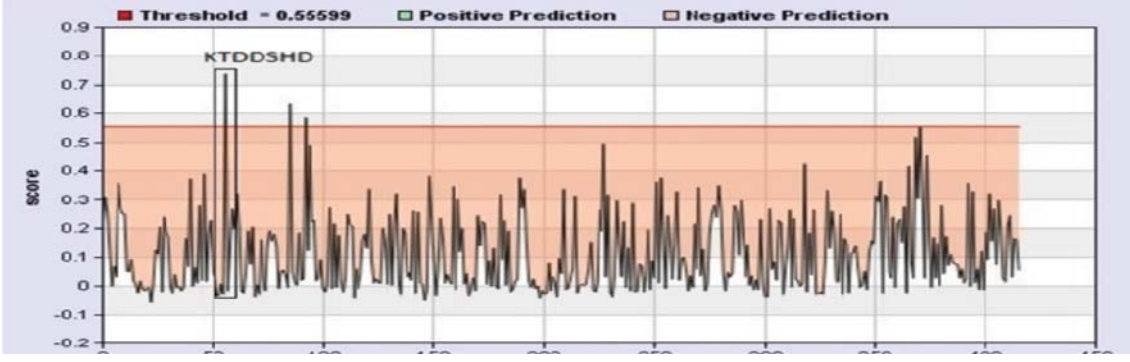


Fig 1. NetCTLpan prediction of T cell epitopes in the E2 region. The most probable T cell epitope (KTDDSHD) is above the threshold line (red) and is boxed.

the most potential region of CHIKV that can interact with host cell surface receptors for viral attachment. The most probable T cell epitope (57KTDDSHD63) and the common T and B cell epitope(84FVRTSAPCT92) were located in domain A which intended to be highly conserved. Most domains comprised of b strands while domain C also consisted of a long a helix (366–422 aa) and comprised the highly conserved ''TPY'' domain (398–400 aa) and the trans membrane domain (365–385 aa) (Fig 2). As shown, many of the mutations were mapped to areas with major secondary structures, and could affect the local protein structure.

The study depicted the circulation of IOL strains of CHIKV in Odisha which was in congruence to our previous study. Several genetic mutations were detected in the CHIKV isolates which had various roles during the evolution of the virus in diverse ecological environments. More importantly we predicted several T-cell epitopes among which peptide ^{57}KTDDSHD^{63} stands out to be the most probable epitope thus opening new options for both diagnostic and prevention of CHIKV infections. The peptide ^{84}FVRTSAPCT^{92} was detected to be the common T and B cell epitope that can induce both T and B cell immune response and hence can act as good vaccine candidates. Such T and B cell epitope based synthetic vaccines would be useful during outbreaks and can become an integral component for achieving protection. The amino acid positions 356–379 and 365–385 were found to be likely trans membrane helices and can be used as drug targets against CHIKV in treatment of the infection. Five positively selected sites (210, 211, 318, 375, and 377) were detected in the E2 region, which were observed to be fixed in most viral isolates.

Interpretation

Structural modelling revealed that E2 gene of CHIKV was composed of three domains and the major

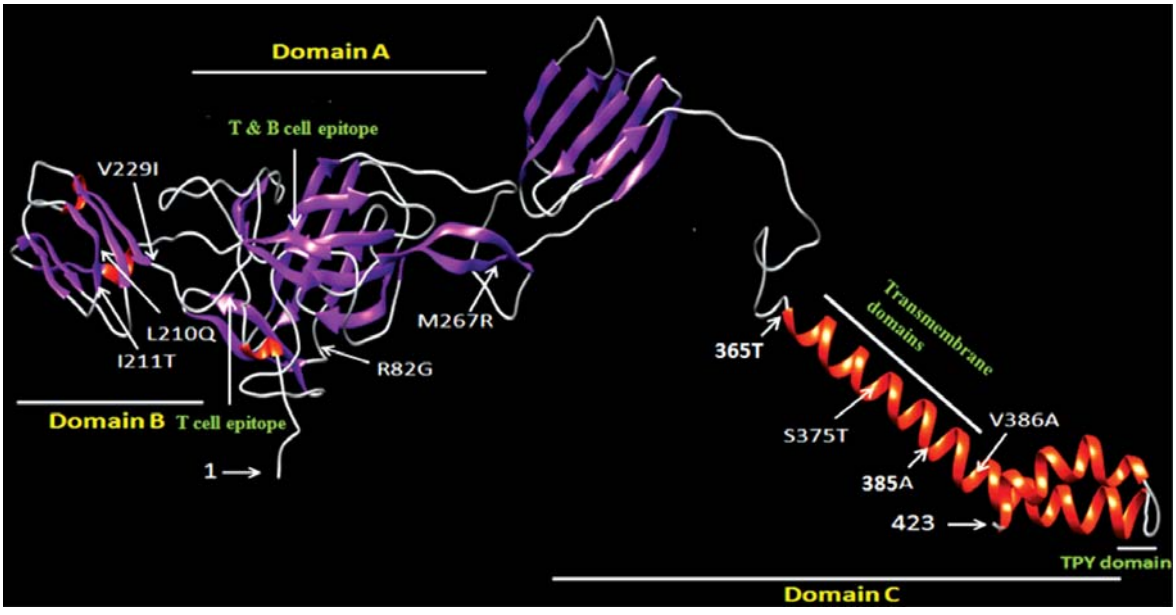


Fig 2. 3-D structure (cartoon style) of E2 protein of CHIKV generated in UCSF Chimera after homology modeling with certain selected mutations, T cell epitope (57–63 aa),TPY (398–400 aa) and trans membrane domains (365–385 aa) mapped. The secondary structure of E2 protein is shown in different colors: blue color denote b strands, red color denote a helix and grey color denote coils. The E2 gene consisted of three domains: A–C. 1 indicates the first amino acid at the N terminal end and 423 indicate the last amino acid at the C terminal end of the E2 gene.

adaptive mutations were detected in domain B of CHIKV, which can thus modulate binding of CHIKV to host cells, while the most probable T cell epitope and common T and B cell epitope was detected in domain A, which intended to be most conserved. Major secondary structure in most domains were b strands while domain C also consisted of a long a helix (366–423 aa) and comprised the highly conserved ''TPY'' domain (398–400 aa) and the trans membrane domains (365–385 aa).

The results of this study can elucidate thedominance pattern of certain lineages / variants and extinction or entry of certain strains of CHIKV in to evolutionary cul-de-sac in due course of time. The present study revealed many interesting facts about the variability of CHIK virus E2 region in an endemic environment. This is the first report from Odisha, India demonstrating a predictive approach to the genetic variations, epitopic regions and the trans membrane helices of the E2 region. Further molecular investigations of the signature residues at the E2 regions in newly evolving CHIKV strains around the globe would help to understand the changing pattern of this re-emerging virus and will aid in the development of vaccine / drug against CHIKV.The resurgence and persistence of CHIKV warrants the need for continuous monitoring and identification of arboviral vectors and genetic divergence of newly evolving variants with a view to plan for appropriate strategies for vector control and vaccine development.

4. Investigations of dengue virus during outbreak in Bhogorai Block of Balasore district, Orissa state, in 2011.

Background

Odisha state of India is divided into four distinct physiogeographical regions; northern plateau, central tableland, coastal plains and eastern ghats. As per the State Health Department, most parts of Odisha had been affectedby periodic outbreaks of dengue since 2011, out of which Bhograi block in Balasore district of coastal region has been the most affected and reported >70 dengue cases from June to August 2012. Bhograi block is a suburban area, located besides the sea, having a rich vegetation of forests with a population of about 40,521 (Census 2011). Due to its proximity to sea, people from nearbyBangladesh and other places often migrate to this region,thus increasing the risk of transmission of vector-borne diseases. The local people are mostly involved in pottery work and manufacture a variety of earthen pots for drinkingand commercial purposes.

Work done

At the request of the State Health Department, detailed entomological investigations focusing mainly on Aedes pupae were carried out by entomology team of Dr. R. K. Hazra from July to August 2012 in the most affected villages, i.e. Nimatpur, Kaubani and Kakada of the Bhograi block. Around 300 houses were inspected in each of the villages after stratifying the areas by attack rates. Adult mosquitoes were collected using battery operated aspirators in the houses surveyed. All water containing indoor and outdoor breeding containers were thoroughly searched for the presence of Aedes pupae, which were collected by using pipettes and dippers. Adult mosquitoes and pupae collected from field were brought to the laboratory. Pupae were left to emerge as adults for species identification. Emerged adults from the collected samples were identified as Aedes species and pooled according to species, sex and the type of container habitat. The data on pupal survey were analyzed and calculated in terms of different pupal indices, i.e. pupal container index (PCI), PPP and PPH. Abundance of indoor and outdoor containers with Aedes pupae at the collection sites was assessed in the study to know the most productive container in the areas surveyed. Productivity of a container type (the number of pupae in the container type divided by the total number of pupae in all the containers)

was estimated for each container that harbored Aedes pupae. Each mosquito pool (<10 mosquitoes), i.e. field collected adults and those emerged from pupae was subjected to laboratory processing for DENV identification by reverse transcription polymerase chain reaction (RT-PCR). The infection rate of each RT-PCR positive DENV mosquito pool was estimated using a maximum likelihood estimate (MLE) statistical method for unequal pool sizes that calculated 95% confidence interval (CI) per 1000 mosquitoes 13.

From the present study it can be concluded that the recent outbreaks of dengue in Bhograi block of Odisha were caused due to the circulation of DENV-2 and DENV-3, DENV-2 being the predominant serotype. DENV RNA was detected in pupae reared mosquitoes, which confirmed the vertical transmission of DENV, which is a major factor responsible for virus persistence and survival in nature for long periods, especially during adverse climatic conditions and inter-epidemic periods when thevector density is low and has an important role in the reemergence of DENV. High DENV infection rate in Ae.albopictus, the most abundant vector in the areas surveyed, rendered it to be the main arboviral vector in this region, also documented to be the major arboviral vector in Odisha in our previous .Aedes species, particularly *Ae. albopictus* was found mainly breeding indoors in all the affected areas, which was quite interesting finding in the study. Since, *Ae.albopictus* prefer to breed outdoors, hence more indoor breeding *Ae. albopictus* during outbreaks suggested change in breeding behaviour of *Ae. albopictus*. Earthen pots proved to be the most ideal indoor breeding spots in all the areas since most pupae wereobtained from earthen pots, thus having high productivity. During the outbreak, vector control measures, specifically targeting the elimination of pupal containers in human dwellings like earthen pots, plastic drums, etc. were undertaken by the local authorities, which generated public awareness among the people regarding DENV and its vector breeding behaviour. The State Health

Table 1. Aedes mosquito species collected, total number of pools, dengue virus positive pools and MLE after RT-PCR analysis in the affected villages of Bhograi block, Odisha.

Mosquitoes collected from the field	No. of specimens (pools)	DENV positive(pools)	Infection rate MLE (95% CI)	DENV serotypes detected
Ae. albopictus female	92 (9)	1	10.87 (0.64, 53.05)	2
Ae. albopictus male	17 (2)	0	0	–
Ae. aegypti female	11 (1)	0	0	–
Ae. aegypti male	8 (1)	0		
Ae. vittatus female	4 (1)	0		
Ae. vittatus male	0 (0)	0		
Total	132 (14)	1		
Pupae reared mosquitoes				
Ae. albopictus	485 (48)	4	8.49 (2.77, 20.36)	2*, 2+, 2*, 2&3*
Ae. aegypti	290 (29)	1	3.45 (0.20, 16.69)	2*
Ae. vittatus	41 (4)	0	0	–
Total	816 (81)	5	0	

*DENV positive pools in indoor breeding spots; +DENV positive pools in outdoor breeding spots; DENV–Dengue virus; MLE–Maximum.

Table 2: Abundance of indoor and outdoor containers with *Aedes* pupae alongwith their productvity in the affected villages of Bograi block, Odisha.

Villages surveyed	Distribution	Container type	No. of water filled containers	No. of containers with pupae	No. of pupae	Productivity of container	PCI	PPH	PPP
		Earthen pots	210	188	272	40.17	0.89		
	Indoors	Buckets	156	96	112	16.54	0.61		
		Plastic drums	110	69	101	14.91	0.62		
		Discarded tires	124	68	88	12.99	0.54		
Nimatpur		Tree holes	65	28	37	5.46	0.43	2.25	0.46
(n = 300)		Cement tanks	55	21	34	5.02	0.38		
(P = 1453)		Discarded small wates	35	16	18	2.65	0.45		
	Outdoors	(<3 litres)							
		Discarded large wastes	23	11	15	2.21	0.47		
		(> 10 litres)			**677**				
		Earthen pots	115	76	90	39.30	0.66		
	Indoors	Plastic drums	75	35	49	21.39	0.46		
Kaubani		Cement tanks	75	31	41	17.90	0.41		
(n = 275)	Outdoors	Tree holes	38	17	34	14.84	0.44	0.83	0.22
(P = 1012)		Discarded small wastes	23	10	15	6.55	0.43		
					229				
	Indoors	Earthen pots	108	66	78	47.85	0.61		
Kakada		Cement tanks	75	30	41	25.15	0.40		
(n = 300)	Outdoors	Tree holes	32	14	23	14.11	0.43	0.54	0.10
(P = 1512)		Discarded small wastes	17	7	13	7.97	0.41		
		Tires	16	6	8	4.90	0.37		
					163				

n= No.of houses inspected, P=No.of people in inspected houses, Productivity of container = No.of pupae in the container type x 100 / total no. of pupae, PPH (Pupae per house) = No. of pupae / No. of houses inspected, PPP (pupae per person) = No. of pupae / No. of people in inspected houses, PCI = Pupal container index = No. of pupal positive containers / No. of containers searched, Numbers in bold indicate the total number of pupae in each village.

Department, Odisha also implemented strategic measures, aiming primarily at source reduction of vectors by eliminating the most productive containers in the affected as well as nearby areas to prevent the further spread of dengue.Hence, the study suggests extensive entomological surveyswith greater emphasis on intradomecile vector control methods for reducing the transmission of DENV to new areas.

5. Hemoglobinopathy detection among patients from Government hospitals of the Odisha.

In addition to the SCD screening activity, Haemoglobinopathy testing of the referred cases from Government Hospital was also carried out with intramural funding with the objective to support Government Medical colleges of the State where there is no facility for these tests. Under this a total of 72 patients have been tested and clinical report has been given to the Hospital authorities.

Result

Out of a total 72 referred patients from Capital Hospital tested for haemoglobinopathies 32 were normal, 19 beta-Thalassemia trait, 13 Sickle cell trait and 5 Sickle cell disease, 1 beta-thal major,1 Hb E trait and 1 was found to be homozygous for Hb D.

6. Tuberculosis activity in RMRC.

Support to State Government on Programmatic Management of Drug Resistant TB

Providing culture and drug sensitivity testing of MDR TB follow up sputum samples for seven tribal districts of Odisha.

Procedure

Sputum samples of MDR TB patients undergoing DOTS Plus treatment were sent to RMRC, Laboratory by the District Tuberculosis Officer from the respected districts using courier services for solid culture and second line anti TB drug testing. On arrival of samples at RMRC, the sputum samples were processed by Petroff's method for culture and a deposit smear was made for ZN staining for AFB. The processed specimen was inoculated to two LJ and one LJ-PNB slants. The growth of mycobacteria were observed up to 8 weeks and colonies showing rough, buff colored colonies were subjected to 2nd line anti TB drug testing with ofloxacin, kanamycin and ethionamide. The results of microscopy, culture and DST were communicated electronically to concerned DTO and state TB officer.

Result

Up to now 40 sputum samples from two districts were received and results of 28 samples were communicated to the concerned DTO. Growth of mycobacteria was observed in two sputum samples so far. The study is in progress.

7. Establishment of a "Biomedical Informatics centre".

Activity on establishment of a "Biomedical Informatics centre" under the project "Biomedical Informatics centre of ICMR" has been taken up. Dr. Sapna Negi is the Principal Investigator for the same. Under this, two rooms has been identified for building up the facility which will have One work station, 5 computers, one printer and one scanner. Space for further development of the centre and for future server has been considered and CPWD has been approached for renovation and electrical fittings. Interviews has been conducted for the recruitment of staff which includes scientist II (one post), Scientist I (one post) and Research Assistant (one post) on 22nd Nov 2013, and recruitments of one RA, Scientist 1 has been done recently.

Works of Ph.D Scholars

SAC expert dicussing with Ph.D Scholars during SAC meeting poster session

1. Malaria parasite development in insecticide resistant mosquito: Role of glutathione S-transferase (GST) in mosquito immunity.

Post Doctoral Fellow (ICMR) : Dr. Asima Tripathy
Mentor : Dr. S. K.Kar, Director
Project started : 22nd May 2013
Project Duration : Two years

Background

Plasmodium development with its mosquito vector is an essential step in malaria transmission. The innate immune system of most mosquitoes is able to completely clear a *Plasmodium* infection, preventing parasite transmission to humans. Previous studies have shown that higher levels of ROS in mosquito hemolymph limit *Plasmodium* development. Our previous study showed that an enhanced ability of the insecticide resistant mosquitoes tolerated oxidative stress by the protective role of Glutathione S-transferase and also showed high level of production of induced GST in blood fed mosquitoes and during gonadial development. The intake of blood meal by the mosquito brings metabolic changes and induces a state of oxidative stress and further it is increased by the presence of *Plasmodium* parasites in the blood meal. So, in both the cases the mosquito passes through a stress management pathway. There might be an association between the response to *plasmodium* infection and insecticide resistance, thus enhancing the importance of further studying their interaction. Till date specific mechanism by which increased levels of GST may lead to parasite development inside the normal and resistant mosquitoes remains to be established.

Objective

To observe the role of GST in development of malaria parasite in normal and resistant mosquito vector

Plan of Work

Anopheles stephensi mosquitoes will be reared in the Department of Entomology, Regional Medical Research Center (RMRC). The female mosquitoes will be exposed to DDT 4% as per the standard WHO protocol and percentage of mortalities will be calculated and analysed on a log-time probit mortality regression. The resistant mosquitoes will be infected with *P. berghei* by feeding on anesthetized infected mice and *P. berghei* midgut infection will be quantified 48 h post-infection by the method of Usui et al, 2011. Simultaneously the *P. berghei* infected mosquitoes will also be exposed to DDT 4% to observe their resistance status. From each experimental groups, including control, mosquitoes will be taken for GST assay and will be correlated further with the susceptibility status of the vector to parasite, host efficiency index of the host vector, gonotrophic cycle, infectivity rate of the parasite inside the host vector, sporozoite rate and sporogony of the vector, survival status and longevity of the mosquito vector. The findings will be analyzed with appropriate tests such as descriptive analysis, t-test, test of proportion, ANOVA. Animal care guidelines will be followed according to the Regional Medical Research Centre (ICMR) Animal Care and Use Committee (ACUC).

Progress of the Work

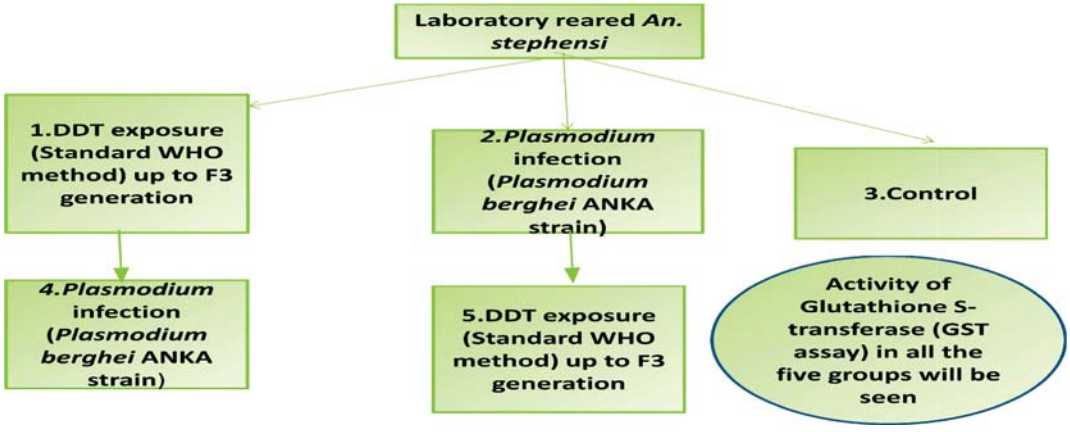
Rearing of mosquito

Anopheles stephensi mosquitoes rearing has already been started and colonies are maintained in the Department of Entomology, Regional Medical Research Center (RMRC), Bhubaneswar at a temperature 28 ± 2^0C and relative humidity 80 ± 10% as per the standard protocol (RMRC Annual Report, 2011).

Ethical Committee clearance

The Animal Care and Use Committee (ACUC) reviewed the study protocol of the project and accorded its permission to utilize Balb / C mice for the project work.

Future Study design



Expected outcome

(a) The will lead to development of smart insecticides (targeting GST) capable of disrupting the protective pathways of *Plasmodium* parasite development and insecticide resistance in mosquito vector.

(b) It will provide some insight into transmission blocking (TB) in vector targeting GST.

2. Study of Aedes mosquitoes in various parts of Orissa with reference to transmission of arboviral disease.

Name : Biswadeep Das
Status : SRF(ICMR)
Date of joining : January 2010.
Guide : Dr. R.K.Hazra

Objectives

1. To study the distribution and bionomics of Aedes mosquitoes involved in disease transmission in different parts of Orissa.
2. To develop a molecular method for identifying the immature stages of different Aedes mosquitoes that will be collected from various parts of Orisaa.
3. Iii.To identify pathogenic viruses in Aedes mosquitoes collected from different regions of Orissa by molecular methods.
4. To study the genetic factors of CHIKV and DENV and their role in mediating vectorial transmissibility in different regions of Orissa.

Work progress

In the previous report, comprehensive entomological survey was undertaken in the coastal areas of Orissa and a multiplex PCR was developed to distinguish the three Aedes species commonly found: *Ae. albopictus*, *Ae. aegypti* and *Ae. vittatus*. Discarded tires were the main Aedes breeding spots and *Ae. albopictus* are the most abundant species. Chikungunya and dengue outbreaks were surveyed in the several areas and studies revealed that both the diseases are transmitted among humans by *Ae. aegypti* and *Ae. albopictus* which are anthropophilic mosquitoes and are peridomestic. In this report, the molecular aspects of chikungunya and dengue virus and arboviral vector are discussed in relevance to transmission of the diseases. Cases with suspected CHIKV infection belonged to the age group 4-80 years with majority of symptomatic cases occurring in the age group 25-50 years. Since maximum number of CHIKV cases occurred in the age group 25-50 years, a group that is actively involved in work during daytime, it suggests a corollary with the day feeding habits of the *Aedes* mosquito. During the first 2 days of fever, viremia was detected by RT PCR in about 90% of patients, whereas IgM antibody was detected

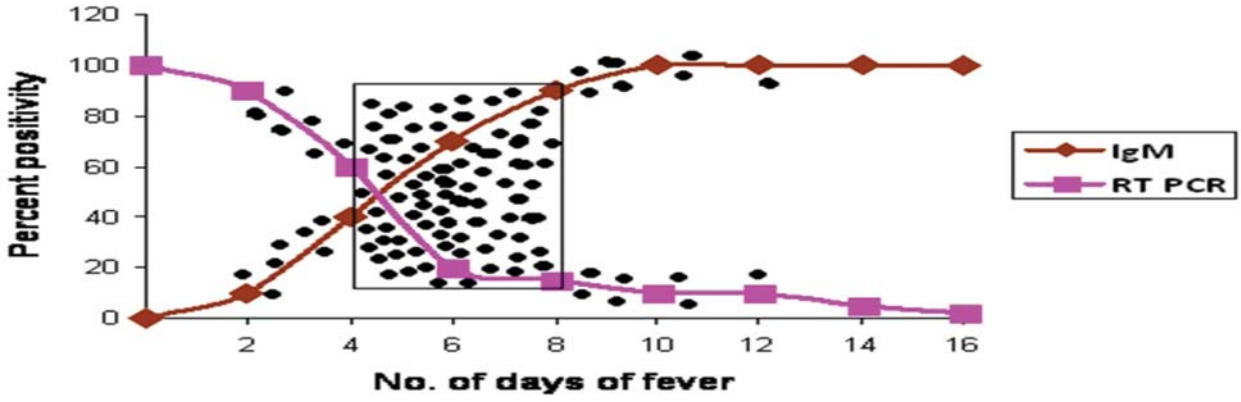


Fig 1. Percentage of CHIKV and DENV +ve sera tested by RT-PCR and IgM against number of days of fever. Most cases (black dots within box) were collected at 4-8 days of fever.

in approximately 10% of patients. The chance of having viremia decreased as the IgM antibody developed. During 4–10 days of fever, the IgM antibody developed in all patients, while CHIKV was still detectable by RT-PCR in approximately 15% of patients. The present study indicated high RT-PCR positivity in comparison to serology which may be attributed to the collection of sera at a very early stage or acute stage of the illness (Fig 1). Thus the RT-PCR technique was useful in detecting the viral infection in the acute / early phase during an outbreak.

Selection analyses within the E1 and E2 genes of CHIKV isolates of Orissa revealed certain codons to be positively selected; thereby indicating that CHIKV evolution in Orissa is probably governed by adaptation of certain sites, which appears to be advantageously selected in the course of evolution of CHIKV. Although factors related to CHIKV pathogenesis remains an unsolved conundrum, by virtue of their transmission capability by different vectors and experiencing radical mutations, these sites under adaptive evolution are presumed to be contributing to E2's role in host and cell tropism, virus yield and transmission through some unknown

Table 1. Sites identiûed to be under positive selection in E2 gene by Datamonkey web interface of the Hyphy package.

Codon position	SLAC method		FEL method		IFEL method		REL method		FUBAR method	
	dN-dS[a]	p-value	dN-dS[a]	p-value	dN-dS[a]	p-value	dN-dS[a]	Bayes factor (p value)	ω=β/α	Posterior probability (p-value)
210L/Q	-	-	0.312	0.058	0.342	0.051	-	-	4.492	1.57
211I/T	-	-	-	-	0.152	0.058	-	-	-	-
318V	5.59	0.077	0.441	0.05	0.764	0.012	0.472	82.897	-	-
375S/T	-	-	0.421	0.071	0.654	0.067	-	-	-	-
377I	-	-	-	-	0.174	0.068	-	-	-	-

'a' indicates Normalized dN-dS values; '-' indicates position not detected. p value < 0.1 / Bayes factor> 50 / posterior probability value > 0.9 was considered to be statistically significant.

molecular interactions in context to host cellular factors of never-ending dynamics of mutant generation and selection driven ûtness testing (Table 1).

Molecular investigations of chikungunya virus during several outbreaks in Odisha revealed the circulation of a unique strain, Indian Ocean Lineage (IOL) which was further subdivided into Indian subcontinent and Indian Ocean clades. IOL strain originated from the ECSA genotype of CHIKV which was supposed to be the predominant strain circulating in India since 2006. It was supposed to be originated from Kenya, 2004. The CHIKV strains from Odisha possessed the primary adaptive mutation, E1-A226V along with second step adaptive mutations, E2-L210Q and E2-I211E that epistatically modulated the E1-A226V mutation to incerese the transmissibility of CHIKV by *Ae. albopictus* by enhancing the rapid dissemination of virion particles in the salivary gland of the vector. Among the structural proteins, E1 and E2 proteins play major role initiating the entry of CHIKV in the host cell. Domains I and II of the E1 protein are involved in E1 trimerization during the viral fusion process at the time of infection. Domain II mediates the E1–E2 interaction during the virus maturation and budding from infected cells. Mutations detected in the E1 gene of Orissa CHIKV isolates were mapped across all functional regions / domains (Fig. 2). The primary adaptive mutation, E1-A226V was located within the fusion loop of the domain II, indicating this region plays a major role in the evolution of CHIKV E1 protein. The T and B cell epitope, located in the domain II, was found to be highly reactive with high solvent accessibility, which indicated it react actively with the immunoglobulins and hence can initiate immediate immune response.

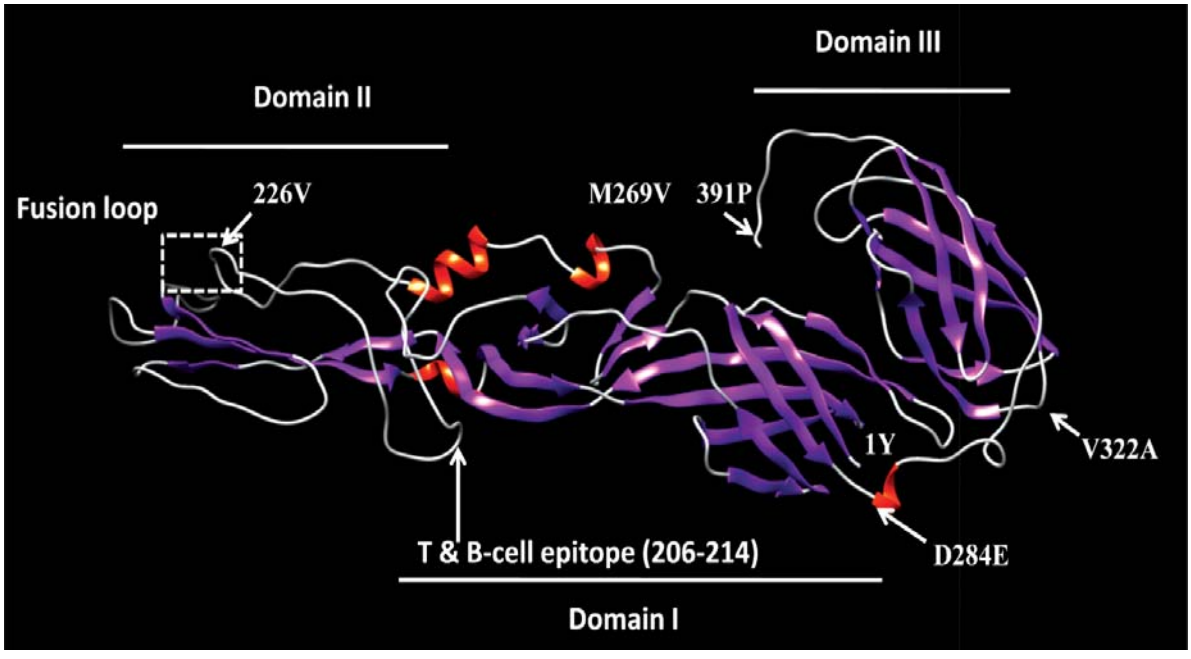


Fig 2. 3-D structure (cartoon style) of E1 protein of CHIKV generated in UCSF Chimera after homology modeling with certain selected mutations, T and B cell epitope (206-214) and major mutations mapped. The secondary structure of E1 protein is shown in different colors: blue color denote â strands, red color denote á helix and grey color denote coils. The E1 gene consisted of 3 domains: I,II and III. 1Y indicate the first amino acid at the N terminal end and 391P indicate the last amino acid at the C terminal end of the E1 gene.

The folding back of domain III and its interactions with the E1 trimer during fusion are critical events in alphavirus entry. Domain III in all the CHIKV isolates was found to be conserved without any mutation, and hence depicted more stability in the 3-D model (Fig 3). In the E2 protein, most of the mutations fell in the region between amino acids 210 and 386. This stretch of the protein (aa 1–364) in alphaviruses forms a highly hydrophilic domain. The T and B cell epitopes in the alpha virus was located in domain B and comprised mainly of non polar amino acids, with high hydrophobicity and without any mutation, which indicated it to be highly conserved and hence can be effectively used as immunogenic maker of CHIKV during early infection.

From the present results, it can be concluded that the recent outbreaks of chikungunya in Orissa have been caused by viral strains of IOL group of the ECSA genotype with adaptive mutations like E1-A226V, E2-I211T and E2-L210Q and several other mutations, which in turn has favored *Ae. albopictus* to be the main arboviral vector in this region. The rapid spread of the infection may be attributed to high viral load in *Ae. albopictus* species, which is the most abundant arboviral vector in Orissa and can efficiently transmit the virus to new areas. This study explored several genetic and immunogenic markers of CHIKV, which can be exploited for the development of anti-CHIKV measures, subject to rigorous experimental validation.

Molecular phylogenetic analyses using C-prM gene of DENV revealed the circulation of Indian lineage of DENV-2 (genotype-IV) and DENV-3 (genotype-III) in vectors and patients' serum during recurrent outbreaks in Orissa (Fig. 4 & 5). DENV-2 was found to be the more prevailing serotype (85 %) as compared with DENV-3 serotype (15%) in the cases.

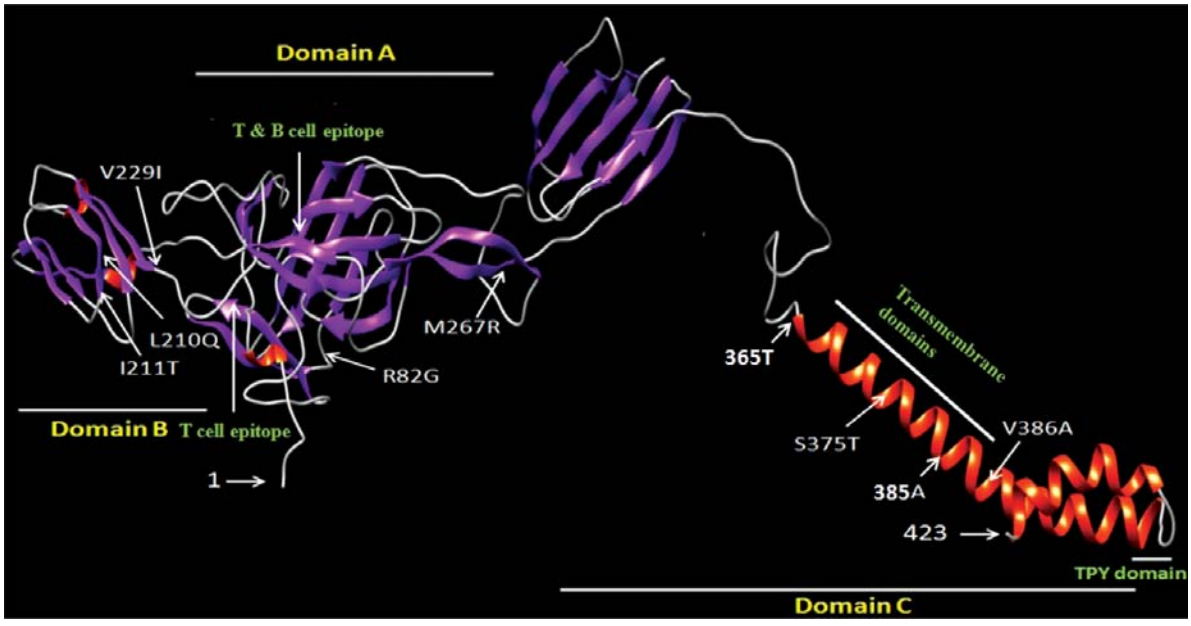


Fig. 3. 3-D structure (cartoon style) of E2 protein of CHIKV generated in UCSF Chimera after homology modeling with certain selected mutations, T cell epitope (57-63 aa), TPY (398-400 aa) and transmembrane domains (365-385 aa) mapped. The secondary structure of E2 protein is shown in different colors: blue color denote â strands, red color denote á helix and grey color denote coils. The E2 gene consisted of 3 domains: A, B and C. 1 indicate the first amino acid at the N terminal end and 423 indicate the last amino acid at the C terminal end of the E2 gene.

Both the genotypes are highly virulent and have been associated with repeated dengue outbreaks in India. Since co-circulation of virulent genotypes have been associated with the occurrence of recurrent DHF epidemics, hence co-circulation of DENV-2 (genotype-IV) and DENV-3 (genotype-III) may lead to more severe epidemics in Orissa unless proper anti-dengue measures are implemented. Selection analyses within the C-prM region of the DENV-2 and DENV-3 isolates revealed that most codons within the C-prM region were conserved, thereby indicating that DENV-2 and DENV-3 evolution in Orissa is constrained by purifying selection. This suggests the possible contributions of other ecological factors such as mosquito density and behavior and susceptible human population to the recurrence of dengue outbreaks in Orissa.

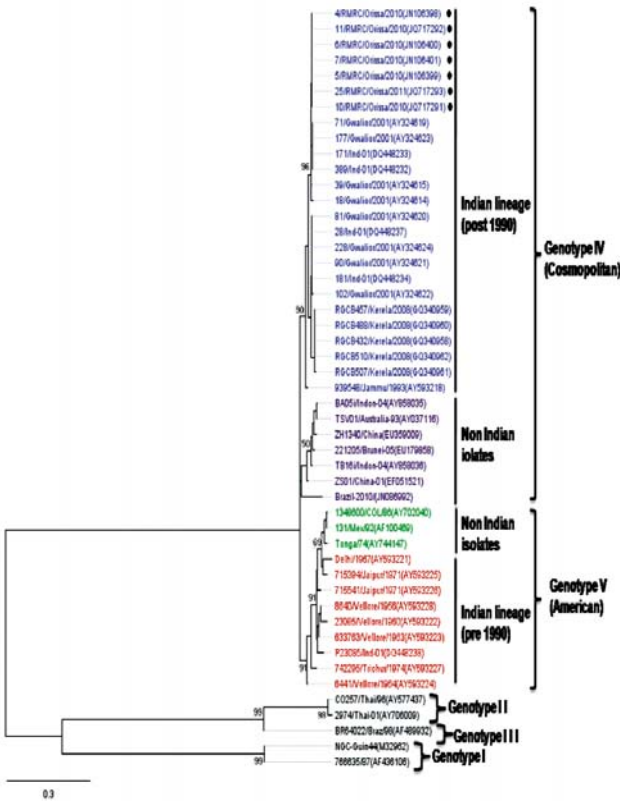


Fig 4. Mid point rooted phylogenetic tree of DENV-2 viruses generated by maximum likelihood method based on C-prM gene. Each strain is identified by its name and the year it was isolated, followed by GenBank accession numbers in parentheses. Solid circles denote viral RNAs isolated and sequenced from mosquitoes and serum obtained from Orissa. The tree depicted 2 broad genotypes containing Indian isolates: Genotype V (American) comprising Indian isolates before 1990 (red) and Non Indian isolates (green) and Genotype IV (Cosmopolitan) subdivided into Indian lineage after 1990 (blue) and Non Indian lineage (dark blue). Bootstrap values are indicated at the major branch points.

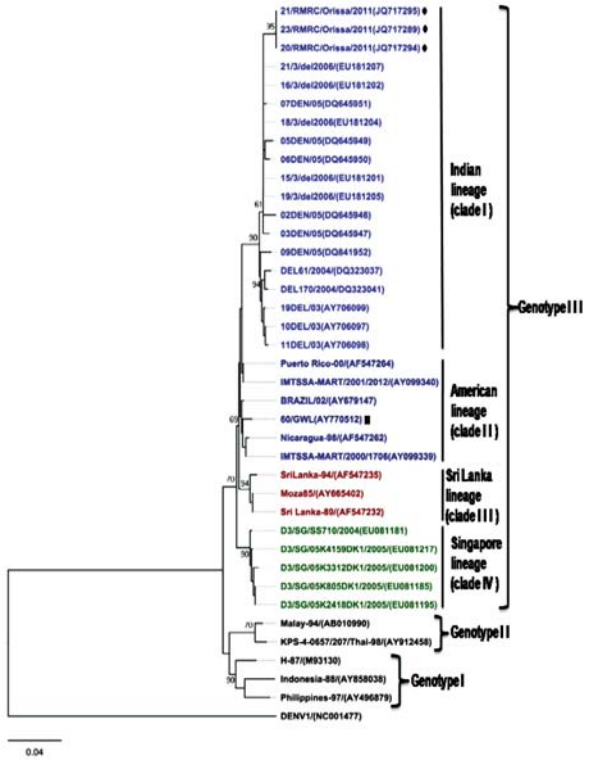


Fig 5. Mid point rooted phylogenetic tree of DENV-3 generated by maximum likelihood method based on C-prM gene. Each strain is identified by its name and the year it was isolated, followed by GenBank accession numbers in parentheses. Solid circle denotes viral RNAs isolated and sequenced from serum and mosquitoes obtained from Orissa. The Indian isolates grouped into genotype III, which was subdivided into 4 clades: clade I represented Indian lineage (blue), clade II represented American lineage (dark blue), clade III represented Srilankan lineage (red) and clade IV represented Singaporean lineage (green). Solid square represented Gwalior-60 isolate of India which was exceptional and grouped within American lineage.

Publications

1. Das B, Sahu A, Das M, Patra A, Dwibedi B, Kar SK, Hazra RK (2012). Molecular investigations of chikungunya virus during outbreaks in Orissa, Eastern India in 2010. Infect Genet Evol. 12 :1094-1101.

2. Das B, Swain S, Patra A, Das M, Tripathy HK, Mohapatra N, Kar SK, Hazra RK (2012). Development and evaluation of a single-step multiplex PCR to differentiate the aquatic stages of morphologically similar Aedes (subgenus: Stegomyia) species. Trop Med Int Health. 17: 235-43.

3. Sahu A, Das B, Das M, Patra A, Biswal S, Kar SK, Hazra RK (2013). Genetic characterization of E2 region of Chikungunya virus circulating in Odisha, Eastern India from 2010 to 2011. Infect Genet Evol. 18C :113-124.

4. Das B, Das M, Dwibedi B, Kar SK, Hazra RK (2013). Molecular investigations of dengue virus during outbreaks in Orissa state, Eastern India from 2010 to 2011. Infect Genet Evol. 16: 401-410.

5. Das B, Hazra RK (2013). Entomological investigations of dengue outbreaks with special attention to pupal indicators of Aedes mosquitoes in coastal Orissa. J Vector Borne Diseases. 50: 147-150.

6. 48 sequences submitted to Genbank : HQ010436 (Aedes vittatus), HM486433 (Aedes aegypti), HQ010437 (Aedes albopictus), dengue virus (JN106398-402), chikungunya virus(JN711127-JN711138) (JQ012933- JQ012946).

3. A study on neurotropic viruses causing encephalitis in adults and children of Odisha.

Research Scholar : Sushil Kumar Rathore
Guide : Dr B. Dwibedi

Introduction

Encephalitis is one of the life threatening diseases. It is the inflammation in the brain parenchyma resulting from direct viral invasion or hypersensitivity initiated by virus or another foreign protein. Sudden fever, stiff neck, photophobia, confusion and convulsions are some characteristic symptoms of viral encephalitis. It can occur in the individuals of all age group. Generally children are more affected than adults, so also adults that have compromised immune system and elderly people. The major causative agents are viruses but bacteria,parasites, protozoa and fungi have also been reported. Virus causing endemic and sporadic

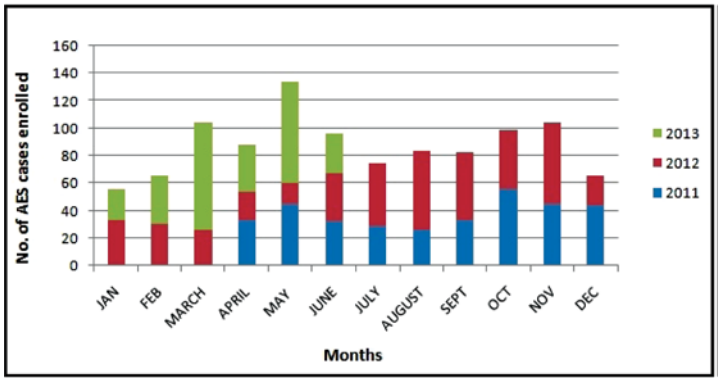


Fig. 1: No. of cases enrolled in every month during the study period.

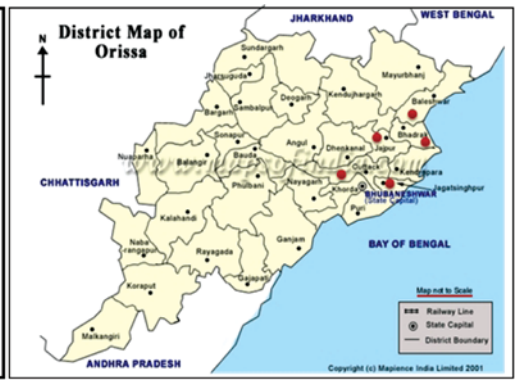


Fig. 2: Catchment area showing majority of cases enrolled with Encephalitis.

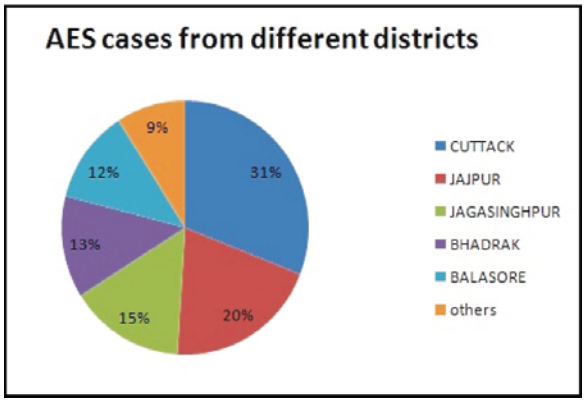


Fig. 3: Districts with higher no. of cases.

Table 1. Clinical features of the cases investigated.

CLINICAL SIGNS/SYMPTOMS	VIRAL AGENTS (%)	
	IDENTIFIED	UNIDENTIFIED
Fever	87.3	27
Convulsion	47	45
Altered Sensorium	28.2	32
Meningeal Signs	31	25.2
Paresis	6.6	2
Cranial nerve palsies	9	1
Vomiting	40.6	32

encephalitis throughout the world are Japanese encephalitis virus (JEV), Herpes simplex virus (HSV), Enterovirues(EV), Myxo / paramyxoviruses and Chikungunya.

Objective

1. To identify the causative viral agents of encephalitis.
2. To study the clinical presentation in encephalitis due to different viruses.

Materials and Methods

1152 patients were enrolled for the study after being physically and clinically diagnosed by the concerned physician. Clinical and demographic information were recorded on predesigned format together with physical examination. Samples (serum / CSF) were collected as per the standard guidelines of venipuncture and lumbarpuncture. Samples were

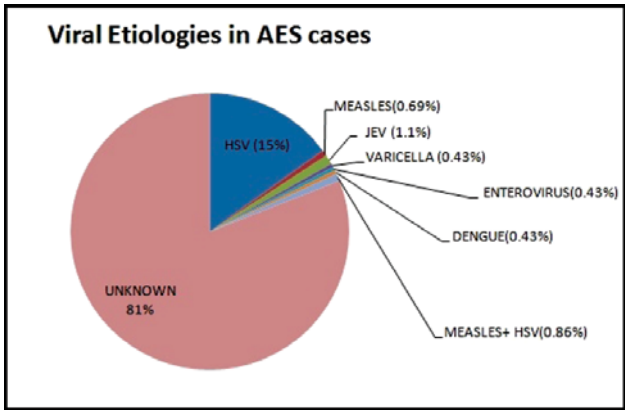


Fig.4 Percentage of Viral agents in AES cases.

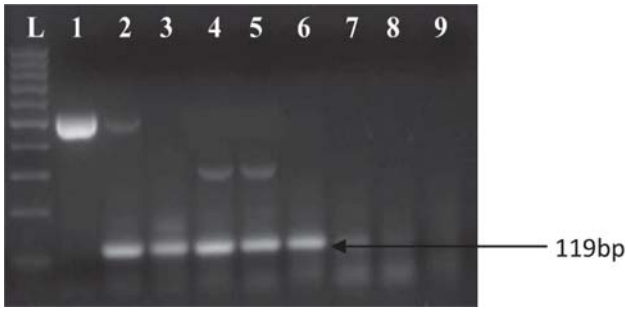


Fig.5 Amplified RNA from serum and CSF (lane 2) samples of Dengue cases. Lane 1 is the first PCR product and lane 2 is nested product at 119bp.

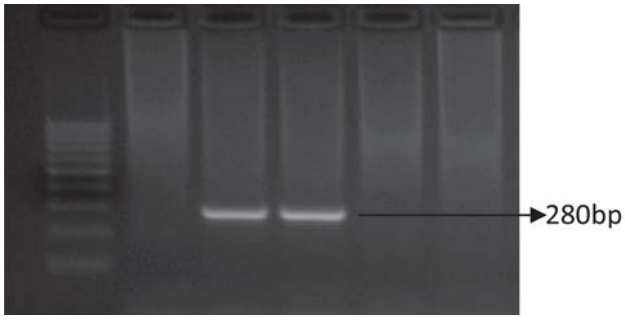


Fig.6 Amplified DNA from CSF samples of HSV encephalitis cases. L (Ladder)1-2 are samples and 3rd is Negative contol.

aliquoted and stored at -20^{0}C and -80^{0}C for serology and PCR respectively. Samples were subjected to serology and PCR. IgM ELISA was done for HSV I and HSV II, Measles, Dengue, Varicella, EV and JEV.

Results

1152 no of cases from six different hospitals were tested. The study group comprised of patients with mean age of 7 ± 3.1 and male to female ratio of 1.08:1 in the patients below 14yrs while mean age 28 ± 2.9 and male to female ratio 1.8:1 in patients above 14yrs.

The cases were found to be distributed throughout the year and month wise distribution is shown in Fig.1. Higher numbers of AES cases were enrolled during September to December, 2012 that falls on post monsoon and early winter seasons in this region.

Most of the hospital cases of encephalitis have been reported from Cuttack. Jajpur, Jagatsinghpur, Bhadrak and Balasore were other districts from where patients had been reported.

Clinical Features:

The clinical features of the identified and unidentified cases have been shown in table no. 1. Patients suspected with measles encephalitis had rash all over their body. Patients with dengue encephalitis had significantly decreased thrombocytes count**.** The patients with post varicella encephalitis had marked features of chickenpox with vesicular rashes before 0-15 days of hospital admission.

Viral Etiology

Viral etiologies were identified in 219 patients out of 1152 enrolled patients. The most common etiology was HSV infection (183, 15.8%) followed by JEV (26, 2.5%) and Measles (18, 1.5%).5 cases of Varicella, EV and Dengue was detected in each. Ten encephalitis patients suffering from Measles were also positive for either IgM antibodies or PCR against HSV (3 HSV PCR, 7 HSV I IgM). Single case of definite dual infection with dengue and JEV was detected.

Conclusion

This study reported viral aetiology of AES in 17.2 % cases attending tertiary care hospitals from Odisha and neighbouring states of eastern India and HSV was found to be the major viral agent. Other viral causes identified were Measles, Varicella, JEV and Enteroviruses. Case fatality rate was10% among viral AES cases while it was 6.2% in AES subjects without viral aetiology. Dual infection of HSV and Measles was observed during the study which is a rare evidence of simultaneous infection of both causing encephalitis. This is the first report on viral aetiology of AES from this region and the findings will be useful for better management of viral AES especially due to HSV and planning of strategies for the preventable infection like vector borne diseases, Measles and Chickenpox. The described clinical presentations will also be useful for syndromic management of AES cases in resource poor settings in the world that lack proper laboratory investigation facility.

4. Bacterial meningitis and pneumonia among pediatric age group in Odisha.

Research Scholar : Chinmayee Priyadarshini Khuntia
Guide : Dr. S. K. Kar
Co-guide : Dr. B.Dwibedi

Background

Bacterial meningitis is an important disease especially of early childhood with high case fatality and risk of neurological disorders. The fatality rate associated with this disease is as high as 20-30% in neonates and children world-wide (Xavier et al., 2003) whereas in India and other developing countries fatality rate has been quoted as 16-30%. A wide range of bacteria are associated with meningitis and 80% of

all cases of bacterial meningitis are caused by Haemophilus influenzae, Neisseria meningitidis, Streptococci pneumoniae (Hart, 2003). Over two third of all the cases of bacterial meningitis occur in children less than 5 years age group. The relative frequency of etiological agents of bacterial meningitis varies with age and geographical region. The burden of disease from bacterial meningitis is higher in low resource setting with poor health infrastructure of developing countries because of high rate of malnutrition generally poor living condition and inadequate access to preventive and curative services which may predispose individuals to infection and opportunities for optimal treatment.

Objective

1. To isolate Streptococci pneumoniae, Haemophillus influenzae and Neissseria meningitidis from hospitalized meningitis children under age group of 5 years.
2. Identification of the isolates on the basis of their biochemical properties and serological characteristics.
3. Surveillance of prevalence of serotypes of Streptococci pneumoniae, Haemophillus influenzae and Neissseria meningitidis in orissa.
4. To assess antibiogram trends in Streptococci pneumoniae, Haemophillus influenzae and Neissseria meningitidis.
5. Detection of species specific virulence genes of Streptococci pneumoniae, Haemophillus influenzae and Neissseria meningitidis using specific primers by PCR assay
6. Genetic lineage and clonality study of Streptococci pneumoniae, Haemophillus influenzae and Neissseria meningitidis isolates by PFGE and RAPD PCR assay.

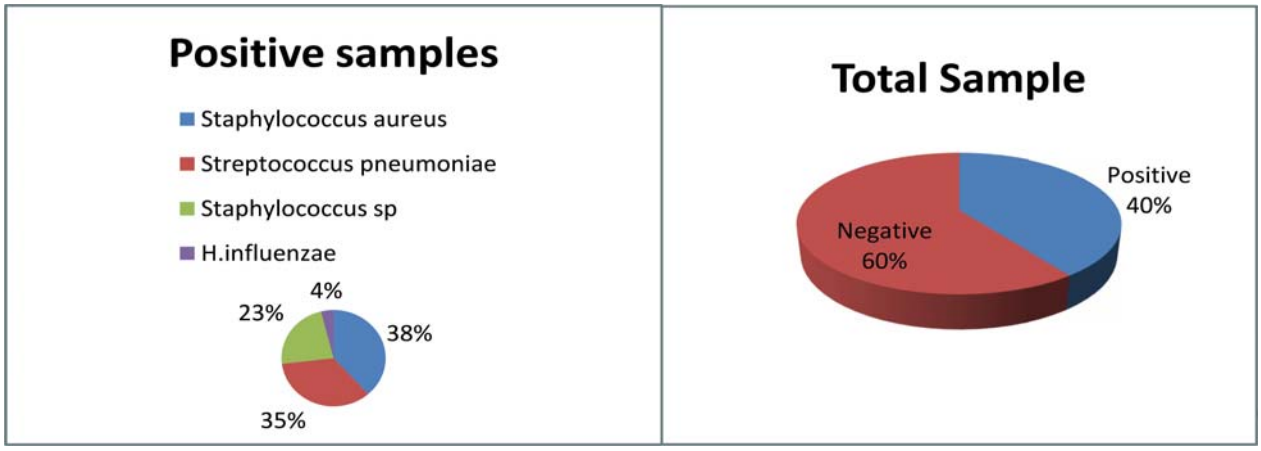
Plan of work

Blood and CSF samples were collected from suspected pneumonia and meningitis cases of different pediatric age group less than 5 years for the isolation of Streptococci pneumoniae, Haemophillus influenzae and Neissseria meningitides.. Isolation and identification of the bacterial isolates wad done as per the procedure for identification (Manual of Medical Microbiology, ASM press). Antibiogram of the isolates of Streptococci pneumoniae, Haemophillus influenzae and Neissseria meningitidis was carried out by well and disc diffusion method (Kirby, 1966). Detection of virulence gene such as capsular transport (ctr A) in case of Neissseria meningitidis pneumolysin gene (ply) in Streptococci pneumoniae and capsulation (bexA) in Haemophillus influenzae by PCR assay. Genetic correlation and clonality study was done by PFGE, RAPD PCR assay, dendogram and sequencing.

Work progress

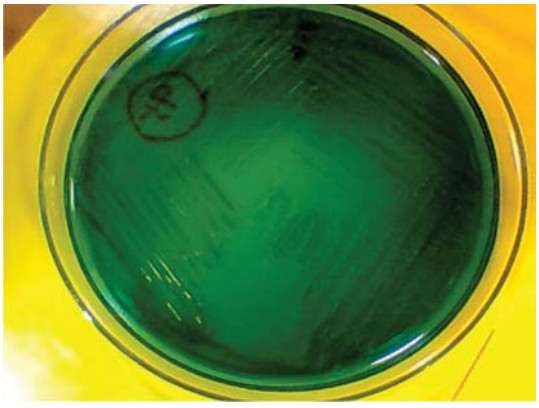
Subject enrolment & Lab Investigation:

- Number of patients attending the hospital i.e. SVPPGIP, Cuttack, suspected of meningitis and no. of hospital admission were recorded during the study period through 24hr surveillance.
- Total 11903 patients were admitted to the hospital during April 2012 to March 2013 and out of total in patients 491(4%) cases were suspected for meningitis cases.
- 632 no of suspected meningitis cases were enrolled in the study those who satisfied the inclusion criteria laid down in the protocol.
- The major presenting illness was fever with convulsion (78.63%). Other associated features were bulging fontanelle (21.36%), neck rigidity (17.2%), and altered sensorium (15.66%).
- Around 35% of patient reported to the above hospital within 24hrs of onset of fever. History



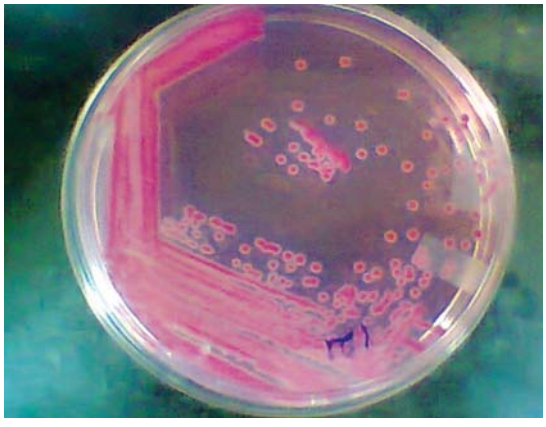
of use of antibiotics before admission was observed in 61% of cases.

- 463 CSF samples and 179 blood samples was collected for investigation. In all cases samples were processed immediately and put into culture (within 15-30 mins).
- Latex agglutination test was done in 379 samples. Out of 379 samples subjected to latex test, 13



Salmonella typhi on HEA agar plate

Biochemical test of Salmonella typhi



Klebeseilla pneumoniae on Mac conkey agar plate

S. pneumoniae on Blood agar plate.

samples were latex positive (five for Hib, seven for S.pneumoniae and one for group B streptococcus).

- CSF cell count varied from 0 to 16500. About 36% of CSF samples presented with cell count more than 10, while only 9% of CSF samples had WBC count more than 100.
- 135 no of CSF samples were analysed by real time PCR, out of which 33 were positive, out of which

Table-1: Different symptom percentage of suspected meningitis cases admitted to the hospital during the study period.

Month	Neck stiffness	Bulging fontanel	Alter sensorium	Seizure/ convulsion
April	3	1	2	11
May	7	7	6	20
June	7	0	4	18
July	7	7	1	19
Aug	12	14	3	41
Sep	2	8	2	25
Oct	6	8	3	21
Nov	4	7	6	28
Dec	3	15	6	40

Table 2: Result of Latex test and Bacterial culture.

Jan	3	5	6	41
Feb	0	4	5	17
March	4	9	4	26
April	6	8	8	37
May	14	15	14	54
June	7	9	10	31
July	11	13	7	31
Aug	13	5	12	37
total	109 (17.2)	135 (21.36)	99 (15.66)	497 (78.63)

Table 3. Month-wise distribution of suspected, probable and confirmed meningitis cases.

Month	Suspected Meningitis	Probable Meningitis	Confirmed Bacterial Meningitis
April	12	4	8
May	28	8	13
June	25	2	4
July	25	2	2
August	52	8	1
September	39	3	0
October	36	5	3
November	47	11	7
December	53	12	7
January	47	4	1
February	21	3	0
March	35	4	0
April	42	2	1
May	60	4	1
June	39	3	1
July	35	3	0
Aug	36	4	0
Total	632	82(12.97%)	49(7.75%)

29 were found to be positive for S.pneumoniae 17 and 4 for H.influenzae type b.

- Out of 179 blood samples processed for culture, 2 were culture positive for Klebseilla pneumoniae and 3 were positive for Pseudomonas aeruginosa. Antibiotic susceptibility done against Klebseilla pneumonia revealed that it was sensitive to Norfloxacin, Cephotaxime, Azithromycin, Chloramphenicol, Ciprofloxacin, Gentamicin, Ceftazidime and resistant to Ampicillin and Neomycin. Pseudomonas aeruginosa was found to sensitive to Cefotaxime, Gentamicin, Ciprofloxacin and resistant to Ampicillin, Ceftazidime and Penicillin.

- Of the total CSF samples subjected to culture 6 samples were culture positive and the causative organism were identified as S. aureus in 3 cases, Salmonella typhi in one case , S.pneumoniae and K.pneumoniae in one case each. S. typhi was found to be sensitive to chloramphenicol and cotrimaxozole.
- 60 cases of suspected pneumonia were enrolled in the study. The children enrolled were different pediatric age group less than 5 yrs as per the inclusion criteria.
- Out of 60 enrolled pneumonia cases, 36 samples (60%) are non-culturable whereas 24 samples (40%) are culture positive. Among the culture positive samples in most of the cases the causative organism found to be Staphylococcus aureus (38%) and Streptococcus pneumonia (35%). The other associated causative organisms are coagulase negative Staphylococcus sp and H. influenzae type b. The association of both Staphylococcus aureus and Streptococcus pneumoniae was confirmed in three cases (5%).
- Male child are more prone to pneumonia than the female child and the diseases is more frequent in children below 1 year (78.3%).
- Antibiotic profile of S. aureus reveals that it was sensitive to vancomycin, ampicillin, erythromycin, chloramphenicol, cefotaxime, gentamicin and resistant to cefazidime wheren as S. pneumponiae was sensitive to chloramphenicol, cefotaxime, penicillin, vancomycin and oxacillin.

Work to be done in next six month

- Isolation and characterization of bacterial isolates from suspected meningitis cases in Rayagada distric.
- Real time PCR assay for the collected sample and study their comparative reliability and efficiency with respect to culture and latex agglutination method.
- Antibiotic trends of these organisms and their resistance marker gene.
- Genetic correlation and clonality study of these organisms.

Expected outcome

The study will not only focus on the etiology of bacterial meningitis but also provide the idea on the prevalence of the specific serogroups of the Streptococci pneumoniae, Haemophillus influenzae and Neissseria meningitidis in this region. Further, this will be helpful in designing of Hib, pneumococcal and meningococcal vaccination program. Molecular assay are more accurate and rapid than conventional methods. Therefore, it will help in better and rapid identification of the etiological agents. The antibiotic profile will throw insight on the drug resistivity pattern and will emphasize the better management practices in hospitalized bacterial meningitis.

5. Studies on the distribution of sibling species of malaria vectors and their role in malaria transmission in Odisha.

Name	:	Mumani Das
Status	:	SRF(ICMR)
Date of Joining	:	May 2013
Guide	:	Dr. R. K. Hazra

Objectives

- Distribution of vectors of malaria in Odisha.
- Identification of sibling species of major vector by molecular methods.
- To study the insecticide resistance of different vectors by WHO method and molecular techniques.

- Vector incrimination and inoculation rates (EIR) in different parts of Odisha.

Work progress

Malaria is highly endemic in Odisha, a coastal state in the eastern region of Indian Peninsula. It affects more than 2.3 billion people, half of the world's population, in more than 100 countries in the tropics from South America to the Indian peninsula. Female Anopheles mosquitoes are the exclusive vectors for malaria. In Odisha, 3 species are considered as the main malaria vectors i.e. *An.culicifacies*, *An. annularis* and *An. fluviatilis*. Species identification is very essential for any vector control programme. For effective malaria control, proper identification of anophelines and their sibling species is very essential in obtaining a clear vision on the scenario of malaria of that area. Four endemic districts were chosen from different physiographical divisions of Odisha according to their endemicity for malaria as evident from the Annual Parasite Index (API). **Ganjam** district from Coastal tract, **Anugul** from Central tableland , **Kalahandi** from Eastern Ghat and **Keonjhar** from Northern plateau were selected for the study. Initially the collection of mosquito was done in two districts i.e. Keonjhar and Kalahandi during the four month of study. Adult mosquitoes were collected from indoor and outdoor resting habitats from 6.00 a.m. to 9.00 a.m. and in the evening from 6.00 p.m. to 10.00 p.m. from

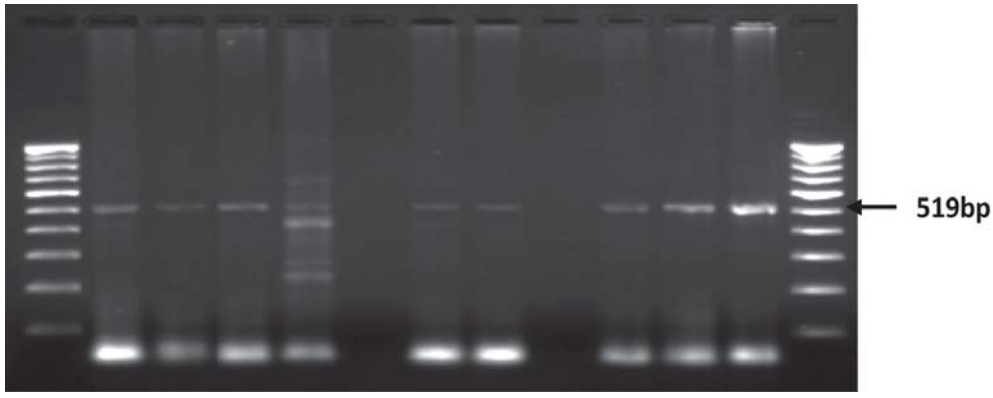


Fig 1. 1.8% agarose gel photo showing the human blood fed in mosquito.

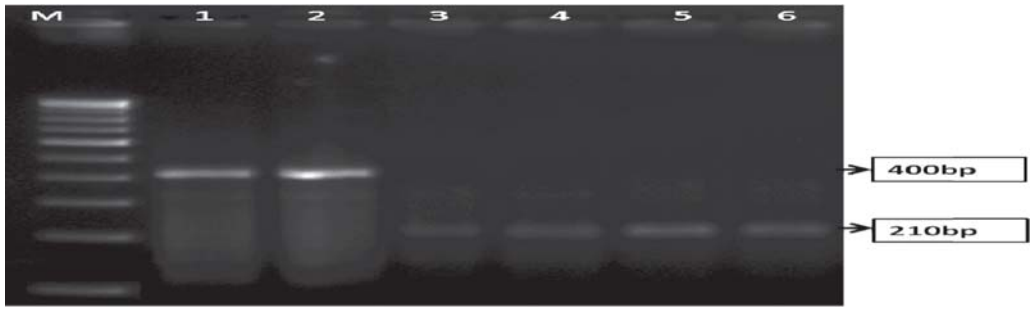


Fig 2. PCR-RFLP pattern for D3 of *An. annularis* mosquito. M represents 100-bp ladder, lane 1 and 2 shows species B which is not digested by BSM AI, and lane 3, 4, 5 and 6 represent species A which is digested by BSM AI .

both human dwellings and cattle sheds by using mouth aspirator and torch. A CDC light trap was installed from dusk to dawn (i.e. from 6 p.m. to 5 a.m.). Genomic DNA was isolated from both head–thoracic region and abdominal region of individual mosquitoes separately by phenol–chloroform method. Morphologically identified *An. culicifacies, An. annualris and An. fluviatilis* were initially amplified using primer from the D3 and COII region. The isolated DNA from both the body parts (head– thoracic region for sporozoite and abdominal region for HBF) was subjected to one multiplex PCR assay to detect the presence of *Plasmodium falciparum* and human blood mosquitoes (Fig 1).From the mosquito collected from kalahandi district during month of June *An. culicifacies* were tested for the presence of Plasmodium sporozoite and human blood fed. Out of 11 *An. culicifacies* 9 shows positive for human blood and no mosquito was found positive for sporozoite.

Achievements

Sequences submitted at GenBank (NCBI):

1. The DNA sequence of the ribosomal D3 and Mitochondrial COII region of Anopheles mosquito were submitted at the GenBank, NCBI. The accession numbers are JX131308, JX131309, JX131310, JX131311, JX131312, JX131313, JX131314, JX131315, JX131316, JX131317, JX131318, JX524874, JX524875, JX524876, JX524877, JX524878, JX524879, JX524880, JX524881, JX524882.

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Publication & Information

Publications

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14. Kumari S., Marai N., Hazra R.K., Kar S.K. and Mahapatra N. Anopheles subpictus B and its role in transmission of malaria in Odisha, India. *Tropical Biomedicine*.2013 Dec; 30(4); 710-7. **(IF = 0.921)**

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HRD Activities

Ph.D Awarded in 2013

1. Role of NO in the pathogenesis of severe Plasmodium falciparum malaria

Investigator: Gunanidhi Dhandamajhi
Guide: Dr. M.R.Ranjit
University: Utkal University

2. Molecular mechanism of rosetting in severe falciaprum malaria

Investigator: Ronaly Rout
Guide: Dr. M.R.Ranjit
University: Utkal University

Pre Ph.D Program

For the year 2012-13, RMRC, Bhubaneswar is functioning as Pre Ph.D nodal Centre of Utkal University in Biotechnology and Life Sciences. The Pre Ph.D course work has already started in RMRC since June 2013. Total 18 students have taken admission in Pre Ph.D couse work in Biotechnology & Life Sciences. Dr. N. Mohapatra, Science-E is the course Cordinator of the Pre Ph.D course work.

M.Sc. Dissertation Program

During the period January to June 2013, total 28 M.Sc. Dissertation students from various Universities of the country have undertaken 6 month project work in Biotechnology, Microbiology, Bioinformatics, and Molecular Biology under scientists of the Centre. The details of M.Sc. dissertation Program is attached herewith.

Sl. No.	Name of the student	Guide	Topic of Dissertation project
1.	Barsa Baisalini Panda Sambalpur Univ.	Dr.B.Dwibedi,Sc- C	Genotype Distribution of Rotavirus Infection And Association With Clinical Severity
2.	Swati Pattnaik OUAT	Dr.A.S.Kerketta, Sc- D	Prevalence of Vibrio cholerae during non-epidemic periods from various water sources of Satyabadi block of Puri district
3.	Anish Shrivastava AMITY Univ.	Dr.SapnaNegi, Sc-D	Comparison of SCD morbidity among patients and normal Individuals and its association with HbF levels
4.	Sandhya Rani Gupta Ravenshaw University, Cuttack	Dr.M. S. Bal, Sc- B	Detection of Circulatory Filarial Antigen (CFA) & Antibody ISO types in Hydrocele Serum & Fluid in the Lymphatic Filariasis.
5.	Ksheerabdhi Tanaya Panda Utkal Univ.	Dr.A.S.Kerketta ,Sc- D	Isolation of vibrio cholerae fromdifferent health facilitiesof Puri.
6.	Samarendra kumar rout	Dr. Sapna Negi,	Incidence of G6pd deficiency among new borns

	OUAT	Sc-D	in Kalahandi district of odisha and association of its status with patient's hematocrit profiled
7.	Rashmi Kanti Patel Sambalpur Univ.	Dr.Namita Mahapatra, Sc-E	Xenomonitoring of Wuchereria bancrofti in vector in MDA coverage area.
8.	Vijeta Mohapatra Sambalpur Univ.	Dr.G Bulliya, Sc-E	Nutritional Status of Lactating Mothers and their children under two in Rayagada district.
9.	Jhansirani Sahoo AMIT, Bhubaneswar	DR.A.K.Satapathy,Sc-E	IgG (isotype) antibody response to carbohydrate antigen in lymphatic filariasis
10	Bhunupriya Dehury Sambalpur Univ.	Dr. R.K. Hazra Sc-D	Isolation of Dengue virus from different pool sizes of larvae and pupae of Aedes mosquitoes.
11	Husne Ara Utkal Univ.	Dr. B. Dwibedi, Sc- C	Level of protective immunity under five children to measles infection.
12	Sarita Pradhan Sambalpur Univ.	Dr. D. Das, Sc- D	PCR based detection of Mycobacterium tuberculosis
13	Banishree Sahoo TACT, Bhubaneswar	Dr. R.K. Hazra Sc-D	Inclusion of anopheles species and their vectorial attributes in multiplex PCR.
14	Subhashree Pathy Ravenshaw University	Dr.Namita Mahapatra, Sc-E	Malaria transmission in Urban areas.
15	Subhashish Mohapatra IPT, Salepur	Dr. D. Das, Sc- D	Second line anti-tuberculosis drug sensitivity in Mycobacterium tuberculosis isolates of Odisha
16	Deeptimayee Rout Ravenshaw University	Dr. Sapna Negi, Sc-D	Incidence of sickle cell disease among newborns in Kalahandi District of Odisha and association of SCD status with patients haematocrit profile.
17	Anima rani Jena Ravenshaw University	Dr. B. Dwibedi, Sc- C	Etiology of acute viral hepatitis in subjects attending hospitals and its clinical manifestation.
18	Subhankar Mohanty MITS, Bhubaneswar	Dr.B.B.Pal,Sc-E	Spectrum of bacterial infection among hospitalized patients
19	Debapriya Mishra MITS, Bhubaneswar	Dr. D. Das, Sc- D	Comparison between ZN and FM microscopy for the detection AFB.
20	Sambit Ku. Beuria TACT, Bhubaneswar	Dr.B.B.Pal,Sc-E	Identification of different bacteria pathogens isolated from different filarial patients.
21	Krishna Kalpana Mishra NOU	Dr.G Bulliya, Sc-E	Study on acute malnutrition among children in Bhubaneswar.
22	Bijayananda Panigrahi Berhampur Univ.	Dr. T. Hussain, Sc-E	Metabolic syndrome and Diabetes among adults in an urban area of Bhubaneswar.
23	Kiran Mohapatra Utkal Univ.	Dr. T. Hussain, Sc-E	Pre-diabetes and t2 diabetes among adults in an urban area of Bhubaneswar.
24	Saraswati Munduri Berhampur Univ.	DR. A.K. Satapathy, Sc-E	A comparative study of microfilaraemia, filarial specific IgG4 and circulating filarial antigens in bancroftian filariasis.
25	Gourabamani Swalsingh Berhampur Univ.	Dr.B.B.Pal,Sc-E	Incidence of different shigella species among hospitalized children from Bhubaneswar area.
26	Poonam Tripathy Pt. Univ. Raipur	Dr. A.S.Kerketta,Sc- D	Contribution of Shigella towards acute diarrhocal disease in coastal area of Odisha.
27	Suryasnata Das S 'O' A Univ.	Dr. M.R.Ranjit, Sc- E	Diagnosis of malaria by multiplex PCR
28	Subhashree Priyadarsini S 'O' A Univ.	Dr. M.R.Ranjit, Sc- E	Study and Analysis of HRP 2 gene polymorphism in Plasmodium falciparum in Odisha Population

Facilities

(i) OPD facility of the centre at Capital Hospital, Bhubaneswar

(Dr B. Dwibedi, Dr A.S. Kerketta, Mr B. N. Murmu, Mr B.N. Sethi & Mr H.S. Nayak)

The centre is providing out patient facility to patients of lymphatic filariasis and haemoglobinopathy. The facility is being utilized for referral investigation & diagnosis of suspected cases of filariasis and haemoglobinopahy from different parts of the state. Besides, the facility is providing treatment to acute and chronic filarial disease including decompression therapy for lymphedema reduction. The facility is also being utilized for collection of clinical information and biological samples for diagnosis and research including viral diagnosis, bacterial meningitis and hypertension related to research projects of the centre as well as PhD programmes.

During the year 415 cases of lymphatic filarial diseases attended the set up, Out of them 70% of cases have chronic filarial disease as grade II - IV lymphedema. Rests were having acute episodes of adenolymphangitis. The cases were examined and ADLA attacks were identified and treatments provided. Lymphedema management was provided with preventive chemotherapy, foot hygiene and intermitted de compression therapy. Around 2 % cases were children below 14 years of age.

307 samples were collected from capital hospital, Bhubaneswar for viral infection diagnosis and 41 no. of samples were collected for bacterial pneumonia diagnosis using the facility by the project staff. 50 cases of hypertension were enrolled from the hospital set up, on which genetic markers studied by our research scholar and the bio chemical test report provided to the patients.

The services offered at the above facility have benefited the patient and the state health department in diagnosis and treatment of the cases. This also supported the research activity of the centre which required clinical facility and clinical information that supplemented the laboratory and epidemiological expertise of the centre.

(ii) Insectorium

Regional Medical Research Centre at present has one Insectoriums where cyclic colony of three mosquitoes species Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus are maintained for different experimental purposes.

Experimental test on reared mosquito's species.

The reared mosquito species were use in to test

- Insecticide susceptibility status.
- Larvicidal and plant bioassay test.
- The development of malaria and filariasis parasite in the reared vector species is done to investigate the transmission and vectorial capacity of the vector.
- The study on interaction between malaria parasite and the vector has been initiated. An.stephensi was fed on infected human blood by membrane feeding technique.
- Gene expressions by monitor up to 10 days till the development of sporozoite stages in the mosquitoes.
- Now we are proposing for conducting virology work on Chikungunya and Dengue for which Ae. aegypti and Ae. albopictus will be maintained.
- The institute also evaluates the Larviciding properties of different insecticides send from other institutes.

Training: Trainings are given on the adult and larval identification of the vector and maintaining the colony to malaria technical Supervisor (MTS), Staffs of BMC and CMC,Insect Collector of state government and students of different universities.

(iii) NNMB (National Nutrition Monitoring Bureau) activities:

There has been a steady increase in urban population in India (28 to 31.3%) and Odisha (15-16.7%) during 2001-2011, largely contributed by migration of population from rural and tribal to urban areas, having an impact on their health and nutrition. Studies have shown that an epidemic increase in the prevalence of diet-related chronic non-communicable diseases (NCD) like overweight and obesity, insulin resistance, diabetes mellitus, hypertension, cardiovascular diseases (CVD) cancers specially in urban population. Major causes of this increase in NCD are especially transition in nutrition, physical activities and lifestyles leading to increased rates of hypernsion, diabetes, dyslipidemia, overweight and adopting risk behaviour like alcohol consumption and tobacco uses. Moreover, undernutrition intrauterine and early childhood periods leads to development of chronic degenerative disease such as hypertesnsion, diabetes and CVD in later life. Hence there is a need to assess health and nutritional status and the magnitude of NCDs of urban population in 16-NNMB states that include Odisha by adopting multistage sampling procedure.

The NNMB-Odisha is carrying out "Assessment of diet and nutritional status of urban population and prevalence and determinants of hypertension and diabetes mellitus and dyslipidemia among urban adults in the selected cities of Odisha". The general objective is to assess diet and nutritional status of urban population, and prevalence and determinants of obesity, hypertension, type-2 diabetes mellitus and dyslipidemia among urban adults (>18 yrs) in 5 cities namely Balasore, Baripada, Bhubaneswar, Rourkela and Sambalpur. The specific objectives are:

1. To assess the current status of food and nutrient intake among different age/gender/physiological/activity groups of urban population,
2. To assess the current nutritional status of all the available individuals in the selected HHs in terms Anthropometry and clinical examination,
3. To assess the history of morbidity during previous fortnight among al the individuals covered for Anthropometry,
4. To assess the prevalence and determinants of overweight and obesity, hypertension, diabetes mellitus and dyslipidemia among the urban adults men and women(>18 yrs)
5. To assess body composition using Bio-electrical impedance assessment (BIA)/skin-fold thickness at 4 sites among adults covered for Anthropometry.
6. To assess knowledge and practices about obesity, hypertension, diabetes and dyslipidemia among adults, and
7. To assess lifestyle patterns and risk behaviours of adults.

Study design

It is a community-based cross sectional study with multi stage random sampling procedure.

Study setting: 15 Municipal Wards selected from each 5 selected cities/towns, which have more than one lakh population.

Work progress

The NNMB-Odisha unit initiated urban survey in September 2012 and so far covered 21 wards that included cities Balasore (15-wards), Bhubaneswar (5-wards) and Rourkela (1-ward). The survey investigations include household & socio economic particulars; nutritional anthropometry; diet survey by 24-hour recall method; measurement of blood pressure (adults >18 years); fasting blood glucose level by glucometer (adults >18 years); infant & young child feeding practicess (IYCFP) for mother of index child (<3yrs); collection of dry blood spot (DBS) for DNA extraction, food frequency questionnaire (adults >18 years); and knowledge and practices of adults about their health, nutrition and lifestyle. The coverage of

urban survey in terms of households and independent parameters since September 2012 till date is presented in table mentioned below and the data is being submitted to Central Reference Laboratory, National Institute of Nutrition, Hyderabad.

Study parameters	Name of selected cities			
	Balasore	Bhubaneswar	Rourkela	Baripada
Number of wards surveyed	15	4	1	&
Number of households	720	192	48	Sambalpur
Diet surveys (HHs)	180	48	12	-
Food frequency (Individuals)	972	229	55	-
Knowledge & Practices (Individual)	1534	388	108	-
IYCF practices	78	17	4	-
Anthropometry(Individuals)	2377	573	166	-
Fasting blood sugar (adults)	1351	345	104	-
Lipid profile (adults)	234	192	51	-
Dry blood Samples (Individuals)	1351	310	104	Yet to be
Status	completed	ongoing	ongoing	Initiated

(iv) Animal House

Animal facility in the center continues to be used for all research projects requiring animal experimentation. Currently Rabbits, M. Coucha, Balb / c mice, and G pigs are available for experimentation. This animal facility has been registered with CPCSEA. All the projects concerning animal use/ experimentation are discussed in Animal ethical committee of the center. The facility is well maintained by animal house attendants. Staff has maintained periodic records of animal house. Pelleted feed procured from NIN, Hyderabad has been provided to the animals. Staff has maintained periodic records such as Form-C, Form-D etc of animal house as per provision of CPCSEA. This facility is maintained regularly with periodic inspection and health monitoring by veterinarian.

Experiment on Laboratory Animal

(v) Library, Information & Publications

Library & Information Centre of RMRC has been the life-line for the research activity of the institute.

At present, it stands as a modern library & Information Centre with a lot of modern facilities with Wi-Fi enabled. It is regarded as one of the best Bio-Medical & Health Science Research libraries in Odisha.

The library has been using the LIBSYS an Integrated Library Management software package with all the modules for the library housekeeping operations. Using LIBSYS OPAC, users can search the Library Online Catalogue by Author, Title, Subject, and keywords.

Regional Medical Research Centre, Bhubaneswar library has been categorized as Category- III library by ICMR in 2010 as per review committee report of Govt. of India. The role of the library is not confined with library activities rather library, Information &

Publication activities of the Centre. The Library serves the research needs of the scientists, researchers, students, doctors and academicians of the state. The services are extended to a number of other organizations like Universities, Medical Colleges, CSIR Lab, ICAR Lab, DAE and DBT Lab of the state.

Library Timing

9.00 Am to 5.30 P.M (Monday to Friday)

Daily Article Service

Library has started a new article delivery service to all ICMR scientists and researchers. In this Daily Article Service every day (Monday to Friday) one current research article from International journals used to send to the scientists in their respective e- mail id. This service will be extended to all medical college faculties in future. Scientists/ Doctors who are interested to avail this service, please send a request to sahoo@icmr.org.in in order to register your e-mail id in our group mail.

Library Apprentice Trainees

Regional Medical Research Centre, Bhubaneswar is one the Biomedical Research Centre of Indian Council of Medical Research (ICMR), Ministry of Health & Family Welfare, Govt. of India. The Centres library is recruiting Apprentice library trainee with fixed stipend amount for the period of one year which is approved by ICMR, New Delhi and Board of Practical Training (BOPT), Kolkata since 2009. The fixed stipend amount Rs. 5000.00 per month has been enhanced to Rs. 11,500.00 per month since 2011. Only current year MLISc pass out students are illegible for Library apprentice trainee for the period of one year. During training the trainees are explored on the following activities.

i. Total Housekeeping operation of RMRC Library (Acquisition, technical processing, Classification in UDC & Serial Control etc.)

ii. Working on Library Automation Software- Libsys.

iii. Institutional Repository (IR) of RMRC Scientific Publications.

iv. Online literature search through, ICMR-EJC, ERMED consortia, JCCC@ICMR & Science Direct for scientists and researchers.

v. Scanning, Digitizing, Resource Sharing and News Clippings on local health news.

vi. Bibliometric analysis and Impact factor of scientific journals.

vii. Learning how to write research papers in Library & information science.

Library Trainee working on Daily Article Servic

Publication Cell

Library works as publication cell of the institute. It regularly publishes Annual Report, News Bulletin, Library News Letter, IEC materials on specific diseases and special publications. During Centenary year of ICMR (2011), the following 7 publications are published by the publication cell.

Coordinating HRD Activities

Library works as coordinator for M.Sc. dissertation program and summer training program

of M.Sc. students from various universities as 6 monthly project works at RMRC Bhubaneswar.

LAN System

RMRC Local Area Network (LAN) is connected to all scientists, divisions and section for Internet & Intranet connectivity. At present, the library is equipped with LAN Server connected with more than 50 computers in the RMRC building. Besided LAN server, Mail-server, Antivirus- server and Libsys Server are also installed in Library for Networking. Two leased likes i.e NKN and NIC for Web OPAC with BSNL leased line and National Knowledge Network (NKN) for Internet connectivity.

New RMRC Website Hoasted

RMRC, Bhubaneswar hoasted its new Website: www.rmrcbbsr.gov.in

Library collection

Books:-3859

Bound Journals:-4608

Foreign Journals (Prints):-31

Indian Journals (Prints):-30

E-resources

Online Journals- Science Direct (87)

CD ROM Databases- MEDLINE

E-Consortia

Presently RMRC have two major consortia for E- resources.

ICMR-EJC

ICMR E-journal Consortia **(ICMR-EJC)** subscribes worlds top 4 weekly journals in Biomedical sciences i.e, Nature, Science, The Lancet and New England Journal of Medicine (NEJM) that can be accessible for all ICMR Institutions of the country. The above four online journals are IP activated by all ICMR Institutions of the Country.

Nature: **http://www.nature.com**

Science: **http://www.sciencemag.org**

The Lancet: **http://www.thelancet.com**

NEJM: **http://www.nejm.org**

JCCC@ICMR

JCCC is J-Gate Custom Content (JCC) for a group of homogeneous consortia members. JCCC-ICMR is an extension of JCC, for the Indian Council of Medical Research (ICMR). It covers 1941 journals received collectively at 29 institutions / centres of ICMR. In addition to these, around 201 open access journals are also covered. In all, journals from 755 publishers are covered on a single platform. JCCC@ ICMR is also IP activated. (Website: **http://icmr.jccc.in**)

Meetings/Seminars/Symposia organized

I. 11th Annual Conference of the Lymphology Society of India (LSI) organized at RMRC, Bhubaneswar.

The 11th National Annual Conference of the Lymphology Society of India "LymphoCon-XI" was organized at Regional Medical Research Centre (ICMR), Bhubaneswar from 13th -14th of December 2013. The theme of the conference was "Overcoming Challenges towards Elimination of Filariasis". The scientific programme was started with the Presidential address of LSI, delivered by Dr. SK Kar,Director, RMRC, Bhubaneswar in the morning session, after the welcome address by the Organising Secretary, Dr. A. Mahapatra, Scientist-E of this Centre.This was followed by the scientific sessions, Dr. Jamal Oration award , Panel Discussions and Guest Lectures. Among the distinguished scientists, Dr. David C Zawieja, Professor of Medical Physiology, Texas, Emeritus Prof. Dato. C.P.Ramachandran, Dr. V. Kumarswamy, Ex-Director, TRC, Chennai. & Dr. S.P.Tripathy, Ex- DG, ICMR.

The conference was inaugurated by the Honourable Minister of Health & Family Welfare, Dr. Damodar Rout and Co-Chaired by The Honourable Minister of Agriculture and Animal Husbandry, Shri. Debi Prasad Mishra, of Government of Odisha. The highlight of the inaugural function was the presentation of the Lifetime Achievement award to Emeritus Prof. Dato. C.P.Ramachandran by Dr. Damodar Raout, Minister of Health & F W , of Odisha. Prof Ramachandran was honoured for his scientific contributions, sustained efforts to the Global and National programme and his support to research efforts in the country and Region. The Honourable Minister of Agriculture and Animal Husbandry, Shri. Debi Prasad Mishra, of Government of Odisha emphasised the importance of this conference at Odisha and he also pointed his association with RMRC since two decades. Dr. S.P.Tripathy, Ex DG ICMR was also honoured on this occasion; during his deliberations he emphasised the vision of RMRC as the first Director of RMRC. Followed by this Ms. Dharitri Panda, The Senior Financial Advisor of ICMR, New Delhi who represented the GD, ICMR gave a heart touching speech on Odisha's need and efforts towards elimination and emphasised the support of ICMR from time to time in this regard. The Secretary General, LSI, Dr. G. Manokaran delivered the Secretarial Report to the house, vividly mentioning the activities of LSI. The President LSI, Dr. SK Kar, gave the Presidential remarks in the house. This was followed by the Chief Guest's address by the Honourable Minister of Health & Family Welfare, Dr. Damodar Rout. During his speech he has emphasised the importance of MDA, achievements of the State and assured all future help needed towards elimination. Finally, Dr. A. Mahapatra, the Organising secretary extended the Vote of Thanks, to the august house, which was followed by a Odisi dance programme and dinner.

Ethical Committee Meeting

The human ethical Committee meeting was held on 8th March 2013 & 24th May 2013 for ethical clearance

Human ethical meeting held at RMRC

of projects undertaken at RMRC, Bhubaneswar. The animal ethical committee meeting was held on 11th June 2013 for ethical clearance on the projects.

Scientific Advisory Committee Meeting

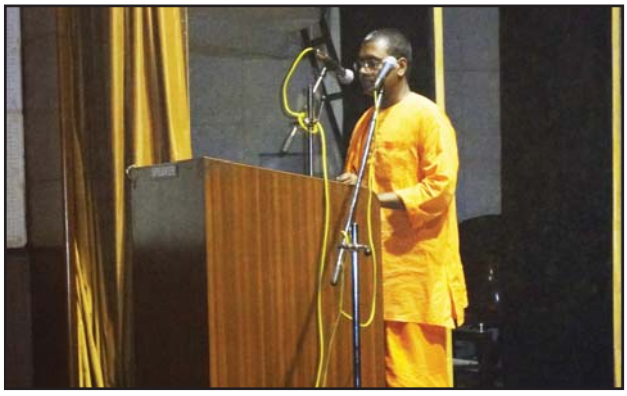
For review of ongoing and completed projects two pre SAC meeting was held at RMRC,

27th SAC meeting in progress

Bhubaneswar. The 27th SAC meeting was held on 6-7 Nov. 2014 under the chairmanship of Prof. D.S. Agarwal. In the SAC meeting total 19 ongoing projects and 7 completed projects were discussed. Besides that total 17 new proposal from various scientists were discussed and reviewed by SAC members for approval.

Guest Lecture

I. Dr.V.Balaji, Prof. & HOD Department of



Talk on the occasion of Swamy Vivekananda Jayanti Celebration at RMRC on 17th Jan 2013

Microbiology, CMC, Vellore delivered a talk on "Quality Control on Microbiological studies" in the seminar hall on 05/04/2013 at 2:30 p.m.

II. A batch of 18 students along with Sri Kunwar Singh Rawat, Lecturer of P.G. Department of Library & Information Science, Utkal University visited RMRC Central Library on 26/08/2013 at 1:30.

Events

I. Swami Vivekananda Jayanti was celebrated in the Auditorium on 17/01/2013.

II. Hindi Day Observation was celebrated in the Auditorium on 20/09/2013.

III. M.Sc Dissertation students given their poster presentation on 24th - 28th June 2013 on their respective project work.

IV. JRFs/SRF Research scholars given their poster presentation on 31/10/2013.

Meetings/Seminars Attended by Scientists

- **Dr. S. K. Kar**

1. Participated a meeting on "Kidney Failure" at Collector Chamber, Cuttack on 12th September 2012.
2. Participated "Pre-SAC review meeting" at RMRC, Bhubaneswar on 17th & 18th September 2012.
3. Participated "Tribal Sub Plan Meeting" at RMRC, Bhubaneswar on 4th October 2012.
4. Participated Joint SAC on Tuberculosis at NJIL & OMD, Agra on 4th October 2012.
5. Participated "SAC Meeting" of NIV Pune on 6th & 7th December 2012.
6. Participated "SAC Meeting" of VCRC, Pondichery on 18th & 19th December 2012.
7. Participated "SAC Meeting" of RMRC, Dibrugarh on 20th & 21st December 2012.
8. Participated "CME Programme" at Medical College, Rajhmuhendry , AP on 3rd January 2013.
9. Participated "Tribal Health Research Forum Meeting" at ICMR Headquarter on 13th January 2013.
10. Participated as expert member in Assessment Board Meeting at ICMR Head Quarters for the post of Scientist-B, Scientist-D, & Scientist-E on 10th-13th Feb.2013
11. Participated in SAG Meeting at ICMR Head Quarters on 20th & 21st Feb.2013
12. Visited Rayagada Field Unit of RMRC for review of the field station on 5th & 6th March 2013.
13. Organized "Rayagada & Kalahandi Research Proposal Meeting" at RMRC, Bhubaneswar on 7th March 2013.
14. Organised "Human Ethical Committee Meeting" at RMRC, Bhubaneswar on 8th March 2013.
15. Participated " Scientist Assessment Board Meeting" at ICMR, Headquarter on 21st & 22nd March 2013.
16. Participated "Scientist Assessment Board Meeting" at ICMR, Headquarter on 30th March 2013.
17. Participated Tribal Health Research Forum Meeting at VCRC, Pondicherry on 15th April 2013.
18. Participated a Meeting " Medicine upto date 2013" at SUM Hospital, Bhubaneswar on 20th April 2013
19. Participated 24th Parasitology Congress at RMRC, Jabalpur on 27th-29th April.2013

20. Participated Malaria Group Meeting at NIMR, Delhi on 3rd May.2013.
21. Participated Meningitis Meeting at Chennai on 6th May 2013.
22. Participated 2nd National Workshop on Lymphatic Filariasis at CRME, Madurai on 5th June 2013.
23. Participated Meeting on Prevention and Management of water born & vector borne deceases in the state Secretary office Chamber on 12th July 2013.
24. Participated Oral Cholera vaccine in Kasipur block of Rayagada meeting in the Chamber of Hon'ble Health Minister on 16th July 2013.
25. Participated CME on Ethical "Drug Development" at AIIMS, Bhubaneswar on 20th July 2013.
26. Participated Review meeting on Malaria Control Activities NVBDCP Conference held on 20th July 2013.
27. Participated Dengue Conference at CRME, Madurai on 25th-26th July 2013.
28. Participated Workshop on TB & Diabetes at Delhi on 2nd-3rd Aug 2013.
29. Participated Tribal Health Forum Meeting at Jodhpur on 9th-10th Aug 2013.
30. Participated Translational Research meeting on Rayagada & Kalahandi Projects on 3rd Sept 2013.
31. Participated Ethics committee meeting of Apollo Hospital, Bhubaneswar on 4th Sept 2013.
32. Participated Translation Research Meeting at ICMR on 3rd Sept 2013.
33. Participated Ethics Committee Meeting at 4:30P.M. At Appolo Hospital on 4th Sept 2013.
34. Participated Workshop on "Research Methodology Scientific Writing" at KIIMS, Bhubaneswar as a Resource Person on 6th Sept 2013.
35. Participated National Seminar on Incidence & prevelance of mendilian traits & diseases in people of Orissa at Adaspur, Cuttack at 16th Sept.2013.
36. Participated Hands on workshop on DNA Diagnostics 2013 at SGPGI, Lucknow on 28th-30th Nov 2013.
37. Participated 33rd APICON, Odisha at VSS Medical College Burla, Chaired Scietific session on 9th-10th Nov.2013.
38. Participated SAC Meeting of VCRC, Pondicherry on 3rd-4th Dec. 2013.
39. Participated Lymphology Conference at RMRC, Bhubaneswar on 13th-14th Dec. 2013.

- **Dr. N. Mahapatra**

1. Attended Symposium on "Biomedical Research in Medical Institute" on 1st April 2012 at RMRC, Bhubaneswar.
2. Attended Meeting on "Tribal Health Research Forum-2012"on 8th August 2012 in the eve of International day of World's Indigenous People at RMRC, Bhubaneswar.
3. Attended meeting on "Vector borne diseases review"on 25th &25th July 2012 at RMRC, Bhubaneswar.
4. As a member attended Subject Research Committee meeting of Utkal University.

- **Dr. M. R. Ranjit**

1. Invited to deliver a lecture on "*Cellular and Molecular Biology of Malaria Parasites*" to the Post Graduate students (Zoology & Microbiology) of Basic Science College, OUAT, Bhubaneswar on 8.12.2012.
2. Invited to deliver a guest lecture on "Climate *Change and Malaria*" to the Students (PG & UG) and Teachers of Udayanath Autonomous College of Science & Technology , Adaspur, Cuttack on the eve of "Silver Jubliee Year" on 19.01.2013 .
3. Attended and deliver a guest lecture on " *Malaria in Odisha: Future Perspectives*" at XII International Conference on Vector and Vector Borne Diseases held from 16th to 18th September 2013 at

MohanLal Sukahdia University, Udaipur, Rajasthan.

- **Dr. A. Mahapatra**

1. Chaired the session Prof. JBS Haldane Memorial Lecture on 5th Nov 2012, at Utkal University, Bhubaneswar.
2. Participated 38th All India Sociological Conference held at M.S. University,
3. Udaipur during Dec 27-29th 2012 presented a paper entitled "Leprosy and the Role of Social Scientists".
4. Attended three - 2012-2013 Institutional Ethical Committee meetings of Indian Institute of Public Health, Bhubaneswar.
5. Attended National Seminar on "Emerging Trends in Anthropology", at Utkal University, Bhubaneswar on 18-19th March 2013.
6. Presented a Paper on "Effect of Choloroquine Chemoprophylaxis on pregnant women: cohort study" in the "XII International Conference on Vector and vector Borne Diseases", 16-18th Sept. 2013 at MS University, Udaipur.

- **Dr. G. Bulliyya**

1. Attended a meeting on "Exploratory study on epidemic kidney disease among rural population in Cuttack district, Odisha" held at Office Chamber of Collector, Cuttack on September 12, 2012.
2. Attended "Nutrition: First 1000 Days of Life" as a Member in Panel discussion organized by Nestle Nutrition Institute, Science for Better Nutrition, held at Hotel Hindustan International, Bhubaneswar on 11th Nov 2012.
3. Meeting regarding discussion on progress of eradication and cause of kidney disease affected people in Baramba and Narasingpur area, organized by Collector & District Magistrate at Conference Hall of Collectorate, Cuttack on 20th December 2012.
4. Attended "Stakeholders Consultation on Improving lives and livelihoods of Rural and Tribal Communities' as a Member in Panel list discussion organized by M.S.Swaminathan Research Foundation, Chennai, held at OUAT, Bhubaneswar on 26th Dec 2012.
5. Attended 'Training of Trainers for Annual Health Survey (AHS) Clinical Anthropometric and Biochemical (CAB) components, held at NIHFW, New Delhi on15-17th April 2013.
6. Attended Sub-Committee for evaluation of projects submitted under Technology Applications for Livelihood Improvement of Scheduled Caste Population (TALIM) organized by DST-New Delhi at NBRI, Lucknow on 22-23rd May 2013 and presented project proposal on Scaling-up evidence-based nutrition-specific and sensitive interventions to improve maternal and under-2 child nutrition (1st 1000 days) among scheduled caste populations in Odisha.
7. Attended 'Training of Trainers for NVBDCP & World Bank Project on Endline Household Survey for Malaria', held at NIMR, New Delhi on 15-16th July 2013.
8. Attended 'Technical Committee Meeting (Research)' to examine modified proposal of Naandi Foundation on Mid-day meal project with iron supplement in Keonjhar district held at TMST Conference hall, Behind Capital Hospital, Bhubaneswar on 24.08.2013.
9. Attended 'NNMB Steering Committee Meeting' held at National Institute Nutrition, Hyderabad on 30th August 2013.

Training Programs Conducted

10. Conducted 1st Batch of Training programe for Health Supervisors and Health Investigators on Clinical, Anthropometric and Biochemical (CAB) component of Annual Health Survey (AHS) held at RMRC, Bhubaneswar from 3rd to 7th August 2013.
11. Conducted 2st Batch of Training programe for Health Supervisors and Health Investigators on Clinical, Anthropometric and Biochemical (CAB) component of Annual Health Survey (AHS) held

at RMRC, Bhubaneswar from 7th to 10th September 2013.

12. Behera S, Dixit S, Bulliyya G, Kar SK. Fat-soluble antioxidant vitamins and iron overload and chronic malnutrition in children with β-thalassemia major. Indian Journal of Pediatrics. 2013; DOI10.1007 / s12098-013-1162-0.

- **Dr. B. B. Pal**

1. Attended as technical expert in state forensic laboratory, Rasulgarh for purchase of different scientific equipments during Jan- Oct. 2013. 29 / 04 / 2013, 23 / 07 / 2013, 12 / 08 / 2013, 09 / 10 / 2013
2. Attended the workshop organized by directorate of water management, BBSR on "Water quality issues, opportunities and socio-cultural concerns of water use in agriculture on 7th Aug. 2013.and presented the key note address on " Overall impacts of using multi quality water sources on soil -water-plant-animal in continuum"

- **Dr. A. S. Kerketta**

1. Participated in Annual meeting of Tribal Health Research Forum during August 2012 at RMRC, Bhubaneswar.
2. Attended Tribal Health Research Forum at ICMR Head quarter during February 2013.
3. Attended the PIs meeting to discuss the progress of Multi-centric study on Migratory Population and develop intervention strategy during February 2013 at ICMR Head quarter.
4. Attended Research and Ethical Committee meeting of State Health Department, Govt of Odisha at Secretariat on 20.02.2013.
5. Attended the tribal health forum meeting at VCRC, 15.04.2013 for participation in the tribal health forum meeting at VCRC, Pondicherry.
6. Training attended: Attended Training on "Management of Rural drinking water & sanitation programmers with focus on IEC" at NIRD campus, Rajendra Nagar from 19th to 24th Nov. 2012.

Training imparted

(a) Delivered TV talk on "Dengue & its Prevention" at Door darshan Kendra, Bhubaneswar on 18.03.13

(b) Delivered Radio talk on "Rational Drug use "in All India Radio, Bhubaneswar on 23.01.2013.

- **Dr. D. Das**

Attended "Indo-US joint working group on prevention of sexually transmitted disease and HIV / AIDS grantsmanship workshop 6-7, February 2013 at India International Centre, New Delhi.

- **Dr. R. K. Hazra**

1. Attended Vector forum meeting held on "Task Force project on Insecticide resistance monitoring in malaria and visceral leishmaniasis vectors" under Vector Science Forum held on 1st November, 2012, ICMR Hqrs., at New Delhi.
2. Vector forum meeting held on "Biology and Bionomics of Vectors" under Vector Science Forum held on 2nd November, 2012, ICMR Hqrs., at New Delhi.
3. Attended a Task Force meeting on "Insecticide resistance monitoring in vectors of malaria, dengue / chikungunya, JE, filariasis and cutaneous and visceral leishmaniasis" under Vector Science Forum to review protocols received under Task Force on Insecticide Resistance Monitoring in different disease vectors on 10th May, 2013 at NIMR, Sector – 8, Dwarka, New Delhi .
4. Attended a meeting on "Brain storming conference on dengue scenario in India: Disease burden, surveillance and control", and presented a paper entitled"Entomological investigations of dengue virus outbreaks with respect to pupal indicators in Odisha, 2012" from 25th to 26th July, 2013.
5. Attended an International conference as an Invited speaker on "Challenges in 21st century: Their global impact & strategic management", and presented a paper on "Present and future arboviral threats" in the XII International

conference on vector & vector borne diseases from 16th to 18th September 2013.

- **Dr. T. Hussain**

1. Attended meeting at National Institute for research in Tuberculosis (NIRT), Chennai with Dr, Makesh Kumar for developing the multi-centric project proposal, "*A Longitudinal Study to Evaluate the Incidence of Tuberculosis in Patients with Type 2 Diabetes*" from 4th-5th December, 2012.
2. Attended workshop on Tuberculosis and Diabetes Mellitus (Funded by Department of Biotechnology) in ICGEB, New Delhi on 2nd-3rd Aug.,2013.
3. 1st Annual Conference of Research Society for the Study of Diabetes in India (RSSDI) - Odisha State Branch held on 18thAugust,2013 at Pramod Convention & Club Resort, Cuttack.
4. Attended Workshop for the Investigators on the proposed multi-centric Task Force study on Tuberculosis in Tribal areas at National Institute for research in Tuberculosis (NIRT), Chennai on 4thOct,2013.

- **Dr. S. Negi**

1. Attended a workshop on "Newborn screening of hemoglobinopathies and G6PD deficiency" organized by NIIH, Mumbai, from March 19 to 20, 2013.
2. Attended a meeting of the Expert Group as Principal Investigators of Biomedical Informatics Centre of Regional Medical Research Centre, Bhubaneswar, to be established under the second phase of task-force 'Biomedical Informatics Centres of ICMR'. The meeting was held on 11th and 12th July, 2013 at Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Sri Nagar, Jammu & Kashmir. A presentation was made on the ongoing activities and proposed activities to be initiated at our Centre in relation to the objectives of Biomedical Informatics centre.

- **Dr B. Dwibedi**

1. Delivered a radio talk on 29th Jan. 2013 on Filariasis in Sustha Bharat programme in AIR, Cuttack.
2. 11th and 12th Jan. 2013: Attended high power committee meeting on Vector borne diseases.
3. 29th Jan. 2013: Participated as a Resource person in the Awareness training programme to ASHA & AWW of Narsinghpur and Badamba blocks at Sahid Bhawan, Cuttack organized by Collector Buttack & Inaugurated by Shri Deviprasad Mishra, Minister of Agriculture & Fisherces, Odisha
4. 21st Mar. 2013: Delivered a talk in Emerging Vector Borne viral infection in the region, as an invited speaker at Odisha Biotech conclave-2013 organized by TRIDENT, School of Biotech sciences, Bhubaneswar.
5. 15th April 2013: Attended tribal health research forum meeting at VCRC Puduchery & presented the Fever management strategy in tribal population.
6. 16th May 2013: Attending Translation Research project for Rayagada & Kalahandi field unit at ICMR.
7. 18th May 2013: Steering committee meeting at state secretariat, Dept. of H & FW- on Polio Eradications certification.
8. 27th May 2013: Attended Research & Ethical Committee at Dept. of Health & FW, Odisha.
9. June 2013: Scientific and Ethical committee meeting at State health dept. for review of proposals submitted by AIPH, Bhubaneswar.
10. 3rd Aug 2013: Meeting review of Dengue outbreak by Minister of Health, Odisha at Collectorate, Cuttack.
11. 9th – 10Aug. 2013: Attending Tribal health research forum meeting at DMRC, Jodhpur.

- **Dr.M.Bal**

Participated in the symposium organised by RMRC, Bhubaneswar on "*Biomedical Research in Medical Institutions*" on 1st April 2012.

- **Dr.B.Sahoo**

1. Participated National Knowledge Network (NKN) Workshop held on Oct 17-19,2013 at IISC, Bangalore.
2. Participated Library Development Programme Organized by KIIT University on April 12-13, 2013.

27th Scientific Advisory Committee

1	Dr. D. S. Agarwal B-24, Swasthya Vihar Delhi 110 092	Chairman
2	Dr. P. L. Joshi Former Director, NVBDCP5 80, Metro View Aptt. Sector 13B, Dwaraka New Delhi 110 075	Member (VBDSF)
3	Dr. P.K. Shrivastava Joint Director NVBDCP, 22 Sham Nath Marg DELHI 110 054	Member (VBDSF)
4	Dr. A. C. Mishra Ex-Director National Institute of Virology MCC Campus, Pashan, Pune 411 021	Member (VBDSF)
5	Dr.Sarala K. SubbaRao Consultant, ICMR Ansari Nagar, New Delhi 110 029	Member (VBDSF)
6	Dr. Nikhil Tandon Deptt. Of Endocrinology & Metabolism AIIMS, Ansari Nagar NEW DELHI 110 029	Member
7	Dr.A.C. Dhariwal Director NVBDCP, 22 Sham NathMarg, DELHI 110 054	Member
8	Dr.V. Rama Baru Prof. of Social Sciences Centre of Social Medicine and Community Health Jawaharlal Nehru University, New Delhi	Member
9	Dr. N. K. Mehra Dept of HLA, AIIMS, Ansari Nagar NEW DELHI - 110 029	Member

10	Dr. R. M. Pandey Deptt. Of Biostatiscs AIIMS, Ansari Nagar New Delhi 110 029	Member
11	Dr.B. Sesikeran Ex-Director National Institute of Nutrition P.O: Jamai Osmania, Hyderabad 500 007	Member
12	Dr. J. Mahanta Director Regional Medical Research Centre N.E. Region, Post Box 105 Dibrugarh 786 001	Member
13	Dr.P. Jambulingam Director, Vector Control Research CentreIndira Nagar, Pondicherry 605 006	Member
14	Dr.Soumya Swaminathan Director National Institute for Reseearch in TB Mayor V.R. Ramanathan Road Chetput, Chennai 600 031	Member
15	Dr. S. P. Tripathy Director NJIL & OMD Post Box No:1101, TajGanj Agra 281 001	Member
16	Dr. S. Chakrabarti The Director National Institute of Cholera & Enteric Diseases, P-33 CIT Road, Scheme XM, Beliaghata, P.O.Box 177 Kolkata 700 010	Member
17	Dr. Neeru Singh Director Regional Medical Research Centre for Tribals, Nagpur Road, P.O. Garha Jabalpur (M.P.) 482 003	Member
18	Dr. Neena Valecha Director National Institute of Malaria Research Sector-8, Dwaraka, New Delhi 110 077	Member

19	Dr. Rashmi Arora Chief, ECD, ICMR Ansari Nagar, New Delhi 110 029	ICMR Representative
20	Dr. Manju Rahi ICMR, Ansari Nagar New Delhi-29	ICMR Representative
21	Dr. D. K Prusty Director of Health Services, Directorate of Health Services Govt. of Odisha, Heads of the Deptt. Building Bhubaneswar	
22	Dr. S. K. Kar Director, RMRC, BBSR	Member Secretary

HUMAN ETHICAL COMMITTEE

1	Dr.Kabi Prasad Misra Sr. Consultant Cardiologist & 55, Ganesh Nagar Gandamunda, Khandagiri, Bhubaneswar 751 030	Chairman
2	Prof.Aruna Mishra Laxmi Vihar PO: Sainik School Bhubaneswar	Co-chairperson
3	Dr. P. K. Dash Director, Medical Education & Training Heads of the Dept Building Govt. of Orissa Bhubaneswar 751 001	Member
4	Mrs Kasturika Pattanayak Ex-Chair Person, Social Welfare Board Govt. of Orissa, 1, Lewis Road Bhubaneswar.	Member
5	Dr P. K.Acharya N-1 A / 10 IRC Village Near CRP Square, Bhubaneswar 751 015	Member
6	Dr.Sisir Kumar Mahapatra Sr. Consultant Physician Surya Nivas, Plot No:B-1 / 91 Lingaraj Vihar, Pokhariput, Bhubaneswar 751 002	Member
7	Sri.Himadri Mohapatra Toshali Plaza, Iind floor Satyanagar, Bhubaneswar	Member

8	Prof. Rita Ray HoD Sociology Utkal University, Vani Vihar, Bhubaneswar 751 004	Member
9	Dr. S. K. Kar Director Regional Medical Research Centre, Bhuabneswar	Member- Secretary

Animal Experimentation Ethical Committee

1	Dr S. K. Ray, Ex-Principal Orissa Coll. of Anim. Husb. & Vet. Sc. Qr.No.M-109 Baramunda H.B. Colony Bhubaneswar 751 003	
2	Prof. Sachidananda Das PG Dept. of Zoology Utkal University Bhubaneswar.	Member
3	Mrs Kasturika Pattanayak Ex-Chair Person, Social Welfare Board Govt. of Orissa, 1, Lewis Road Bhubaneswar.	Member
4	Dr. M. R. Ranjit Scientist-E Regional Medical Research Centre Bhubaneswar	Member
5	Dr. R. C. Patra Prof. & Head, Dept. of Veterinary Medicine OUAT, Bhubaneswar – 751 003	Member
6	Dr R. K. Hazra Scientist-D Regional Medical Research Centre Bhubaneswar	Member
7	Dr. Kishore Chandra Mohapatra Plot No: 17, Gautam Nagar PO: BJB Nagar, BBSR 751014	Member (CPCSEA)
8	Dr. Dwarikanath Mohanty Plot No: 1215 / 1654, Khandagiri, Bari Bhubaneswar 751 030	Member(CPCSEA)

9	Dr. A. K. Satapathy Scientist-E Regional Medical Research Centre Bhubaneswar	Member- Secretary
10	Dr. S. K. Kar, Director, Regional Medical Research Centre, Bhubaneswar.	Convener

Technical Equipment Purchase Committee

1	Dr. A. K. Sahoo Principal Scientist CIFA, Kausalyagunj, Bhubaneswar- 751 002	Chairman
2	Dr. P.Das Sr. Scientist CIFA, Kausalyagunj Bhubaneswar- 751 002	Member
3	Dr. N. K. Debata Prof. Microbiology SUM-Hospital, Bhubaneswar	Member
4	Dr. M. R. Ranjit Scientist - ERMRC, BBSR	Member
5	Dr. B. Dwibedi Scientist-C RMRC, Bhubaneswar	Member
6	Mr. G. Behera Accounts Officer RMRC, Bhubaneswar	Member
7	Dr. A. K. Satapathy Scientist-D RMRC, Bhubaneswar	Member –Secretary

Budget and Resource Generation

The total sanction budget in respect of the Centre (Non-Plan & Plan) for the year 2012-13 is **Rs. 844.20** lakhs. The head wise expenditure for 2012-13 of the budget is shown below. The resource generation during 2012-13 is 10.10 Crore.

BUDGET OF RMRC (12-13) (In Lakhs), SOURCE : ICMR

RMRC Main Budget 2012-2013 (Rupees in Lakhs).

Establishment	Administrative Expenses	Contractual Service	Others	Equipment	Capital
492.05	111.5	80.50	0.95	39.55	120.00

Staff Position (As on 31st Dec. 2013)

Scientists

1.	DR. S.K. Kar, MD, Dip. Clin. Epid.	Scientist-G & DIRECTOR
2.	Dr. (Mrs.) N. Mahapatra, M.Sc., Ph.D.	Scientist-E
3.	Dr. M.R. Ranjit, M.Sc., Ph.D.	Scientist-E
4.	Dr. A. Mahapatra, M.Sc., M.Phil., Ph.D.	Scientist-E
5.	Dr. A.K. Satapathy, M.Sc., Ph.D.	Scientist-E
6.	Dr. G. Bulliyya, M.Sc., Ph.D.	Scientist-E
7.	Dr. B.B. Pal, M.Sc., Ph.D.	Scientist-E
8.	Dr. Taziba Hussain, M.Sc., Ph.D	Scientist-E
9.	Dr. (Mrs.) A.S. Kerketta, M.B.B.S.	Scientist-D
10.	Dr. Dasarathi Das, M.Sc. Ph.D	Scientist-D
11.	Dr. R.K. Hazra, M.Sc., Ph.D.	Scientist-D
12.	Dr. Sapna Negi, M.Sc., Ph.D	Scientist: D
13.	Dr. Bhagirathi Dwibedi, M.B.B.S, M.D	Scientist-C
14.	Dr. Madhusmita Bal, M.Sc.M. Phil, Ph.D	Scientist-B
15.	Dr. Nilam M. RaoSomalkar,MBBS ,MD	Scientist-B

Research & Technical Staff

1.	Mr. P.K. Jangid, M.Sc.	Technical Officer-A
2.	Mr. R.K. Das, M.Sc.	Technical Officer-A
3.	Dr. A.S. Acharya, M.Sc., M.Phil, LL.B.,Ph.D	Technical Asst.
4.	Mrs. G. Mallick, M.Sc.	Technical Asst.
5.	Mr. R.C. Parida, M.Sc.PGDCA	Technical Asst.
6.	Mr. N.S. Marai, M.Sc., LL.B.	Technical Asst.
7.	Mr. D.P. Hansdah, M.Sc.	Technical Asst.
8.	Dr. N. Mandal, M.Sc., M.Phil., B.Ed.	Technical Asst.
9.	Dr. P. K. Sahoo, M.Sc., Ph.D.	Technical Asst.
10.	Mr. B. Murmu, M.Sc., M.Phil.	Technical Asst.
11.	Dr. H.K. Khuntia, M.Sc. Ph.D	Technical Asst.
12.	Miss. Sujata Dixit, M.Phil, M.Sc	Technical Asst.
13.	Mr. H.K. Tripathy, B.Sc, PGDME	Technical Asst.

14.	Mr. R. N. Nayak, B.A.	Technical Asst.
15.	Mr. B. N. Sethi, Dip. MLT	Technical Asst.
16.	Mr. H.S. Naik, Dip. MLT	Technician-C
17.	Mr. S.C. Rout ,ITI	Technician-C
18.	Mr. T. Moharana	Technician-C
19.	Mr. C.R. Samantray	Technician-B
20.	Mr. K.C. Dalai, B.A., ITI	Technician-B
21.	Mr. B.K. Kanhar	Technician-B
22.	Mr. G. D. Mansingh	Technician-B
23.	Mr. B. Pradhan	Technician-A
24.	Mr. C. S. Tripathy, B.Com. LL. B.	Technician-A
25.	Mr. S.S. Beuria	Technician-A
26.	Mr. G. Simhachalam	Technician-A
27.	Mr. K. C. Parichha	Technician-A
28.	Mr. N. N. Pattnaik	MTS (Technical)
29.	Mr. K. C. Jena	MTS (Technical)
30.	Mr. S. K. Mallick	MTS (Technical)
31.	Mr. H.K.Jena	MTS (Technical)
32.	Mr. Banamali Nayak	MTS (Technical)
33.	Mr. Baburam Behera	MTS General)
34.	Mr. K.G.Samal	MTS Technical Engineer

Library & Information

1.	Dr. B. Sahoo, M.L.I.Sc., Ph.D.	Library & Information officer
2.	Mr. Chakradhar Nayak	Technician-A
3.	Miss. Dhara Sharma, M. Lib & Inf. Sc.	Apprentice Library Trainee
4.	Miss. Nasima Begum, M.Lib & Inf. Sc.	Apprentice Library Trainee
5.	Mr. Mukesh Kumar Bhoi, M. Lib & Inf. Sc.	Apprentice Library Trainee
6.	Mr. Rajim Sur Rai	MTS(General)

Administration & Accounts

1.	Mr. G. Behera	Accounts Officer (Admn. Officer Incharge)

2.	Mr. B. Sutar, M.Com	Section officer
3.	Mr. P.C. Nayak, B.A.	Personal Assistant
4.	Mr. R.C. Muduli, B.A.	Assistant
5.	Mr. A.P.Parida, B.A	Assistant
6.	Mrs. R. Varghese	Personal Asst
7.	Mr. S.K. Satapathy	U.D.C.
8.	Mr. B.S. Rao	U.D.C.
9.	Mr. R. Rath	UDC.
10.	Mr. D.K.Mohanty, B.A	Steno
11.	Mr. S. Nayak	L.D.C
12.	Mr. S.K. Das, B.Com.	L.D.C.
13.	Mr. S.K. Majhi, M.A., LL.B.	L.D.C.
14.	Mrs. S. Beuria, M.A	L.D.C
15.	Mr. R.C. Dash	MTS(General)
16.	Mr. Sankar P Sharma	MTS(General)
17.	Mr. M.B. Thappa	MTS(General)
18.	Mr. T. Bahadur	MTS(General)
19.	Mr. D.C.Rao	MTS(General)
20.	Mrs. Triveni Nayak	MTS(General)

Director's Office

1.	Mr. L.S. Rao, B.A.	PS to Director
2.	Mr. K.C.Nayak	MTS(General)
3.	Mr. R.K. Hembram	MTS(General)
4.	Mr. Pandaba Sahoo	MTS(General)

Workshop & Maintenance Staff

1.	Mr. B.K. Biswal	Technician-A (E)
2.	Mr. S. Sutar	Technician-A (E)
3.	Mr. J. Behera	MTS Technical Engineer
4.	Mr. B.K. Moharana	MTS Technical Engineer
5.	Mr. Sankar Bisoi	MTS(General)

Animal House Staff

1.	Mr. A. Senapati	MTS (Technical)
2.	Mr. S.K. Das	MTS (Technical)
3.	Mr. Jaladhar Naik	MTS (Technical)
4.	Mr. Banamali Sahoo	MTS(General)

Transport Staff

1.	Mr. Md. Daulat Khan	Driver
2.	Mr. Sibaram Patra	Driver
3.	Mr. Anakar Nayak	Driver
4.	Mr. A.R. Khan	Driver
5.	Mr. P.K. Behera	Driver

Ph.D Students

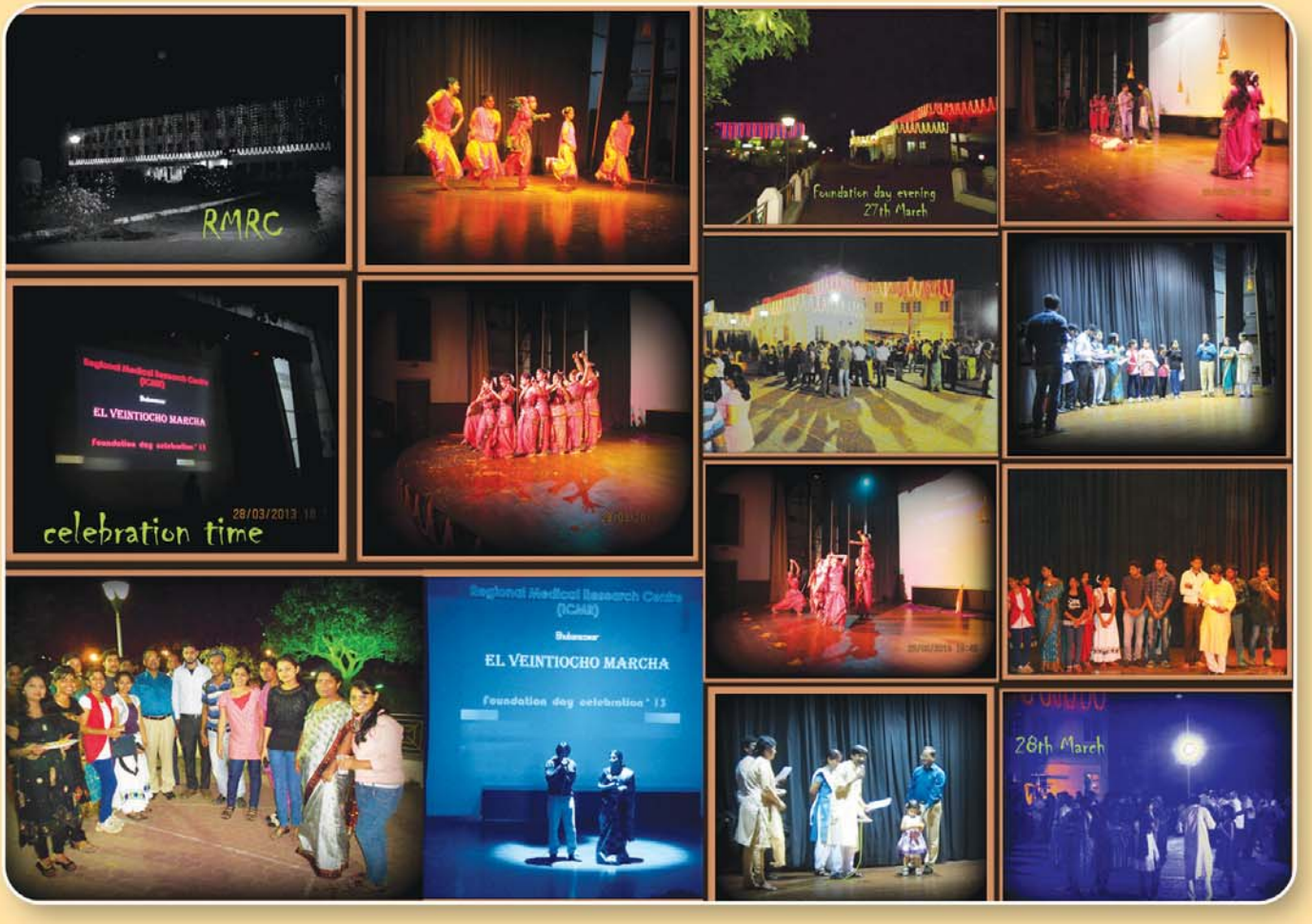
1.	Manisha Patnayak, M.Sc	JRF(CSIR)
2.	Biswadeep Das, M.Sc	JRF(UGC-CSIR)
3.	Chinmayee P Khuntia, M.Sc	SRF (ICMR)
4.	Susil Kumar Rathore, M.Sc	JRF (ICMR)

Staff of NNMB Unit

1.	Mrs. S. Paikray	Asst. Research Officer
2.	Dr. A.R Mohanta	Asst. Research Scientist
3.	Mrs. Haraprava Sahu	Social Worker
4.	Mr. G.C. Mantri	Lab. Technician
5.	Mr. R.K. Sahoo	Driver
6.	Mr. Santosh Kumar Juharsing	Field Attendant.

Holi Celebration among RMRC Staff in 2013

Drawing competion among staff students of RMRC



RMRC Foundation Day Celebration-2013

Regional Medical Research Centre

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