Annual Report 2006-07

ICMR

Indian Council of Medical Research

REGIONAL MEDICAL RESEARCH CENTRE

(Indian Council of Medical Research)

Bhubaneswar

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Annual Report
2006-07

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REGIONAL MEDICAL RESEARCH CENTRE
Bhubaneswar

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Preface

The year focused on strengthening the link with local Govt. in augmenting national and state run Programmes, developing strategies for public health importance, tools/products that can assist diagnosis or prophylaxis. Besides research, the human resource and infrastructure were strengthened by recruiting fellows from reputed organizations like UGC, CSIR or others. Several modern lab equipments procured with modernization of lab.

Research was addressed in areas of vector borne diseases like filariasis, malaria and chikungunya and diarrhoeal disorders. Research was addressed in basic applied as well as in operational areas. Out of 26 projects undertaken during the year, 8 were completed and 13 were extramural that helped resource generation with financial support from National or International agency.

To assist national Programme, issues related to Mass Drug Administration (MDA) in filariasis were addressed; strategy to improve drug compliance in urban areas was developed. Important issues on Malaria are addressed to help the current national programme. Clinical trials were addressed to assist the key issues. Studies in protective immunity in filariasis were undertaken to address host parasite interaction and to develop immunoprophylaxis against filariasis. Multiplex PCR assays developed to facilitate quick diagnosis of *V.Cholerae* infection and vectorial status identification. On request from Health Services, Govt. of Orissa, outbreaks of chikunguniya, diarrhoea and hepatitis infection were investigated. On request from Orissa State AIDS Control Society, Govt. of Orissa, diagnostic facility is being provided twice a week for CD4 cell count of referred cases of AIDS of this region.

The centre has proposed to add newer areas of research i.e., developing infrastructure for TB culture facility and virus culture lab. Since several epidemics are reported from this region, council has proposed to have virus culture facility. For TB, no culture facility is currently available in the State.

Human resource development was stressed to help train manpower in advanced biomedical field in this region. Around 46 M.Sc./M.Phil. students from various reputed Universities completed their six monthly dissertation work and 17 Ph.D. scholars pursuing their PhD under different scientists. Medical Officers of the Eastern region serving under Govt. Health Services were imparted training in Epidemiological investigation methods. Summer training was imparted to 20 students in area of Biotechnology.

Several scientists were sent to reputed institutes in India and abroad to acquire higher knowledge, training and for attending conferences and meetings.

National Conference of Lymphology Society of India was organized by this Centre. Besides several other meetings like Human and Animal Ethical Committee, symposiums, lectures from outside experts organized. The linkages and collaboration with upcoming local institutes like Institute of Physics (DAE), RRL (CSIR), KIIT University, Medical Colleges were strengthened. Regular weekly seminars are being conducted. Six monthly news bulletin and library news letter are published.

The newly constructed buildings like Auditorium, guest house, Scholar's hostel and animal house were inaugurated by Prof. N.K. Ganguly, Director General of ICMR on 16th Dec, 2006.

During the year, the Centre generated funds of Rs. 1 crore through extramural projects, research fellowship & equipment grants from outside sources other than the budget. The centre published 13 research papers in 2006 of which 9 are SCI publications. Besides, in 2007 till date 10 research papers have been published or in press of which 9 are covered in Science Citation Index (SCI). Library subscribes 34 foreign and 37 Indian biomedical print journals for 2007. Online accesses of full text journals are made accessible to all computers of the centre. During this year, library has procured more than 1000 research books & monographs on biomedical & health science subjects. Library subscribes 5 online journals through ICMR library consortium. In addition to that, our library has started e-journal consortium between NML, ICMR & AIIMS, where 1505 medical journals will be accessed freely through ERMED-India e-journal consortium of Directorate General of Health Services (DGHS), Ministry of Health & Family Welfare, Govt. of India. Library facility has been extensively used by outside institutions besides our scientists and staff.

There are 97 staff in position including 15 scientists with various expertises who catered to accomplish the output.

The scientists and staff of this Centre have made continuous effort and contributed to significant output of the Centre. I sincerely thank scientists and staff for their endeavour and contributions. I am also thankful to the State health department and other agencies and collaborating institutes for their assistance and co-operation. I extend my deep gratitude to Council for its continuous support, guidance and encouragement. With all round support, the Centre can continue its endeavour to achieve its goal.

Dr.S. K. Kar
Director

Research Highlights (2006-07)

Malaria is considered as important public health problem in Orissa. Therapeutic efficacy of Chloroquine (CQ) was evaluated as per the WHO guidelines, in two endemic blocks i.e. Adenigarh block of Boudh district and Madanpur-Rampur block of Kalahandi district; indicated that more than 90% of cases studies failed to show adequate clinical and parasitological response (ACPR) to CQ. and large proportion of cases detected with Early Treatment Failure (ETF). In vitro resistance to CQ was studied from blood samples of severe and uncomplicated clinical cases reported from various endemic districts.

The frequency of Pfcrt (76T) and Pffmdr1 (86Y) point mutations responsible for the CQ resistance was found to be 68.7% and 53.8% in this region. When the occurrence of Pfcrt and Pfmdrl point mutations were combined total 8 genotypes were found to be circulating among the parasite population of Orissa. Amongst them Pfcrt 76T + Pfmdrl 86Y genotypes are more prevalent (41.26%) than other genotypes. This indicates that the CQ resistance P.falciparum are more frequent than wild types in Orissa. Survey on SP drug resistance in malaria endemic zone of Orissa, indicates that nearly, 40.5% and 9.6% of the parasite population harbours the 108 point mutation in DHFR and DHPS genes respectively, yet only 1.05% of the parasite population harbours both the mutation.

Both Infant Mortality and Maternal Mortality Rates in this region are high. Besides, high prevalence of anaemia was recorded by our earlier studies undertaken amongst adolescent girls and pregnant mothers residing in malaria endemic region. Hence, CQ chemoprophylaxis amongst pregnant mothers was evaluated prospectively in a cohort in 17 Gram Panchayats of Nayagarh district of Orissa. No significant difference was observed in the malaria incidence between compliant and non-compliant mothers. Placental blood specimens are being examined for comparison. CQ compliant mothers were found to be low (36%).

Novel primers were designed from the D3 and ITS2 region of A.anularis, a malaria vector of this region An.annularis sequences are designed to carry out SSCP to find out the intra-specific differences between the sibling species of malaria vectors. Multiplex PCR method was developed for simultaneous detection of sporozoite, blood meal analysis and sibling species from single mosquito. An. Culicifacies sibling species- 'A & D' was reported for the first time from this region. Three plant extracts are under laboratory trial showing promising results for the development of a Potent Larvicide.

Toll like Receptors (TLRS) have been studied in innate recognition of the P.falciparum: The glycosyl-phosphatidylinositol anchor induces signaling in host cell via TLR-2 and TLR-4. The influence ofTLR-4 polymorphisms in complicated and uncomplicated cases in human malaria were studied. The TLR-4 ASP 299 Gly variant occurred at a frequency of 17.6% in complicated malaria and was significantly more in complicated malaria ($P<0.03$). These findings suggest that TLR-4 (Asp 299 Gly) polymorphism may be contributing to development of severity in clinical manifestations of malaria.

Currently, Diethylcarbamazine (DEC) used in three dose ranges in different age groups annually during Mass Drug Administration (MDA) to eliminate lymphatic filariaisis. The programme show lower compliance rate largely due to side reactions reported.

Open clinical field trial being carried out in three matched population groups with three different but uniform doses of DEC (ie. In 100, 200 or 300 mg single dose) irrespective of age indicated comparable effectiveness at 3 month post DEC follow up between the groups receiving various doses associated with lowest frequency and severity of side reaction in 100mg dosage

The Centre developed an innovative strategy of drug delivery in urban areas for mass drug administration in filariasis programme using partnership strategy. MDA with local decision-making and local leadership is quite effective in urban communities. This innovative approach has potential to achieve desired levels of results in different strata of urban communities. Govt. of Orissa has adopted above strategy in year 2007 for urban areas.

Glutathione-s-transferase (GST) purified from the adult cattle parasite Setaria digitata was studied for its protective role in human filariasis. Broad single band of 26-28kda antigen was detected by PAGE. Antibody response to this purified fraction is being analysed. More than 90% of infected individuals were sero positive for IgG antibody indicating its potential as an immuno-diagnostic antigen. Microfilaria clearance ability of this fraction is being evaluated in experimental animal after immunization with GST.

The apoptosis of embryogenic stages in female filarial worms indicated decreased production of Mf. The parasite stages like RI (Mf), R2 & R3 (eggs) of S.digitata were found to be more apoptotic in worms collected from amicrofilariamic cattles(+/-) in comparison to worms from microfilariamic (+/+) animals. These results indicate that apoptosis of developing intrauterine developmental stages could be a contributing factor for absence of circulating Mf in amicrofilaiaemic animals.

A community based open intervention trial in a total of 800 tribal adolescent girls aged 12-18 years is being undertaken in 34 villages covered under 12 Gram Panchayats in Gumma block of Gajapati district. The anthropometric measurements increased consistently with increase in age of the adolescent girls. Out of 518 girls studied so far, the prevalence of moderate and severe grade of anaemia was found to be high in girls of younger age groups. The proportion of girls with inadequate iron stores i.e. serum ferritin level <15ng/ml was 39.5% indicating poor iron storage. Out of253 cases examined 8.3% were confirmed for malarial parasite. Their peripheral smears & intestinal helminths are examined before initiating intervention regimes in 4 different matched groups.

Weekly surveillance to detect the pattern of diarrhoeal disorder was undertaken in cases admitted to referral hospitals of Puri, Bhubaneswar & PHCs around Puri indicated 44.2% of 451 rectal swabs culture positive.Of these 54.4% were E. coli, 25.5% were V.cholerae 01 (Ogawa-20, Inaba-24) 4.5% Shigella and 15.5% Aeromonas species. Using specific primer for E.coli we detected 7% ETEC, 6% EPEC ANS 5% EAggEC corresponding to the detection of virulent gene - elt, eae and ast respectively. Antimicrobial susceptibility pattern of V.cholerae strains isolated, showed uniform resistance to Co-trimoxazole, Furazolidone and Nalidixic acid.

A quadriplex PCR assay was developed in a single tube reaction for simultaneous detection of serotype (01 &I or 0139), biotype (Classical or El tor), toxigenic potential and top regulating factor of V.cholerae.

The health consequences of domestic violence were studied with reference to reproductive health, in sampled population of both rural and urban areas in eastern region of India. While the Prevalence was found to be 60%, 30% of women were exposed to violence during their pregnancy; which was associated to the outcome of their reproductive health, in terms of pre term births, stillbirths and spontaneous abortions.

Presence of Chlamydia trachomatis (CT) genital infection in symptomatic females attending Gynaecology OPD was studied in 103 cases by PCR test confirmed 7% positive, thus establishing the diagnosis set up in medical college.

Outbreak of Chikunguiniya infection was investigated in three districts namely Cuttack, Kendrapara & Nayagarh in around 10,000 populations indicated 32.5 seropositivity for chick IgM antibodies. The incriminating vectors of both species Ae. aegypti and A.abopictus were identified in the affected villages in high density.The tribe specific prevention of hepatitis viral infection studied amongst four primitive tribes of Orissa indicated high prevalence of HBS Ag (25%) HCV (12%) in Mankidia tribe of Mayurbhanj district risk factor for infection is being studied.

1 On Going Studies

1.1 Molecular analysis of drug resistance genes and prediction Of treatment outcome in P falciparum infections in Orissa.

Status : Extramural (NVBDCP)
Investigators :
Dr M R Ranjit
Dr G P Chhotray
Dr A S Acharya
Starting date : March 2006
Cosing date : August 2009

Objectives:

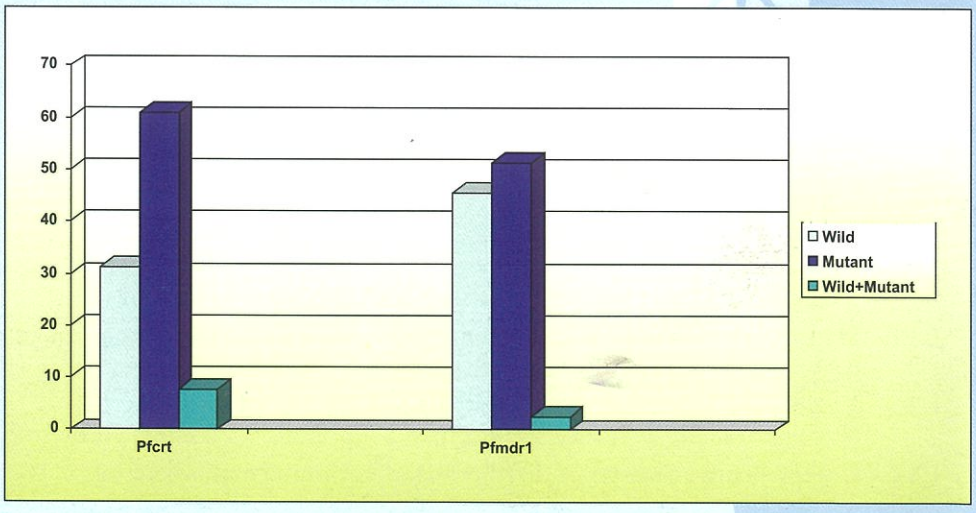
1. To observe the frequency of the genotypes of Pfcrt/Pfmdr1 and DHFR-DHPS associated with Chloroquine and Pyremethamine-Sulphadoxine resistance in natural *P falciparum* parasite populations of Orissa.
2. To study the origin and spread of resistance alleles through the parasite population in this region.

Progress:

Total 269 blood samples positive for P falciparum infection has been collected randomly from the patients attending the malaria clinics at peripheral hospitals (PHCs) of Malkangiri, Phulbani, Anugul, Keonjhar and Sundargarh districts.The parasite genomic DNA was isolated and purified by phenol extraction and EtOH precipitation. The point mutations responsible for developing resistance to the corresponding drugs in Pfcrt/ Pfmdr1 and DHFR/DHPS genes were analysed by PCR-RFLP methods using the primers described elsewhere and standardized in our laboratory.

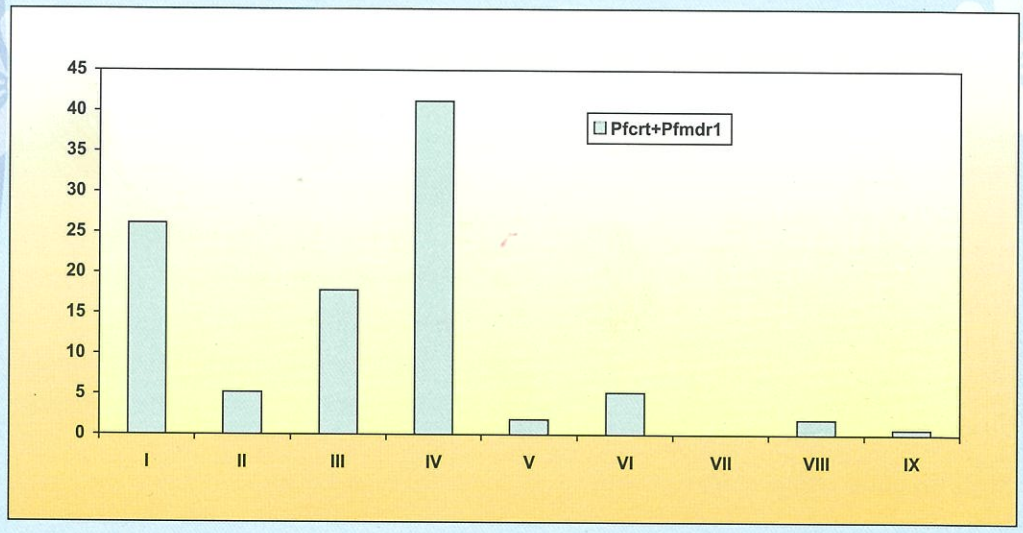
The analysis of molecular data indicates that the frequency of Pfcrt(76T) and Pfmdr1(86Y) point mutations responsible for the CQ resistance is about 68.7% and 53.8% respectively among the studied samples (Fig 1).

Fig1: Prevalence of Pfcrt & Pfmdr1 point mutations responsible for CQ resistance



Taking Pfcrt and Pfmdr1 point mutations together total 8 genotypes were found to be circulating among the parasite population of Orissa. Amongst them Pfcrt 76T + Pfmdr1 86Y genotypes are more prevalent (41.26%) than any other genotype (Fig 2). This indicates that the CQ resistance P falciparum isolates are more frequent than wild types in Orissa.The result correlated with the finding of high frequency of cerebral malaria cases / severe malaria in Orissa.

Fig 2: Prevalence of different genotypes of P falciparum based on Pfcrt& Pfmdr1 point mutation



There are total 4 point mutations in DHFR (codon 51, 59, 108 and 164) and DHPS (codon 436, 437 ,540 and 581) genes are responsible for the development of resistance to pyremethamine and sulphadoxine drug combinations. Among these point mutations 108 in DHFR and 437 in DHPS are the primary mutations responsible for S-P drug resistance while occurrence of other mutations increases the degree of resistance to the drug. In our study though 40.5% and 9.6% of the parasite population harbours the 108 point mutation and 437 point mutation in DHFR and DHPS genes respecrtively, yet only 1.05 % of the parasite population harbours both the mutation (Table 1).

Table 1: Frequency distribution of DHFR and DHPS alleles in P falciparum isolates of Orissa.

	DHFR				DHPS			
	51 No(%)	59 No(%)	108 No(%)	164 No(%)	436 No(%)	437 No(%)	540 No(%)	581 No(%)
Wild	197(73.2)	129(47.9)	160(59.9)	253(94.1)	251(93.3)	238(88.5)	240(89.2)	222(82.5)
Mutant	62(23.1)	135(50.2)	109(40.5)	16(5.9)	18(6.6)	26(9.7)	26(9.7)	26(9.7)
Mixed	10(3.7)	5(1.9)	0(0.0)	0(0.0)	0(0.0)	5(1.9)	3(1.1)	21(7.8)

While analyzing the different combinations of point mutations in DHFR and DHPS genes it is observed that quadruple mutation combination is nil. Only double mutation combination has been observed in 4.4 % of the parasite population. This indicates that the parasite population of Orissa has not developed resistance to S-P drug combination and the drug can be introduced safely. The study is in progress, the microsatellite analysis is to be carried out to analyse the spread of the resistance parasite populations in Orissa.

1.2 Molecular characterization of Anopheles annularis complex. Development of species specific diagnostic markers and microsatellite markers.

Status : Extramural
(CSIR)
Investigators :
Dr. R. K. Hazra
Dr. N. Mahapatra
Starting date : March 2006
Cosing date : September 2008

Objectives:

1. To compare cytotaxonomic technique with new molecular technique likePCR, PCR-RFLP and SSCP specify to establish the accurate identification of the sibling species.
2. To develop microsatellite markers for *Anopheles annularis* species for population genetics analysis.
3. To develop multiplex PCR technique to achieve simultaneous detection of sporozoite identification, blood meal analysis and sibling species identification from single mosquito.

Work Progress:

As a base line study the mosquito samples of *Anopheles annularis* were collected from Keonjhar, Angul, Kendrapara, Boudh, Kandhamal, Mahanga, Gajapati area of Orissa. The number of *Anopheles annularis* collected from different localities of Orissa was 215. The *An.annularis* collected from different localities of Orissa was first screened for D3 and ITS2 region of ribosomal DNA to verify the sequence variation among the individual species. Novel primers were designed from the D3 region of 28S rDNA of *An.annularis* for carry out Single Strand Conformational Polymorphism (SSCP) to see the differences between the sibling species. We observed two marked differences in the lane 1 and 15 in Fig.1 and 3 marked differences in lane 1, 2, 4 and 7 of Fig. 2 of SSCP gel.

To know the sequence variation all samples were sequenced for both D3 and ITS2 region of rDNA. Total 45 samples were sequenced for the ITS2 (~475 bp) and D3 (~400 bp) region of 28S rDNA for An. *annularis* collected from different localities of Orissa (Table1). Sequencing was done in both directions for the primers. Alignment of the sequence with the database NCBI information using BLAST searched confirmed that the sequences are from the D3 and ITS2 region of the rDNA of *An.annularis* and their closely related group. The D3 and ITS2 sequences were edited manually and all additional relevant sequences of the two loci will be soon submitted to Gene Bank.

From the sequence data it is revealed that mosquito collected from Keonjhar, Gajapati, Angul, Choudwar, Baudh did not show any intraspecies sequence variation and were identical to each where as some samples showed interspecies variation within the *An.annularis* group. The majority of the specimens from Orissa showed identical sequence to that of the *An. annularis A* (considered as "species A") where as some species which was misidentified as *An.annularis* which showed marked difference in the SSCP gel was found as An.philippinensis, *An.pallidus* after sequencing. Further species differentiation is going on by using PCR-RFLP, species specific multiplex PCR.

Standardization of human host preference and presence of parasite by PCR was done (Fig.3). A new primer was designed from human and parasite genome respectively. This process is modified and easier than previous PCR detection of *P.falciparum* and gel diffusion technique of human blood. By the new method fifty DNA samples of *An.annularis* were tested and two were found positive for *P.falciparum*.

Table1 : Sequencing result of *An.annularis* complex

Sl.No.	Area of collection	Amplified region	Type of species	No.Sequenced
1.	Angul	D3	*An.annularis A*	4
2.	Gajapati	D3	*An.annularis A*	2
3.	Choudwar	D3	*An.annularis A*	1
4.	Keonjhar	D3	*An.annularis A*	2
5.	Boudh	D3	*An.annularis A*	4
6.	Kandhamal	D3	*An.pallidus*	2
7.	Gajapati	ITS2	*An.annularis A*	1
8.	Angul	ITS2	*An.annularis A*	4
9.	Boudh	ITS2	*An.philippinensis*	1

Figure 1 (SSCP assay)

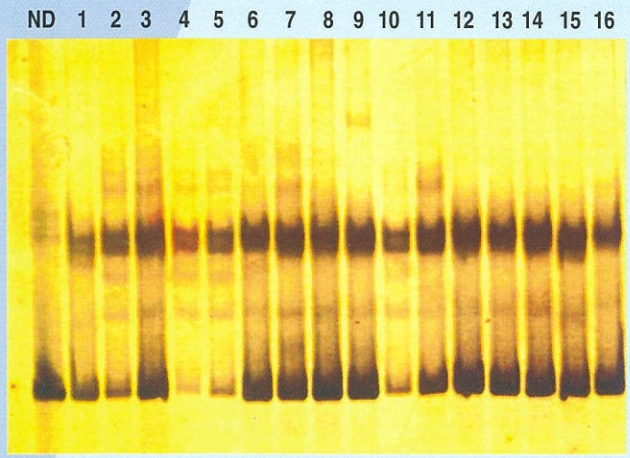


Figure 2 (SSCP assay)

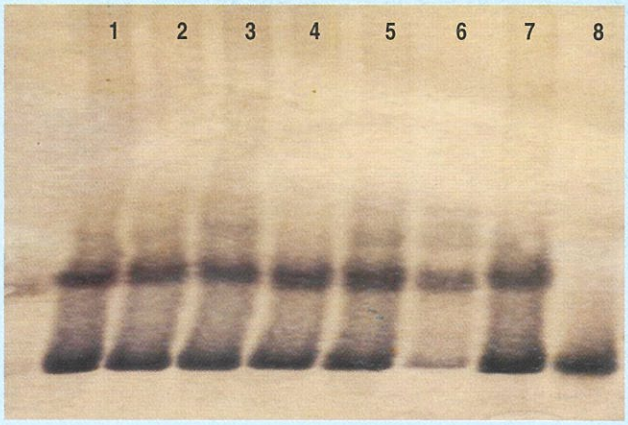
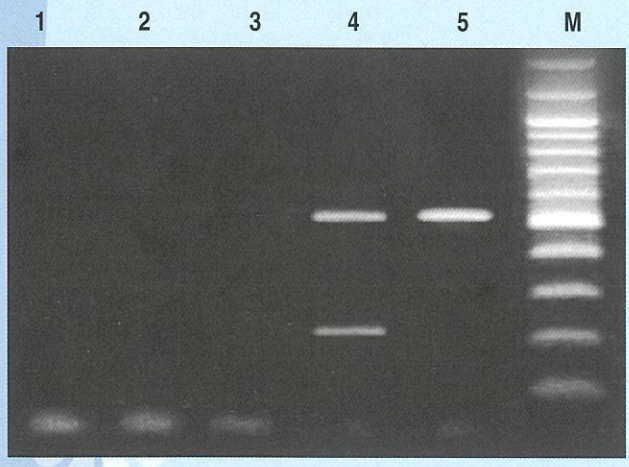


Fig 3



PCR standardization of sporozoite and human blood presence in An.annularis species. Lane 1-3: Test sample of An.annularis species, Lane 4:P.falciparum positive human blood, Lane 5: Normal human blood.

1.3 Development of potent mosquitocidal agents from natural sources

Status : Extramural
(ICMR Task force)
Investigators :
Dr. N. Mahapatra
Dr. R. K. Hazra
Dr. S. K. Parida
Dr. U. V. Mallavadhani (Collaborator)
Regional Research Laboratory, BBSR
Stating date : March 2005
Closing date : July 2007

Objectives :

1. Identification, collection and extraction of potent natural sources (Terrestrial plants, mushrooms, high altitude taxa like lichens, orchids and ferns.
2. Generation of abundantly available natural products and analogues.
3. Mosquitocidal screening of the natural sources and natural products/analogues against mosquito vectors, *An. stephensi* (malaria), *Cx.quinquefasciatus* (filariasis), *Ae.aegypti* (dengue).
4. Development of potent natural mosquitocides

Procedure for collection and extraction of natural sources.(carried out by RRL)

Natural sources such as plant ,Lichen, mushrooms etc were collected from out location and wild sources. Species were identified .The collected plant product were washed with fresh water, shed dried and powdered in pulverizer. The powder sources material were packed in soxhlet extractor and extracted with various polar and nonpolar solvent such as; n-Hexane, Ethylacetate, and Methanol. Concentration of these extract under reduced pressure yielded the respective residues. These residues are then screened for mosquitocidal activity.

Initially methanol, chloroform, ethylacetate and water soluble extract of Cinnamon species and methanol and ethylacetate extract of Euphorbia and Dispirus species respectively were supplied by RRL for screening for mosquitociddal activity.

Bioassay test:

The bioassay test of the plant extracts were carried out in the laboratory condition against the 3 species of mosquitoes viz.,*An.stephensi*(vector of malaria), Ae.aegypti (vector of Dengue) and Cx.quinquefasciatus (vector of filariasis) following standard WHO procedure (WHO 1981) . A known amount of the extract were dissolved with a known volume of solvent to give the stock solution ,appropriate amounts of which were added separately to 100ml of water in 500ml beakers to give different test concentration(0.01to1ppm) . Each concentration were replicated five times. After addition of the test material, the water were stirred vigorously and left for about 30 minutes for evaporation of the solvent. Around 15 healthy laboratory bred late 3rd instar larvae were released into each beaker for assay. The mortality and behaviour of the larvae were observed .A pinch of yeast tablet were given into each beaker for Bioassay test:

The bioassay test of the plant extracts were carried out in the laboratory condition against the 3 species of mosquitoes viz.,An.stephensi(vector of malaria), Ae.aegypti (vector of Dengue) and Cx.quinquefasciatus (vector of filariasis) following standard WHO procedure (WHO 1981) .

Results:

We have screened 9 coded products out of 17 extracts given to us by RRL, Bhubaneswar for doing bioassay test of the plant extract against 3species of mosquitoes viz., An.stephensi, Ae.aegypti and Cx.quinquefasciatus

following standard WHO procedure (WHO 1981) The product, RRL 010 gave 100% mortality at 50 ppm for Cx. quinquefasciatus, RRL 002 and 003 showed 30% and 40% mortality at 10 ppm after 96 hours.

LD 50 and LD 90 values were calculated for 010,012,023 products for Cq quinquefasciatus and the values are 1.56 ± 0.28 4.25 ± 2.12, 0.98 ± 0.25, 1.50 ± 0.47, 1.83 ± 0.21, 2.81 ± 0.43 (in ppm) respectively. LD 50 and LD 90 values were 1.50 ± 0.12, 2.24 ± 0.26, (in ppm) for An stephensi of the product 010. The study is in progress

Some of the products which showed delayed adult emergence and abnormality in the larval structure will be tested whether it has some insecticidal growth regulatory hormonal effect. The study is in progress.

Further work is also going on to generate more extracts and fractions to identify the potent natural mosquitocidal agents.

Justification for the Extension of the Project (One Year)

Some of the products which showed delayed adult emergence and abnormality in the larval structure will be tested whether it has some insecticidal growth regulatory hormonal effect present or not.

The screening of five high altitude taxa (Ferns, Lichens, Mushrooms) and five potential triterpinoides needs to be carried out.

Further work is also needed to find out the active compound by silica gel chromatography.

1.4 A study on immunoregulation and genotyping for cytokine polymorphism in human cerebral malaria

Status : Extramural
(ICMR Task force)
Investigators :
Dr. A. K. Satapathy
Dr. B. Ravindran (P.I)
Collaborators :
Dr.Shobona Sharma, TIFR, Mumbai
Dr. B. K. Das, SCB Medical College, Cuttack
Starting date : Jan 2006
Closing date : Dec 2008

Objectives:

1. To study B-cells responses (IgG and IgE) to malarial phospoproteins, Viz. PfPO, Pf2, Pf9 and MSP1, MSP3, AMA 1 and GPI in cerebral and/or in multiorgan dysfunction in human P.falciparum malaria
2. To quantify T-regulatory cells a) CD4+ CD25+ b) CD4+ CTLA 4+ cells in circulation and CSF in human cerebral malaria
3. To type the following host gene polymorphism and to correlate predisposition to develop cerebral and/ or Multi-organ dysfunction in P.falciparum malaria: a) TLR-4; b) TGF-b; c) TNF-a d) inos and e) IFN-g

One of the severe pathological manifestations observed in *P.falciparum* infections is cerebral malaria and more crucially patients developing multiorgan dysfunction involving renal and hepatic dysfunction along with cerebral symptoms. However, only a subset of *P.falciparum* infected patients suffer from such clinical symptoms. The factors responsible for development of cerebral malaria amongst *P.falciparum* patients are not yet clearly identified. Host genetic predisposition as well as an immune response that is dominated by inflammatory responses have been attributed to development of cerebral malaria. Several immune responses and host genetic polymorphism have been implicated in naturally acquired immunity and in cerebral malaria. In the current study an attempt has been made to study the host gene polymorphism involved in several arms of the immune response have been investigated for correlation with the clinical manifestation of *P.falciparum* malaria. Innate immune recognition of

Plasmodium and subsequent release of cytokines and inflammatory mediators are important for parasite clearance but may also contribute to disease severity. The family of toll-like receptors (TLRs) has been identified as key host molecules in the induction of innate immune responses to microbial ligands. The Toll Like Receptor (TLR) family is a group of pattern recognition receptors. TLR-4 is found on surface of mammalian monocytes, macrophages and neutrophils. It recognizes endotoxin (lipopolysaccharide present in cell wall of gram negative bacteria) as its ligand and results in induction of inflammatory molecules. Two known mutations in TLR4 gene (Asp299Gly, Thr399Ile) have been known. Glycosylphosphatidylinositol (GPI) molecules of the parasites have been identified as dominant toxins involved in the pathogenic process. These molecules initiate the production of excess levels of the cytokines tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), leading to a systemic inflammatory cascade, renal failure, multiorgan dysfunction, hypoglycemia, lactic acidosis, and death. Purified malarial GPIs also increase expression of cell adhesion molecules (ICAM-1, VCAM-1, and E-selectin) and nitric oxide production in human vascular endothelial cells through cytokine-independent pathways. Since, recently, *P. falciparum* GPI was reported to induce signaling via both TLR-2 and 4, we hypothesized that modified recognition or signaling via variants of TLR-4 could influence susceptibility to and manifestation of malaria.

Progress of work done:

Patients reporting at the out–patient department and/or admitted to the department of Medicine at SCB medical college, Cuttack with short history of fever associated with unarousable coma were assessed clinically. Based on the status of clinical manifestation the patients were divided in to complicated and uncomplicated malaria group.

The project was initiated in Jan '06. About 130 blood samples of clinically non-complicated *P.falciparum* malaria, complicated malaria with renal or hepatic involvement-multiorgan dysfunction were collected at SCB Medical College, Cuttack as reported in previous year annual report. About 300 blood samples have been collected so far. DNA were purified from the leukocytes and preserved for typing genetic polymorphism.

1. TLR-4 polymorphism:

The TLR-4 (Asp 299Gly) is caused by substitution of A>G in the coding region at position 896. This mutation was detected by allele specific PCR assay. Purified DNA from leucocytes was used for typing genetic polymorphism of TLR-4. Two mutations in TLR4 gene (Asp299Gly, Thr399Ile) in the human population have been known. We typed the mutation by PCR assays using the (wild type) Forward primer-5'-CTTAGACTACTACCTCGATGA-3', and (mutant) Forward primer 5'-CTTAGACTACTACCTCGATGG-3', (allele specific interaction at the 3' end) with a common anti-sense Reverse Primer- 5'-TAAGCCTTTTGAGAGATTTGA-3'. PCR assay was performed as follows: 12 cycles of 10 seconds at 95^0 C followed by 60 seconds at 65^{0}C, followed by 17 cycle of 10 seconds at 95^{0}C, 50 seconds at 60^{0}C and 30 seconds at 72^{0}C. The final extension was 7 min at 72^{0}C. The final amplicon of PCR product is 192 bp. The amplified products were analyzed by electrophoresis on 2.5% agarose gels stained with ethidium bromide.

To check the possibility of host factors playing a role in the clinical outcome of malaria infection, the prevalence of TLR4 (ASP 299 GLY) genotypes was assessed in about 250 patients with malaria displaying different clinical manifestations. As shown in Table-I the distribution of A and G allele was found to be 82% and 18% respectively. The frequency of heterozygous genotype (AG) was found to be more when compared to wild type genotype (GG). The genotype frequency for TLR-4 (299) mutation was significantly different between complicated

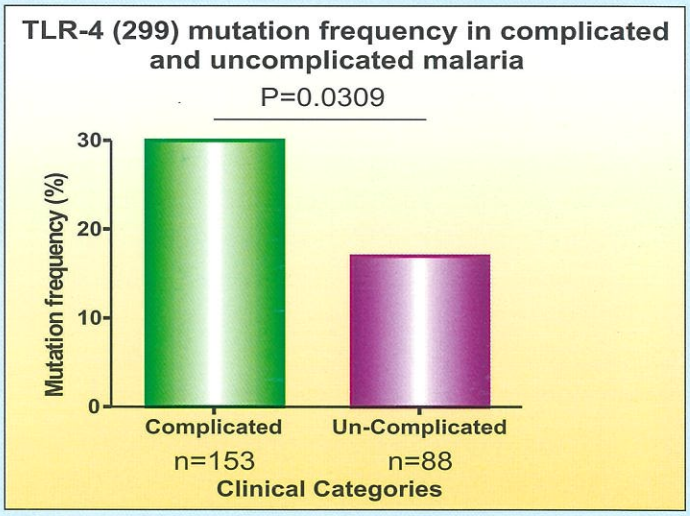
and uncomplicated malaria – the mutation was significantly high in complicated malaria cases in comparison to non-complicated cases suggesting a genetic predisposition of subjects with this mutation to clinical complications associated with human malaria. (p=0.0309) (Fig-1).

Table-1

.	SNP	Allele	Frequency (%)	Genotype	Frequency (%)
TLR4	Asp299Gly	A	395/480 (82)	AA	179/240 (75)
		G	85/480 (18)	AG	37/240 (15)
				GG	24/240 (10)

Genotype and Allele frequencies for TLR-4 (Asp299Gly) mutation in clinical manifestation of *P.falciparum* malaria

Fig-1



2. TGF- polymorphism:

The anti-inflammatory properties of TGF-β in malaria are well documented. In mice, TGF-β in regulated quantities promotes protective immune responses, with slower parasite growth during early infection, increased quantities appear to down regulate pathology during late infection. Circulating levels of TGF- β were found to be low during lethal infections with *P. berghei* ANKA in mice. In contrast resolving infections with the non-lethal parasite *P. chabaudi* and *P. yoelli* were correlated with a significant TGF- β production. Further, data from human malaria infections suggest that TGF- β inversely correlated with malaria severity. Although host genetic polymorphisms have been implicated in naturally acquired immunity and in cerebral malaria, the status of polymorphism of TGF- β gene has not been examined and the current study was undertaken to fill this lacuna.

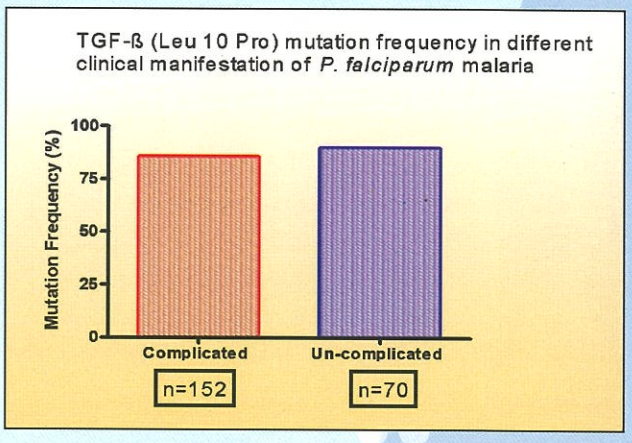
The TGF-β (Leu 10 Pro) mutation is caused by a T to C substitution at codon 10 of exon-1 region. This mutation was detected by PCR-RFLP with sense primer 5'-GCC TCC CCA CCA CAC CAG-3' and antisense 5'-GCC GCA GCT TGG ACA GGA-3'. The parameters were denaturation at 95^{0}C for 5 min, followed by 30 cycles, denaturation at 95^{0}C for 30s, annealing at 60^{0}C for 30s and elongation at 72^{0}C for 45s. The final elongation was at 72^{0}C for 5 min followed by cooling at 4^{0}C. The 237bp length amplicon was digested at 37^{0}C with MspA1I for 4 hrs resulting in fragments that either cut into three fragments of 104bp plus 92bp plus 41bp (allele C) or 133bp plus 92bp plus 12bp (allele T). These fragments were analyzed by electrophoresis on 3.5% agarose gel and stained with ethidium bromide.

Table - 2

Gene	SNP	Allele	Frequency (%)	Genotype	Frequency (%)
TGF-β	Leu 10 Pro	T	184/444 (41)	TT	28/222 (12)
		C	260/444 (59)	TC	128/222 (58)
				CC	66/222 (30)

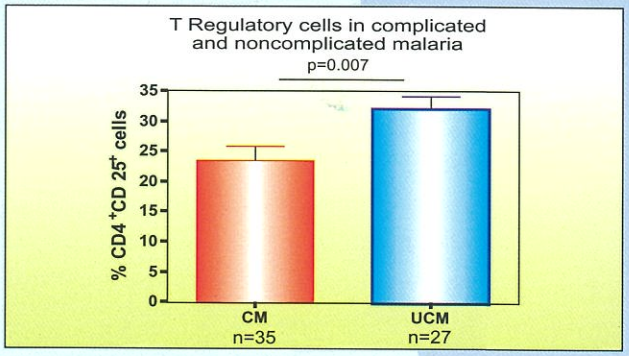
As shown in Table-II the distribution of T and C allele were 41% and 59% respectively. The frequency of heterozygous genotype (TC) was found to be more as compared to wild type genotype (TT). We assessed the association between TGF- β (Leu 10 Pro) polymorphism in complicated and uncomplicated *P.falciparum* malaria and the results are shown in Fig-2. There was no significant difference between complicated and non-complicated malaria cases suggesting that Leu 10 Pro mutation TGF- β gene does not play a major role in determining the clinical outcome in human malaria.

Fig-2



The factors responsible for precipitation of cerebral malaria amongst *P. falciparum* patients are not yet clearly identified. Various T-cells associated factors have been ascribed to precipitation of and/or protection from cerebral malaria. Development of cerebral malaria (in both humans and mice models) is attributed to inflammatory Th1 types of responses leading to production of high levels of TNF- α. Such hyperactivity of T-cell responses are now known to be down-regulated by regulatory T lymphocytes which have been characterized in recent years and phenotypic markers on these cells have been identified. In this context the study of T regulatory cells in human cerebral malaria has crucial implications. Attempts were made to measure T-regulatory cells (CD4+andCD25+cells) in complicated and non-complicated malaria and the results are shown in Fig-3. CD4+ and CD25+ cells were found to be significantly low in complicated malaria in comparison to non-complicated malaria indicating a role for inflammation observed in complicated malaria.

Fig-3



Further, the transcription factor FOXP3 is known to play a key role in CD4+CD25+ regulatory T cell function. These cells are anergic to proliferative responses in vitro and do not express key cytokines including IL-2 or IFN-γ in response to stimulation. In the absence of reliable antibody reagents, FOXP3+ cells are normally identified by virtue of high levels of expression of CD25+ on CD4 T-cells. The CD 4+ T cells with the highest level of CD 25+ (CD 4+ CD

25+ high) have been shown to appear as a tail to the right from the major population when T-cells are double stained with anti-CD4 and anti-CD25 antibodies. Thus, the CD4+CD25+ 'high' populations were gated as shown in Fig-4 and the cells in the gate were quantified .We quantified the T regulatory cells expressing high levels of CD25+ in non-complicated and complicated human malaria with a view to study the relationship of T regulatory cells with inflammatory response observed during malaria. The results are shown in Fig-5. The CD4+CD25+ 'high' cell population was significantly low in complicated malaria in comparison to clinically uncomplicated malaria.

Mononuclear cells from human blood were stained with anti CD4 and anti CD25 antibodies. The CD 4+ CD 25+ high populations were gated (R3) and scored.

Fig-4

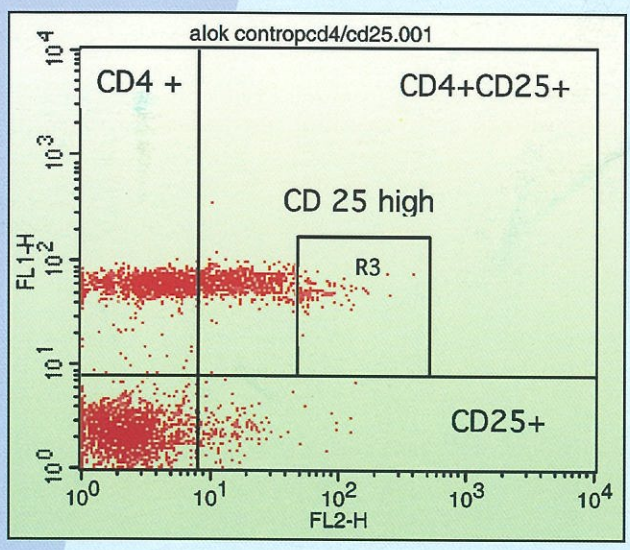
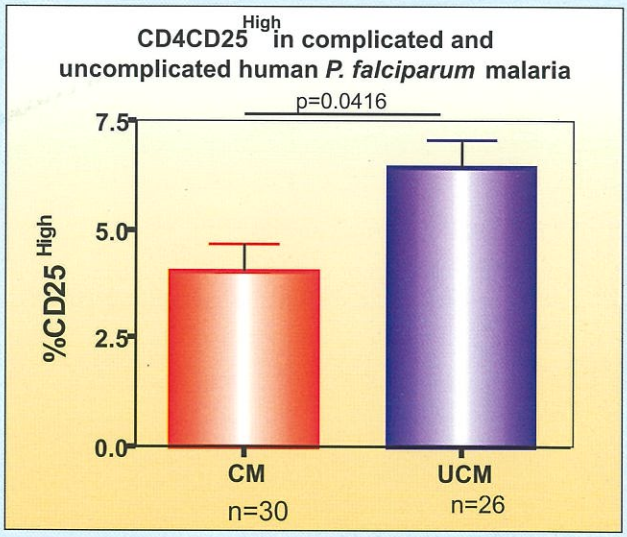


Fig-5



1.5 The effect of choloroquine chemoprophylaxis during pregnancy among the compliant and non - compliant mothers of Orissa.

Status : Intramural
Investigators:
Dr. Amarendra Mahapatra
Mr. D.P. Hansdah
Mr. H.S. Naik
Mr. B. Purohit
Stating date : March 2006
Closing date : February 2008

Background Information:

Evidences reveal that *P. falciparum* is a major cause of anemia in pregnancy, especially in primigravidae. Effective measures aim at prevention of malaria and anaemia in pregnancy, especially in primigravidae, that would significantly reduce anaemia and its deleterious effects on both the mother and the baby.

Under the IMR mission Government of Orissa is supplying weekly 600mg of Choloroquine chemoprophylaxis, to all the pregnant mothers

in the State since 2002, under the National malaria control programme. All the pregnant women are registered for immunization and ANC at the Angan Wadi Center in the village. After first trimester of the pregnancy the AWC worker gives the chemoprophylaxis to all the pregnant women weekly till 6 weeks following child birth. This has not been evaluated earlier. The study attempts to assess the compliance rate and compare the pregnancy outcomes between the compliant and non-compliant mothers. The reasons for non-complaint will also be documented.

It is necessary to base best practice on the best available evidence for the particular situation. The acceptability of the intervention will depend on its value as perceived by the providers and the consumers of care. Health education in the context of local cultural and behavioral factors can assist in creating awareness of the importance of antenatal care and of the benefit of specific interventions. Further the risks associated with parity and malaria in pregnancy appears to be greatest in young women.

Objectives:

1. To assess the frequency of malaria among the pregnant women between complaint and non complaint mothers.
2. To assess the drug compliance pattern.
3. To enumerate the reasons of non compliance.

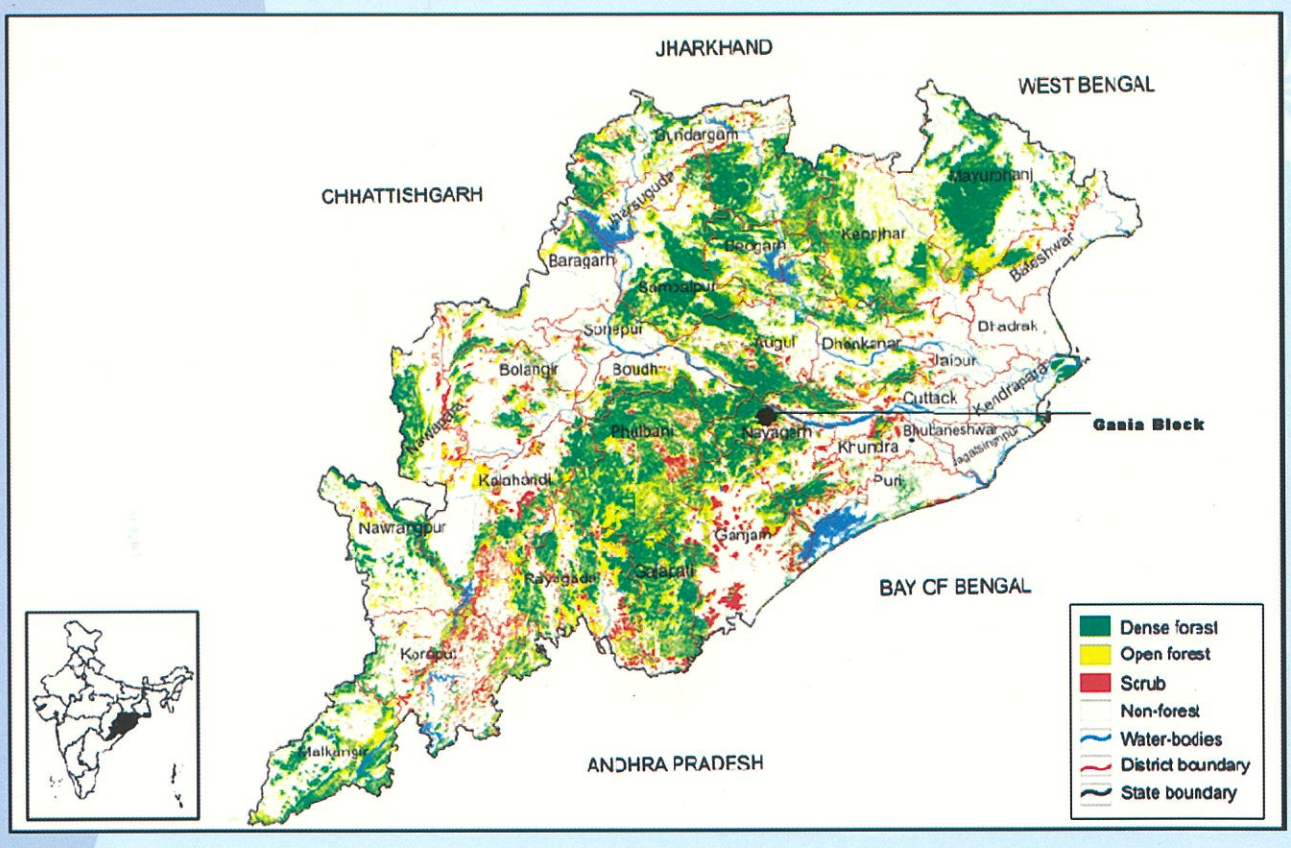
Method:

A cohort of 500 pregnant women in their first trimester will be registered and followed up prospectively in each subsequentrimster and after child birth. Door to Door follow up schedule 2nd, 3rd trimester and at child birth) includes assessment of (i) past obstetric and malaria history (ii) peripheral blood examination by finger prick for malaria parasite detection by both slide microscopy and RDT (Rapid Diagnostic Test Kit, (iii) Hb% status (Cyanohoemoglobin method). At child birth both placental and cord blood will be cfollected for parasite and chloroquin resistance status of parasite for Pfcrt & pfmdr point mutation. The data on baby born will be recorded. The antenatal attendance of mothers to clinics will be recorded. Based on above data, pregnant women will be devided in two groups (i) CQ compliant (full dose taken) (ii) CQ non-compliant. While follow up local anganwadi workers help will be taken. The data will be compared between two groups, using SPSS statistical package.

Area:

Gania and Daspalla Blocks of Nayagarh district of Orissa is selected as study sites. The area is situated in the Mahanadi basin of Nayagarh—Phulbani forest range. Nayagarh District is highly endemic for P.falciparum malaria, with 34 malaria deaths in 2002 and 6 in 2004. The malaria data related to Gania PHC for period 2003-2006 is given in Table1.

A Map of the area :



Tab-1: Epidemiological information on Malaria in Gania PHC.

Year/ Epidemiological Parameter	2003	2004	2005	2006
SPR	5.4	9.2	8.2	9.2
Pf%	83.1	82.2	87.2	85%
API	11.9	22.6	22.2	22.7
ABER	22.09	24.39	26.94	24.4
Deaths	2	2	6	0

Result:

This is an ongoing study and registration of the pregnancies in first trimester is carried out at present. Till date 219 pregnant cohorts have been registered from 16 Gram Panchayats, 35 villages & hamlets of Gania Block, in Nayagarh district, of Orissa. Till date 77 pregnancies were revisited for follow-up.

Among 78 registered cases, 18 had history of malaria (Table-3). In the follow-up during 2nd trimester, 1 out of 5 women reported malaria. Out of 78 pregnancies registered 31 were in their 1st Parity, the rest 47 Pregnancies

reported a Bad Obstratics history (BOH) of last pregnancies. They are as follows: Still birth-2, Abortion- 4 and Infant death due to Malaria - 2 cases. In another 2 cases infant death occurred in the 4th and 7th day but the reasons of cause of death could not be ascertained. Required number of samples will be collected and analysed in due course of time, for any conclusive results.

The preliminary results reveal an association of gravidity with that of haemoglobin level (Hb %). At present it suggests that with an increase of gravid status the mean Hb% decreases.

The mean Hb% among these subjects at baseline was 9.3%; Out of 219 pregnant cases covered 80.0% were anemic; Out of anaemic cases 10.0% were graded as severely anemic (Tab-2).

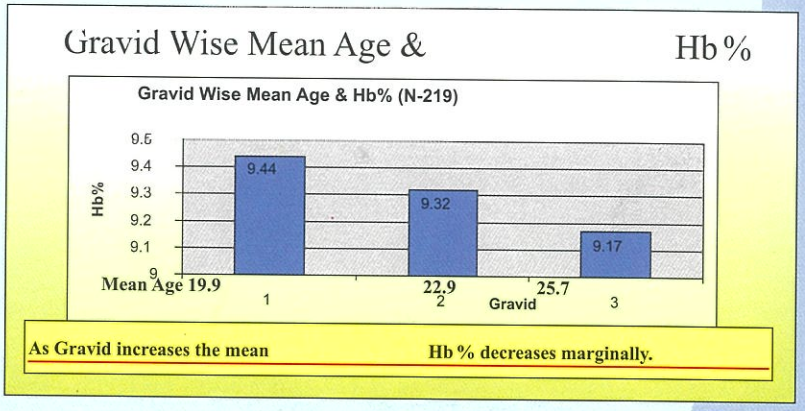


Table –2: Distribution Hb% among the pregnant women (n=266)

Anemia level	Normal >11	Mild 9-11g/dl	Moderate 7-9g/dl	Severe < 7	Total No.
No.	44 (20.0%)	87	66	22 (10.0%)	219

Table: 3 Epidemiological Detaild of all subjects:

Parity / Number	1st	2nd	3rd +	TOTAL
No.of Preg. Women	97	78	44	219
Malaria History	17	17	14	48(22%)
Hb%	9.44%	9.32%	9.17%	9.3%

1.6 Malariogenic stratification of Anugul district of Orissa using sibling species prevalence of malaria vectors.

Status : Intramural
Investigators :
Dr. N. Mahapatra
Dr. R. K. Hazra
Dr. S. K. Parida
Mr. N. S. Marai
Mr. H. K. Triparthy
Starting date : October 2003
Cosing date : September2007

(Details of status report submitted by the scientist will be presented)

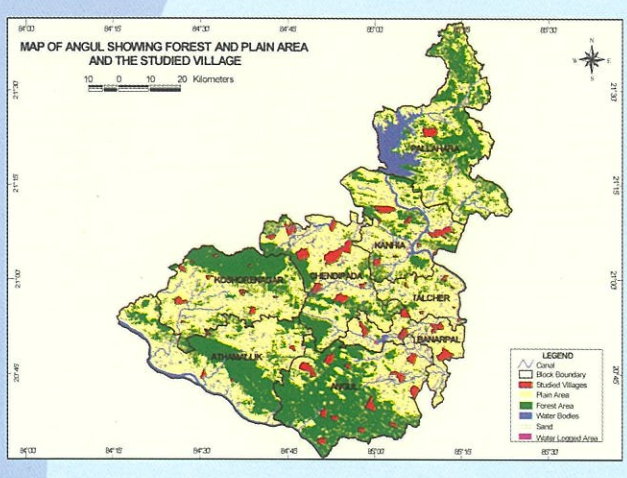
Objectives:

1. To study the prevalence of different sibling species complex of malaria vectors and their susceptibility status to insecticides in Anugul district of Orissa.
2. To study the bionomics of the complex like resting, feeding and biting behaviour, anthropophilic indices, gonotrophic cycle, and preferential breeding habit.
3. Malariogenic stratification of the district basing on the above parameters

Results:

The entomological studies carried out from eight PHCs of Angul district reveals the presence of 13 species of mosquitoes belonging to four genera i.e. *Anopheles, Culex, Aedes and Armigeres.f Anopheles* species collected were *An.aconitus, An.annularis,, An.culicifacies ,An.fluviatilis ,An.hyrcanus, An. maculatus, An. pallidus, An. pseudojamsei, An. subpictus ,An.splendidus, An. tessellatus, An. vagus and An. varuna* in the district. Among them three vectors viz. An.culicifacies, An.fluviatilis and An.annularis were found to play important role in transmission bases on their density and infection level detedted.

Out of the above three vectors, two species namely An.culicifacies ,and An.fluviatilis were processed for identification of sibling species by both polytene chromosomal method and molecular methods. An.culicifacies was composed of sibling species A, B, and C where as An.fluviatilis was composed of S and T. The remote sensing data, land use and land cover maps were collected and analyzed for forest coverage and elevation from the sea level. Based on forest coverage (0-10%,11-29%,more than 30%) the district was divided in to 3 zones. These three ecozones tally with the altitude from sea level (Fig1). In each ecozone all the three vectors were present with seasonal variation observed (fig-2) .The sporozoites are also detected in all the three species with different sporozoites rates Fig-3. An.culicifacies A & D was were reported first time in Orissa and also An.annularis plays an important role as secondary vector.



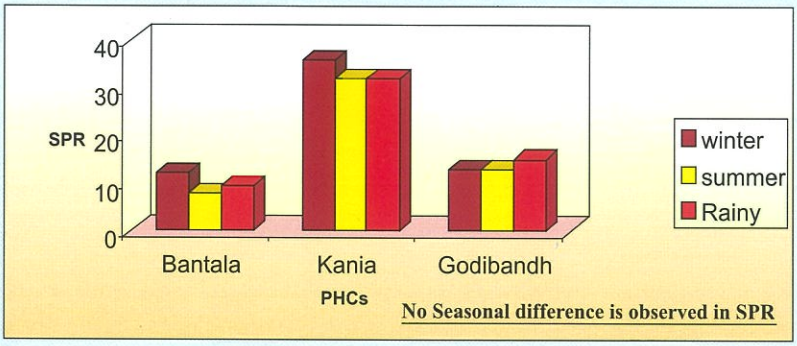
Index : Pink=600-1200 mts MSL ; Green= 300 – 600 mts MSL
Yellow = less than 300 mts MSL.

Based on GIS, taking forest coverage, and height & water bodies Angul district has been divided in to three ecozones.

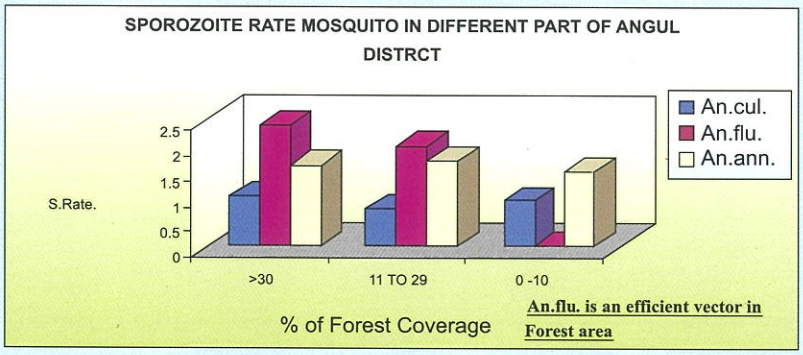
(i) High forest coverage (ii) medium & (iii) low that correspondes to elevation from sea level as well. The data on vector pattern density and infectivity are correlated with forest coverage since mosquito habit is biologically influenced by ecology. Higher the forest coverage higher was found the vector density of *An.fluviatilis* and infection rate.*An.culicifacies* played the major role in all ecozones particularly in rainy season and in area with lowest forest coverage. *An.fluviatilis* density was higher in winter season particularly in higher forest covered areas in foothill region. *An.annularis* played secondary but important role in transmissions in all ecozones, particularly in winter. Epidemiological data indicates perennial transmission. Comparative analysis is being carried out to correlate the vector abundance and its parasite infection status in three seasons in different ecozones.

Ongoing Studies

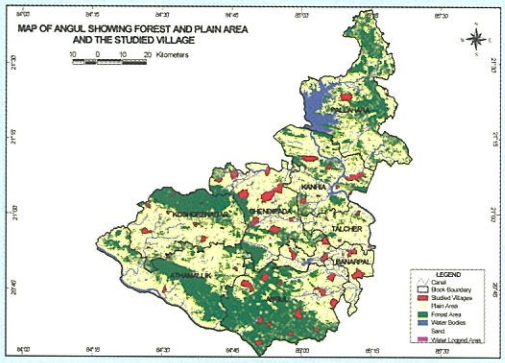
Malaria incidence of three PHCs in different season



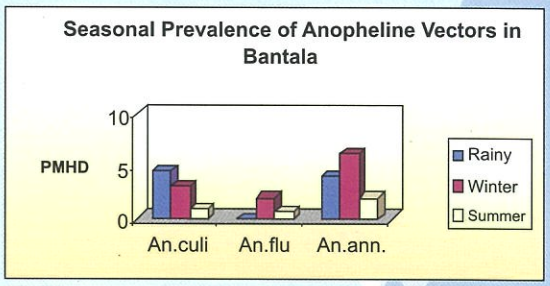
No Seasonal difference is observed in SPR

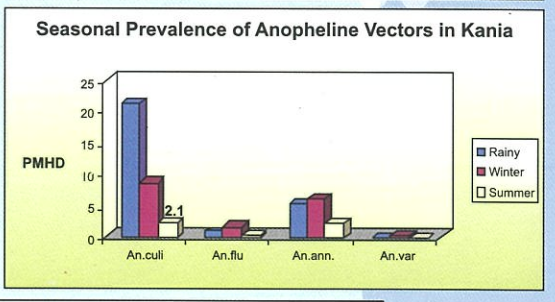


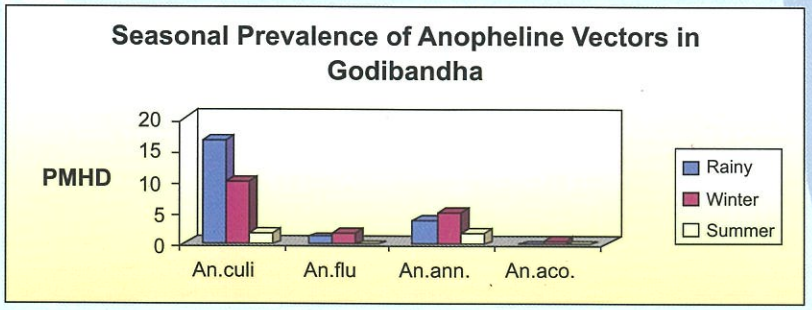
An.flu. is an efficient vector in Forest area



Vector prevalence map of 3 different PHCs with different forest coverage







Susceptibility Test :

The susceptibility status of *An.culicifacies sibling species* B and C to insecticides showed that both B and C are resistant to DDT but susceptible to Deltamethrine 0.5%. *An.fluviatilis* is sensitive to DDT.

1.7 A randomized clinical trial with chloroquine and alternate drug regimens to study the comparative efficacy, in treatment of uncomplicated P.falciparum malaria in two endemic districts of Orissa".

Status : Extramural
Investigators :
Dr. A. S. Kerketta
Dr. M. R. Ranjit
Mr. P. K. Jangid
Starting date : March 2006
Closing date : February 2008

Objective of the study

Primary objective:

To study the comparative efficacy of four antimalarial drug regimens in treatment of uncomplicated P. falciparum malaria.

Secondary objective:

(i) To differentiate the recrudescence and re-infection by using molecular marker.

Trial regimens

1. Chloroquine (10mg/kg on Day 1 and 2 followed by 5mg/kg on Day 3) over three days.
2. Single Oral dose of SP(25mg/kg BW of Sulfadoxin +Pyrimethamine 1.25mg/kg BW).
3. Single Oral dose of SP(on Day 0) + Oral Artesunate for three days (Sulfadoxine 25mg/kg BW, Pyrimethamine 1.25mg/kg BW, Artesunate 4mg/kg BW) once daily.
4. Lumerax (fixed oral dose of combination of 20 mg of artemether and 120 mg of lumefantrine) at 8 hourly dose schedule.

Inclusion criteria:

1. Age > 6months –65yrs and both sex
2. P. falciparum mono infection with parasite density of 1000-100000-parasite/µl of blood.
3. Axillary temperature >37.5 and <39.5° C
4. Ability to come for the stipulated follow up visits 5. 5.Informed consent by the patient or by parent/ guardian for children.

Exclusion criteria:

1. One or more of danger signs or any signs of severe and complicated malaria
2. Presence of severe malnutrition.
3. Pregnancy

Ongoing Studies

Work progress

On the pilot basis the study was initiated intramurally & was undertaken at PHC Nuagaon of Kandhamala district of Orissa in close collaboration with the PHC medical officer. The average of past 3 years (2004-2006) malariogenic surveillance data of the PHC undertaken by state health department show SPR of 9.53%, SFR 9.38%, API 26.94% and Pf prevalence of 98.36%. The block has several endemic villages, of which one highly endemic village situated close to the PHC was taken for the study. The total household in the village is 204 and total population 1568. Central clinic was organized at the anganwadi centre of the village. History of fever was recorded with simultaneous recording of body weight and axillary temperature. Detail clinical examination was done and finger prick blood was collected after obtaining written consent. A total of 117 fever cases screened, of which Pf infection found among 98 (83.8%) cases. Similarly Pv infection was found in 6 (5.1%) cases and mixed infection of Pf and Pv was marked in 9(7.7%) cases. Four (3.4%) cases were found to be free from malaria infection. The subject selection follows the inclusion and exclusion criteria of WHO guideline for assessment of therapeutic efficacy of different antimalarials. A total of 62 eligible cases were included in the study. Of these 40 (64.5%) were female and 22(35.5%) were male (table-1). The age rages from 7 months to 56 years. The above mentioned trial drug regimens were administered to the patients according to permuted block randomization table. The drugs were instituted to the patient under direct supervision of the study team. Thus 16 cases allocated the regimen arm 1 and 2 that is Cloroquine and Sufladoxine pyremethamine and 15 each in arm 3 and 4 that is SP+ Artesunate and Lumerax (Lumefantrine +Artemether). Good clinical practices (GCP) guideline was followed strictly during the study. The cases were closely monitored daily for first three days and thereafter on 7th day i.e on 7th ,14th ,21st and 28th day.

Out of total cases studied 2, 1, 2 cases were lossed for follow up in regimen arm 1, 2 and 3 respectively. One case each in regimen 1 and 3 found having mixed infection during follow up microscopy. Thus out of the cases enrolled, the total study population could followed up till 28 days of study period were 13(81.25%), 15(93.8%), 12(80.0%), 15 (100%) in all the above drug regimen groups respectively. The efficacy of four-drug regimen was classified as per WHO guide line for assessment of therapeutic efficacy of antimalarials in intense malaria transmission area (WHO/MAU96.1077).

Classification of therapeutic response:

Assessment of therapeutic efficacy of antimalarials in intense malaria transmission area (WHO/MAU96.1077)

1. Early treatment failure (ETF)-Development of danger signs or severe malaria on Day 1, Day 2,or 3 in presence of parasitaemia
   - parasitaemia on Day 2 higher than Day 0 count with axillary temperature >37.5°C.
   - Parasitaemia on Day 3 > than 25% of the D 0 count
   - Presence of parasitaemia on Day 3 with axillary temperature >37.5°C.
2. Late treatment failure (LTF)- Development of danger signs or severe malaria in presence of parasitaemia on any day from Day 4 to Day 14.
   - Presence of axillary temperature>37.5°C in presence of parasitaemia on any day from Day 4 to Day 14 without previously meeting any of the criteria of ETF.

3. Adequate clinical response (ACR)- Absence of parasitaemia on Day 14 irrespective of axillary temperature, without previously meeting any of the criteria of ETF or LTF.

- Axillary temperature <37.5°C irrespective of presence of parasitaemia without previously meeting any criteria of E/LTF.

Out of the cases followed, four cases (30.8%) in regimen 1, three (20.0%) cases in regimen 2, and two cases (13.3%) in regimen 4 showed early treatment failure (ETF) and shifted to the near by government hospital for alternative and indoor treatment. Late treatment failure (LTF) was marked in three cases (20.0%) in regimen 2 and 1(8.3%) in regimen 3. The adequate clinical response was marked in 9 cases (69.2) in regimen 1, 9 cases (60.0) in regimen 2,11cases (91.7) in regimen 3 and 13 cases (86.7) in regimen 4.

Table -1 Delails of study population

	Chloroquine	Sulfadoxine-Pyrimethamine	SP+Aretesunate	Lumefantrine+Artemether
MALE	9 (56.2)	11(68.8)	10((66.7)	10(66.7)
FEMALE	7(43.8)	5(31.2).	5(33.3)	5(33.3)
Average Age	15.6	23.1	18.4	25.2
Range of Age	2.5-35	7m-52	1.5-50	3-56

Table-2 Therapeutic response to different antimalarials

	Reg I (CQ) N=13	Reg II (SP) N=15	Reg III (SP+ART) N=12	Reg IV (Co- ARTEM) N=15
ETF* N (%)	4 (30.8)	3 (20.0)	0	2 (13.3)
LTF* N (%)	0	3 (20.0)	1 (8.3)	0
ACR* N (%)	9 (69.2)	9 (60.0)	11 (91.7)	13 (86.7)

* ETF: Early treatment failure LTF: late clinical failure ACR: Adequate clinical response

1.8 Vector mapping with its susceptibility status to insecticides in high risk districts of Orissa

Status : Extramural (NVBDCP)
Investigators :
Dr. R. K. Hazra
Dr. N. Mahapatra
Dr. S. K. Parida
Mr. N. S. Marai
Mr. H. K. Tripathy
Stating date : November 2006
Closing Date : October 2008

Background :

Mosquito fauna that transmits malaria and its insecticidal susceptibility status in Orissa has not been precisely studied , although few reports exists from patchy regions of state. In view of high morbidity reported in Orissa due to malaria it is essential to identify the vector responsible for transmissions of malaria in different region and its susceptibility to different insecticides so as to plan for appropriate insecticide spray and IEC to curtail the spread. As requested by State health Department, seven high risk districts are being studied to describe the above.

Ongoing Studies

Objectives

- To study the vector fauna, their habits and density and vector infection rate in the sample sites of seven high district of Orissa.
- To study the susceptibility status of malaria vectors to different insecticides used in public health Programme.

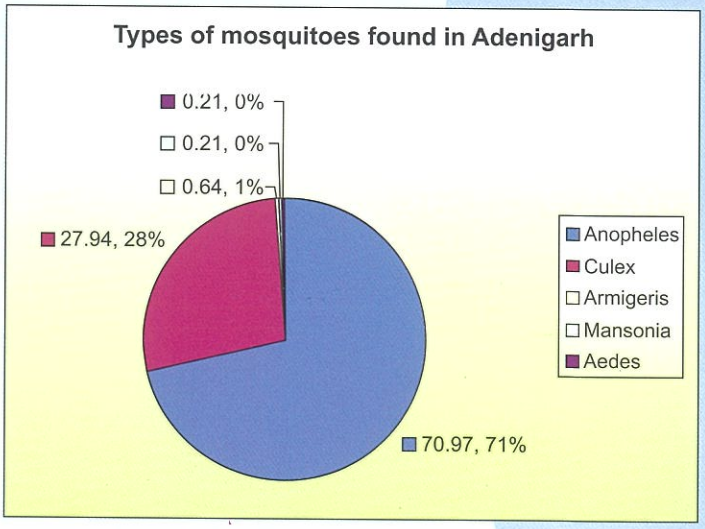
Work Progress:

Out of seven districts, Baudh, Gajapati, Kandhamal and Keonjhar district were surveyed. One time mosquito collection were done. This will be repeated in all seasons.

Vector survey at Adenigarh block of Baudh district.

Adenigarh PHC consists of 18 Sub Centres. We visited nineteen villages of five sub centre from where malarial deaths was reported in 2005. The five Sub Centres are Sarsara, Dholpur, Kharbhuin, Charichwak and Mahalik pada. Twenty six species belonging to five genera of mosquito were collected from sampled household of study villages. Ten percent of household from each study villages was subjected for vector survey and a total of 132 houses was included. Out of 26 species of mosquitoes, nineteen species were from genus Anopheles, the rest were Culex, Armegeres, Mansonia and Aedes. Total 1392 mosquitoes were collected among which 988 were Anophelines. Among nineteen anopheline species collected six were identified as known vectors of malaria they are An.annularis, An.culicifacies, An.fluviatilis, An.varuna, An.minimus and An.philipinnensis. The number collected and percentages with total anophelines were An.annularis 149(15.08%), *An.culicifacies* 213(21.55%) *An.fluviatilis* 147(14.87%), *An.varuna* 57(5.76%), *An.minimus* 5(0.5%) and *An.philipinnensis* 1(0.1%). All these were collected from both Human dwelling and Cattle Shed. Three species was found to be playing major role in transmission of malaria. Sporozoites were detected from *An.fluviatilis* out of 147 samples tested by PCR only 2 show sporozoite positive. No sporozoite was detected in other vectors tested.The susceptibility status of *An.culicifacies*, *An.fluviatilis* and *An.annularis* revealed that *An.culicifacies* and *An.annularis* were resistance to DDT but *An.fluviatilis* was susceptible to DDT (Table 2). All the species were observed susceptible to synthetic pyrethroid .Two important species those are known to be very efficient vectors of malaria were found, they are *An.minimus* and *An.philippinensis*. The periodical information on vectorial status for malaria transmission like identification of vectors and its species complex, bionomics, feeding habits and susceptibility status in these districts of Orissa are essential to help evaluation of efficacy measures in the control Programme.It is proposed to increase the sample size of house hold coverage to obtain adequate number of vectors for sporozoite detection as normally sporozoite positivity are usually detected in very low proportion of vector.

Fig.1



Name of the PHC/ Village	Anopheles species tested	Insectie cid	Death of Mosquito				% of control mortality	% of test mortality	Corrected mortality
			Control		Test	Dead/ Alive			
			1 hr.	24 hrs.	1 hr.	24 hrs.			
Adenigarh Kalimati	*An.fluviatilis*	DDT4%	Nil	Nil	10/10		Nil	100%	100%
Adenigarh Pitchukudi	*An.culicifacies*	DDT4%	Nil	Nil	1/10		Nil	10%	10%
Adenigarh Kambhun athpur	*An.annularis*	DDT4%	Nil	Nil	2/10		Nil	20%	20%
Adenigarh Kambhun athpur	*An.annularis*	Pyrethroids	Nil	Nil	10/10		Nil	100%	100%
Adenigarh Hariharpur	*An.culicifacies*	DDT4%	Nil	Nil	0/10		Nil	0%	0%
Adenigarh Hariharpur	*An.culicifacies*	Pyrethroids	Nil	Nil	10/10		Nil	100%	100%
Adenigarh Pitchukudi	*An.culicifacies*	Deltamet hrin 0.05%	Nil	Nil	8/10		Nil	80%	80%
Adenigarh Pitchukudi	*An.culicifacies*		Nil	Nil	0/10		Nil	0%	0%
Adenigarh Kalimati	*An.fluviatilis*	DDT4%	Nil	Nil	10/10		Nil	100%	100%
Adenigarh Kalimati	*An.fluviatilis*	Deltame thrin 0.05%	Nil	Nil	0/10		Nil	0%	0%
Adenigarh Kalimati	*An.fluviatilis*	Deltame thrin 0.05%	Nil	Nil	10/10		Nil	100%	100%
Adenigarh Lundryjore	*An.annularis*	DDT4%	Nil	Nil	6/10		Nil	60%	60%
Adenigarh Lundryjore	*An.annularis*	Deltame thrin 0.05%	Nil	Nil	10/10		Nil	100%	100%
Adenigarh Mahalikpada	*An.culicifacies*	DDT4%	Nil	Nil	0/10		Nil	0%	0%
Adenigarh Mahalikpada	*An.culicifacies*	DDT4%	Nil	Nil	0/10		Nil	0%	0%

Table1: Susceptibility Status of Malaria vector in Baudh districts of Orissa

Fig.2

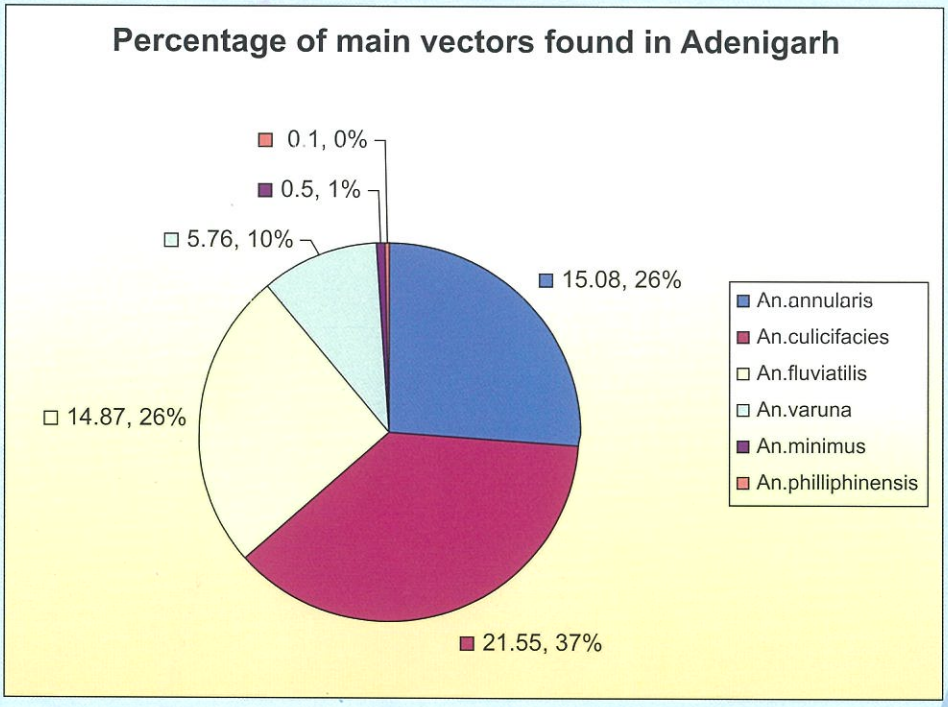
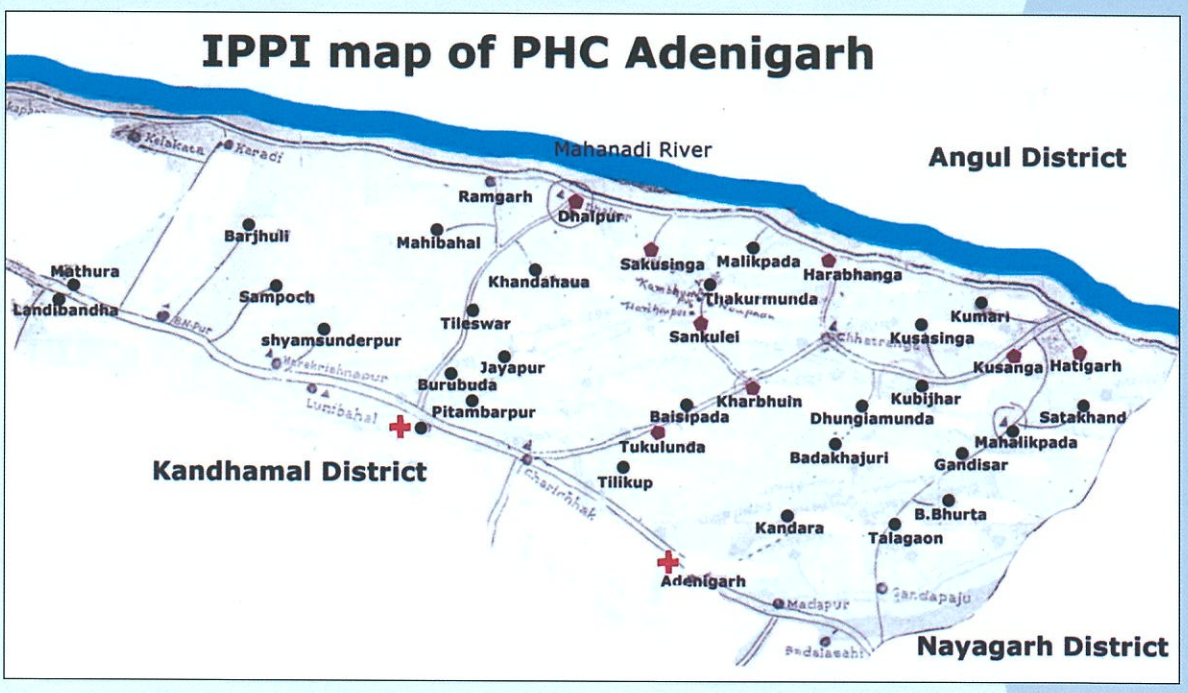


Fig.3



Out of 6 vectors of malaria collected only 3 vector species were subjected to insecticide susceptibility test.

Vector survey at Mohana block of Gajapati district

Gajapati district comprises seven PHC including district hospital, they are Kashinagar, Gumma, Rayagada, B.K.Pada, R.Udayagiri, Mohana, Gurandi and Parlakhemundi. Mohana PHC was selected as the area of the PHC is maximum and highest no. of malaria cases were recorded from this PHC. Distribution Anopheline vector species is depicted in this report. Eleven species belonging to two genera of mosquito were collected from 14 villages, nearly ten percent of house hold ie. 141 house hold were surveyed for vector collection.. Out of which eight species were from genus Anopheles, the rest were Culex. Total 307 mosquitoes were collected among which 264 were *Anophelines* .Among eight anopheline species collected three were identified vectors of malaria they are *An.annularis*, *An.culicifacies* and *An.fluviatilis*. The number collected and percentages with total anophelines was 17% of the total mosquitoes collected .The abdomen of major species showed half gravid (HG), full fed (FF), and unfed. An.culicfacies and An.annularis was collected from both cattle shed and human dwelling.

The susceptibility status of *An.culicifacies, An.fluviatilis* and *An.annularis* revealed that An.culicifacies and *An.annularis* were resistance to DDT but *An.fluviatilis* was susceptible to DDT (Table 2). All the species were observed susceptible to synthetic pyrethroid .Two important species those are known to be very efficient vectors of malaria were found, they are *An.minimus* and *An.philippinensis*. As the collection was done in the month of March and April ,the no. of mosquito were found in very low number. We will collect the mosquito in all seasons with increasing the no.of house hold for increasing the mosquito number.

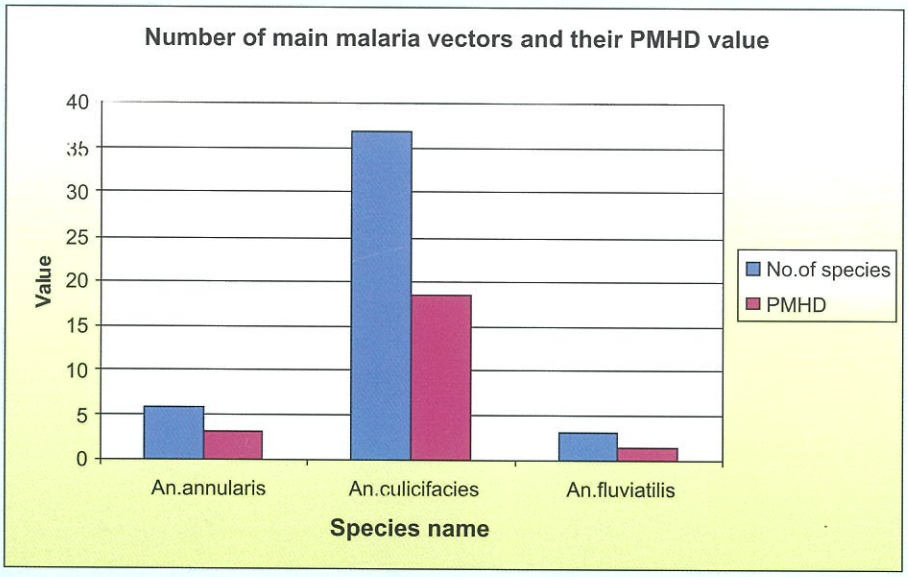
Table 2

Total number of species collected

Serial No.	Name of species	Total number
1.	*An. annularis*	6
2.	*An. culicifacies*	41
3.	*An. fluviatilis*	5
4.	*An. subpictus*	135
5.	*An. vagus*	60
6.	*An.tasselatus*	4
7.	*An.splendidus*	10
8.	*An.superpictus*	2
9.	*Cx.vishnui*	32
10.	*Cx.quinquefasciatus*	9
11.	*Cx.tritaneorhyncus*	2
	Total	307

Mohana is highly endemic among all the blocks of Gajapati district

Fig.4



Susceptibility status to insecticides

Table 3

Vectors	DDT		Synthetic Pyrethroid	
	Susceptible	Resistance	Susceptible	Resistance
An.annularis	+	+		
An.culicifacies		+	+	
An.fluviatilis+		+		

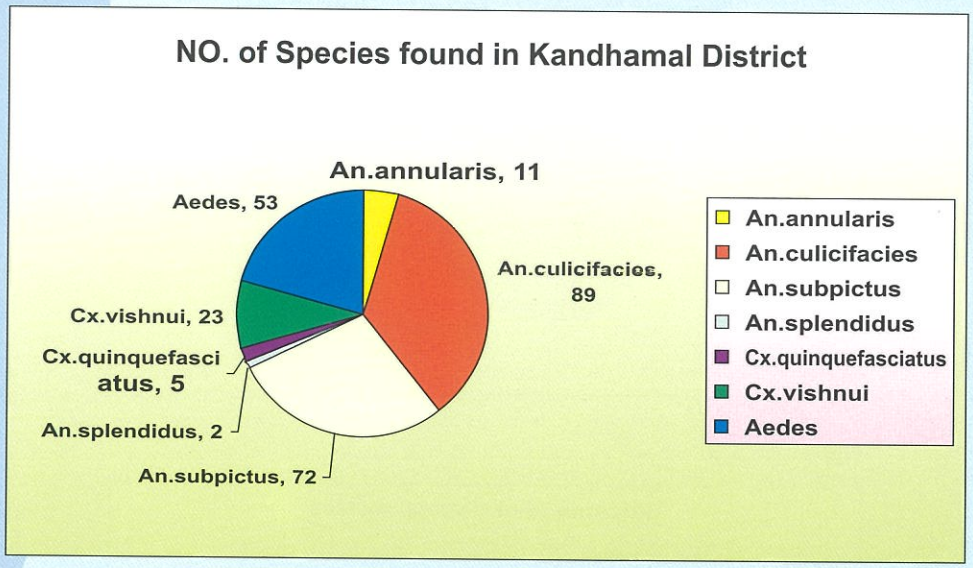
Vector survey at Kandhamal district

Seven species belonging to two genera of mosquito were collected. Out of which four species were from genus Anopheles, the rest were Aedes and Culex. (Fig 5). Total 255 mosquitoes were collected among which 174 were Anophelines. Among four anopheline species collected two were identified vectors of malaria they are *An.annularis* and *An.culicifacies*. The number collected and percentages with total anophelines was 68.2% of the total mosquitoes collected .The abdomen of major species showed half gravid (HG), full fed (FF), and unfed. An.culicfacies and *An.annularis* was collected from both cattle shed and human dwelling. As the collection was done in summer and rain just started during the period of collection the mosquito density was very low. In future more household will be taken with minimum 20% coverage of household of sample village will be done for avoiding the error.

The susceptibility status of *An.culicifacies* and *An.annularis* revealed that *An.culicifacies* and *An.annularis* were resistance to DDT (Table 4). All the species were observed susceptible to synthetic pyrethroid.

AR-54

Figure 5



Susceptibility status to insecticides

Table 4

Vectors	DDT		Synthetic Pyrethroid	
	Susceptible	Resistance	Susceptible	Resistance
An.annularis		+	+	
An.culicifacies		+	+	

Vector survey at four blocks of Keonjhar district

The study was under take in four PHC of Keonjhar districts, they are PHC – Ghatagaon, Jhumpura, Padmapur, Padmapur. Twelve species belonging to three genera of mosquito were collected from six villages covering 103 house hold.. From the total mosquito collected Anopheles are 83.5%, Culex 16.1% and Mansonia 0.28% (Fig 6). Ten species were from genus Anopheles, the rest were Mansonia and Culex. Total 711 mosquitoes were collected among which 596 were Anophelines. Among ten anopheline species collected five were identified vectors of malaria they are An.annularis, *An.culicifacies, An.fluviatilis, An.varuna* and *An.philippinensis*.(Fig7). The percentages of anophelines was 83.8% of the total mosquitoes collected .The abdomen of major species showed half gravid (HG), full fed (FF), and unfed. The main malaria vectors were collected from both cattle shed and human dwelling.

The susceptibility status of *An.culicifacies* and *An.annularis* revealed that An.culicifacies and *An.annularis* were resistance to DDT but *An.fluviatilis* was susceptible to DDT (Table 5). All the species were observed susceptible to synthetic pyrethroid.

Figure 6

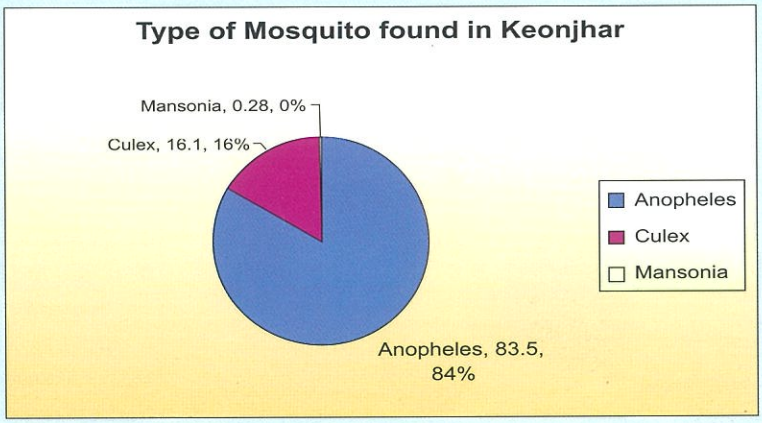
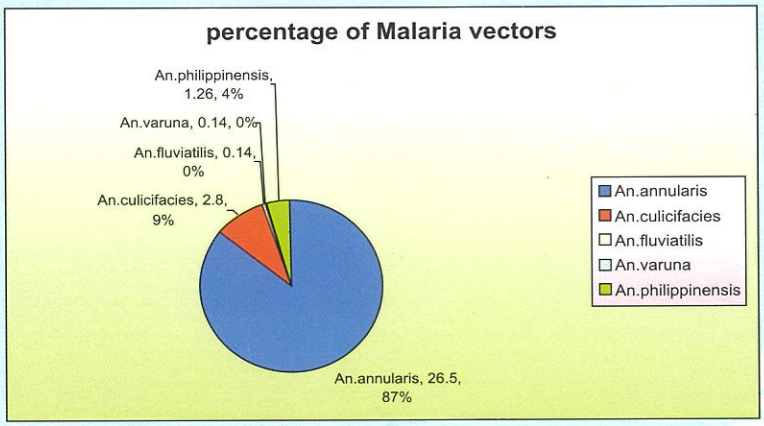


Figure 7



Susceptibility status to insecticides

Table 5

Vectors	DDT		Synthetic Pyrethroid	
	Susceptible	Resistance	Susceptible	Resistance
An.annularis		+	+	
An.culicifacies		+	+	

Future work

The periodical information on vectorial status for malaria transmission like identification of vectors and its species complex, bionomics, feeding habits and susceptibility status in these districts of Orissa will essential to help evaluation of efficacy measures in the control Programme. In future study these studies will be undertaken and mosquito will be collected in three seasons. From each district minimum four blocks will be selected taking in view of ecological condition and malarial incidence and the sample villages will be selected by taking malaria incidence and from each village mosquito will be collected from 20% of the house hold.

1.9 Mapping of vector habitats for filariasis through remote sensing and geographical information system (GIS).

Status : Extramural
(ICMR Task Force)
Investigators :
Dr. N. Mahapatra
Dr. R. K. Hazra
Dr. S. K. Parida
Collaborator : ORSAC, BBSR
Stating date : March 2007
Closing date : February 2010

Objectives :

1. Mapping of vector habitats of filariasis in two endemic districts of Orissa through Remote Sensing and GIS
2. Development of filariasis risk map.

While National Health Policy targets to eliminate filariasis by 2015 in Orissa, with high endemicity of disease needs to develop a filariasis risk map to help accelerating strategy for elimination. The available data on distribution of filariasis of the state is inadequate. Morbidity control and reduction in transmission are two strong pillars for the elimination of filariasis, it is essential to map the vector habitats for filariasis in Orissa. This can be used for developing filariasis risk map so that the health authorities can efficiently monitor surveillance and control effect.

District	Name of the blocks	
	Endemic	Non-endemi
Khurdha	Jatni	*
Puri	Satyabadi	*
Angul	*	Angul

* To be decided based on GIS data and state data

Selection of study area.

Three districts such as Khurda, Puri and Angul are covered in two scenes of LISS-III (Multi spectral sensor) and five scenes of Pan chromatic sensor of IRS-IC/ID Satellite. Basing on preliminary work done by the investigators in some villages, the following endemic and nonendemic blocks are selected from each of the above districts.

Time Frame of the Study;

Tout of two years study period phase wise time framing was done for 1st year below to show the research activities to be carried out during the stipulated time .

Ist Year

Phase-I	Selection of village from the preliminary R.S.based survey	4 Months
Phase -II	Epidemiology Survey ,Entomological Survey,	4 Months

Remote Sensing Survey

Phase - III	Entomological Survey, Remote Sensing Survey	4 Months

GIS based analysis:

In all the above study areas mosquito collections were done. Vector Cx.quinquefasciatus is identified and dissected to calculate vector density and infection and infectivity rate. The study has been initiated recently. The work is in progress.

1.10 Development of strategy for optimizing the accessibility and utilization of the government operated malaria control programme (EDPT) – an operational research study for control of malaria through formative research.

Status : Intramural
Investigators :
Dr. A. Mahapatra
Dr. A. S. Kerketta
Starting Date : August 2007
Closing Date : July 2009

Background:

Orissa, at present contributes to the highest number of malaria cases and deaths in the country. With 3.8% of India's population, Orissa accounts for 15 -20 % of total malaria cases, 35-40% of Plasmodium falciparum infection and 36% malaria deaths of the country.

Malaria transmission is high in most of the districts of Orissa, covered largely by hilly forested areas inhabited by mostly tribal population constituting 22% of population of state. Even in costal districts also moderate to high transmission recorded. Wide spread CQ resistance perennial transmission, illiteracy, undernutrition also prevent in many areas. Large number of sevier malaria cases reposted from this region. In spite of malaria program operating in the state malria spread to new areas and deaths still continues. While the program perform better result in some areas in many remote and rural sectors the accessibility to malaria care and utilisation of available resources provided by the programme are not optimum. While it is important to address several other important issues related to malaria appropate drug, diagnostic or pathogenesis of disease progression, it is also important to ensure how the available knowledge is accessed to need who can optimally use so as to curtail transmission early reporting and adherence to full drug dosage be achieved using best possible & feasible strategy with no additional cost.

It is observed that with different population setup, with varied cultural practices, stigmas & at varied awareness level and literacy, the early reporting of disease, full utilisation of programme services, and adherence to full course of antimalarial treatment is lacking in spite of drug and diagnosis facility is provided by the programme with fever treatment depot, sub centres at grassroot level. There seems to be gap between health providers and community in certain areas where bridging the gap may be more plausible. Improving community awareness and motivational level of grassroot workers be evaluated, where our services can be offered based on health need or by creating the demand at community level

Study design :

The study is being be carried out in two phases that include

Phase – 1 : Formative research and strategy development – 4 months

Phase – 2 : Implementation and evaluation of the strategy – 18 months (Consolidation – 2 months)

Study area :

The study will be undertaken in one community development block of three districts situated in 3 ecozones of Orissa and endemic for Malaria with high morbidity.. The selection of the block is based on the following criterion: poor accessibility, poor economy, lesser development, poor health infrastructure, low literacy and malaria endemicity.

Sampling :

Stratified random sampling of the villages will be undertaken. 3 PHC villages, 3 Health sub-centre villages, and 8 villages with no health facility (4 with Angan wadi centre and 4 with out them) will be chosen.

Sample size and Survey method :

Qualitative methods like illness narratives and in-depth interviews will be undertaken. About 20-25 illness narratives from the patients or their care givers, who have suffered from fever and/or convulsions in the preceding two weeks and about 8-10 in-depth interviews from the formal and non-formal care providers will be collected per village, there by generating about 280 illness narratives and 80 in-depth interviews. Data on community need perception, community behaviour, facilities & services availability and potential for community participation will be obtained. Limiting factors will be recovered. Similarly from grassroot servicing level, the services available, motivational level, perception and activity under programme will be recovered.

Data analysis :

The data will be analyzed using SPSS software.

Progress :

Formative Research has been initiated. The Community and the PRI members were apprised and their cooperation was sought. The Health system has also sensitized for this purpose at Cuttack district.

1.11 Evaluation of Intervention of Malaria Mitigation Measures in the Command Area of Rengali Left Bank Canal System

Status : Extramural
Investigators :
Dr. A. Mahapatra
Dr. A. S. Kerketta
Dr. R. K. Hazra
Starting date : August 2007
Cosing date : July 2009

Evolution of the project :

The system of Rengali Left Bank Canal (LBC - II) from RD29.177km to 71.313km is under construction by department of Water Resources with loan assistance from Japan Bank for International Cooperation (JBIC). Under this system, Malaria Mitigation Measures (MMM) are being undertaken over a period of three years, commencing from 01.04.06. It is proposed to conduct post evaluation study of the scheme, at end of the period after 31.03.2009. Chief Engineer, Department of Water Resources, Government of Orissa has requested Regional Medical Research Centre to undertake the post evaluation study wide letter number 12,408 dated 18/08/2006 from the office of the Chief Engineer & Basin Manager, Brahmani Left Basin, Samal.

Background :

Rengali dam was commissioned over the Brahmani River during 1974-85 with a barrage constructed 34 km downstream at Samal. Two canals take off from the barrage. The left bank canal now is seen in three parts.

LBC – I constructed over a distance of 0 to 30 km. LBC – II is being constructed over a distance of 31 – 70 km and LBC – III is extending beyond 70 km. The command area of LBC – II mainly constitutes 4 blocks of Dhenkanal district (Kamakhyanagar, Bhuban, Parjang and Kankadahada).

EMCP is implemented in Kankadahada block of Dhenkanal district as a part of the Enhanced Malaria Control Programme, which is operational in hyper-endemic areas covering a total of 240 blocks in 21 districts in Orissa. Other three blocks are not covered under the programme. Malaria Mitigation Measures under the JBIC assistance are being undertaken in these three blocks; where as IEC component of MMM will be implemented in all the four blocks.

The main goal of the Malaria Mitigation Measures Project is to reduce the morbidity and the mortality due to malaria in the project area with the following objectives:

1. Strengthening infrastructure and testing facility of the existing institutions
2. Adopt integrated vector control measures
3. Increase awareness on prevention of malaria, early detection, appropriate management and preventing complications
4. Promote medicated mosquito nets
5. Train and build capacity of the malaria workers, AWWs and community leaders.

At the commencement of the Malaria Mitigation Measures, there is no available base line data, except for the secondary data available with the current malaria control programme of the state government. In order to have a baseline data, which further can be compared with the data to be collected during the end line survey after 31.03.2009, RMRC has proposed for an additional baseline survey by the end of 2006.

Objectives :

1. To assess the epidemiological situation of Malaria in the Dhenkanal district
2. To conduct malaria survey at the house hold level and to assess the KABP of the head of the house hold in the sample population
3. To study the mosquito fauna, vector prevalence, pattern, density, vector infestation rate and their susceptibility status

Study area :

The study will be conducted in the four blocks of Dhenkanal district. Villages will be selected from these blocks.

Data collection :

Data would be collected from both the primary and secondary sources. Data collections from the primary sources include House-to-House fever survey, KABP study of the head of the house hold and mosquito collection from the selected villages. During the fever survey, cases of fever at the time of visit or during the past two weeks would be recorded. Blood slides will be collected from all the suspected malaria cases. A suspected malaria case refers to cases of fever without symptoms of cough or cold (ARTI), skin rashes (Eruptive illness), burning micturition (UTI), skin infections, painful swelling joints and ear discharge.

Sampling:

Stratified random sampling method would be used for selection of the villages on both sides of the LBC in the four blocks of Dhenkanal district. All the house holds of the selected villages will be included in the survey. The number of mosquitoes to be collected from each of the villages will be decided in consultation with the statistician.

Progress:

The Base line data has been collected. This base line information has to be compared yearly for 3 years for the assessments on malaria mitigation measures taken.

1.12 Mapping of P.falciparum susceptibility to Chloroquine, in Malaria Endemic Districts of Orissa.

Status : Extramural (EMCP)
Investigators :
A. S. Kerketta
Mr. P. K. Jangid
Stating date : November 2006
Closing date : November 2008

Objective

To study the therapetic efficacy of chloroquine in the treatment of uncomplicated P.falciparum malaria

Work Progress

Out of 7 study districts named Kandhamala, Boudh, Ganjam, Gajapati, Nawrangpur, Raygarda and Keonjhar district the study was initiated in Adenigarh PHC of Boudh District of Orissa. The health facilities under the PHC area are, one area hospital, two PHC new and 18 sub-centres. As per the previous three years surveill ance data of EMCP, the area is having SPR more than 5% it indicates that there is high malaria transmission in the communities of the area and Pf prevalence of more than 90% indicating increase in severe and complicated malaria.

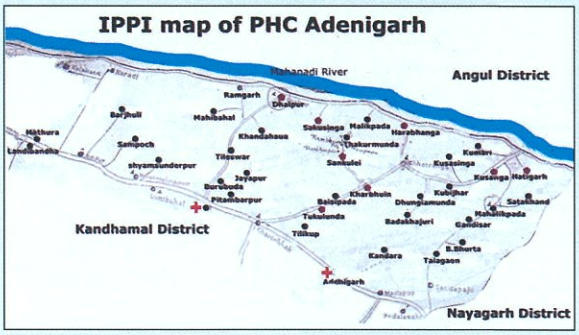


Table-1: Malaria situation in PHC area as per last five years surveillance data by EMCP

Year	2000	2001	2002	2003	2004	2005
ABER	7.78	9.13	11.54	8.89	10. 08	11.49
API	8.04	8.90	9.25	4.54	5.71	6.42
SPR	10.33	9.75	8.01	5.06	5.66	5.59
SFR	9.32	8.32	7.14	4.67	7.5	6.42
Pf%	90.22	85.38	89.07	92.37	94.49	96.25
Total population	110738	109116	110858	112623	114447	116279
BSE	8615	9963	12795	10114	11536	133365

The team led by the physician, comprised of research assistant, two technicians and two more technicians from the state health department and an attendant undertook the field study for 28 days cycle of treatment in all cases studied. The health worker of the particular sub-centre also accompanied the study team to the study villages. Based on malaria incidence in different sub-centres only two sub centres with highest endemicity and death were selected. In order to select the villages in each sub- centre the team made visits to following villages to ascertain the village information and co-operation etc. The villages named Palsakhuntuni and Khajurinali, Tentulipada of Dholpur sub-centre, Belapadar of Sakusingha, Atalsara and Hatigada of Kusanga, Sankulei of Sankulei, Pakhala-Ponka of Tukulunda, Harbhanga and Khairbhuin and Taila sahi of Khairbhuin were visited. Out of the ten villages, in Belapadar, Atalsara, Hatigada, Sankulei, and Pokhalo Panka only one/two or no fever cases were found during the visit. In other four villages that are based in the foothill areas fever cases reported almost in every house- holds. Thus these villages named Palsakhuntuni, Khajurinali, Tentulipada, Khairbhuin and Talia sahi were selected for the study of therapeutic efficacy of Chloroquine covering a total population of 1330 in 147 households. Initially to select the eligible case a rapid fever survey was done by door-to-door visit. In a centre place of the village the camp was organized and all the fever cases were brought there. A careful and precise registration of address of each fever cases was done. Prior to the enrolment the patient and his/her attendant were briefed in details about the aim, procedure and the benefits of the study. The informed and written consent was obtained from each subject. Each patient underwent physical and clinical examination with a careful history of the duration of fever and on treatment sought. The axillary temperature was recorded with the help of an electronic thermometer and the body weight was measured with the help of a calibrated scale-weighing machine. A finger prick blood was collected from each patient for a thick and a thin smear in duplicate and also for rapid diagnostic kit test (RDK). Simultaneously the thick and thin films of peripheral smear were prepared and were air dried rapidly. The thin smear was fixed with anhydrous methanol. The smears were stained with Giemsa stain 3% and at pH 7.2 and examined on same day. The parasite count was done against 200 WBC in thick smear. Thin smears were used to confirm the parasite species. The cases found to have either P. vivax infection or mixed infection with P.falciparum and P.vivax were given treatment and disposed up. Taking consideration of inclusion and exclusion criteria, the eligible cases were selected for the study. The expected study population was 132 in four sub-centres. But the fever cases were not available in four sub- centres during the study period since it was not the peak transmission season for malaria, only sporadic cases were reported in most of the sub-centres. Therefore the study populations of 49 could be drawn from only five villages of two sub-centres and included as the study population (the detail age/sex is given in Table-2).

Table-2 : Age and Sex distribution of the study population

Age in years	Male	Female	Total
<5		1(2.0)	1(2.0)
5-14	3(6.1)	13(26.5)	16(32.7)
15 and above	8 (16.3)	24(49.1)	32(65.3)
Grand total	11(22.4)	38(77.6)	49

A total of 49 cases included as the study population. Each case was administered the drug Chloroquine 150 mg base (Brand name Lariago, manufacturer Ipca Pharma Pvt Ltd) on the spot and under supervision of the medical team, after noting down the Batch No, MFD and EXP date. The dose schedule followed the WHO recommendation (10mg/Kg Body weight on days 0 and 1 and 5mg /kg on day 2) after or with food. The study subjects were monitored daily by the medical doctor for initial 3 consecutive days. They were advised not to take any other drug during the study period with out informing investigator.

Follow-up

On the follow up day 2, 7, 14, 21 and 28, the study subjects were followed up with blood smear for parasite count. Besides blood smears collection the clinical examination was done on each day for recording for danger signs as per WHO guideline i.e. not able to drink or feed, repeated vomiting, convulsions during present illness, lethargic or unconscious and unable to sit or stand. The case found to have treatment failure with CQ was given the second line of drug Sulfadoxine- pyrimethamine according to the standard NAMP guideline. In all cases the health of the study subject was given priority over the test.

Dropouts

Out of total study population 5 (10.2) dropped out from the study. Of which one case did not want to continue as study subject and denied to give blood sample on day 2. Three children were given treatment from third party. One moved to the place outside reach of active follow-up. Thus a total of 44 (89.8) subjects could be followed up for whole 28 days study period.

The therapeutic response classification

The therapeutic response was classified according to the WHO guideline (2001).

1. Early treatment failure (ETF)-Development of danger signs or severe malaria on Day 1, Day 2,or 3 in presence of parasitaemia and parasitaemia on day 2 higher than Day 0 or Parasitaemia on day 3 > than 25% of the D 0 count.

2. Late treatment failure (LTF) - 1. Late Clinical Failure (LCF)
   - 2. Late Parasitological Failure (LPF)

   LCF -Development of danger signs or severe malaria after Day 3 in presence of parasitaemia OR presence of axillary temperature >37.5°C on any day between Day 4 to Day 28 without previously meeting any of the criteria of ETF.

   LPF - Presence of parasitaemia on any of the schedule return on Day7, Day14, Day 21 and Day 28 with axillary temperature less than 37.5°C without previously meeting any of the criteria of ETF or LCF.

3. Adequate clinical and parasitological response (ACPR)- Absence of parasitaemia on Day 28 irrespective of axillary temperature, without previously meeting any of the criteria of ETF, LCF or LPF.

Results

A total of 97 fever cases were screened clinically and all suspected malaria cases screened for malaria parasite by the ICT test i.e. SD Pf / Pv malaria kit for quick identification of mixed infection cases. Out of these 58

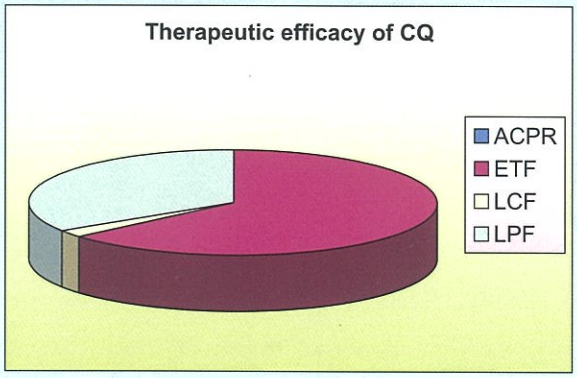
(59.8%) were P. falciparum mono infection, 11(11.3) P.vivax mono infection, 23 (23.7) had mixed infection of P. falciparum as well as P.vivax infection and 5 (5.2) did not have any malaria parasite infection. Out of the P falciparum cases, 2 patients had very high parasite count and were difficult to count the exact number of parasite and 7 patients had very low parasite count. The PV and mixed infection cases were treated as per the NAMP drug schedule and disposed after wards. Thus 49 patients included in the study. The baseline information of the study sample is given in Table-3.

Table 3: Mean and range of baseline information of study population

	Mean	Range
Age	23.71	2- 65
Axillary Temp	37. 8621	37.5 – 39.0
Parasite Count on Day 0	3475.66	1000 - 42400

The data obtained from the study shows that, out of 44 patients who continued till the end of the study, none showed adequate clinical and parasitological response (ACPR). A total of 28(63.6) showed Early treatment failure (ETF), of which one case 3 (6.8) had parasitaemia on Day 2 more than Day 0 and 25(56.8) had parasitaemia level on Day 3 more than 25% of the D0 parasitaemia level. Late clinical failure (LCF) was marked in 1 case (2.3%) that developed severe malaria on Day 6, become unconscious and had convulsion. She immediately shifted to the District Headquarter Hospital. A total of 15 (34.1) showed late parasitological failure (LPF) with parasitaemia on Day 7.

Therapeutic response of malaria parasite to CQ



Inference:

The drug treatment remains to be the mainstay for eliminating the infective focus and parasite carriers in a given community and the drug to be an ideal weapon should have 100% cure rate within the recommended doses. But the present study has revealed 100% treatment failure with standard dose of first line of drug for uncomplicated P.falciparum malaria in the 44 eligible cases studied in Adenigarh PHC area of Baudh district. When the CQ resistance status of present study area is compared with that studied in M. Rampur block of Kalahandi district in the year 2005, Adenigarh data shows conspicuous increase in ETF proportion. This is indicative of ineffectiveness of first line drug CQ in malaria in the area. It warrants requirement of urgent step to change to the second line of drug Sulfadoxine-Pyrimethamine or combination therapy of artesunate and SP compound in the control programme.

1.13 Efficacy and Tolerability of single dose DEC of 100mg, 200mg & 300mg strength in filariasis endemic community in Orissa.

Status : Extramural
Investigators :
Dr. B. Dwibedi
Dr. S. K. Kar
Stating date : March 2006
Closing date : March 2007

Objective:

1. To compare efficacy of single dose mass administration of DEC in 100mg, 200mg and 300mg strength in three defined filarial endemic population.
2. To observe the side reactions in three dosage levels as above.

Application of the Research for National Health Policy

Mass drug (DEC) administration is running in the country for the target of elimination of lymphatic Filariasis by 2015. the programme is based on mass administration of single annual dose of DEC in the recommended dosage of 6mg/kg body weight averaged for the age groups of populations i.e. 2-5, 5-14 & >14 years. But the population compliance of the programme is limited mainly by the fear of side reaction to the drug and also by confusion of the drug dosage in the above age groups. The present study aims at generating evidence for effectiveness of a lower but uniform dose of DEC that may influence side reaction and easy distribution while retaining efficacy. This study may help the national programme for elimination of Filariasis in increasing the population compliance of the drug.

Work progress :

Different villages in endemic parts of Cuttack, Khurda & Puri district were visited. Clinical and MF survey was done in those areas for selection of the study sites. Three (3) sites were selected from the endemic region for imparting three regimens namely, Bhatimunda & Kandarakana of Cuttack and Retanga from Khurda district with approximate population of 2000 in each site. The village population enumeration and MF status of the population in the villages during pilot survey is presented in the following table.

Pre Drug Assessment:

Population census & village mapping was undertaken in all the 3 sites followed by clinical examination, microfilaria survey & ICT test for presence of W.infection and in each site before institution of DEC. The survey included both clinical & laboratory parameters. The Clinical assessment include history & examination of Acute & chronic filariasis, history of other chronic diseases and anthropometry (body weight). Besides, the above laboratory investigation stool microscopy for intestinal helminths was undertaken in sub samples in each study sites.

Children below 2yrs, pregnant women and critically ill individuals were excluded from the study.

Ongoing Studies

Baseline Investigation & Clinical Parameters

Parameters		Retanga	Bhatimunda	Kandarakana
Total Population		1808	2124	1907
Eligible Population		1493	1710	1613
Population tested		1127	1191	1225
Age Group wise population				
	2–5 yrs	78 (6.92%)	67 (5.63%)	50 (4.08%)
	6–14 yrs	266 (23.60%)	265 (22.25%)	226 (18.45%)
	>14 yrs	764 (67.79%)	838 (70.36%)	900 (73.47%)
ICT +Ve		264(23.4%)	185(27.3%)	210(17.1%)
Mf +Ve		86(7.64%)	98(8.23%)	82(6.69%)
Mf Density(Geometric Mean)		5.46	4.669	4.659
Lymphoedema Cases		42(2.4%)	89(4.3%)	84(4.5%)
Hydrocele Cases		93(5.3%)	101(4.8%)	96(5.2%)
Haemoglobin <10gm/dl		44.14%	40.32%	54.04%
Entomological Index				
Mosquito Density		32.8	28.4	25.8
Vector Infection Rate		1.8	2.8	3.7
Vector Infectivity		1.8	1.5	1.8

IEC Activity & Community motivation before MDA:

Awareness on pathogenesis, spread & effect of the filarial infection in the community was created in the three study sites by conducting group discussions in presence of village heads, local medical officers & Para-medical personnel along with the PRI members. Information on the disease, its treatment & prevention was disseminated through leaflets, posters, Audio-visual demonstrations & lectures in different settings. Motivation camps as well as door-to-door visits were made to explain about the necessity of mass administration of DEC for elimination of filariasis in their community through transmission block. This was quite essential to make the people understand the role of MDA for the individual & the community.

The community was convinced of management of any side reaction occurring after drug consumption by a team including physicians to be present during & after drug administration in the field site for the first week after MDA and during follow up period.

Drug Distribution, Supervised Consumption & follow-up of for side reaction:

Prior to the study permission was obtained from state health department and ethical committee of the centre .No other antifilarial intervention was done in any of the study sites .The regimens were allocated randomly to the three study sites for uniform single dose mass administration. The children below 2 years, pregnant women & those who are critically ill were excluded for DEC consumption. DEC tablets in the recommended dosage (100mg/200mg/300mg) were distributed in a priorly informed date to the community. DEC 100mg was given in

all the study sites in children between two to five yrs age and those below two yrs age were not given DEC dose as similar to the national programme of MDA .The team members distributed the tablets by door-to-door visit in presence of the local volunteers. DEC consumption was ensured by supervised/ direct observation method.

The team visited all the households during and after consumption of DEC to observe occurrence of side effect if any which were managed by providing treatment at the doorstep. Daily follow-up visit was done for first 7 days. Any side reaction persisting beyond this time was also managed. Detail note of the appearance & persistence of different side reactions were recorded.

Tolerability & Effectiveness to Three Strengths of DEC

DEC Consumption & Side reaction			
Parameters	**100mg**	**200mg**	**300mg**
Overall DEC consumption	1289(86.33%)	1455(85.08%)	1300(80.59%)
DEC intake among tested population	87%	89.1%	82.1%
Side reaction (%)	6.2%	7.9%	10.46%
Post DEC follow-up investigation at 3 months			
Mf Clearance (%)	48.7	39.28	41.11
Cases with Mf Count Reduction (%)	64.86	75.7	50
Mf Density (Geometric Mean)	2.67	2.17	2.17
Mf Incidence (%) Out Of Baseline Mf -Ve	1.22	4.34	2.12

Present status :

Entomological survey:

Entomological survey was included in the study for evaluating the vector density & transmission of filarial larvae through the vector in the community. This is considered important to see the effect of DEC on community transmission of filariasis. First round of insect collection & filarial infection in the mosquitoes was conducted during the time of drug administration which is to be followed up for one year post treatment to evaluate transmission block imparted by 3 regimens.

DEC tablet : Supervised Consumption

Follow up of the community for microfilariamia & antigenemia:

The community need to be followed for microfilaria clearance & reduction of antigenemia at 6 and 12 months level to compare the efficacy of the three DEC regimens

In order to know the effect 0f transmission in the community at three dosage level evaluation of both parasitological & entomological parameters need to be studied to complete one year post MDA follow-up, which is quite essential. Extension (further 8 months) of the project activity for completing the above task will be required to know the one year post follow up result which is essential.

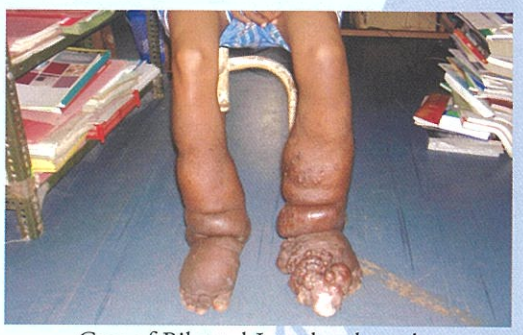
Justification for Extension of the Project:

Initiation of the project was delayed because of maintaining a clear gap of one year from the previous MDA round under taken by the state although their compliance level were found to be below 40% in the villages selected The drug distribution was undertaken in 3 sites in a date earlier to the next round of MDA run by the state and no intervention was done by state run MDA in the study sites.

The comparative drug compliance among the 3 regimens shows encouraging results in form of low frequency and severity of side reactions in 100mg regimen in comparison to 200 & 300 mg dosages. Microfilarial examination during the first follow-up investigation at 3 months following the DEC intake shows identical Mf clearance in all the 3 regimens; showing possible effectiveness of DEC at lower dose i.e. 100mg. Further follow-up of the communities for a total period of 1 year is essential to observe the period of sustained amicrofilarimia and reappearance in the three dosage levels or for conclusive documentation of the drug effectivity in one year.

If the 100mg uniform dose is found effective, it will be advantageous to the programme in (1)-Increasing the drug compliance by reducing side reaction, (2)-Avoiding dose confusion among three different age groups and (3)-To reduce the cost of the drug to 1/3rd of the present expenditure.

So it is proposed to extend the study for another 8 months to complete the follow-up investigations as well as clinical follow-up of the community.



Case of Bilateral Iymphoedema leg

1.14 Immunochemical characterization of filarial glutathione S-transferase and its protective potential in experimental filariasis.

Status : Extramural (DST)
Investigators :
Dr. M. K. Beuria
Dr. M. K. Das
Dr. M.S. Bal
Starting date : March 2005
Cosing date : February 2008

Objectives:

1. To determine recognition pattern of anti-Glutathione-S- transferase (GST) antibodies (SDS-PAGE and immunoblotting) in filarial sera.
2. To determine the cytokine responses specific to GST in filariasis.
3. To evaluate the protective potential of GST to clear microfilariae in experimental infected animal.

Background:

Glutathione-s-transferases are essentially detoxification enzymes helps in parasite survival against host-induced damage. These enzymes have been used as component of anti-parasitic vaccine in Schistosomiasis, Fascioliasis and in Chaga's disease. In this study we have purified Glutathione-s-

transferase from cattle filarial parasite *Setaria digitata* to evaluate it's role in human filariasis. We have earlier determined the IgG and IgM antibodies to Glutathione-s- transferase in individuals living in areas endemic for *Wuchereria bancrofti* infection.

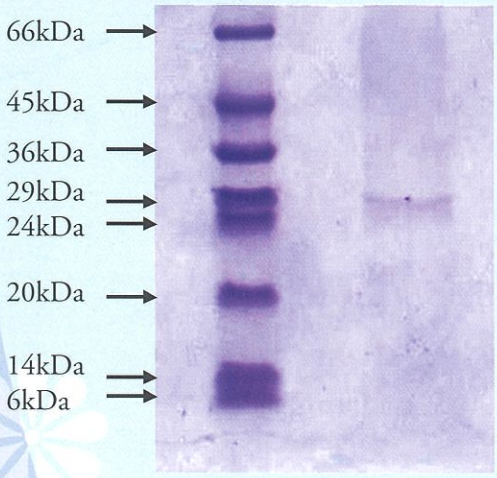
Results:

Glutathione binding protein with GST activity was purified from soluble extracts of adult cattle parasite Setaria digitata through Glutathione Agarose column. On SDS-PAGE it revealed a broad band of 26-28 Kda. Antibody recognition pattern to this fraction is being checked by both ELISA and Immunoblot assay. Antibody response to GST were determined in asymptomatic microfilaraemics (AS), Chronic filariasis patients (CP), endemic normal subjects (EN) living in areas endemic for bancroftian filariasis. More than 90% subjects were positive for IgG antibody compared to 45% in endemic normals. IgM positivity of about 50% was observed in endemic normals compared to 90% in infected subjects. Sera collected from individuals with other helminthes infection from non endemic area were negative for any isotype indicating the filarial specificity of GST. Further IgG antibody isotype was determined in circulating filarial antigen (CFA) positive and negative individuals. There was no significant difference observed between the two groups. Western blotting is being done to evaluate the binding of GST with filarial sera. Sera from EN, AS and CP individuals identified both the two bands. Sera from non endemic normal individuals could not recognize GST by immunoblotting.

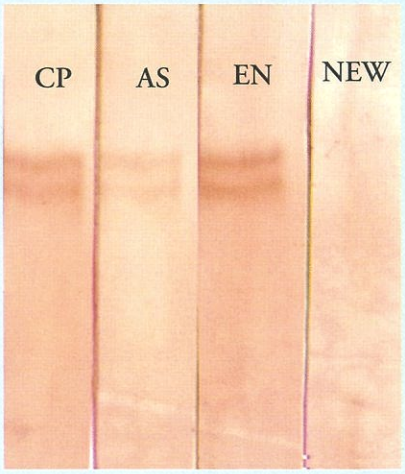
The Setaria digitata – Mastomys model is standardized in order to study the clearance of microfilaria following GST immunization. The course of appearance of microfilaria in S.digitata implanted mastomys was observed taking 20 µl of blood from tail vein. Microfilaria was detected in the peripheral blood on day 4 of post implantation. It was observed that microfilaria could persist in the circulation up to day 160 of post implantation with a peak on day 20 in control group of mastomys. The effect of immunization with GST on appearance of microfilaria is being determined. Initial experiments indicate that the suppression of microfilaria in GST immunized mastomys.

Cell culture experiments for T cell proliferative response and cytokine production are being done taking peripheral blood mononuclear cells from endemic subjects stimulating with GST.

Analysis of GST by SDS-PAGE



Recognition of GST by different groups of filarial sera



1.15 Human Bancroftian Filariasis: Identification of Immunological markers of morbidity in Hydrocele and Elephantiasis

Status : Intramural
Investigators :
Dr. A. K. Satapathy
Dr. A. S. Kerketta
Dr. P. K. Sahoo
Dr. B. Ravindran
Stating date : March 2006
Closing date : February 2009

Objectives:

1. To evaluate filarial specific as well as mitogen induced T-cell proliferative responses in hydrocele and lymphoedema patients
2. To quantify inflammatory cytokines and chemokine levels in patients with hydrocele and lymphedema and correlating with severity of chronic manifestation.
3. To type genetic polymorphism of TNF receptors (TNFR I & TNFR-II) in hydrocele and lymphedema patients.

Work Progress:

Hydrocele and elephantiasis are two major clinical manifestations associated with chronic Bancroftian filariasis. The more important question still remains as to why some individuals develop one form of pathology, and other develop another form of pathology. Although hydrocele and lymphedema / elephantiasis are two diverse forms of chronic manifestation, immunological data distinguishing patients with elephantiasis or hydrocele have not been very successful. An attempt has been made to address these issues in this study.

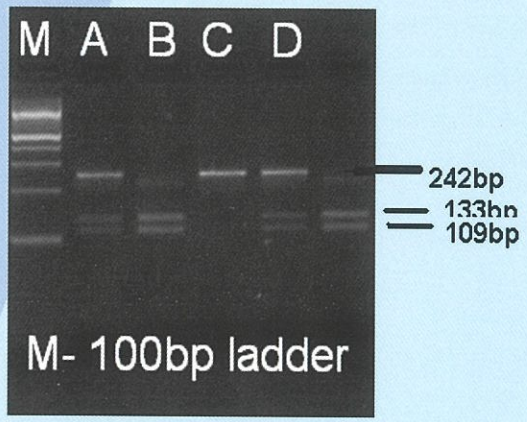
As mentioned in the previous annual report we had compared antibody levels the following five different recombinant filarial proteins in Elephantiasis and Hydrocele cases. The filarial recombinant proteins were Abundant Larval Transcript- 1) ALT-1; 2) ALT-2; 3) Serpin –2 (SPN-2); 4) Cystein Protease inhibitor –2 (CPI-2) and VAL. The first two molecules are produced by infective larval stages while SPN-2 is synthesized only by microfilarial stages and CPI-2 is present on the surface of adult filarial worms. The IgG antibodies responses to CPI-2 (surface of adult worm) and SPN-2 (Microfilarial stage) were significantly more in Hydrocele cases in comparison to elephantiasis patients. Isotype analysis showed IgG1 antibody to ALT-1 and CPI-2, IgG2 antibodies to SPN-2 and IgG4 antibodies to CPI-2 were significantly high in hydrocele in comparison to lymphedema indicating significant differences in humoral immune responses of the two diverse forms of chronic diseases.

Our earlier investigations on cytokine levels in circulation revealed very clear differences between the two chronic manifestations of filariasis – patients with elephantiasis were found to have elevated levels of IL-6 and TNFR-75 receptor while hydrocele cases displayed elevated levels of TNFR-55. Increased TNF-a was a feature found only in acute filariasis and not in patients displaying chronic forms of the disease. TNF receptors are known to mediate inflammatory signaling of TNF-α. The biologic activities of TNF are mediated by two structurally related but functionally distinct receptors TNFR-I and TNFR-II, belonging to the TNFR gene family. There was a dichotomy in plasma levels of two TNF receptors between infected subjects and patients with filarial disease. TNFR-I was found to be significantly elevated in patients with disease manifestations while TNFR-II on the other hand was significantly elevated in subjects with patent infection. The two forms of chronic filarial diseases were significantly different from each other when the ratio of TNFR-I and TNFR-II were analyzed – elephantiasis patients displaying a significantly high ratio in comparison to hydrocele cases indicating that these immunological markers may be useful in differentiating the two forms of chronic disease manifestation.

TNFR-II Polymorphism

TNFR-II gene is localized on 1p36.2 and comprise of 10 exon. A polymorphism at position 196(ATG>AGG) in exon 6 change Arginine(R) to Threonine(M). This mutation was detected by PCR amplification of involved region with sense primer 5'-ACT CTC CTA TCC TGC CTG CT-3' and antisense primer 5'-TTC TGG AGT TGG CTG CGT GT-3'. PCR was performed by using the following condition: an initial denaturation at 95°C for 5 min followed by 30 cycle of denaturation at 95°C for 1 min anneling at 64°C for 1 min and elongation at 72°C for 1 min. the final elongation was at 72°C for 7 min followed by cooling at 40C. The PCR products of 242bp were digested at 37°C with Nla III for 4 hrs and the cleavage products were analyzed by electrophoresis on 3% agarose gel stained with ethidium bromide. The amplicon which either cut into two fragments of 133bp and 109bp (196M) or it remain 242 bp uncleaved (196R) as shown in Fig- 1.

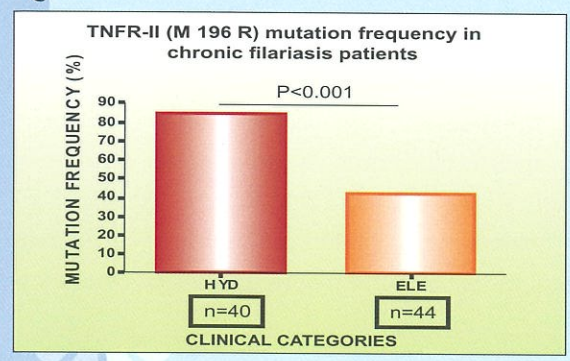
Fig-1



Wild type (RR) -C
Homo mutant (MM)- B
Heterozygous (RM)- A,D

We analyzed genetic polymorphism of TNFR-II (M 196 R) gene in the two major forms of chronic manifestations of filariasis. Fig-2 shows the frequency of TNFR-II (M 196 R) mutation in hydrocele and elephantiasis subjects - there was a significant difference between the two forms of chronic disease. TNFR-II (M 196 R) mutation frequency was significantly more in hydrocele (85.0%) in comparison to patients with elephantiasis (43.0 %). This result suggests that TNFR-II mutation might predispose patients for development of hydrocele. We are currently studying the association, if any, between this polymorphism and plasma levels of TNFR-II in the two forms of chronic filariasis.

Fig-2

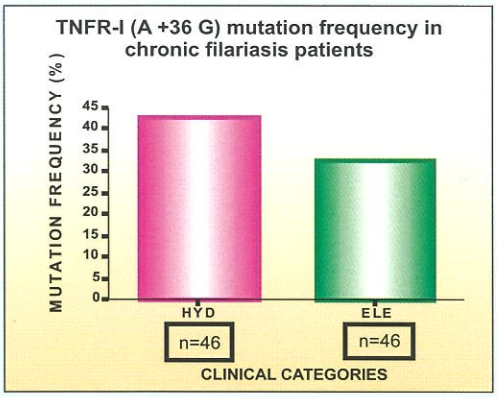


TNFR-I Polymorphism

A common A>G gene polymorphism of TNFR-I is found at +36 of exon1. This mutation was detected by PCR-RFLP method. This mutation was detected by PCR amplication of involved region with sense primer 5'-GAG CCC AAA TGG GGG AGT GAG AGG-3' and antisense primer 5'-ACC AGG CCC GGG CAG GAG AG-3'. PCR was performed by using the following condition: an initial

denaturation at 95°C for 5 min followed by 35 cycle: denaturation at 95°C for 1 min anneling at 60°C for 1 min and elongation at 72°C for 1 min. The final elongation was at 72°C for 7 min followed by for a cooling at 4°C. The PCR product of 182bp were digested at 37°C by MspA1 I for 4 hrs resulting in fragment that either cut in to two fragment of 75bp and 107bp (allele G) or were not restricted (A allele). These fragments were analyzed by electrophoresis on 3% agarose gel stained with ethidium bromide.

Fig-3



We analyzed genetic polymorphism of TNFR-I (A to G) gene in the two major forms of chronic manifestations of filariasis. Fig-3 shows the frequency of TNFR-I (A to G) mutation in hydrocele and elephantiasis subjects - there was not a significant difference between the two forms of chronic disease. These finding suggest that TNFR-I (A to G) mutation may not have significant role in development of chronic disease manifestation.

In this study mitogen as well as filarial antigens induced T-cell proliferative responses in lymphedema and hydrocele patients is being evaluated.

1.16 Epidemiology of viral hepatitis in tribal populations of Orissa, Madhya Pradesh/ Chhatisgarh and Jharkhand, India – a multicentric study

Status : Extramural (Tribal Task Force)
Investigators :
Dr. S. K. Kar
Dr. B. Dwibedi
Dr. A. S. Acharya
Dr. B. V. Babu
Dr. A. Mohapatra
Collaborator : S.C.B. Medical College, Cuttack
Stating date : March 2006
Closing date : February 2009

Objectives:

The project aims at studying the epidemiology of viral hepatitis in six primitive tribal populations of Orissa. The objectives of the project are

1. To determine the prevalence of hepatitis A, B, C, D & E viruses along with the circulating genotypes of HBV & HCV.
2. To assess the risk factors of transmission of hepatitis viruses
3. To study the pre-core and basal core promoter mutants of HBV

Rationale of the Project:

Nearly 22% of population of orissa are tribal. Of 62 tribes in the state, 13 are primitive who reside in isolation; mostly in hilly terrains of southern, northern & Western Ghats. It is estimated that their population has been decreasing over last few decades. These tribes have their unique culture, beliefs, stigmas and accordingly their practices differ within the tribe or from general community. Since they are geographically isolated and shy of contact with the general community, the development of these tribal groups have been difficult. Their social pattern and living condition and practices significantly influence their health status. With lower life expectancy and high infant and maternal mortality rate, these tribes suffer from various communicable diseases with very high mortality. To address above, one of such issues attempted to study is the hepatitis viral infection, its prevalence and risk factors of transmission .Out of few such studies reported amongst tribals, that reported from Andaman was found to have very high prevalence.

Viral hepatitis is caused by different viruses that belong to different taxonomical families and genera. Among them HAV & HEV are transmitted by faecal-oral root, HBV & HCV are blood borne viruses transmitted through contaminated blood, blood products and through improperly sterilised needles/ syringes. The prevalence of these viruses in tribal areas of India mostly remain unknown. The present study would like to access the prevalence of theses viruses in different tribal areas and to find out any risk factors for transmission. It is assumed that certain risk factors would be unique to practices followed in certain tribes because these are primitive tribes and geographically isolated and quite shy of contact with community at large. Among all hepatitis viruses, HBV & HCV lead to chronic infections and super infections with other hepatitis viruses' increases severity of the disease. Vaccines against HAV & HBV are now available but not yet been included in universal programme of immunisation due to its high cost. However, understanding of the risk factors and prevalence rates might lead to inexpensive and appropriate intervention measures. Distribution of viral genotypes can also be useful in context of transmission within the tribes and by comparing the data with available information at the national level

Summary of progress

The study was initiated in the highest tribal concentrated district of Orissa, i.e. Mayurbhanj, where 5 primitive tribes reside distributed in various blocks. Out of total primitive tribes, sampled population from each tribe are being studied with approximate sample size of 2500 in total. Depending on prevalence of hepatitis infection in population the exact size will be recalculated later in course of study.

The demographic information, origin, living & settlement pattern, cultural practices and general morbidity pattern of all these tribes are being assessed. To achieve objective I & II- i.e. determine prevalence of infection & associated risk factors for transmission, both field and lab studies are initiated.

Study area & Population Covered

Baseline information on existing population, health, conditions & risk factors/ behaviour for transmission of enteral and parenteral transmission of Hepatitis viruses in the primitive tribes of the study area were collected. Lodha & Saora primitive tribes of the selected study site were included during the first year for clinical assessment and blood sample collection. Field studies were initiated in 2 blocks namely Morada & Suliapada, covering population of 2 & 3 villages of above respectively. Attempt was taken to cover all age groups and both sexes of total population of villages. So far 411 subjects were covered by clinical examination, assessment of risk behaviour and collection of blood specimens for testing. Village map was prepared enumerating the dejurae population. The population under study are very primitive in their social, cultural and other behaviours of day to day activity and livelihood. The population is totally scared of the developments in the socio-economy, education, health awareness and health facilities of the present society.

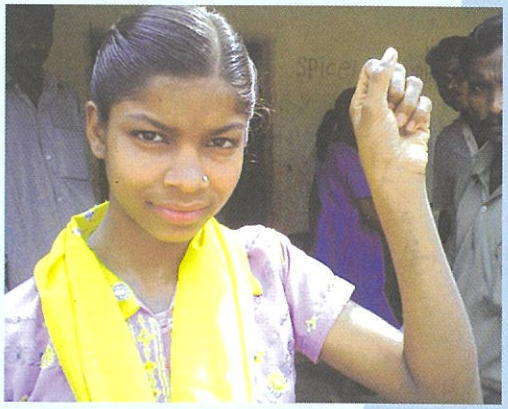
IEC activity

As a result the primitive tribal group are still at the lowest level of literacy and health awareness thus their knowledge on viral hepatitis and its transmission, consequences is poor. Before collection of blood samples from the primitive tribal population, IEC activity was undertaken covering whole community under study. Permission was obtained from the department of tribal welfare, Government of Orissa seeking their cooperation during study activity. Community meetings were organised in the study villages of Morada and Suliapada block of Mayurbhanj district in presence of the village leaders & field level staff and officials of Integrated Tribal Development Agency,

Govt. of Orissa, working for the above communities. Demonstration on the communicable diseases, especially viral hepatitis, diarrhoeal disorders and respiratory infections was made using audio-visual aids as well as direct interactive sessions with the community group. Cause & spread of viral hepatitis, presentation and complications of hepatitis was explained to the community using audio visuals (video shows) in the respective villages.

Prevailing risk factors for transmission of Hepatitis infection

Factors relating to both enteral & parenteral routes for transmission of hepatitis viruses were looked in. Unsafe drinking water, unhygienic preservation of cooked food (Rice & Kanji), was observed as a common in all the tribes. Consumption of different meats like poultry, mutton, perk, jungle birds, reptiles (godhi) & rodents (wild rat) were noted which can be associated with subsequent investigation result. Risk factors leading to parenteral transmission of HBV & HCV are prevalent in the form of tattooing both in males & females; even the tattooing process is continuing within the adolescent age groups. Sharing of needle while tattooing is the practice. Besides, shaving by in the barber with common knife/ blade, body piercing, & body marking were the other common practices noted.



Tattooing in Forearm of an Adolescent Girl

Prevalence of Risk Factors for Enteral Transmission (HAV/ HEV)

Parameters		Number N=411	Percentage
Unsafe Drinking Water		403	98
	Perk	37	9
	Chicken/Mutton	354	86.1
Meat Eating (Type)	Beef	4	0.9
	Godhi	100	24.3
	Rat	45	10.9
Full Boiled Meat		411	100
Semi Boiled/Raw Meat		0	0
Toilet use	Constructed Latrine	4	0.9
	Open Field Defecation	407	99
Hand washing after toilet	Mud/sand use	330	80.2
	Water only	70	17
Alcohol intake	Regular (129)	Male	82 63.5
		Female	47 36.4
	Occasional (120)	Male	65 54.1
		Female	55 45.8
Pet Animal (200)	Pig	3	1.5
	Goat	129	64.5
	Dog	2	1
	Chicken	60	30
	Cattle	64	32

Prevalence of Risk Factors for Parenteral Transmission (HBV/ HCV)

Parameters		Number N=411	Percentage
Polyandry/ Polygamy		Nil	0
Scarification		67	16.3
Body Piercing		108	26.2
Blood Letting		36	8.7
Tattooing		47	11.4
Shaving by Barber		127	30.9
Drug Use	Smoking	102	24.8
	Inhalational	1	0.2
	Injectable	0	0
Other Drugs	Ganja	3	0.7
	Pan	41	9.9
	Tobacco	131	31.8
H/o Multiple Injection		149	36.2
H/o Surgery		79	19.2

Clinical examination, Blood sample collection & Primary health care

In the selected study villages, population census was made and the family heads were informed about the day of examination and sample collection. Willing individuals/ participants were enrolled into study after getting informed written consent from the individuals/ parent/ guardians (in case of the minors) in presence of their village leaders. The designed questionnaire was filled in by the investigator after interrogating the individual or the guardian. Detailed clinical examination was done by the physician, which was recorded in the clinical Proforma. The participants were given treatment for common ailments complained/ identified during the above examination by doctor. Subsequently venous blood sample were collected asceptically interrogated by physician using disposable needles & syringes and the samples were coded & preserved.

Sample preservation & laboratory test.

Blood samples were collected from 411 participants and serum separated & preserved in fridge in the field. These are transported to Laboratory, made in to 3 aliquots & kept in -70^{0}C deep freeze. Kits used for various tests were as per recommendation of the coordinator as decided in meeting by all partners. Serological (ELISA) tests conducted revealed the following results

Observation

Study population covered all the ages and both sexes, however, lesser representation from adolescent girls was noted and care will be taken in this regard in subsequent visits. Majority (2/3rd of the population) were of low socio-economic with monthly income below Rs.1000/-. Govt of Orissa has initiated settlement programmes or these tribes, hence the classical Chawl/ hut housing structure has been replaced by Khapar/ Tile roof in most of the houses but almost all the families live in a single room house, which is utilised for living, dining, kitchen & store

etc. the verandah or even the living home is also used for keeping pet animals like goat, poultry & cattle. Tattooing, body piercing & scarification, sharing of blades/ knife in the barber shop were the major potential parenteral modes for HBV & HCV transmission. More than 90% of individuals were observed to consume unsafe drinking water and have the habit of open field defaecation which are the probable routes for HAV & HEV spread among the population. Besides, consumption of alcohol was reported by more than 60% of the population.

Combined prevalence if HBV & HCV infection was found to be high which also includes children below the age of 5 years. Exposure to HAV & HEV infection in form of IgG antibody was found to be very high i.e. 79% and 23% respectively.

Subsequent plan of activity

450 individuals from Khadia (360) & Mankidia (90) tribe from Karanjia & Jashipur blocks have already been enrolled into the study and the laboratory tests are ongoing.

Tribe wise sero-positivity for Hepatitis markers

Tribe	Test	HBsAg population	Anti HBS	Anti HBc IgG	Anti HCV	Anti HAV	Anti HEV
Lodha	199	8 (4%)	5 (2.5%)	4 (2%)	2 (1%)	179 (90%)	49 (25%)
Saora	212	9 (4.24%)	6 (2.83%)	3 (1.4%)	0	181 (83.3%)	48 (23%)
Mankidia	45	2 (4.4%)	11 (24.4%)	23 (51%)	6 (13.3%)	34 (75.5%)	10 (24.8%)
Khadia	205	2 (0.97%	12 (5.85%)	10 (4.75%)	4 (1.95%)	162 (79.6%)	46 (22.4%)

It has been planned to cover a sample of around 1500 during the 2nd year of the study & serological tests will be conducted on all the samples. Training on genotyping, viral load estimation & mutant analysis will be imparted to the SRFs at NIV, Pune and the procedures will be standardised at RMRC. Attempt will be taken to undertake these tests independently.

1.17 Comprehensive analysis of diarrhoeal and environmental isolates of *Vibrio cholerae* from a cholera endemic area of Orissa.

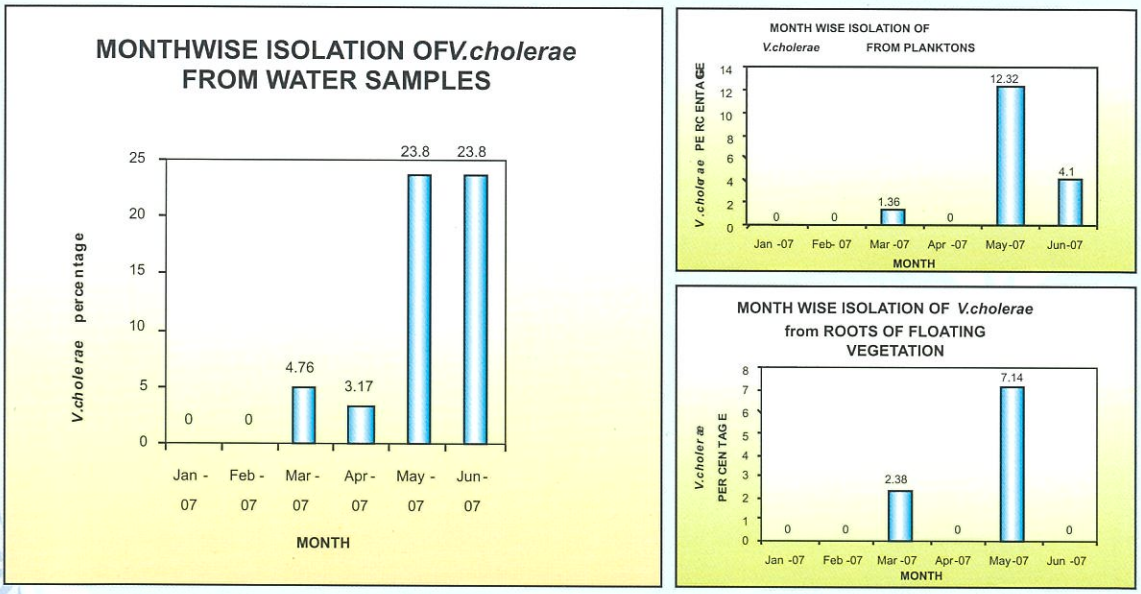
Status : Intramural
Investigators :
Dr. B. B. Pal
Dr. G. P. Chhotray
Dr. H. K. Khuntia
Mr. S. K. Samal
Dr. A. S. Acharya
Stating date : October 2006
Closing date : September 2009

Objectives:

1. Phenotypic characterization of *Vibrio cholerae* strains isolated from diarrhoea patients and environmental samples (water, sediment, plankton and roots of the floating vegetation) from selected areas of Puri district.
2. To find out the correlation between environmental and clinical isolates of *V. cholerae* by different molecular techniques such as PCR assay, RAPD, PFGE and ribotyping for the detection of virulence and regulatory genes.
3. To find out the critical environmental factors like pH of the sediment and water, salinity and temperature of different water bodies.

The proposed project was initiated in Capital Hospital, Bhubaneswar, Pipili CHC, Mangalpur CHC, Sakhigopal Hospital and ID Hospital Puri. The stool samples were collected in CBT medium and transported to RMRC Laboratory for bacteriological analysis (WHO Manual, 1987). The stool samples and the environmental samples were collected weekly from the study areas. During this period 652 stool/ rectal swabs were processed; out of which *E. Coli* were 171(52.1%), *V. cholerae* 107(32.6%), Ogawa 67(20.4%), Inaba 32(9.8%) and O139 serogroups -8(2.4%), *Salmonella* spp. 5(1.5%), *Shigella* spp. 5(1.5%), *Aeromonas* spp. 40(12.1%, Figure -1). Similarly 315 environmental samples (water, planktons and roots of floating vegetations) were processed following the standard techniques for the isolation of *V. cholerae*. Sixty three (20%) *V. cholerae* non-O1 non-139 strains were isolated all together from the environmental samples. It was observed that more number of *V. cholerae* was isolated from Mangalpur, Sakhigopal, Biraharekrushnapur and Teishpur respectively. More number of *V. cholerae* were isolated from water and planktons during the month of May and June 2007; where as the roots of the floating vegetations yielded least no of Vibrio isolates. The temperature of the environment was very high during May-June, which might be responsible for the multiplication of the *V. cholerae* in the environment (Fig 2). It was interesting to note that the outbreak of cholera was reported in the Matipokharisahi of Mangalpur village towards the end of July 2007; from where highest no of *V. cholerae* non-O1 non-O139 were isolated from the pond of Mathipokharisahi. The clinical isolates of *V. cholerae* O1 Ogawa were showing resistant to ciprofloxacin, norfloxacin, neomycin, nalidixic acid, furazolidone, ampicillin, and streptomycin and sensitive to tetracycline, gentamicin, azithromycin and chloramphenicol. Where as the environmental isolates were showing sensitive to ciprofloxacin, norfloxacin, tetracycline, gentamicin and azithromycin. Some selected strains of clinical and environmental isolates of *V. cholerae* were subjected to quadriplex PCR assay. The clinical isolates of *V. cholerae* were positive for *ctx* A and *tcp* A gene showing biotype El Tor and *tox* R positive. Where as the environmental isolates of *V. cholerae* non O1 non O139 were only positive for *tox* R gene. More no of environmental isolates of *V. cholerae* is necessary to look for the presence of *ctx* A gene along with clinical isolates for their molecular analysis.

Fig.2 Monthwise Isolatiion of V. Cholerae from Environmental Samples



1.18 Effectiveness of combined regimens with iron-folic acid, vitamin B_{12} supplementation, deworming and nutrition education in control of anaemia in tribal adolescent girls in Gajapati district of Orissa.

Status : Extramural
ICMR Task Force)
Investigators :
Dr. G. Bulliyya
Dr. A. S. Kerketta
Dr. B. Dwibedi
Mrs. G. Mallick
Stating date : November 2006
Closing date : October 2008

Objective:

To carry out an interventation study on the effectiveness of combined regiments with iron-folic acid, vitamin B_{12} supplementation, deworming and nutrition education in control of anemia in tribal adolescent girls.

Background

Anaemia is a significant public health problem in Orissa and iron deficiency is considered as the major cause due to low intake and bioavailability of iron in the diet. Orissa has the highest rates of infant mortality and maternal mortality, and anaemia becomes the key cause. Prevalence of anaemia in adolescent girls and pregnant women ranged 81-96%. Pregnancy is too short period of time to reduce pre-existing anemia, when women do not seek prenatal care until 2-3rd trimester, although intervention channels already exist to target iron supplementation. Adolescence, as a period of growth and development, is considered the best time to intervene in order to assist in physical and mental development, and to prevent later maternal anemia. A community-based intervention trial is proposed based on the hypothesis that iron-folic acid along with nutrition education and/or other approaches like deworming or B_{12} may be better alternative and effective strategies in reducing iron deficiency anaemia in adolescent girls in a tribal block of Orissa. To achieve the goal, a 4-arm regimen approach is being adopted to compare the efficacy of iron-folic acid administration when combined with deworming, vitamin B_{12} and nutrition education implemented through routine monitoring by the existing ICDS network.

Methodology for Baseline survey

Gumma is one of the seven community-development blocks in Gajapati district, selected for the study because its large tribal population. The study block consist tribal and primitive tribal groups (77.6%) with low literacy rate (Figure-1).

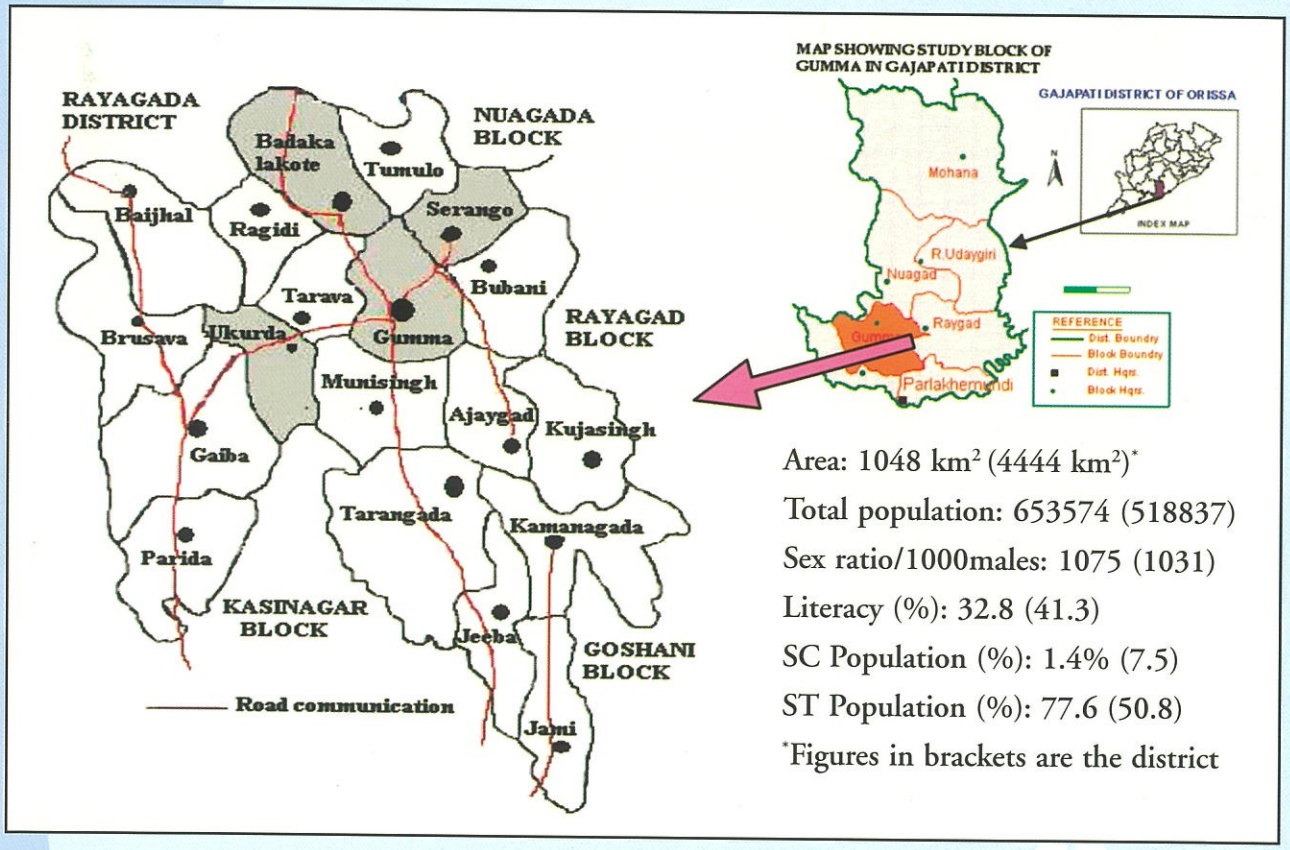
Calculation of sample size is based on the anticipated increase in mean haemoglobin level by 1.0 g (taking mean Hb= 10.7 g/dl and standard deviation [SD]=1.46 g/dl at 95% confidence and 80% power) for a period of one year, the required sample number is 52. This number is multiplied by 2 for allowing analysis to examine between age groups (12-14y and 15-18y) separately, added 20% dropout to follow-up, and expecting about 5% would have excluded and treatment for severe grade of anaemia, thus, a total of 750 girls are included, i.e, 150 in each group.

A pre-tested questionnaire was used to collect the household, socioeconomic data of parents, adolescent girl's age, sexual maturity, personnel hygiene practices, morbidity, clinical examination, KAP on anaemia and its

control measures, food and consumption pattern. Anthopometric measurements were taken using standard equipment and procedures. Finger prick blood samples were collected for haemoglobin and blood smear (thin and thick) for detection of malaria. Serum samples were collected on a sub-sample of girls after centrifuging venous blood for ferrtitin, transferrin receptor and C-reactive protein levels. The serum ferritin concentration was analysed by ELISA technique using commercial kits (UBI Magiwell, USA). Anaemia was considered to be present if the Hb value was below 12 g/dL and its magnitude was classified further as severe (<7 g/dl), moderate (7-10 g/dl), or mild (10-12 g/dl) category. Ferritin values less than 15 µg/L were considered to indicate depleted body iron stores. Stool samples were collected for intestinal worm infestations.

Results

Figure 1. Study areas showing Gram Panchayats in Gajapati district



A total of 840 adolescent girls aged 12-18 years were studied for base line data from 34 villages coving four Gram Panchayats in Gumma block of Gajapati district. Majority of study population belonged to ST (88%), while 6% SC community. About 18% of the adolescent girls were having education. Electricity was available in 17% and piped water was available in 4% of households, while sanitary latrine available in less than 5% of the

households. The study areas are comparable in terms of education, average family size, community, electrification, drinking water facility and sanitation.

In general, awareness about anaemia found to be very low at 13% and a negligible proportion of adolescent girls reported manifestation of anaemia. Around 1.6% of girls stated that dietary inadequacy was the cause of anaemia, while none attributed it to iron deficiency. A considerable proportion of them reported that they had seen IFA tablets (16%), while 2.8% aware of its use for anaemia control and 10% already consumed IFA tablets. Frequency of weekly consumption of iron-rich foods like pulses, eggs, milk and meat products and foods that enhance iron absorption such as fruits and green leafy vegetables were grossly deficient.

The mean anthropometric measurements of body weight, height, body mass index and mid arm circumference shown in Table 1. There were no significant differences in mean body measurements between the four intervention groups.

Table 1: Mean characteristics of adolescent girls

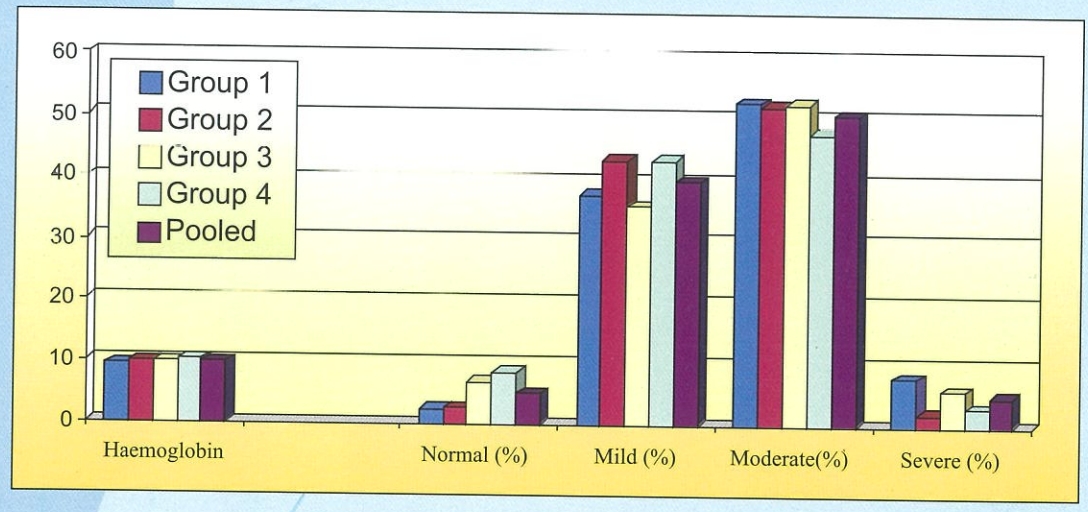
Study Area	N	Age years	Body weight kg	Height cm	BMI kg/m^2	MUAC cm
Area 1	244	14.4+2.00	38.0+7.46	145.0+6.95	18.0+2.61	21.9+2.66
Area 2	181	14.3+1.99	37.6+7.66	143.9+6.97	17.9+2.63	21.8+2.83
Area 3	194	14.7+2.20	38.0+8.00	144.6+7.80	17.9+2.58	21.8+2.80
Area 4	221	14.2+2.02	37.1+7.97	144.2+7.41	17.6+2.60	21.4+3.00
P-value	-	0.010	0.130	0.083	0.085	0.086
Pooled	840	14.4+2.09	37.7+7.78	144.4+7.30	17.8+2.60	21.7+2.89

BMI=body mass index, MUAC=mid-upper arm circumference

Iron deficiency anemia

The mean haemoglobin concentration of adolescent girls was 9.73 g/dl, ranged between 9.59 g/dL and 10.1 g/dL (Fig-2). Overall, 95% of adolescent girls had some level of anaemia. The proportion of girls having mild, moderate and severe grades of anaemia were 40%, 50% and 5% respectively. The proportion girls having moderate and severe grades of anaemia was relatively higher in younger age (12-14 y) than in older age (15-18y) group.

Fig 2. Mean haemoglobin (g/dL) and prevalence of anaemia among adolescent girls



The mean ferritin concentration was found to be 34ug/L in a sample of 232 adolescent girls. The proportion of girls with inadequate iron stores as per WHO criterion (< 15 ng/ml) was 40%, which is being considered the cut-off value for defining hypoferriteinanaemia.

Prevalence of malaria

Microscopic diagnosis of malaria thick and thin blood smears revealed that out of 466 slides examined, 9.2% were found to be positive for malaria parasite. Of total positive for malaria, 4.5% were identified as having P. vivax and 2.8% as P. falciparum infections, while 2% of slides were interpreted as having mixed P. vivax and P. falciparum infections.

Prevalence of intestinal parasites

Out of 122 stool samples examined, the prevalence of intestinal parasites was 16%, of which 8.3%, 2.8% and 4.8% had protozoan, hookworm and roundworm infections respectively. Mixed infestation was seen in 6% of positive samples.

Pattern of Anaemia

Blood samples collected from study subjects are being examined for type of anaemia through peripheral blood smear. Subsamples are analysed for presence of any genetic haematological disorders like thalassemia, sickle cell anaemia or G6PD. Subsamples from each site of study arm are also being tested for more sensitive parameters like soluble transferrin receptor to detect level of anaemia. C-reactive protein (CRP) in study cases are being studied to exclude cases with infection and inflamatiuons that will help in interpretation of serum ferritin levels.

Ongoing Studies

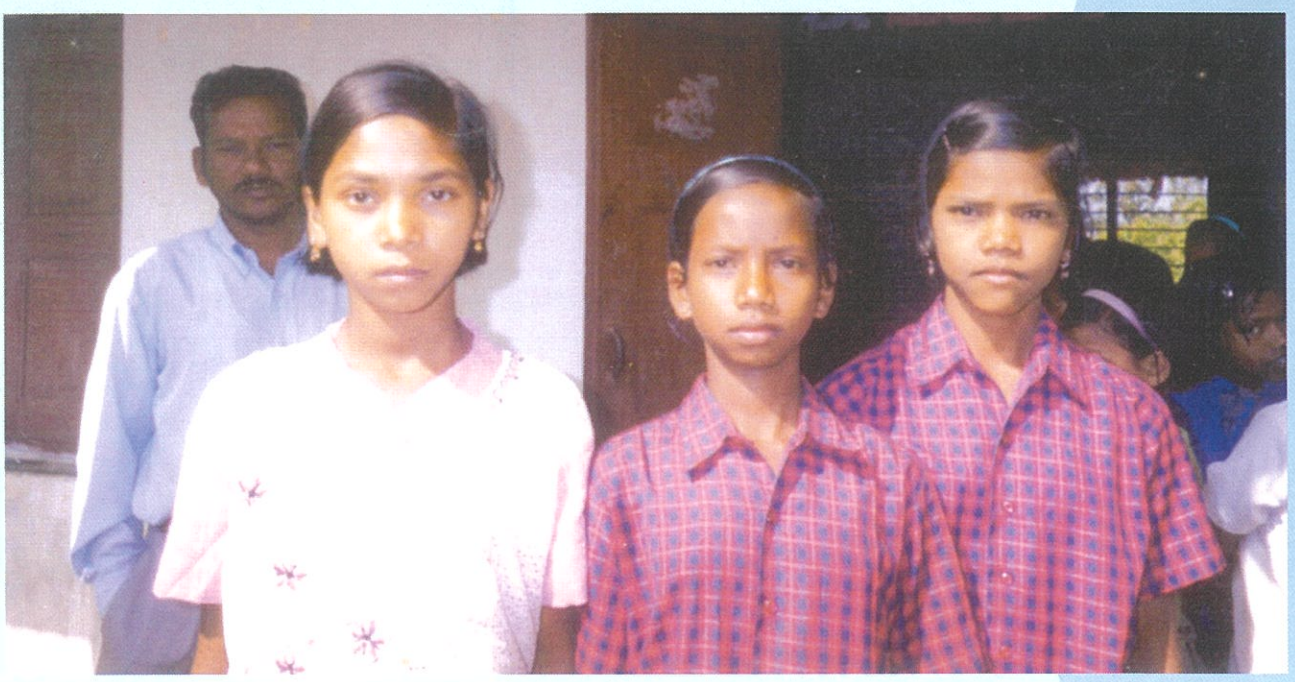
Clinical features

Each regimen group will be clinically examined for features of anaemia, avitaminosis or presence of any other associated clinical signs.

The study is in progress. The intervention on 4-arms are planned this year after completing the baseline survey.

Field work on adolescent girl anaemia

Collection of blood samples in tribal village



Adolescent girl having pallor in Gajapati district

2 Completed Studies

2.1 Post–DEC reactions in Human Bancroftian filariasis: An Immunobiological study in Orissa, India

Investigators :
Dr. A. K. Satapathy
Dr. B. Ravindran (P.I)
Dr. S. K. Kar
Collaborator :
Dr. Achim Hoerauf,
Bernhard Nocht Institute of Tropical Medicine, Hamburg, Germany
Funding :
Extramural Indo-German
Starting Date : March 2003
Closing Date : December 2006

Objectives:

1. To study the role of endosymbionts Wolbachia in mediating reactions after administration of DEC in infected human subjects.
2. To study the role of endosymbionts Wolbachia in mediation of inflammatory responses in human filariasis during acute disease episodes.

Summary of observations and major conclusions:

The project addressed the issue of reactions observed in human communities after administration of Diethylcarbamazine citrate, the anti-filarial drug being currently used for control of lymphatic filariasis. It is generally believed to be associated with microfilarial density in the subject although empirical data for this is not available. Since post-DEC reactions often appear similar to LPS mediated inflammation and an endobacteria such as Wolbachia are known to reside in Mf, the current study was undertaken to investigate the association between Wolbachia density and post-DEC reactions. The underlying principle is that Wolbachia are susceptible to tetracyclines / Doxycyclines and DEC mediated reactions should be preventable in Mf carriers by pre-treatment with the above antibiotics. Two strategies were followed; first, to treat Mf carriers with Doxycycline for different duration and then administer DEC to monitor reactions both clinically as well as sub-clinically by measuring inflammatory molecules viz. TNF-α, IL-6 and RANTES as well as wolbachia to analyze correlations between them; second, to treat cohorts of subjects, (with and without patent infection) with DEC and analyze the correlations as described above. The first approach is being pursued independent of the second approach; it is being done in three phases- in each phase 4 groups of Mf carriers are being used, one placebo and three treated with doxycycline for different durations (5, 10 and 21 days) and subsequently treated with DEC to monitor reactions. The following is the summary of results for the second approach: 1) Pre-treatment TNF-α levels were significantly more in Mf carriers (AS) and patients with chronic filarial disease (CH) (free of detectable infection) in comparison to subjects with cryptic infection (CR) (amicrofilaraemic with filarial antigenemia only), 2) Post–DEC reactions were significantly more in AS and CH cases as compared to CR cases and prevalence was comparable in the two (AS and CH) groups, 3) post DEC reactions were associated with significant elevation of TNF-α only in AS cases and not in CH cases, 4) conversely, significantly elevated levels of RANTES was observed only in CH cases and not in AS cases after administration of DEC, 5) plasma IL-6 levels were found to be significantly elevated in AS cases in comparison to CR and CH categories (pre drug administration) and after DEC administration, the levels of IL-6 decreased significantly in CR and CH cases and not in Mf carriers, and 6) plasma Wolbachia levels (as shown by real-time PCR) significantly decreased within 24 hrs after DEC consumption in CR and CH groups and not in the AS group.

Fig-1

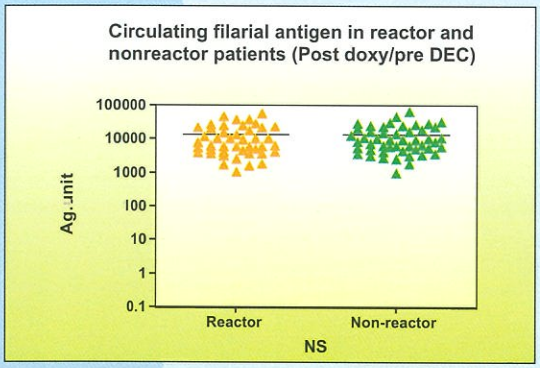


Fig-2

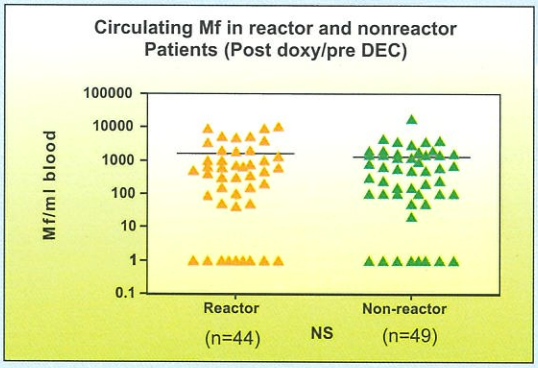


Fig-3

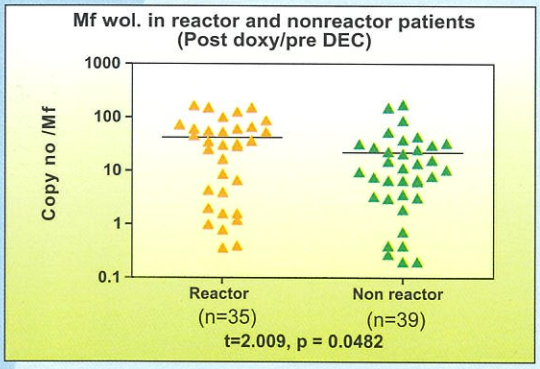
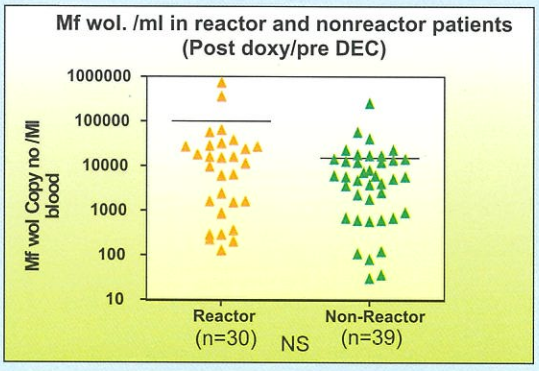


Fig-4



Four groups of Mf carriers were treated with Doxycycline for varying periods of duration - there was no significant change in circulating filarial antigen levels as observed among different groups. The Mf density decreased significantly following Doxycycline treatment for 21 and 10 days. There was a significant decrease in Wolbachia levels in Mf as shown by real-time PCR. Treatment for 21 days and 10 days were effective while 5 days treatment did not significantly eliminate intra-cellular Wolbachia in Mf.

Cumulative clinical scoring of post-DEC reactions in each case was monitored as described in our earlier Annual report. Based on the clinical observations the individuals were divided in to reactor and non-reactor. There was no significant change in circulating filarial antigen levels and Mf was observed between reactor and non-reactors (Fig-1 & 2). Decreased Wolbachia density in Mf (as a result of Doxy treatment) results in significant decreased of post-DEC reactions (Fig-3). The post-DEC reactions correlated with Wolbachia density further emphasizing the importance of Wolbachia endosymbionts in mediating reactions.

Since post DEC reaction correlated significantly with higher levels of wolbachia in mf, we assumed that TLR-4 mutation could potentially influence the post DEC reaction in human filariasis. To check this possibility of host genetic factors playing a role in post DEC reaction, the prevalence of TLR 4 genotypes were assessed in all the Doxy/DEC treated cases to correlate with side reactions. TLR 4 mutation frequency was significantly more in non-reactors (37.70%) in comparison to reactors (17,07%). This indicates that TLR4 mutation could protect human hosts from side reactions associated with DEC treatment.

2.2 Genetic polymorphism of host molecules involved in immunity and immunoregulation in human filariasis

Investigators :
Dr. A. K. Satapathy
Dr. B. Ravindran (P.I)
Collaborator :
Dr. S. Sharma, TIFR, Mumbai
Funding :
Extramural (ICMR Filariasis TF)
Starting date : March 2005
Closing date : August 2006

Objectives:

1. To type polymorphism of human genes that play a role in innate and/or adaptive immunity in human filariasis

2. To correlate genetic polymorphism with clinical and parasitological status in human filariasis

In lymphatic filariasis endemic areas, a spectrum of clinical manifestations can be observed in human communities. A large proportion of infected subjects are free of overt disease manifestations and most of the patients with chronic disease are free of current patent infection. Unlike several other infectious diseases, there is no clear consensus that patent infection would necessarily lead to development of chronic disease in human lymphatic filariasis. Longitudinal epidemiological studies indicated that development of chronic disease need not necessarily be a definitive consequence of patent infection in a given host conversely evidence exists for development of chronic disease with out prior experience of patent filarial infection. These observations point towards the possibility of host genetic factors playing a significant role in the clinical as well as parasitological outcome in the exposed population.

Summary of observations and major conclusions :

In this project an attempt has been made to correlate genetic polymorphism with clinical and parasitological status in human filariasis. The Toll-like receptors (TLRs) are a part of the innate immune defense, which help in the recognition of conserved patterns on microorganisms. Human TLR4 and CD14 are known to be components of the lipopolysaccharide receptor complex and results in induction of inflammatory molecules, which play a critical role in innate immunity against bacterial infections. Since development of overt chronic manifestation in filariasis has been associated with involvement of inflammatory reaction, and filarial parasites contain an endosymbiont Wolbachia with LPS like molecules, we assumed that TLR-4 mutation could potentially influence clinical as well as parasitological status in human filariasis and thus studied TLR-4 polymorphism in different clinical groups of human Bancroftian filariasis. Two known mutations in TLR4 gene (Asp299Gly, Thr399Ile) have been known.

To check the possibility of host genetic factors playing a role in the clinical as well as parasitological outcome in the exposed population, the prevalence of TLR-4 Asp299Gly genotypes was assessed in filariasis with different clinical manifestations. The frequency of Asp299Gly TLR4 mutation did not significantly differ in individuals with various categories in comparison to endemic controls. Mutation frequency in males and females were analyzed separately. Patients with hydrocele were found to display lower frequencies than endemic controls; however the percentage of mutation was not statistically significant between different categories. These finding suggest that TLR4 mutation (Asp299Gly) in endemic population does not play a major role in determining the clinical or parasitological outcome in human lymphatic filariasis.

The association between TLR4 (Thr399Ile) polymorphism and parasitological outcome/chronic manifestations of filariasis in a population from filarial endemic areas has been analyzed. TLR-4 (399) mutation frequency was significantly more in endemic controls (28%) in comparison to patients with chronic disease (7.1%) in males. Similarly, the frequency mutation of TLR 4(399) in patients with active infection was significantly high in comparison to patients with chronic manifestations. This indicates that TLR-4 mutation (Thr399Ile) could protect human hosts from developing chronic filarial disease in males. Hydrocele, the most common chronic manifestation in filariasis is restricted to only males and the observed difference in elephantiasis (observed in both males and females) does suggest that this mutation plays a role only in males and not in females. Such an association of TLR-4 (399) mutation with disease development was not observed in females.The inflammatory cytokine produced by male and female did not differ significantly.

The functional polymorphism -260 C > T in the LPS sensing TLR4 co-receptor CD14 gene enhances the transcriptional activity and results in a higher CD14 receptor density. Individuals carrying the T/T genotype also have significantly higher serum levels of soluble CD14. We investigated the role of the CD14 -260 C>T polymorphism in the clinical as well as parasitologocal status of human lymphatic filariasis.

To determine the effect of CD14 –159 C>T on different clinical and parasitological status, the prevalence of CD14 (-159) polymorphism was assessed in filarial patients. The distribution of C and T allele was found to be 49.5 % and 51.5 % respectively. The frequency of heterozygous genotype (CT) was found to be more when compared to wild type genotype (CC) in the study population. The CD14 –159C>T mutation frequency was not significantly different among clinical categories suggesting that CD14 (-159) may not have any role in human filariasis.

MBL is a C type collectin that participates in pathogen recognition, opsonization, phagocytosis and complement activation. A common set of structural polymorphism, known as the B, C and D alleles (together known as the O alleles) within a 15-bp span in exon 1 disrupt the function of MBL. Low MBL levels are associated with susceptibility to certain infection in contrast high MBL levels might exacerbate the renal complications of diabetes. Since the development of chronic manifestation in filariasis has been associated with involvement of inflammatory reaction, we assumed that MBL mutation might have an association in the clinical as well as parasitological status in human filariasis.

We analyzed the distribution of genetic polymorphism of MBL 52 D gene against parasitological and clinical outcomes following exposure to filarial parasites in a W. bancrofti area. The frequency of MBL 52(D) codon was found to be very rare in this population. The distribution of structural variants known as alleles D of exon 1 in chronic vs. uninfected subjects did not differ significantly by stat. analysis.

We also analyzed the mutation frequency of MBL codon 54 B among patients with filariasis. This mutation was detected by allelic specific PCR method. The frequency was not found to be significantly different between control subjects and patients with acute diseases. However there was a significant difference in mutation frequency between acute disease cases and asymptomatic Mf carriers. MBL 54 B mutation frequency was significantly low in asymptomatic parasite carriers in comparison to acute cases and endemic normals in male.

A Th2 type of immune response is associated with decreased T–cell proliferative response and high IL-10 and low IFN g production. PBMC of Mf carriers has been shown to release higher levels of IL-10 than in patients with chronic lymphatic pathology, thus attributing a role for this cytokine in down regulating effectors immune

responses in Mf carriers. Therefore we assumed that there might be some association between these polymorphism and clinical/ parasitological status in filariasis. We analyzed the mutation frequency of IL-10 (-1082) among patients with filariasis. The genotype frequencies for IL-10 (-1082) mutation did not differ significantly between clinical and parasitological categories. These finding suggest that IL-10 (-1082) mutation may not have significant role in determining the clinical or parasitological outcome in human lymphatic filariasis.

2.3 Innate Immune recognition of filarial parasites by phagocytes

Status : Extramural (ICMR Task Force)
Investigators :
Dr. B. Ravindran
Co-Investigator :
Dr. Vineeta Bal, National Institute of Immunology, New Delhi
Funding :
Extramural (ICMR Filariasis TF)
Starting date : January 2003
Closing date : December 2005

Objectives:

1. To analyse the effect of Btk deficiency on macrophage phagocytosis of microfilaria in terms of cytokine production and effector functions.
2. To identify and characterise cell surface molecule/s involved in the uptake of mf by macrophages.
3. To compare the effects of such molecules on phagocytosis in vitro and parasite clearance in vivo.

Background:

Most of the investigations in both human and animal models of filariasis generally address adaptive immunity and innate mechanisms if any are yet to be investigated. There are instances in animal models of differential susceptibility to filarial infections and it is presumed that innate mechanisms could be operational in those instances and result in the observed 'resistance'. This study proposes to address some of these issues in filarial immunity.

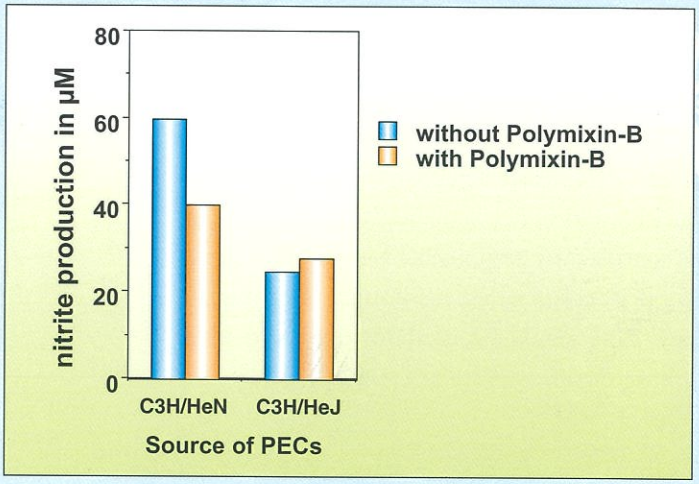
Results and Conclusions:

Investigations indicated that non-MHC related factors could contribute to microfilarial clearance at least in murine models of filariasis. Clearance of microfilaraemia in two different strains of mice was variable and was independent of MHC haplotype.

We had demonstrated that macrophages from x-linked immunodefficient mice (xid) lacking functional Bruton's Tyrosine Kinase (Btk) show poor NO induction and enhanced IL-12 induction contributing to delayed clearance of injected microfilaraemia. Since DBA/2 mice are more susceptible to microfilaraemia than BALB/c mice although having same MHC haplotype (H-2d) investigations were undertaken to study the macrophage function in these two strains of mice with a view to correlate their differential susceptibility with macrophage activity. It has already been shown that inflammatory responses induced by filarial nematode are mediated by LPS like molecules from endosymbiots, Wolbachia bacteria by signaling through the TLR-4 receptor. Based on this background, we have attempted to investigate the role of NO and macrophage effector functions as well as involvement of Toll like receptors in microfilarial clearance in DBA/2 mice. We have used C3H.HeJ [HeJ] and C3H.HeN [HeN] as two strains of mice. While C3H.HeN are normal, C3H.HeJ show natural mutation for toll-like receptor [TLR] 4 because of which C3H.HeJ mice do not respond to LPS, a ligand for TLR4.Our work shows

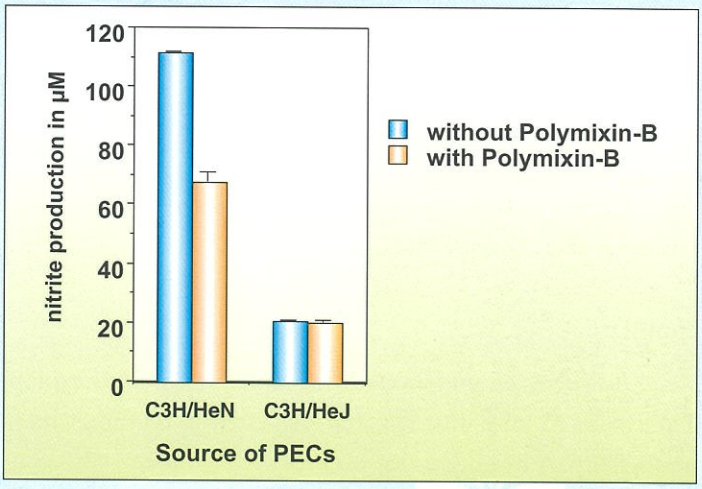
that S. digitata mf extract can stimulate peritoneal exudates cells [PECs] from HeN mice to produce higher levels of nitrites as compared to those from HeJ mice in a dose dependent fashion. Since these mf are collected from adult stage parasites which are transported from Cattle in the abattoir with a potential for contamination with LPS, we added polymixin B as an inhibitor of LPS activity and performed the assay again. Presence of polymixin significantly brings down nitrite production significantly from HeN macrophages, however, the response does not reach baseline level, whereas nitrite production from HeJ macrophages is unaltered by polymixin B [Figure 1].

[Figure 1]



We next used a purified antigen of mf, AgW, to stimulate PECs from HeJ and HeN strains. The objective was to identify the filarial antigen (since Mf extract contains several antigens) that stimulates macrophages thro TLR 4. AgW vigorously stimulates HeN macrophages to produce nitric oxide read out as nitrites. This is again partially inhibited in presence of polymixin B. However, AgW cannot stimulate HeJ macrophages to produce nitrites above background level [Figure 2]. This seems to suggest that in the absence of TLR4 AgW cannot stimulate macrophages to produce nitric oxide. Inhibition with Polimyxin B in HeN macrophages is however incomplete and the nitrites levels do not decrease to levels observed in HeJ mice. This indicates the possibility of a non-LPS component in AgW that can stimulate HeN macrophages thro TLR 4.

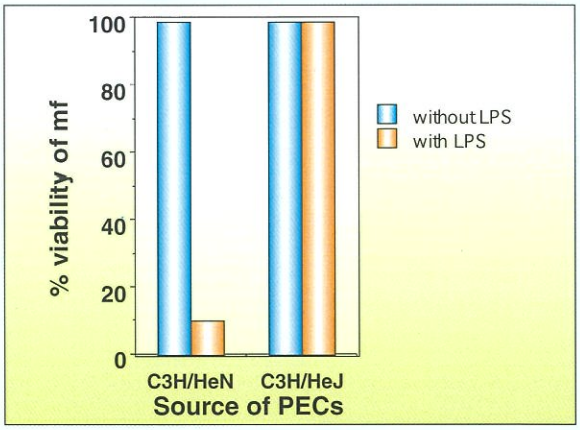
[Figure 2]



We next looked at the ability of activated macrophages to kill microfilaria in vitro in these two strains in presence or absence of LPS. At 24 and 48 hours nearly all mf added to the culture were viable. Hence data at 72 hours is shown here [Figure 3].

While in the absence of LPS both HeN and HeJ macrophages failed to kill mf even at 72 hours [as shown], addition of LPS in cultures resulted in efficient killing of mf in LPS-responder HeN strain macrophages. These data clearly demonstrate that presence of LPS and hence signaling through TLR4 is significantly responsible for mf killing. It is still not clear whether stimulation through any TLR would bring about enhanced mf killing or this is peculiar to LPS.

[Figure 3]



2.4 Development and evaluation of community development and partnership strategies for drug delivery for the control of lymphatic filariasis in urban areas of Orissa, India

Status : Extramural

Investigators : Dr. B. V. Babu, Dr. A. S. Kerketta, Dr. S. K. Kar, Prof. D. K. Behera, Sambalpur University

Funding :

UNICEF/UNDP/World Bank/WHO's Special Programme on Research and Training in Tropical Diseases (TDR), World Health Organisation (WHO), Geneva, Switzerland

Starting date : September 2003

Closing date : August 2006

Objective :

To develop and test alternative innovative strategies for mass drug administration, which would achieve the desired high treatment coverage in urban populations necessary for elimination of lymphatic filariasis (LF).

Summary :

Initially, we undertook a formative research to explore and identify opportunities in urban communities. The results of formative research suggested to go for an intervention strategy with community participation and partnership. A health services driven MDA strategy with community partnership and participation approach was developed and experimented in two endemic urban areas, namely, Choudwar and Dhenkanal in Orissa, India. The following components of intervention are tried out in study areas.

Stakeholders' active involvement in planning and decision-making:

In this programme, municipality and local health institutions played key roles. The municipality consisted of elected representatives of wards (geographical and political units in urban area) called councillors and a chairperson. This body succeeded to form coordination committee by including many stakeholders like bureaucrats of municipality, private practitioners, practitioners from other governmental and non-governmental hospitals,

community based organizations (CBOs) like youth clubs, women clubs and residence associations, non-governmental organizations (NGOs), journalists, representatives of industries, prison and schools, and representatives of religious and ethnic groups in the program. This committee met periodically. The research team initially advocated for the program to different stakeholders and shared the results of formative research. The coordination committee took all decisions on planning including social mobilization and drug distribution. However, the committee received some directions and suggestions from the local health institution, which was responsible for providing drugs and other resources.

Advocacy:

Advocacy was done both among the populations and key partners. This task was performed by the research team for the coordination committee and the latter did the advocacy among the local groups and the community.

Initiating the intervention:

Having designed the plan for the entire urban area, the coordination committee identified ward level partners, who are suitable to undertake activities related to community mobilization and drug distribution. The strengths and weaknesses of these groups were assessed by the coordination committee and necessary inputs were given. In most of the wards the concerned councillors led the ward level activities.

Partnership:

Thus the partners were involved in various stages of intervention. Also some of the stakeholders got involved in resource mobilization. The micro-level planning at the municipality and ward level were made by the coordination committee and the ward level committee, respectively. A few medical practitioners from the coordination committee were grouped into four teams for adverse side reactions surveillance and management as an integral part of the distribution process. In addition, different sub-groups were identified for the separate differential treatment:

Special strategies

1. **Sub-group approach**: Special strategies were developed for better coverage and compliance among the sub-groups like religious and linguistic minorities, inmates of jail and school children.
2. **Use of local practitioners**: The services of local practitioners including private practitioners and physicians from government sector were utilized during management of adverse side reactions, and ward level community mobilization activities.
3. **Sensitization of media personnel**: Through a process of mobilization, media personnel were requested to highlight the positive aspects of the program ignoring the bare instances of some adverse reactions at the time of reporting. For achieving their support, some of them were made members of the coordination committee.
4. **Communication and community mobilization activities**: Intensive community mobilization activities (e.g., rallies by school children and house-to-house visits) were organized by involving different partners during the period of environment building. Various IEC materials also were used.

MDA :

The strategy ultimately resulted in local decision-making in consultation with health institution with regard to execution of drug distribution. The following were the major components in MDA.

1. **Selection and training of community drug distributors (CDDs)**: The ward level committee identified some local volunteers, designated as CDDs, to distribute the drugs. Some para-medical staff of the local health institutions were included as supervisors to monitor the MDA process. The CDDs and supervisors underwent one-day training, organized by local health institution with the help of the coordination committee.

2. **Drug distribution**: The distribution process initiated as planned jointly by the coordination committee and ward level committee. The distribution was carried out by the CDDs either individually or in groups. In some wards, the members of coordination committee, particularly the councillors and members of ward level committees, monitored the activities. The supervisors monitored the activity of the drug distribution and assisted the adverse reactions management team by identifying cases along with CDDs. The distribution process continued subsequently for three more days, but adverse reactions management teams did not conduct mobile operation during those days due to practical exigencies. However, the CDDs, supervisors and ward level committees were directed to bring such cases to respective hospitals of these physicians.

Evaluation:

The intervention was evaluated by using several qualitative and quantitative methods, of which some are used for this report. Household coverage survey was undertaken in intervened urban area, and other urban and rural areas, where MDA was undertaken without such intervention. Three indicators namely coverage (percentage of eligible people who received tablets), compliance (percentage of eligible people who swallowed tablets) and household coverage (percentage of households visited by CDD or health worker during MDA) were used to evaluate the intervention. The intervened urban area recorded significantly higher coverage ($P<0.001$), compliance ($P<0.001$) and household coverage ($P<0.01$) than that of the non-intervened urban area, but nearer to that of the non-intervened rural area.

Key findings:

1. Partnership approach involving various stakeholders is an innovative alternative method for addressing the problem of low MDA coverage and compliance in urban India.
2. The MDA with local decision-making and local leadership is quite effective in urban communities.
3. This innovative approach has potentiality to achieve desired levels of results in different strata of urban communities.
4. It takes into account the specificity of the situation and thereby tries to address the socio-cultural peculiarities of different sub-groups.
5. Seems to be suitable for implementation in other urban areas in India.

Next step

Our evaluation revealed that this approach is feasible and sustainable in urban areas of India in general and in small towns in particular. The approach can be scaled up to larger urban areas with necessary modifications through experimentation. More efforts must be made for raising the community awareness level of the risk of getting the disease, benefits of the program, and perceived health needs. Finally the strategy with necessary revision may be integrated into the national program.

2.5 Studies on prevalence of 76Tcrt / 86Ymdr1 Plasmodium falciparum isolates in severe malaria cases of Orissa and its biological advantage.

Status : Intramural
Investigators :
Dr. M. R. Ranjit
Dr. G. P. Chhotray
Stating date : April 2005
Closing date : March 2006

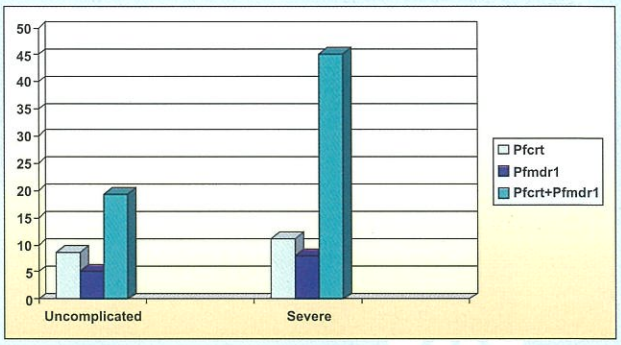
Objectives :

1. To investigate the prevalence of 76Tcrt / 86Ymdr1 Plasmodium falciparum isolates in severe malaria cases of different geographical regions of Orissa.
2. To test the drug sensitivity pattern of these isolates by in-vitro assay.
3. To study the multiplication pattern of these isolates in different blood groups

Observations

Total one hundred and eleven blood samples from severe falciparum malaria cases admitted in S C B Medical College Hospital, Cuttack , IGH, Rourkela and about 81 blood samples from uncomplicated cases belonging to Athagarh, Jajpur, Anugul, Dhenkanal, Khurda, Keonjhar and Sundargarh area has been collected for analysis. The genomic DNA of P falciparum was isolated by phenol extraction and EtOH precipitation. The PfCRT(K76T) and PfMDR1(N86Y) point mutation was analysed by PCR-RFLP method. Amongst the total samples subjected to molecular analysis 57 from uncomplicated and 62 from severe malaria cases showed amplification for both the genes. Of the total severe cases 7(11.3%) cases were found to harbour PfCRT(K76T) point mutation, 5(8.1%) PfMDR1 (N86Y) point mutation and 28(45.2%) both PfCRT(K76T) and PfMDR1(N86Y); while amongst the uncomplicated cases 5(8.7%) cases were having PfCRT 76T point mutation, 3(5.2%) with PfMDR1 86Y and 11(19.3%) having both PfCRT 76T and PfMDR1 86Y point mutations (Fig 1).

Fig 1: Prevalence of CQ resistance molecular markers among uncomplicated and severe falciparum malaria cases in Orissa

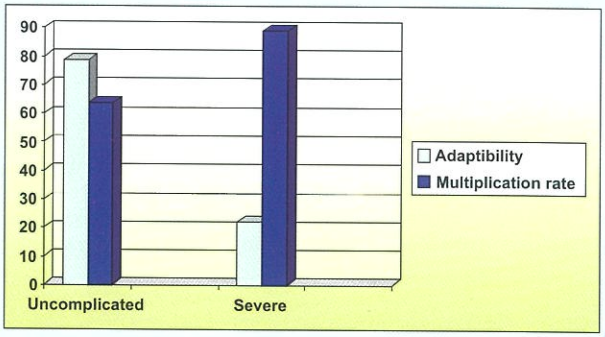


The retrospective analysis of the history of drug consumption revealed that majority of the severe cases (>80%) have consumed the full course of the choloroquine before developing the severe complication. This indicates that the high incidence of severe malaria might be due to the development of resistance to the chloroquine by the P falciparum isolates of Orissa. To determine the parasite growth 24 wild type parasites isolated from uncomplicated cases and 11 double mutant (Pfcrt + Pfmdr1) parasites isolated from severe malaria cases were cultivated in RPMI 1640 medium supplemented with 15%(v/v) human serum for 48 hours following the candle jar method. The parasites were counted per 5000 erythrocytes and the multiplication rate was calculated using the formula logMF = logN (day 2)-logN(day1) .It was found that out of 24 wild parasite isolates 19(79.2%) isolates and among the 11 mutant parasite isolates 2(18.2%) adapted to the in vitro culture system. The multiplication rate was, however observed to be significantly high among the mutant parasites than the wild types (Fig 2).However no significant association was found between blood group antigen and development of severe malaria.

Fig 2: The adaptability and multiplication rate in wild and double mutant P falciparum parasite isolates

Conclusion

1. The majority of the severe malaria cases in Orissa are due to CQ resistance P falciparum isolates.
2. The multiplication potential of the mutant parasites is significantly high among mutant parasites compared to the wild parasite population.



2.6 Epidemiological Characterization of bacterial entropathogens among the children suffering from acute diarrhea – Ahospital based study.

Status : Intramural

Investigators :
Dr. B. B. Pal
Dr. G. P. Chhotray
Dr. H. K. Khuntia
Mr .S. K Samal

Stating date : September 2005

Closing date : August 2006

Objectives:

1. To isolate and identify the various bacterial enteropathogens like E.coli, Salmonella, Shigella, Vibrio cholerae from hospitalized diarrhoea patients of all age groups . from Capital hospital, Bhubaneswar, Sishu Bhaban, Cuttack, and ID hospital, Puri.
2. To type the various bacterial enteropathogens by specific antisera and to study their antibiogram.
3. Detection of toxic genes of pathogenic E.coli and V. cholerae by PCR assay

Summary :

During the study period September 05 to March -06 ,179 rectal swabs were processed from paediatric age groups of diarrhoea patients which yielded E.coli-83(82.2%),V.cholerae O1-16(15.9%),Shigella spp 2% only .The V.cholerae O1 Ogawa and Inaba serotypes were isolated together .The multiplex PCR assay on V.cholerae revealed that all were positive for ctxA and tcpA genes showing biotype El Tor. The PCR assay on 50 E. coli isolates revealed that 2.7% and 2% were positive for EPEC and ETEC strains.

Three hundred and eighty-nine rectal swabs were also collected from ID hospital, Puri.from the adult age group of diarrhoea patients The predominant enteropathogens isolated were E,coli (68.7%)V.cholerae(25.1%) and Shigella spps (3.9%) respectively. Among the V. cholerae O1 strains Inaba sero types dominated over the Ogawa serotypes. and there were few V. cholerae O139 strains isolated.The multiplex PCR assay on V. cholerae revealed that all were positive for ctx A and tcp A genes showing biotype El Tor. The outbreak at Cuttack town during March 2006 was due to V,cholerae O1 Ogawa which showed that this serotype may cause the future outbreak of cholera in Orissa.

2.7 A 6-year's Prospective study of the risks of death by cause from tobacco and alcohol use among 2million Indian men and women, A multi-centric study.

Status : Extramural
(Centre for Global health and research, Toronto, Canada)
Investigators :
A. S. Kerketta
Mr. P. K. Jangid
Dr. A. S. Acharya
Collaborator :
Director of Census Operation, Orissa.
Stating date : Jan 2003
Closing date : December 2006

Objective:

To study all cause mortality and cause specific mortality (Tuberculosis, cancer, vascular disease, asthma, chronic obstructive pulmonary disease, and other causes) by age, gender and socioeconomic group in relation to tobacco and alcohol use among 2 million Indian adults surveyed for their tobacco and drinking patterns in 1998.

Aim:

To improve overall cause of death reporting and to add analytic epidemiological question to Sample Registration System (SRS) by introducing Verbal autopsy (VA) in SRS and ascertaining cause of death (COD) and subsequent coding with ICD-code 10.

Summary and Conclusion:

The present study is a multicentric study undertaken in collaboration with Centre for Global Health and Research (CGHR) and Registrar General India (RGI). It has been carried out within the sample frame of SRS of India.

The study envisages the following activities 1. Training and retraining of the SRS supervisors on Verbal Autopsy methodology 2.Resampling of 10% of VA of SRS supervisors 3. Assigning of cause of death as per ICD-10. Orissa SRS covers 36.7 million people; spread over 405 units and having 51 supervisors to carry out survey on vital statistics every half yearly. The training of the supervisors of DCO Orissa was conducted, on Verbal autopsy methodology after which for first time the VA was implemented in SRS, during 2nd Half Yearly Survey (HYS) of 2002 and 1st HYS of 2003. Repeated refresher's training was organized for the SRS supervisors especially on the use of newer modified manual, use of single page VA form and symptom list .In the Special Survey of death (SSD) covering period of 2001-2003 (except the HYS mentioned earlier), the VA has been conducted. Thereafter from 2004, the VA is in use on regular survey of SRS. After conducting VA, the data entered into the computer by Census department and sent to the office of RGI. The physicians from Government Medical College were imparted training on assignment of COD and ICD-10 code. The scanned copy of each VA form is sent back to two physicians independently. The web- based assignment of cause of death has been initiated and so far around 7000 deaths has been assigned COD and have been coded as per ICD-10. But till date only maternal death data of all over the country has been analysed centrally by RGI. The cause of death data for the maternal death has been collected by the SRS supervisors by using specially designed and well structured VA instrument with recording most common signs and symptoms and assignment of cause of death by medical professionals. The results show

there is substantial declining of nearly 24% of Maternal Mortality Rate (MMR) during the period 1997-2003 (as per the data available with RGI). The important cause for the decline in MMR is found to be delivery conducted by skilled attendance and institutional delivery. The leading cause of maternal death is found to be haemorrhage (38%) includes both ante-partum and post- partum followed by sepsis (11%) and abortion (8%) which substantially contributing towards maternal death. In-contrast the haemorrhage is a much less common cause of maternal death in urban area reflecting better access to emergency obstetrical care. Death data for all other age group is under progress. After assignment of COD & ICD-10 coding the base line survey data of 1997 which was carried out by SRS of Orissa was traced with the personal habits of alcohol and tobacco use of the particular deceased. The result revealed that tobacco smoking is a major cause of premature adult mortality in Indian men. The tobacco use patterns and disease patterns vary considerably within India; so as the expected consequences of prolonged tobacco use will also likely vary in India's large and heterogeneous population. However considerably more research is needed in order to document the links of smoking and tuberculosis, respiratory mortality among non-smoking females and children in relation to passive exposure to smoke and indoor air pollution.

Students working in mordernised clinical laboratory

3

Other Scientific Activities

3.1 Effect of Annual single dose of DEC in filariasis transmission

Investigators:
Dr. N. Mohapatra
Dr. R. K. Hazra
Dr. S. K. Parida

DEC is highly effective microfilaricidal drug. Use of these drugs at community level results in reduction of human infection and consequently transmissions. In some areas due to high vector density effective reduction in transmission does not occur. Choudwar town of Cuttack district though a semi urban area is highly endemic for filariasis. Single dosage of DEC mass drug distribution was done on15.9.04.Baseline data on vector density, infection rate, infectivity rate and infective stage of parasite per mosquito (l3 load)were collected before and after the mass drug distribution. The monthwise vector density(PMHD) of Culex quinquefasciatus is presented in fig 5. It varies from 18.3 to 58.6 in different months of the year. Figure 6 depicts the infection and infectivity rate of Cx. quinquefasciatus. It indicates that soon after the drug distribution no infective larvae could be detected upto three months (October to December).However ,from the month of January, 2005 infection in the vector appeared. There was 45.7% and 26% reduction in infection and infectivity rate compared to the base line data. Still the infection could be detected upto March. The study is in progress. High vector density & Mf rate & transmission was observed in Khurdha in comparison to Chowdwar. Effect of MDA on the transmission parameter is more effective in Chowdwar compared to Khurda. Vector density and Mf rate plays an important role in eradication of filariasis by MDA.

Comparision of Transmission Parameters after MDA in Khurda & Chowdwar

Fig-15

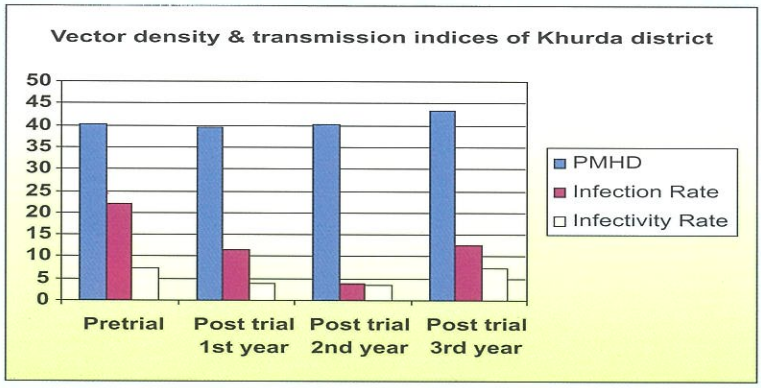
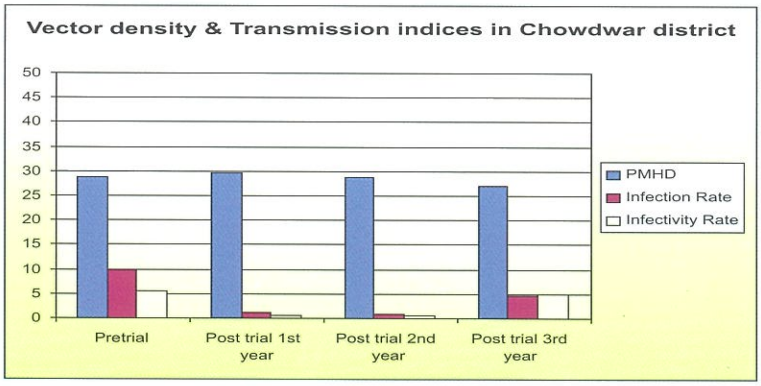


Fig-16



3.2 Vector Survey at Mahanga P.H.C, Cuttack for Malaria.

Investigators :
Dr. R. K. Hazra
Dr. N. Mohapatra

Background :

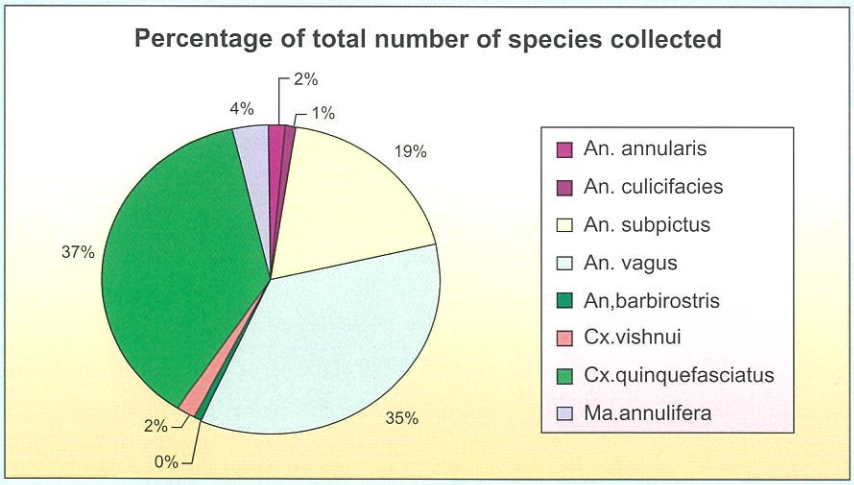
With request from NVBDCP Orissa, vector survey was carried out in Mahanga PHC . Due to sudden reporting of more malaria cases in the area and to check the spread, the vector susuptibility to insecticide was studied.

Objectives:

1. Investigate the case that might have been acquired locally.
2. Entomological investigation to know the vector prevalence, density, habits and mosquito breeding spot with larval density.
3. To prevent a small focus of malaria cases from becoming a source of sustained transmission.

Study Report:

The distribution Anopheline vector species is depicted in this report. Seven species belonging to three genera of mosquito were collected. Out of these five species were from genus Anopheles, the rest were Culex, and Mansonia. Total 245 mosquitoes were collected among which 139 were Anophelines (Table 1). Among five anopheline species collected randomly from households two were identified vectors of malaria they are *An.annularis*, *An.culicifacies*. Total anophelines collected were *An.annularis* 4 (1.6%), *An.culicifacies* 3 (1.22%) (Table1). All these were collected from Human dwelling and Cattle Shed. The mosquito collected from individual villages reveals that two major vectors i.e. An.annularis, An.culicifacies was found in the village. The Per Man Hour Density (PMHD) of total mosquito collected was 16.95; the vector density in terms of PMHD and Critical density were 2 and 1.3, 1.6 and 3.3 of An.annularis, An.culicifacie, respectively (Table 1). The abdomen of major species showed half gravid (HG), full fed (FF), and unfed. An.culicfacies was collected from cattle shed while one An.annularis was collected from human dwelling and three from cattle shed. All the vectors were dissected and sporozoite was not found. The lower number of collection was due to spray activity was already in operation by state while survey took place.Both these vector species were found sensitive to deltamethyne and resistance to DDT.



3.3 Referral Services Rendered for Hemoglobinopathies

Investigators :

Dr. R. S. Balgir, Dr. G. P. Chhotray,
Mr. B. Murmu, Mr. S. S. Nisank,
Miss. S. Dixit and Mr. B. N. Sethi

Referral services were provided in the Centre for diagnosis to the cases referred from local and periphery PHCs, hospitals and Medical colleges in Orissa along with genetic/marriage counselings to the affected persons and families.

In one series diagnostic services were provided to 3 families referred during period from April 2006 to March 2007, for electrophoresis, a total of 8 subjects were screened. Out of 8 cases, 2 (25%) were diagnosed as homozygous sickle cell disease, 3 (37.5%) sickle cell traits, and 3 (37.5%) cases were found normal. Of the 8 cases, 3 (37.5%) and 5 (62.5%) respectively, belonged to general castes and scheduled castes. Genetic/marriage counselings were given to affected families.

In other series, 310 cases were referred to this Centre from different medical colleges & hospitals of the state for diagnosis of type ofanemia, , hemoglobinopathies, G6PD deficiency . Hematological analysis revealed that 70.3%(n=218) had hemoglobinopathies which showed distribution of 15.4% cases(n=48) β-thal minor, 15.4% cases(n=48) β-thal major, 9.6%cases(n=30) HbE- β-thal , 6.7% cases(n=21) HbS-β-thal, 8.0% cases(n=25) sickle cell trait(AS), 14.8%(n=46) sickle cell disease (SS) . Besides about 13.5% cases (n=42) showed G6PD deficiency disorders but about 16.1% cases (n=50) were found to be negative for hereditary haematological disorders.

3.4 Investigation on Chikungunya Out Break.

Investigating Team :

Dr. B. Dwibedi, Dr. A. S. Kerketta,
Dr. E. V. Rao, Dr. N. Mohapatra,
Dr. R. K. Hazra, Dr. M. K. Beuria
Dr. S. K. Parida, K. Dhal, T. Maharana,
R. N. Nayak, N. S. Marai,
C. S. Tripathy, B. Pradhan,
H. K. Tripathy, S. S. Beuria

Introduction:

Chikungunya outbreak was reported in October 2006 affecting many parts of the state. It has created a great public health problem posing difficulty both in diagnosis & treatment of the cases. The doctors of the locality having no prior experience of handling such a disease in the past have also faced much difficulty in managing such cases. Upon request from the state health department the centre undertook clinico-epidemiological as well as entomological investigation to support the health authorities in managing the cases and preventing the spread. One such outbreak investigation is detailed below.

Outbreak period, Site and Population:

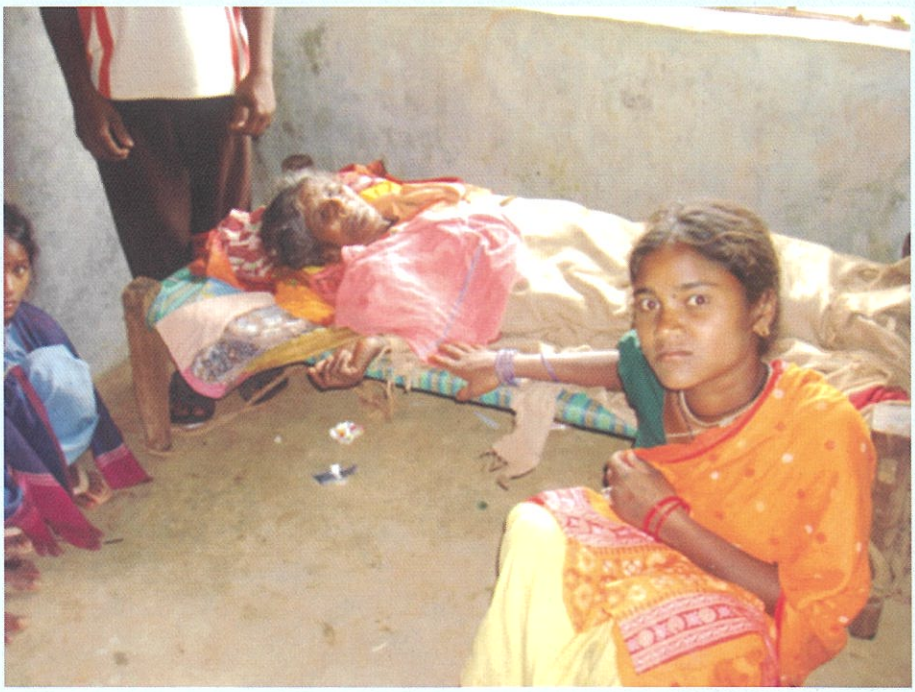
A large no of population from three villages of Cuttack district and one village of Kendrapada district were affected with the common symptom of sudden onset of fever with Joint pain &/or swelling in the months of September & October 2006 during post monsoon period. The period was post monsoon with the paddy crops growing and jute harvesting started.

The affected village Badkul, of Kendrapada district is situated on a delta formed by branches of river Mahanadi in east coast of Bay of Bengal. Total population in the affected area was 2495 covering 422 households. The affected 3 villages of Cuttack district were Bajpura, Meripur & Dobandhia which are contiguous villages narrowly separated by rivulets/river branches.

Field camp during chikungunya outbreak

Materials & method:

The outbreak was investigated by a team comprising of epidemiologist, clinicians, entomologists, laboratory technicians, census takers & insect collectors. Population census of the villages was made by house to house visit & suspected cases of Chikungunya (CHIK) fever were enlisted. A suspect case was defined as an individual suffering/suffered from either of the symptoms of fever, joint pain/ swelling, rash, conjunctival congestion during the affected period September-October-2006. Individual respondents were interviewed and examined clinically. Data on socio-demography, history of illnesses & physical signs (general & systemic) were recorded in a pre-designed format. Symptomatic individuals were provided treatment at the doorstep. Blood samples were collected from the individuals for serological investigation.



Field study : Woman bedridden with high fever and excruciating joint pain

Sera were tested for CHIK & Dengue IgM antibody using IgM antibody capture ELISA method (NIV, Pune - ICMR Kit) from suspected cases. Presence of IgM antibody provided evidence of recent infection.

Entomological Survey:

Entomological survey was done for finding out the vector breeding, adult density, type of breeding places in all the households of the affected villages. Adults were collected in the morning from 11AM to 2PM from human dwelling, cattle shed and outdoor collection. The adults were brought to laboratory and identified. Almost all the containers having water nearby each household were surveyed for larval presence by using dip method or by pasteurette pipette if it is a small container. The larvae and pupae brought to the laboratory were allowed to hatch inside cages and after the adult emergence the species of those mosquitoes were identified and recorded.

Results

Clinico-epidemiological survey:

Sudden onset of cases with fever & joint pain were reported from 2nd week of September in both the areas of Kendrapada & Cuttack District.

Epidemiological Parameters	Kendrapada	Cuttack			
	Badkul	Meripur	Dobandhia	Bajpura	Total
No of household at risk	422	91	96	113	300
Total population at risk	2495	511	617	704	1832
No of clinical cases752	123	168	246	537	
% of people affected (Clinical case)	30.14	24.07	27.22	34.94	29.31

Overall case prevalence rate was 30.14% in Kendrapada and 29.31% in Cuttack. All the ages & both sexes were affected during the epidemic and there was no predilection for any age group or gender.

Fever of acute onset or joint pain was the initial symptoms to present with. Common complains noted were Fatigue (91.98%), Fever (89.18%), Headache (74.44%), Head reeling (79.10%), Joint pain with/without swelling (63.81%), Maculopapular rash (33.96%), and conjunctival ingestion (21.46%), Haemorrhage manifestation (3.36%) was unusual. Anorexia, Nausea and pain abdomen were the other associated minor symptoms. Majority of the symptoms subsided within 3-5 days except joint pain and swelling which was persisting beyond a week. All the individuals were successfully treated symptomatically with Paracetamol & Cetrizine etc. No mortality was reported from the affected areas during the outbreak.

Serological investigation

The sample collected (n=217) were tested for Chik and Dengue by ELISA. Overall sero-positivity for Chik IgM was 63% and 40% for Kendrapada and Cuttack sites respectively. The samples were negative for Dengue IgM.

Entomological investigation

The results of the mosquito fauna of Kendrapada and Mahanga district are given in table 1.

Adult mosquito density (PMH) of affected village

Sl. no	Name of species	District-Kendrapada (Dahimaccha)	District-Cuttack		
		Village- Badkul	Village-Bajapura	Village-Dobandhia	Village-Kurala*
1	Ae.albopictus	1.6	——	1.0-	-
2	Ae.aegypti	0.2	——		
3	Ae.vittatus	0.2	——	-	-

* - Village reported of a large number of cases with fever during the same period; was investigated, but clinical symptoms & signs were in favour of common cold & Acute Respiratory Infection (ARI). Serological tests were negative for Chik IgM.

Conclusion:

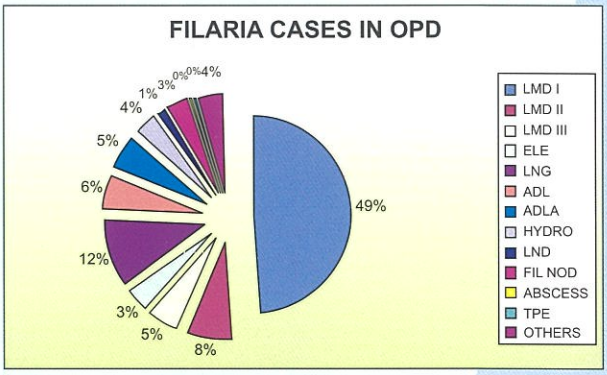
The epidemic was confirmed to be due to Chikungunya virus evidenced by sero positivity to Chik specific IgM antibody. The spread of infection was supported by presence of the Aedes vector in the affected areas. The rapid spread affecting a large number of people from all the ages & both sexes indicate absence of previous immunity to the virus. It creates a possibility of transmission of the disease to newer areas where the vectoral density is suitable for the same. Hence public health measures need to be initiated and doctors in the region be appraised about the clinical pattern & management for early diagnosis out of suspicion, effective management & arresting spread to other areas.

3.5 Service Rendered at Activity In Filaria OPD, Capital Hospital, BBSR

Investigators :
Dr. A. S. Kerketta
Dr. B. Dwivedi
Dr. L. Ho, Mr. R. K. Das
Mr. K. Dhal

During the reporting year a total of 1120 filariasis patient attended to the filaria Out patient department, at Capital Hospital. Of which 711(63.5 %) cases reported for follow-up. Of which 407 (57.2%) cases were male and 304(42.8%) were female. The rest 409 (36.5%) new cases reported with different clinical presentations. Out of total new cases attended 218(53.3%) were male and 191(46.7) were female. The commonest clinical presentation encountered was filarial lymphoedema of different grades, which was marked in 250 (64.5%) cases. The lymphoedema grade- I was found in 198 (48.4%) patients and was marked more among the patients of age group 15-30 and 31-64. The lymphoedema Grade- II found in 32(7.8), Grade III in 20 (4.9) and elephantiasis in 14(3.4%) of the cases. was marked more among the male patients of age group 31-64 years. The Lymphangitis was reported by 10.8% and was marked more among the female patients. Cases with acute attack found in around 11% of patient. Of which acute Adenolymphangitis (ADL) that is patients having acute attack with fever was found in 5.9% and Adeno Dermatolymphangioadenitis (ADLA) was in 5.4% of cases and was more prevalent among the patient aged above 30 years. A total of 16(4.0 %) male patients reported with Hydrocele or orchitis. The other symptoms like inguinal lymphadenitis, filarial nodule other than inguinal lymph node, arthritis, myalgia, abscess, urticaria and Tropical pulmonary eosinophilia found in 1.2%, 3.4%, 1.7%, 2.0 %, 0.5, 0.5, and 0.5% of patients respectively. All the lymphoedema patients were given advise on proper foot care management, limb elevation and bandaging. Only 25 cases were given pneumatic decompression therapy for oedema size reduction.

Inaugatation of OPD Extention service at Capital Hospital, BBSR



The Details of the clinical conditions of the OPD cases

Diagnosis*	Number /(%)
LMD I	198 (48.4)
LMD II	32 (7.8)
LMD III	20 (4.9)
ELE	14 (3.4)
LNG	44 (10.7)
ADL	24 (5.9)
ADLA	22 (5.4)
HYDRO	16 (4.0)
LND	5(1.2)
FIL NOD	14 (3.4)
ABSCESS	2(0.5)
TPE	2(0.5)
OTHERS	16(3.9)

* LMD: Lymphoedema grade I, LMD II: lymphoedema grade II, LMD III: Lymphoedema grade III, ELE: Elephantiasis, LNG: Lymphangitis, ADL: Adenolymphangitis, ADLA: Adenodermato lymphangio adenitis, Hydro: Hydrocele, LND : Lymphadenitis, Fil Nod: Filaria nodule, TPE: Tropical pulmonary eosinophila

3.6 Outbreak of cholera in Panasa Patna village

Investigators :
Dr. B. B. Pal
Dr. G. P. Chhotray
Dr. H. K. Khuntia
Mr. S. K. Samal

Sixty eight people were affected in the month of September 2006 due to severe diarrhoea. The etiological agent was *V. cholerae* O1 biotype El Tor and serotype Inaba and all were positive for *ctx* A and *tcp* A genes. The antibiotic sensitivity pattern of *V. cholerae* O1 Ogawa was Fr Co Na and O1 Inaba was Fr Co S Cf Na Nx N respectively, where as the resistance profile of O139 sero group was Co Na respectively. All the *V. cholerae* O1 Ogawa and Inaba serogroups were sensitive to A, Co, T, G and C. This time the Inaba serotypes were showing dominance over Ogawa serotype.

Development of quadriplex PCR assay:-

A quadriplex PCR assay was developed in a single tube reaction for simultaneous detection of serotype (O1 and/or O139), biotype (Classical or El tor), toxigenic potential and top regulating factor of *V. cholerae* (Fig 1A and 1B).

Multidrug resistance *V. cholerae* isolated from clinical diarrhoeal samples between 1995-2005 in Orissa:-

Antimicrobial susceptibility pattern of *V. cholerae* strains isolated from hospitalized diarrhoea patients and outbreaks areas of different parts of Orissa, India for 10 years were analyzed to determine the changing trends. 700 *V. cholerae* strains isolated during 1995 to 2005 were retrospectively analyzed. It includes 615 *V. cholerae* O1, 79 O139 strains and 6 non O1 non O139 strains. The *V. cholerae* O1 strains showed uniform resistance to co-trimoxazole (Co), furazolidone (Fr) and nalidixic acid (Na) all throughout the decade except 2005, where there is a marked decrease in resistance to Co. Ampicillin (A) was showing about 100% resistance during 1999, 2001 and 2003. The *V. cholerae* O139 strains exhibited 100% resistance to A, Fr and Streptomycin (S) during 1999 and 2001. The common pattern of antibiogram profiles of *V. cholerae* O1 and O139 strains were Fr Co Na and Fr respectively observed throughout the study period. Most of the serogroups of *V. cholerae* O1 and O139 strains exhibited low percentage of resistance to tetracycline (T), ciprofloxacin (Cf) and gentamicin (G). Long term surveillance programme are warranted to identify the changes in antimicrobial resistance pattern of *V. cholerae* strains in this region.

PCR Assay on *E. coli*:

Stool samples collected from the Sishu Bhaban, Cuttack; Capital hospital, Bhubaneswar and Infectious diseases hospital, Puri during 2006, through the active surveillance programme were screened for the detection of diarrhoeagenic E. coli by PCR technique. Of the 580 stool samples 162 (28%) was found culture positive for E. coli. Using specific primers, we detected 7% ETEC, 6% EPEC and 5% EAggEC (Fig 2 and 3) corresponding to the detection of virulent genes as elt, eae and ast respectively. The common clinical features of the patients were watery diarrhoea associated with vomiting,

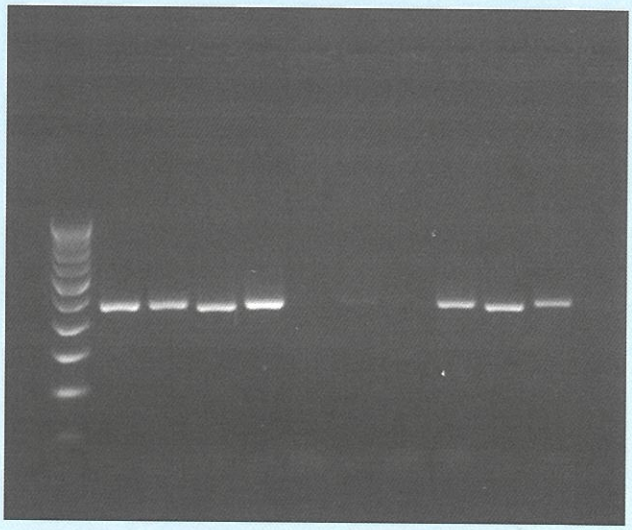


Fig 2:.Figure showing *elt* and *eae* genes of *E. coli* isolated from diarrhoea patients. From (Left to Right)

Lane 1: 100 bp ladder, 2-5: *elt* gene (450 bp) of ETEC, 9-11: *eae* gene (454 bp) of EPEC.

abdominal cramping and nausea etc. These strains were resistance to many common antibiotics prescribed for diarrhoea including nalidixic acid, furazolidone and co-trimoxazole.

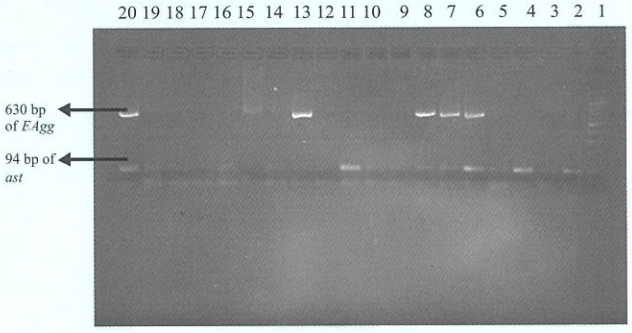


Fig 1A and 1B. Quadriplex and simplex PCR assay of different toxic genes (*tox R*, *tcp A El Tor*, *tcp A* Classical, *rfb* O139, *rfb* O1 and *ctx A*) of *V. cholerae* O1 and O139 isolates isolated from hospitalized diarrhoea patients.

Lane M: 100 bp ladder, 1-9: Quadriplex PCR of different toxic genes, 10-14: Simplex PCR of different toxic genes.

Fig-1A

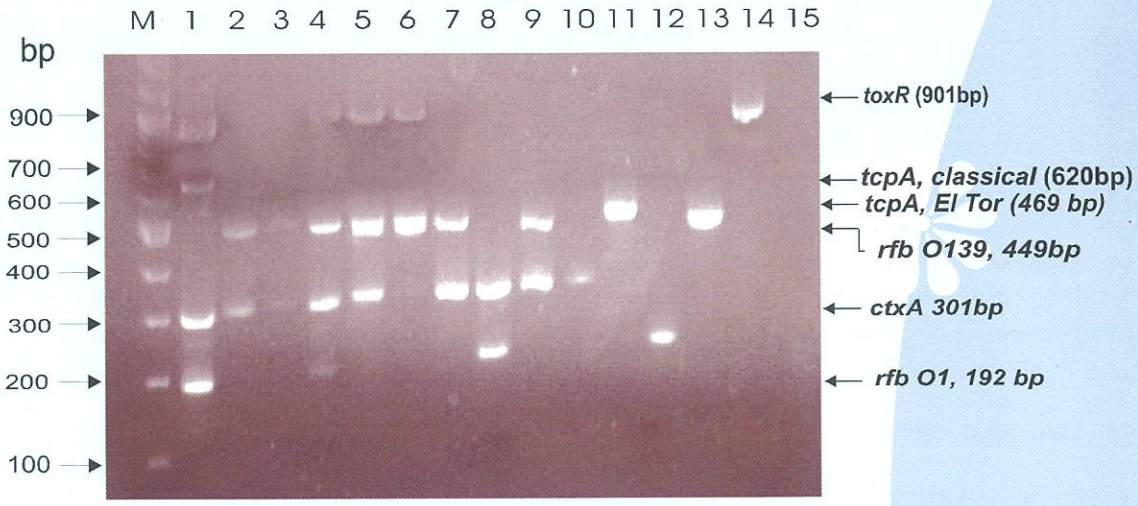
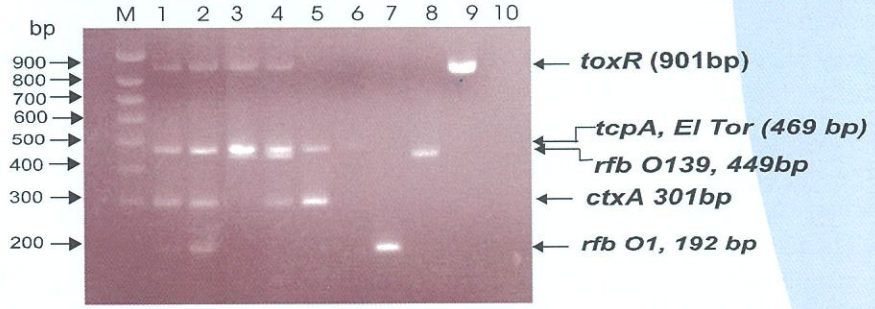


Fig-1B



4

General Information and Publication

General Information and Publications

4.1 Publication

Publications- 2006

1. Babu BV, Behera D K, Kerketta A S, Mishra S, Rath K, Swain BK, Mishra S and Kar S K. Inclusive partnership strategy to increase compliance of mass drug administration during the programme to eliminate lymphatic filariasis in urban areas of India. *Annals of Tropical Medicine and Parasitology*.2006, 100(7): 621-630.

2. Babu BV, Nayak AN, Rath K, Kerketta AS. Use of the Dermatology Life Quality Index in filarial lymphoedema patients. *Trans R Soc Trop Med Hyg*. 2006, 100(3): 258-63.

3. Babu BV, Rath K, Kerketa A S, Swain B. K, Mishra S and Kar S.K. Adverse reactions following mass drug administration during the programme to eliminate lymphatic Filariasis in Orissa state, India. *Trans R Soc Trop Med Hyg*. 2006, 100: 464-469.

4. Babu BV, Swain BK and Rath K. Impact of chronic lymphatic filariasis on quantity and quality of productive work among weavers in an endemic village from India. *Trop Med Int Health*. 2006; 11(5): 712-7.

5. Mahapatra N, Marai NS, Ranjit MR, Parida SK, Hansdah DP, Hazra RK, Kar SK. Detection of plasmodium falciparum infection in anopheles mosquitoes from Keonjhar district, Orissa, India.*J Vector Borne Dis*. 2006, 43(4):191-194.

6. Mand S, Supali T, Djuardi J, Kar SK , Ravindran B & Hoerauf A . Detection of adult Brugia malayi filariae by ultrasonography in humans in India and Indonesia. *Tropical Medicine and International Health*. 2006; 11(9):1375-81.

7. Mishra S, Raj DK, Hazra RK, Dash AP, Supakar PC. An efficient PCR—SSCP-based method for detection of a chloroquine resistance marker in the PfCRT gene of Plasmodium falciparum. *Trans R Soc Trop Med Hyg*. 2006 ,100(3):243-7.

8. Pal BB, Khuntia HK, Samal SK, Das SS and Chhotray GP. Emergence of Vibrio cholerae O1 biotype El Tor serotype Inaba causing outbreaks of cholera in Orissa, India. *Jpn. J. Infect. Dis*..2006, 59: 264-266.

9. Parida S K. Hazra RK Marai NS .Triphathy HK and Mohapatra N. Host Feeding patterns of Malaria vectors of Orissa, India. *J Am Mosq. Cont. Association*. 2006, 22(4):629-34

10. Rath K, Nath N, Mishra Shaloumy, Swain BK, Mishra Suchismita and Babu B V. Knowledge and perception about lymphatic Filariasis: a study during programme to eliminate lymphatic filariasis in an urban community of Orissa, India. *Tropical Biomedicine*. 2006, 23(2): 156-162.

11. Satapathy AK, Sartono E, Sahoo P. K, Dentener M.A, Michael E, Yazdanbakhsh M, Ravindran B. Human bancroftian filariasis: Immunological markers of morbidity and infection. *Microbes and Infection*. 2006, 8:2414-2423.

12. Swain BK and Mishra S. Immunization coverage among migrant tribal children inhabiting slums of Bhubaneswar city, Orissa, India. *Indian Paediatrics*. 2006; 43 (11): 1011-1013.

13. Balgir RS. Do tribal community show reverse relation between sickle cell disorder and G6PD in malaria endemic areas of central eastern India. *Homo*. 2006;57(2)163-176.

Publications- 2007

1. Babu B.V., Mishra, S., Swain, B.K. Personal-protection measures against mosquitoes: a study of practices and costs in a district, in the Indian state of Orissa, where malaria and lymphatic filariasis are co-endemic .*Annals Trop Med Parositol*. 2007; 101 (7):. 601-609.

2. Balgir RS. Infant mortality and reproductive wastage associated with different genotypes of haemoglobinopathies in Orissa, India. *Ann Hum Biol*. 2007 ;34(1):16-25.

3. Balgir RS. Genetic burden of red cell enzyme glucose 6 phosphate dehydrogenase deficiency in two major scheduled tribes of Sundergarh district, North western Orissa, India. *Current Science*. 2007; 92 (6): 768- 773.

4. Mishra K, Raj DK, Hazra RK, Dash AP, Supakar PC. The development and evaluation of a single step multiplex PCR method for simultaneous detection of Brugia malayi and Wuchereria bancrofti. *Mol Cell Probes*. 2007; 21(5-6):355-62.

5. Mohanty A, Kar P, Mishra K, Singh DV, Mohapatra N, Kar SK, Dash A P and Hazra R K. Multiplex PCR assay for the detection of anopheles fluviatilis species complex, Human host preference and Plasmodium Falciparum sporozoite presence, using a unique mosquito processing method. *Am J Trop Med and Hygiene*. 2007; 76(5) : 837-843.

6. Rath K, Nayak AN and Babu BV. Community's knowledge and perceptions about filarial elephantiasis and hydrocele in coastal Orissa, India. *Asia Pacific Journal of Public Health*. 2007; 19(1): 28-33.

7. Sethy PGS, Bulliyya G, Mallick G, Swain BK, Kar SK. Prevalence of goitre and iodine deficiency among school children in urban slums of Bhubaneswar, Orissa. *Indian Journal of Pediatrics*. 2007 ; 74(10): 917-921.

8. Bulliyya G, Mallick G, Sethy GS, Kar SK. Haemoglobin status of non-school going adolescent girls in three districts of Orissa, India. *International Journal of Adolescent Medicine and Health* . 2007; 19(4): 395-406.

In Press

1. Babu BV, Nayak A N and Rath K. Utilisation of primary healthcare services: experiences and perceptions of rural community of East Godavari district, South India. *Indian Journal of Social Work*. (In Press).

2. Kerketa A.S. Assesment of therapeutic efficacy of chloroquine in the treatment of uncomplicated malaria in a tribal block of Kalahandi district of Orissa, India. *Tropical Doctor*. (In press)

Published in Book Chapter:

1. Bulliyya G. Environment and health status of primitive Paudi Bhuiyan tribe in northeastern part of Orissa. In: P. Dash Sharma (Edition ISBN:81-8387-007-4), Anthropology of Primitive Tribes of India. Serial Publications, New Delhi. 2006; 336-368.

2. Bulliyya G. Nayak J.K, G.Mallick G. Indigenous health care practices among the Kondh tribal communities of Orissa, India. In: Indigenous Health care and Ethno-Medicine. Swarup & Sons Publisher, New Delhi. 2006; 54-73. (ISBN 81-7625-725-9).

3. Bulliyya G. Public policies and legislations on land and forest rights of the tribals in Orissa: an overview. In: R.M.Sarkar Edition. Land and Forest Rights of the Tribals Today. Serial Publications, New Delhi. 2006. (ISBN 81-8387-059-7)

4.2 Meeting & Seminar organized

1. Regional Medical Research Centre, Bhubaneswar organized a one-day symposium on " **Morbidity management on Filariasis**" on 18th May 2006 under chairmanship of Dr. S.P. Tripathy, Ex- D.G , ICMR, New Delhi participated by experts from various organisations. Dr. P.K.Senapaty, DHS, Govt. of Orissa, Prof. S.K. Acharya, HOD, Dept of Gastro-Enterology, AIIMS, New Delhi, Dr. P.K.Das, Director, VCRC, Pondichery, Dr. A.P.Dash, Director, NIMR, New Delhi, Dr.V.Kumarswamy, TRC, Chennai, Dr. A.C.Dey, Joint Director, Malaria & Filaria, Govt. of Orissa, Dr. S.K.Mohapatra, Municipal Hospital, Bhubaneswar, Dr. B. N Mohapatra, SCB Medical College, Cuttack, Dr. A. Sahoo, ADMO, Khurda and Dr. Suprabha Das, DD, DHS, Bhubaneswr are among those who attended the Symposium. Dr. S.K.Kar, Director , RMRC Bhubaneswar presented the role of RMRC in filariasis morbidity management in this region. All the scientists and researchers are also attended the symposium.

2. A **Symposium on AIDS** and Protocol Development meeting was organized on 26th March 2007 at RMRC, Bhuaneswar.The meeting was organised at RMRC , Bhubaneswar to discuss on the issues related to priority areas of Research in the HIV/ AIDS in presence of Authorities/ representative from Orissa State AIDS Control Society, NARI, Pune, three Medical Colleges of the State, Anti Retroviral Therapy Centres/ HIV Diagnostic Centre of the State and Scientists from RMRC, Bhubaneswar. Two major areas were identified i.e (a) CD4, CD8 count and response to antiretroviral therapy in identified HIV patients and apportunistic infection preveling in the AIDS patients of the region (b) Socio behavioural assesment in different at risk population and treatment sicking behaviour in the HIV patients.

3. The **project review committee** meeting was held from 16th - 17th May 2006 to discuss the on-going projects and new proposals undertaken by scientists of the Centre. Dr. S.Pattnayak, WHO expert on Malaria, New Delhi chaired the meeting . Various expers from outside participated the review meeting. All the project proposals of RMRC scientists were discussed and interim recommendation made.

4. **Research co-ordination Committee** meeting was organized by RMRC, Bhubaneswar on 29th May 2006 under the chairmanship of Principal Secretary and Commissioner, Health , Govt. of Orissa to discuss the research need this region that can guide the area of research to be taken up by RMRC. Principals/Professors from three medical colleges, Director Medical Education and Training, Director, Health Services, Govt. of Orissa, and other experts like Dr. S.Pattnaik, Dr. B.B.Tripathy, Director, Tribal Welfare and experts from Nutrition Bureau have participated the coordination committee meeting. Dr. S.K.Kar, Director of the Centre presented the activities of the Centre and the committee discussed on the important research issues related to the need of the state and assured collaboration ,support and recommendations.

5. The **10th National Annual Conference of Lymphology Society of India** *(LymphoCon-10)* was organised by the Centre from 15-16 Dec. 2006 at NALCO auditorium, Bhuabneswar. The Conference was inaugurated by the Honorable Chief Minister of Orissa Shri Naveen Patnaik on 15th Dec 2006. The Honourable minister for Tourism and Excise, Shri Debi Prasad Mishra was the Guest of Honour on

Workshop at RMRC, on MDA against Filariasis.

the occasion. Prof. S. Jamal, President Lymphology Society of India delivered the presidential address and Dr. S.K.Kar, Director RMRC, Bhuaneswar and President Elect for Lymphocon-10 delivered the welcome Address.

On 15th the entire day was devoted to different scientific Presentations no Lymphatic filariasis. These topics were also well discussed in the hall during the question-answer session. The CME on MDA & Morbidity management was undertaken in the afternoon session on 15th Dec. 2006. On 16th Decmber the morning session was devoted to Scientific presentation and afternoon session was on live demonstration and interactive surgery on 'nodo-venus-stunt' which was at Kalinga Hospital, Bhubaneswar. with the Consulting Surgeon Dr. Manokaran of Apollo Hospital, Chennai and Prof. S. Jamal. The conference discussed on various approaches of morbidity management of Filariasis and surgical approach were demonstrated in three grade IV lymphoedema for imparting training to surgens of this region . Several physicians , serguns, plastic surgens , Ayurvedic doctors, scientists participated in the program.of this region

6. **20th SAC Meeting:** The 20th Scientific Advisory Committee (SAC) Meeting of Regional Medical Research Centre for the year 2005-06 was held during 25-26th September, 2006. Dr. Sandip K. Basu was the Chairman of 20th SAC.

7. **Inauguration of New Facility:** An auditorium, Scholars Hostel, Guest House and Experimental Animal House were inaugurated by Prof. N.K.Ganguly, Director General, Indian Council of Medical Research on 16th December 2006 in a colourful function in RMRC Campus. Shri Mihir Kumar Mohany, Mayor of the Bhubaneswar Municipal Corporation was the chief guest and Prof. Trilochan Pradhan , ex- Vice chancellor, Utkal University was the guest of Honour in the inaugural ceremony. Among the other dignitaries, Dr. S.P.Tripathy, Ex- D.G, ICMR, Prof. L.N. Mohapatra, Ex- Director, RMRC, Dr. S. Patnaik, WHO consultant, Bhubaneswar, Dr. A. P. Dash, Director, Malaria Research Centre, New Delhi, and Financial Advisor to ICMR Mr. P.K.Seth and many more.

8. A meeting was organized for establishment of Virology Laboratory at RMRC, Bhubaneswar on 20th June 2007 at RMRC, Bhuaneswar. A team of scientists comprising Dr. U.C.Chaturvedi and Dr. D.A.Gadkiri from National Institute of Virology (NIV, Pune) have visited RMRC for setting up diagnostic Laboratory for Viral diseases at RMRC , Bhuabneswar.

4.3 Seminar/ workshop attended

1. Dr S.K.Kar, participated Multicentric drug trial meeting to study the effect of Daflon in treatment of Lymphatic Filariasis at ICMR Hqrs on 4th April 2006.

2. Dr S.K. Kar participated as Guest Speaker in the National seminar on New Vistas and practical adaptability of Para-surgical methods of Ayurveda at Institution of Engineers Auditorium and delivered a talk on "Morbidity Management in filariasis and need for surgical interveion" organized by Central Research Institute (Ayurveda) on 22nd April 2006.

3. Dr S.K.Kar, participated World Veterinary Day at Orissa Veterinary Council as Chief Speaker and delivered talk on "Role of animals in human diseases" at 5 pm on 29th April 2006.

4. Dr S.K.Kar, participated Investigators Meeting on "Viral Hepatitis" at NIV, Pune on 14th July 2006.

5. Dr S.K.Kar, delivered talk on "Research issues in vector Borne diseases to participants of Professional Development Course attendees of MOS from various Eastern States organized by State Institute of Health & Family Welfare at RMRC Seminar Hall on 21st July 2006.

6. Dr.R.S.Balgir, participated as a Member in the Annual Meeting of Board of Studies in Human Genetics, Andhra University, Waltair held on 18th July 2006 at Visakhapatnam.

7. Dr S.K.Kar, participated 5th Professional Developmental Course at SIH & FW as Guest Speaker and delivered a talk on "Epidemiology of malaria in Orissa – Current Perspective" on 4th Aug. 2006 at 10.30 am.

8. Dr S.K.Kar, attended Hindi Pakwada as a Guest Speaker at Water Technology as Chief Guest on 14th Sept. 2006 & delivered a talk on "Importance of Hindi in Scientific Activities".

9. Dr. N. Mohapatra invited as guest faculty to give lecture on malaria and its transmission at Zoology Department of Utkal University during August 2006.

10. Dr S.K.Kar, attended 2nd Governing Body Meeting of API at SCB Medical College, Cuttack on 17th Sept. 2006.

11. Dr S.K.Kar, attended meeting on use of Traditional remedies in Filariasis on 20th Sept. 2006 at 10 AM in Conference Hall of ICMR.

12. Dr.G.P.Chhotray Attended XXXIV annual conference of IAPM(Orissa Chapter) at V.S.S. Medical college, Burla on 9th Sept 2006 .

13. Dr. N. Mohapatra invited as guest faculty to give talk on mosquito borne diseases and its control at Zoology Department of Utkal University during September 2006.

14. Dr S.K. Kar, attended State Level Workshop on MDA in filariasis and delivered talk on "Clinical management and side reaction of DEC in MDA" at Hotel Marion on 4th Oct. 2006.

15. Dr S.K.Kar, attended SAC Meeting at VCRC, Pondicherry on 11th & 12th Oct. 2006.

16. Dr S.K.Kar, attended International Symposium (Trend in Genomic & Proteomic Sciences) at NIRRH, Mumbai on 15th - 17th Oct. 2006.

17. Dr. AK Satapathy attended DBT sponsored workshop on Joint Disease Biology on 14th Oct. 2006 held at Sanjay Gandhi Institute of Medical Sciences, Lucknow.

18. Dr.G.P.Chhotray invited to attend National Symposium on Tribal Health held at Jabalpur on 19th &20th Oct 2006 and delivered a guest lecture on " A Comprehensive health status of some primitive tribes of Orissa " .

19. Dr.B.B.Pal Attended the 30th annual conference of IMM held at Nagpur during October 2006 and presented a paper entitled "Switching over of Ogawa to Inaba Serotypes of Vibrio cholerae causing outbreaks of cholera in Orissa, India".

20. Mr.P.K.Jangid participated and presented a paper entitled "Tribal Health: Perspective issues" in National Symposium on Tribal Health held at Regional Medical Research Centre for Tribals (ICMR), Jabalpur, (MP) during 19-20 Oct'2006.

21. Dr. G. Bulliyya attended'National Symposium on Tribal Health' held at Regional Medical Research Centre for Tribals, Jabalpur on October 19-20, 2006, and presented a paper on 'Iodine nutrition in school-age children and community knowledge regarding iodine deficiency disorders in Dongria Kondh primitive tribal group of Rayagada district"

22. Dr. R.S.Balgir, presented a paper Entitled "Epidemiology, Population Genetics and Phenotypic Diversity of Sickle Cell Disease in India" in the Indo-US Symposium on "Genetic Disorders: Focus on Hemoglobinopathies" held during 29-31st October 2006 at Banaras Hindu University, Varanasi.

23. Dr.G.P.Chhotray Attended ISHTM, 47th National Annual Conference of Indian Society of Hematology & Transfusion medicine held at Gauwahti from 24-26th November 2006and presented a scientific paper " Nutritional anemia & Haemoglobinopathies amongst some primitive tribes of Orissa ".

24. Dr. G. Bulliyya attended 'XXXVIII Annual Conference of the Nutrition Society of India' held at All India Institute of Hygiene and Public Health, Directorate General of Health Services, Kolkata during 4-6, November 2006.

25. Dr. R.S.Balgir, participated by presenting a paper entitled "Monozygotic Twin Pair with Carrier Status of beta-thalassemia Major in a Dudh Kharia Tribal Family of Orissa" in 10th Orissa Bigyan Congress held during November 4-5, 2006, Bhubaneswar.

26. Dr. R.S.Balgir, as invited speaker and presented a paper entitled "Health Scenario of Major Tribals of Orissa in Relation to Human Growth, Development and Nutrition" in the UGC-SAP National Conference on "Biosocial Aspects of Human Growth, Development and Nutrition" held during 20-21st November 2006 at the Department of Anthropology, Panjab University, Chandigarh.

27. Dr. R.S.Balgir, presented a paper entitled "Disease Burden and Health Scenario of Tribes in Crisis: An Orissa Plight" in the National Seminar on "Tribes in Crises: Bio-Cultural Perspective", held during 28-30th November 2006, School of studies in Anthropology, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh.

28. Dr S.K.Kar, attended 20th Annual Function of Rajdhani College, Bhubaneswar as Chief Guest on 20th December 2006 & delivered a talk on Women's Health.

29. Dr. A K Satapathy presented a paper entitled "Genetic polymorphism of TLR-4 genes and association with chronic disease manifestation in Bancroftian filariasis" in the 10th Annual conference of the Lymphology Society of India held at Bhubaneswar from 15th –16th Dec. 2006.

30. Dr. Mrs. AS Kerketa attended National conference on Lymphatic filariasis "Lymphocon-10" at Bhubaneswar from December 15-16th, 2006.

31. Mr.P.K.Jangid participated and presented a paper entitled 'Chronic diseases: An Urgent Need for Action' during 24th Annual National Conference of Indian Society for Medical Statistics held at PSG Institute of Medical Sciences & Research, Coimbatore during 1-3 Dec,2006.

32. Mr.P.K.Jangid participated and communicated a paper entitled 'Statistical Quality Control' in the 10th Annual Conference of Lymphology Society of India held at NALCO Auditorium, Bhubaneswar during 15-16 Dec'2006.

33. Dr S.K.Kar, participated in the review of malaria activities in State in the Hon'ble Chief Minister's Conference Hall on 20th January 2007.

34. M.K.Beuria, M.S.Bal, N, N, Mandal and M.K.Das. Filarial antigenemia in young children living in areas endemic for bancroftian filariasis in Orissa, India. 33rd Annual Conference of Indian Immunology Society, 28-31, January 2007, New Delhi, India.

35. Dr. N. N. Mandal attended the 32nd workshop on Parasite Immunology and Immunogenetics at Mahaboloswar from 7-11 Jan 2007.

36. Dr S.K.Kar, attended International Conference on Emerging Trends in Haematology and Immunohaematology at IIH, Mumbai on 31-1st February 2007.

37. Dr. R.S.Balgir, was an invited speaker and presented a paper "Aberrant Heterosis in Hemoglobinopathies with Special Reference to beta-Thalassemia and Structurally Abnormal Hemoglobins E and S in Orissa" in the National Seminar on "Anthropology and Society: Issues and Applications" held during February 1-2, 2007 at Panjab University, Chandigarh.

38. Dr. R.S.Balgir, presentsed a paper entitled Genetic Burden of Red Cell Enzyme Glucose-6-Phosphate Dehydrogenase in Two Major Scheduled Tribes of Sundargarh District in Northwestern Orissa, India" in the 32nd Annual Conference of Indian Society of Human Genetics (ISHG) and International Symposium on "Deconstructing Diseases: The Genomic Advantage" held during 14-16th February 2007 at Kolkata.

39. Dr S.K.Kar, attended sensitization workshop on vector Borne Disease Control Programme in urban area at Hotel Pushpak on 19/3/2007 and delivered talk on MDA - Challenges.

40. Dr S.K.Kar, participated in the National Dissemination Workshop on Health Sector Policy Reform Options Database (HS-PROD) of NIHFW, New Delhi on 23rd March 2007.

41. Dr.G.P.Chhotray Attended National Seminar on Social Disparity in Development : Issues & Challenges of Tribal Development in Orissa on 29th & 30th March 2007 at Hotel Crown ,Bhubaneswar & delivered a lecture on " A comprehensive health status & carrying out intervention programme to augment health care delivery in some primitive tribes of Orissa ".

4.4 Events

Ethical Committee Meeting:

The Animal Ethical Committee meeting was held on 4th August 2006 in the RMRC Seminar Hall under the chairmanship of Dr. S.K.Ray and Dr. S.K.Kar, Director, RMRC, was the convener of the meeting. The committee discussed various issues of the ongoing projects for ethical clearance. Mr N.R. Mansingh, (Nominee of the CPCSEA), Dr. M.K Das, DD (SG) and Dr. N. Mohapatra, AD and Experts from other Institutions were also participated the meeting.

Signing of MOU:

RMRC signed a MOU with KIIT University on 24th June 2007 for Collaborative research in the field of Biomedical Research and Biotechnology

National Science Day:

National Science day was observed on 28th February 2007 at RMRC Bhubaneswar. Dr. Kabi Prasad Mishra, eminent Cardiologist and Director, Medical Research, Kalinga Hospital, Bhuabeswar was invited on this occasion. He delivered a talk on *"Stress & Heart: A holistic Approach"* on the eve of National Science Day observation. All the staff, students and scientists were present during the deliberation.

Guest Lectures:

1. Dr. G.B.Nair, Director ,Lab Sciences Division, ICDDRB, Dhaka delivered a talk on Diarrhoeal Disorders on 18th July 2006 at RMRC Seminar Hall.

2. Dr. Kabi Prasad Mishra, Eminent Cardiologist and good orator on Vedas and Purans was invited on 29th March 2007 as guest lecturer.

3. Dr Y,.D.Sharma, Prof. & Head, Dept. of Biotechnology, AIIMS, New Delhi, delivered a lecture on Molecular Epidemiology of drug resistance malaria in India" in the seminar Hall of RMRC on 2/8/2006 at 11 am.

RMRC Foundation Day

26th RMRC Foundation Day was celebrated on 29th March 2007. During the morning session eminent cardiologist Dr. K.P.Mishra and Director, Medical Research, Kalinga Hospital, Bhuaneswar delivered an oration on Veda and Heart Diseases. Competations and cultural program was arranged in evening. The dance, song and drawing competition was held among the children and colorful program was organized in the campus followed by prize giving ceremony.

4.5 Human Resource Development

Ph.D Awarded:

Dr. N.N.Mandal, , R.A awarded Ph. D on "Studies on the Immuno protective potential of detergent soluble and lipid antigens of filarial parasite in Lymphatic Filariasis" under Utkal University, Bhubaneswar in April 2007. He was working under Dr. M.K.Das, Deputy Director(SG), RMRC, Bhubaneswar.

Poster Presentation of Ph.D scholars during SAC meeting.

Ph.D. Scholars:

Presently total 17 Ph.D. scholars are undertaking their research work in various departments of RMRC, Bhubaneswar. Most of the scholoars are with CSIR, UGC or Lady tata fellowship holders.

M.Phil. Programme:

Presently 4 M.Phil students from Utkal University & Sampalpur University are undertaking their dissertation work in various departments of RMRC, Bhubaneswar.

M.Sc. dissertation Programme:

During this period, total 46 M.Sc. (Biotechnology) students have completed their 6 months dissertation work under the guidance of various scientists of the centre on various topics. The summary and topics of the dissertation are being stored in library for other readers for reference.

Summer training

Summer training programme was organized by the centre for B.Sc. (Biotechnology) & M.Sc. (Biotechnology) students from various Organizations & Universities during this period.

Training:

- Biswanath Murmu R.A & Miss. Sujata Dixit, R.A have gone to IIH Mumbai for 10 day training from 25.06.2007 to 04.07.2007 in Thalassemia and Sickle cell Disease. They were imparted training on diagnosis of thalassemia and sickle cell disease by CAM electrophoresis and Bio-Rad variant, DNA isolation by phenol chloroform method, purity of DNA, PCR amplification of DNA and Covalent Reverse Dot Blot (CRDB) technique and learned the diagnosis of G-6PD deficiency by Dichlorophenol-Indophenol dye method and quantitation of G-6PD deficiency.
- Dr.N.N. Mandal, RA, Immunology obtained training at CDRI, Lucknow on Animal feeding of vectors in July 2007.
- Mr.R.N. Nayak & Mr.K. Dhal under took training at IRMS, Delhi on field epidemiology in 2006.
- Mr.T. Moharana had under gone training on Hepatitis at NIV, Pune in 2006-07.

Foreign Visit:

- Dr M.R.Ranjit, Asst. Director has visited Johns Hopkins Bloomberg School of Public Health, Baltimore, MD21205, USA from 1st March 2007 to 31st August 2007 uinder Dr.Nirbhaya Kumar

for obtaining higher studies on Functional Genomics of malaria parasite under ICMR international fellowship programme.

- Dr. A.S.Acharya has undergone WHO Fellowship on "Epidemiology and Biostatistics" at Khonkaen University, Thailand from 1st July to 10th August 2007.

4.6 Facilities

A. Library, Information & Publication

Library & Information Centre of Regional Medical Research Centre, Bhubaneswar houses an exclusive collection of books, foreign and Indian journals, databases, reprints, etc. on various subjects of biomedical sciences. For the calendar year 2007, the library subscribed 34 foreign journals and 37 Indian journals and procured 1500 books.

The Library serves the research needs of the scientists, researchers, students, doctors and academicians of the state. Besides its services are extended to a number of other organizations like Universities, Medical Colleges, CSIR Lab, ICAR Lab, DBT Lab and DAE Lab of the state. The Library has been catering to the needs of the postgraduate research scholars from various Universities/ Institutions of the country who are undertaking their M.Phil/M.Sc. dissertation work under the guidance of our scientists.

The Library resources stand at about 10,000 volumes including books, back-volumes of periodicals, theses, Reports and WHO publications. The Print Journal subscription goes beyond 100. While more than 1500 journals are available in online through ICMR e-journal consortia and NML-ERMD consortia of National Medical Library, DGHS, New Delhi.At present, the library is equipped with Mail-server, Antivirus-server and multiple computers interconnected through LAN with BSNL broadband Internet connectivity.

ICMR e-Consortia:

The following five journals are accessible online through ICMR e- Consortia. *BMJ, Lancet, Nature, NEJM, Science*

NML-ERMED Consortia : In this consortium total 1515 Medical journals are accessible. (www.nmlermed.in) . It can be accessed through any terminal of RMRC LAN system. All journals in this consortia has been activated through RMRC IP address.

Internet : Internet plays vital role in library and information services. The traditional reprint request for scientists through card has been shifted to on-line reprint request. After installation of LAN system in the library and BSNL Broadband network in RMRC library, on-line reprint requests are being done for the scientists and researchers through Internet. The library is also sending our reprints to the requester through

e-mail by scanning the articles of our scientists. Internet facility is available for all ICMR scientists for searching various on-line databases, full text journal articles, and abstracts and subject searches for scientists for presenting papers and preparing scientific talks and seminars. E- mail facility is open for all scientific staff of the centre.

Resourse Sharing

All ICMR librarians have formulated an ICMR Librarians group mail (*icmrlibrarians@yahoogroups.com*) for resource sharing. Any article available in any ICMR library can be obtained and delivered to scientists through this group mail services. This service is being provided to all ICMR scientists as resource sharing among ICMR libraries of the country.

Off- Line MEDLARS

Library possess MEDLINE-CD ROM database from the period 1966-2002. This facility is extended to all library users as off-line MEDLARS services. The researchers and scientists and doctors from various medical colleges are making use of this facility. This facility is provided free of cost to all users. This MEDLINE database carries abstracts of more than 6000 Biomedical journals covered in Index Medicus.

Photocopying

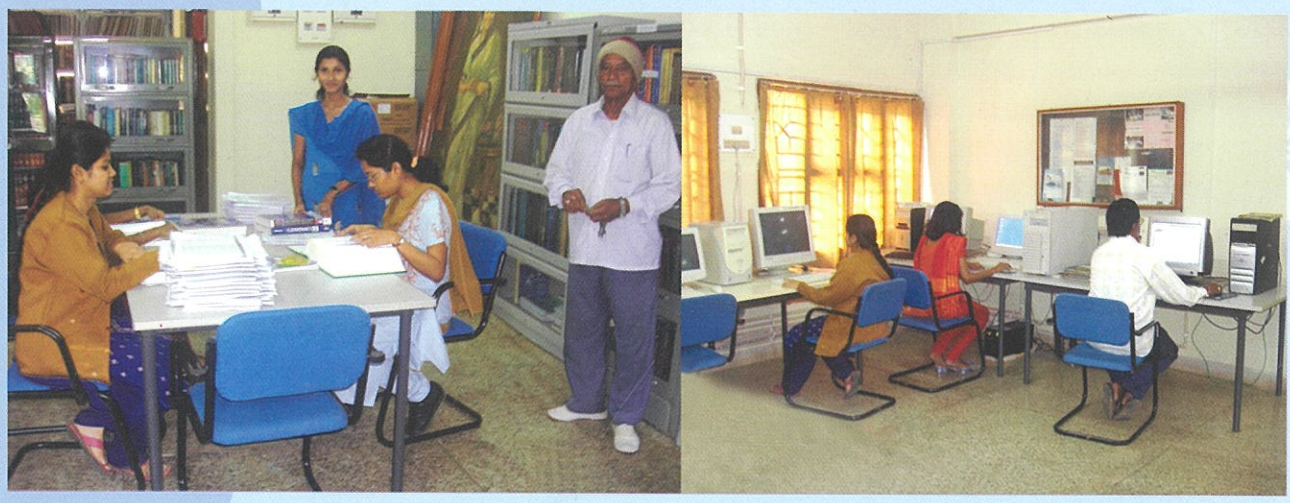
Photocopy facility is available for scientists and researchers of ICMR free of cost. For non-ICMR library users Rs. 0.75 paisa per page has to be deposited for photocopy charges. A digital copier cum- LAN printer is installed in library for better reprographic services.

Publication Cell:

The library & Information Centre is doing publication activities of the Institute. RMRC News Bulletin and Library News Letter are being published from this division. IEC materials on various diseases are also made on local languages for distribution to the public. Besides, the division looks after the publication of Centre's Annual Report.

The following are the publications from RMRC being published from library division.

1. RMRC News Bulletin
2. Library News Letter
3. IEC Materials on various diseases on Malaria, Filariasis, Sickle cell diseases, IDD, in regional language.
4. Posters on recent advances in Fiariasis, Malaria, Sickle cell diseases, and diarrhoea for children.



Students doing literature search in RMRC library

B. Insectorium

The Centre maintains the insectorium facility at the Entomology division by rearing of various stages of vectors used for laboratory studies. Cyclic colonies of three mosquito species i.e. Aedes aegypti (black eyed Liverpool strain), Anopheles stephensi and Culex quinquefasciatus are being maintained. This year attempt is being made to rear Aedes albopictus and Anopheles culicifacies. Cyclic colonies are maintained for conducting different experiments such as development of different strains and species of filarial worms that will help in the selection of proper animal model and conducting bio-assays of different plant products for observation of its insecticidal properties. Different stages of mosquitoes are also supplied to Universities, other laboratories and state Govt. for giving training g for identification of species. Students from various universities, Research laboratories also come for training to know the technique of rearing of mosquitoes.

C. Animal house

Animal facility in the center continues to be used for all relevant on-going projects. Currently M. Coucha, Balb/c mice, Guinea pigs and Rabbits are available for experimentation. All the projects concerning animal use/ experimentation are discussed in Animal ethical committee of the center and work progress also review periodically by the committee. Staff has maintained periodic records of animal house. This facility is maintained regularly with periodic inspection monthly and health monitoring by veterinarian and visit by CPCEA nominee.

D. OPD at Capital Hospital:

The Centre runs twice a week OPD at Capital Hospitsal for referral cases of filariasis where cases are diagnosed and treated. During March, 2007, another OPD room was allotted to the Centre by the hospital

for extending referral facilities malaria and hepatitis cases diagnosis. This arrangements meet both service component as well as research. Regular blood samples are collected with clinical history from Capital Hospital and S.C.B. Medical College in collaborative research programme.

E. Activities of NNMB Unit, Orissa, Bhubaneswar

The NNMB Unit, Orissa under NIN, Hyderabad functioning at RMRC, Bhubaneswar at present is undertaking Second Repeat Tribal Survey for "Assessment of Diet & Nutritional Status of Tribal Population" along with assessment of Prevalence of obesity and hypertension among adult men and women of >= 20years of tribal population.

The objectives of the Survey are as follows:-

1. To assess the food and nutrient intake among different age/sex/physiological groups of tribal population living in the Integrated Tribal Development Agency/ Project (ITDA/P) areas in NNMB states.
2. To assess their nutritional status of all the available individuals in terms of Anthropometry, clinical examination and to study the time trends in the nutritional status.
3. To assess the history of morbidity during previous fortnight among all the individuals covered for anthropometry.
4. To assess the prevalence of obesity and hypertension among the adult men and women (>=20years) in tribal population.
5. To assess awareness about hypertension and diabetes among adults (>= 20years) of the tribal community.

Till September 2007 the Unit has covered 81 villages in 8 districts where 3244 households are covered. Clinical Examination, Anthropometry and morbidity status was done in 10567 individuals.

Diet Survey was completed in 810 households using Individual dietary food intake method. KAP on Hypertension and Diabetes Mellitus with Blood Pressure measurement, Waist Circumference & Hip Circumference measurement was done in 1907 individuals. The data is being sent to NIN, Hyderabad for analysis.

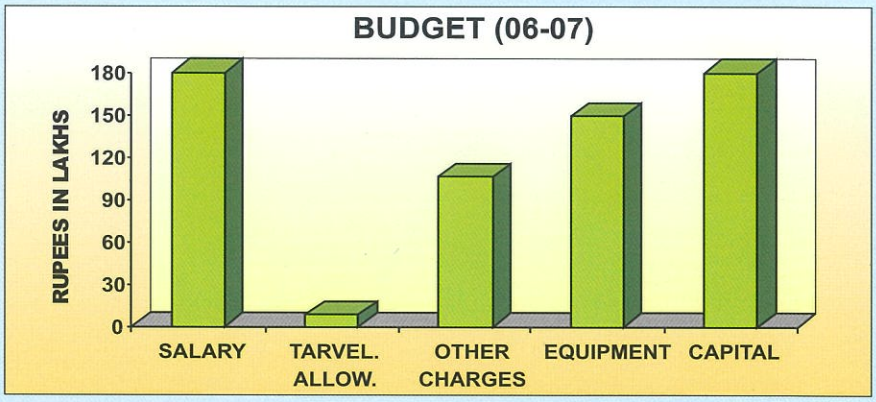
The Unit has also conducted a Survey for assessment of Nutritional Status of <=5 years children in Koraput district of Orissa in the GIO-UNICEF Programme with the NIN Team during August 2006.

The Unit is also doing a Survey for early detection of Chronic Renal Diseases among tribal population of Orissa where some important data like their age at marriage, any infant mortality, history of Oliguria, Anuria, Haematuria, Pyuria, Diabetes Mellitus, Hypertension are collected along with Blood Pressure

measurement and examination of Urine for Albumin /Sugar/Micro albumin among >= 15 years individuals. The data is handed over to clinical Division of RMRC,BBSR for analysis.

4.7 Budget and Resource Generation:

The total sanctioned Budget in respect of the Centre (Non-Plan & Plan) for the year 2006-07 is 7.34 Crore. The head-wise expenditure of budget is shown below in the graph. The resource generation during this period 61.68 from extramural grants and Ph.D program through UGC and CSIR.



4.8 Committees

21st Scientific Advisory Committee

1	Dr. Sandip K. Basu Director, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110 067.	Chairman
2	Dr. Satish Gupta National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi-110 067.	Member
3	Dr. K. Ramachandran Consultant, National Institute of Epidemiology, Chennai.	Member
4	Dr.S.K.Sarin Professor & Head, Department of Gaestroenterology, Room no 201, Academic Block, G.B.Pant Hospital, New Delhi – 110002.	Member

5	Dr. B.S. Ramakrishna Prof. Dept. of Gastroentorology Christian Medical College, Vellore- 632002	Member
6	Dr.V.Kumaraswami Deputy Director (SG), Tuberculosis Research Centre, Chennai – 600031.	Member
7	Dr. S.K. Kar Director, Regional Medical Research Centre, Bhubaneswar-23	(Member Secretary)
8	Dr. Dipali Mukherjee DDG (SG) & Chief, ECD, Indian Council of Medical Research, Ansari Nagar, New Delhi 110 029.	ICMR Representative
9	Dr. Rashmi Arora DDG (SG), ECD-II, Indian Council of Medical Research, Ansari Nagar, New Delhi 110 029.	ICMR Representative
10	Dr. Ira Ray B 265 GKI, New Delhi-110 048.	Spl. Invitee
11	Dr. D.S. Aagarwal B-24, Swasthya Vihar, New Delhi 110 092.	Spl. Invitee
12	Dr.Sarala K.Subbarao Ex-Director, MRC, Consultant (ECD), ICMR, New Delhi – 29	Spl. Invitee
13	Dr.P.R.Narayanan Director,Tuberculosis Research Centre, Chennai – 600031.	Spl. Invitee
14	Director of Health Services, Govt. of Orissa, Heads of the Dept. Building, Bhubaneswar.	Spl. Invitee

Human Ethical Committee:

1.	Justice (Mrs.) A.K. Padhi Former Judge, Orissa High Court 10, Bhasakosh Lane Nimchouri, Cuttack-753 002	Chairman
2.	Dr. B B. Tripathy Retd. Prof. of Medicine Saradiya Mission Road, Cuttack-753 001	Member
3.	Dr. (Mrs.). P. Mohanty Hejmadi Former V.C., Sambalpur University GM-8, VSS Nagar, Bhubaneswar-751 004	Member
4.	Mrs. Kasturika Pattanayak Ex-Chair Person, Social Welfare Board Govt. of Orissa, 1, Lewis Road, Bhubaneswar	Member
5.	Dr. (Mrs.) Manorama Das C/o. Prof. G.C.Das, Santiniketana, Mathasahi, Cuttack	Member
6.	Dr.P.K.Acharya, Ex-Director of Health Services, Govt. of Oriss.a	Member
7.	Dr. S.K. Kar Director Regional Medical Research Centre, Bhubaneswar	(Member- Secretary)

Animal Ethical Committee:

1.	Dr. S.K. Ray Professor & Head, Dept. of Veterinary Medicine Orissa College of Animal Husbandry & Veterinary Sciences, O.U.A.T., Bhubaneswar – 751 001	Chairman
2.	Dr. G.B.N. Chainy Professor & Head, Dept. of Zoology Utkal University, Bhubaneswar – 751 004	Member

3.	Prof. P.C. Supkar Institute of Life Sciences Bhubaneswar-751 023	Member
4.	Mr. N.R. Mansingh Inspector, SPCA C/o. CDVO office, Puri – 752 002	Nominee of the CPCSEA
5.	Dr. M.K. Das Deputy Director (Sr. Gr.) RMRC, Bhubaneswar	Biological Scientist
6.	Dr. A.K.Satpathy Assistant Director RMRC, Bhubaneswar	I/C Animal facility
7.	Dr. (Mrs.) N. Mohapatra Assistant Director RMRC, Bhubaneswar	Biological Scientist
8.	Dr. S.K. Kar Director RMRC, Bhubaneswar	Convener

Technical Equipment Purchase Committee:

1.	Dr. A.K. Sahoo Principal Scientist CIFA, Kausalya gang Bhubaneswar- 751 002	Chairman
2.	Prof. P.C. Supakar Director-in- Charge Institute of Life Sciences Bhubaneswar-751 023	External Member
3.	Dr. P. Das, Senior Scientist, CIFA Bhubaneswar	External Member
4.	Dr. N.K.Debata Pathologist, SUM Hospital Bhubaneswar	External Member

5.	Mr. A.K. Mohapatra AO, RMRC, Bhubaneswar	ember
6.	Mr. R.V.Rao, ACO, RMRC, Bhubaneswar	Member
7.	Dr. M.K. Das, DD (SG) Bhubaneswar Secretary	Member- RMRC,

Technical Building Maintenance Committee:

1.	Mr. D. N. Tripathy Chief Engineer, CPWD (Retd.)	Chairman
2.	Mr. P. K. Pattanik Sup. Eng. (Elect.), PWD (Retd.)	Member
3.	Mr. P. Kapoor Jt. Director (Agriculture), Orissa (Retd.)	Member
4.	Dr. B. B. Pal Assistant Director, RMRC	Member
5.	Dr.B.Dwibedi Research Officer, RMRC	Member
6.	Mr. A.K.Mohapatra Admn. Officer, RMRC	Member
7.	Mr. G. Behera Section Officer, RMRC	Member

4.9 Staff Position (up to Oct. 2007)

DIRECTOR

DR. S.K. Kar, MD, Dip. Clin. Epid.

IMMUNOLOGY DIVISION

Dr. M. K. Das, M.Sc., Ph.D.	Deputy Director (Sr. Gr.)
Dr. B. Ravindran, M.Sc. Ph.D	Deputy Director (SG) Deputation to ILS Post transferred to RMRC, Belgaon.
Dr. M. K. Beuria, M.Sc., Ph.D.	Asst. Director
Dr. A. K. Satapathy, M.Sc., Ph.D.	Asst. Director

Dr. N. N. Mandal, M.Sc., M.Phil. Ph.D.	Research Assistant
Dr. P. K. Sahoo, M.Sc., Ph.D.	Research Assistant
Dr. (Mrs.) M.S. Bal, M.Sc., M.Phil., Ph.D.	Research Assistant
Mr. H.S. Naik, Dip. MLT	Lab. Technician
Mr. K.C. Parichha	Insect Collector
Mr. S.C. Das	Lab. Attendant
Students :	
Mr. Alok Das Mohapatra, M. Sc.	SRF (UGC)
Mr. Santosh Kumar Panda, M. Sc.	SRF (UGC)
Mr. Aditya Kumar Panda, M. Sc.	SRF (CSIR)
Miss. Madhumita Panda, M.Sc.	SRF (UGC)

PATHOLOGY AND MICROBIOLOGY DIVISION

Dr. G.P. Chhotray, M.D.	Deputy Director (Sr. Gr.)
Dr. M.R. Ranjit, M.Sc., Ph.D.	Asst. Director
Dr. B.B. Pal, M.Sc., Ph.D.	Asst. Director
Dr. H.K. Khuntia, M.Sc., Ph.D.	Research Assistant
Mr. R.K. Das, M.Sc.	Research Assistant
Mr. B.N. Sethi, Dip. MLT	Lab. Technician
Mr. K.C. Dalai, B.A., ITI	Lab. Assistant
Mr. B.K. Kanhar	Lab. Assistant
Mr. C.R. Samantray	Lab. Assistant
Mr. K.C. Jena	Laboratory Attendant
Mr. S. K. Mallick	Lab. Attendant
Students	
Mr. Sudhansu Sekhar Nishank, M. Sc.	SRF (CSIR)
Miss. Ronali Rout, M.Sc.	JRF (UGC)
Miss Upasana Sahoo, M.Sc.M.Phil	SRF (RMRC)
Mr. S.K.Samal, M.Sc.	SRF (RMRC)
Mr. Gunanidhi D Majhi, M.Sc.	JRF (UGC)

CLINICAL, EPIDEMIOLOGY AND STATISTICS DIVISION

Dr.B.V.Babu, M.A., Ph.D., PGDAS	Deputy Director
Dr. A. Mohapatra, M.Sc., M.Phil., Ph.D.	Asst. Director
Dr. (Mrs.) A.S. Kerketta, M.B.B.S.	Senior Research Officer
Dr. Bhagirathi Dwibedi, M.B.B.S, M.D (Paed)	Research Officer
Mr. P.K. Jangid, M.Sc.	Technical Officer
Dr. A.S. Acharya, M.Sc., M.Phil, LL.B., Ph.D	Research Assistant
Mr. D.P. Hansdah, M.Sc.	Research Assistant
Mr. R.C. Parida, M.Sc.PGDCA	Research Assistant
Mr. S.C. Rout	Lab. Technician
Mr. T. Moharana	Lab. Assistant
Mr. R.N. Nayak, B.A.	Census Taker
Mr. K. Dhal, B.A.	Census Taker
Mr. N.N. Pattnaik	Laboratory Attendant
Mr. H.K.Jena	Field Attendant
Mr. K.C. Nayak	Sweeper
Students:	
Miss Prajyoti Sahu, M.Sc. M.Phil	SRF (RMRC)
Biswa Ranjan Purohit, M.A	SRF (RMRC)

MEDICAL ENTOMOLOGY DIVISION

Dr. (Mrs.) N. Mohapatra, M.Sc., Ph.D.	Assistant Director
Dr. R.K. Hazra, M.Sc., Ph.D.	Senior Research Officer
Dr. S.K. Parida, M.Sc., Ph.D.	Technical Officer
Mr. N.S. Marai, M.Sc., LL.B.	Research Assistant
Mr. H.K. Tripathy, B.Sc, PGDME	Technical Assistant
Mr. G.D. Mansingh	Insect Collector
Mr. B. Pradhan	Insect Collector
Mr. C.S. Tripathy, B.Com. LL. B.	Insect Collector

Mr. S.S. Beuria	Insect Collector
Mr. G. Simhachalam	Insect Collector
Mr. Banamali Nayak	Field Attendant
Students:	
Miss. Sunita Swain M.Sc.	JRF (Lady Tata Fund.)
Miss. Asima Triparthy, M.Sc.	SRF (RMRC)
Miss. Swati Kumari	JRF (Lady Tata Fund.)

HUMAN GENETICS DIVISION

Dr. R.S. Balgir, M.Sc. (Hons.), Ph.D.	Deputy Director (Sr. Grade)
Dr. G. Bulliyya, M.Sc., Ph.D.	Asst. Director
Mr. B. Murmu, M.Sc., M.Phil.	Research Assistant
Mrs. G. Mallick, M.Sc.	Research Assistant
Miss. Sujata Dixit, M.Sc	Research Assistant
Mr. Baburam Behera	Sweeper- cum- Attendant
Students	
Mr.Basant Kumar Swain, M.S.W	SRF (RMRC)
Mr.Priyadarsi Girija Shankar Sethy, M.Sc.	SRF (RMRC)

LIBRARY & INFORMATION

Dr. B. Sahoo, M.L.I.Sc., Ph.D.	Asst. Lib. & Inf. Officer
Mr. Chakradhar Naik	Sweeper-c-Attendant
Mr. R.S. Bahadur	Watchman

ADMINISTRATION

Mr. A.K. Mohapatra, B.A., LL.B.	Administrative Officer
Mr. B. Sutar, M.Com	Assistant
Mr. R.C. Muduli, B.A.	Assistant
Mr. P.C. Nayak, B.A.	Personal Assistant
Mr. Abani K. Nayak, B.Com.	Personal Assistant

Mrs. R. Varghese	Steno
Mr. B.S. Rao	U.D.C.
Mr. S. Nayak	L.D.C.
Mr. R. Rath	L.D.C.
Mr. S.K. Das, B.Com.	L.D.C.
Mr. S.K. Majhi, M.A., LL.B.	L.D.C.
Mr. R.C. Dash	Office Attendant
Mr. M.B. Thappa	Watchman
Mr. R.S. Rai	Watchman
Mr. Som P. Sharma	Watchman
Mr. T. Bahadur	Watchman
Mr. D.C.Rao	Sweeper

DIRECTORS' OFFICE

Mr. L.S. Rao, B.A.	Private Secretary
Mr. K.G. Samal	Attender
Mr. R.K. Hembram	Field Attendant

ACCOUNTS

Mr. G. Behera, M.A.	Section Officer
Mr. A.P.Parida, B.A	UDC
Mr. S.K. Satapathy	UDC
Mr. Sankar P Sharma	Watchman

WORKSHOP, INSTRUMENT & BUILDING MAINTENANCE

Mr. B.K. Biswal	Electrician
Mr. S. Sutar	Generator Operator
Mr. J. Behera	P.H —Wireman
Mr. B.K. Moharana	Plumber-c-Carpenter
Mr. Banamali Sahoo	Gardener
Mr. Sankar Bisoi	Cook-cum-Guest House Attd.

General Information and Publications

ANIMAL FACILITY

Mr. A. Senapati	Animal House Attendant
Mr. S.K. Das	Animal House Attendant
Mr. Jaladhar Naik	Animal House Attendant
Mr. Pandav Sahoo	Animal House Attendant

TRANSPORT

Mr. Md. Daulat Khan	Driver (Special Grade)
Mr. Sibaram Patra	Driver (Grade-I)
Mr. R. Pradhan	Driver (Grade-I)
Mr. Anakar Nayak	Driver (Grade-II)
Mr. A.R. Khan	Driver (Grade-II)
Mr. P.K. Behera	Driver

NATIONAL NUTRITION MONITORING BUREAU (NNMB) STAFF

Dr. S.K. Das, MBBS	Research Officer (Medical)
Mrs. S. Paikray	Asst. Research Officer
Mrs. Haraprava Sahu	Social Worker
Mr. D.K. Mohanty	Steno-C-Office Asst.
Mr. R.K. Sahoo	Driver

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Printed by : Textbook Bureau, BBSR