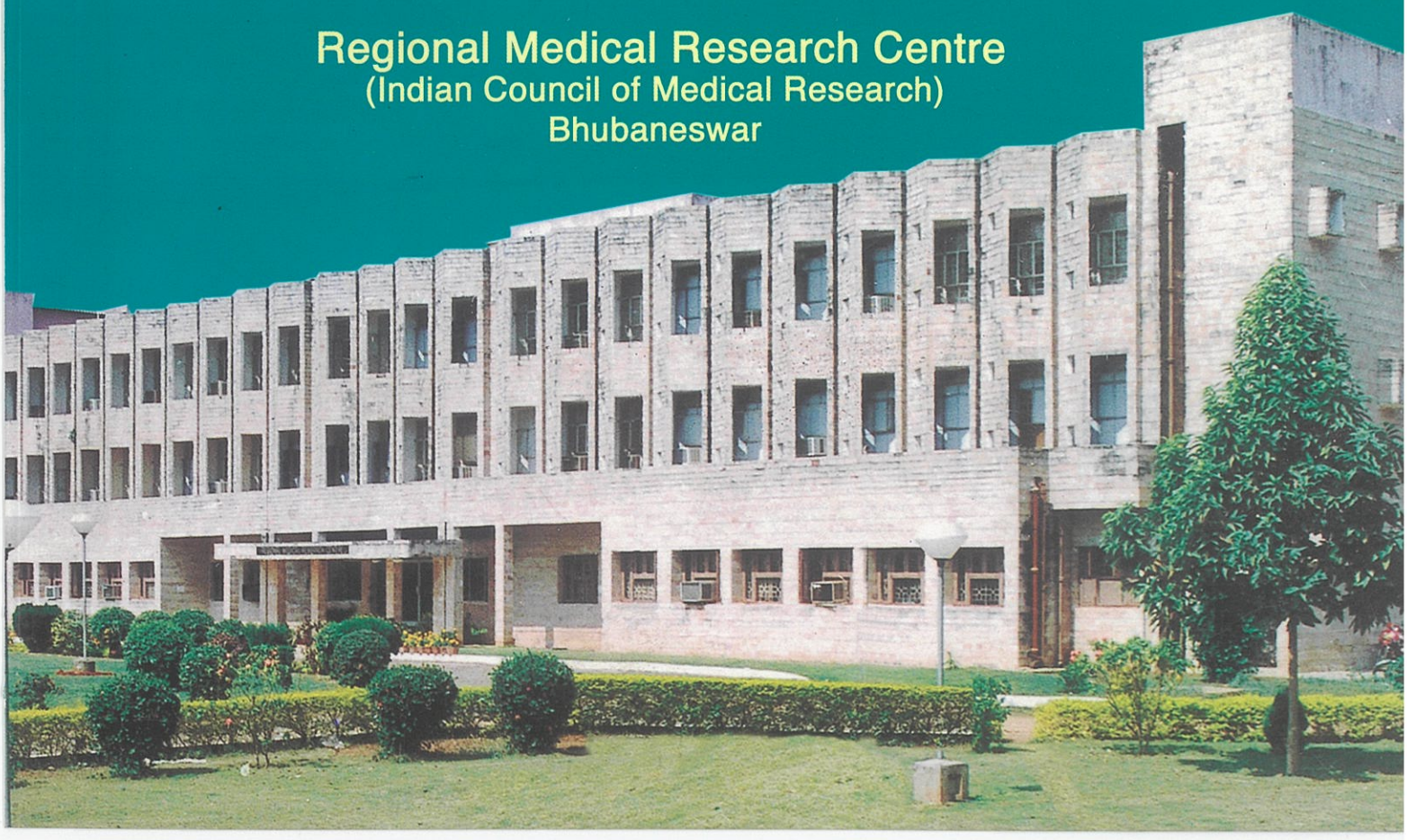
Annual Report
2003-04

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



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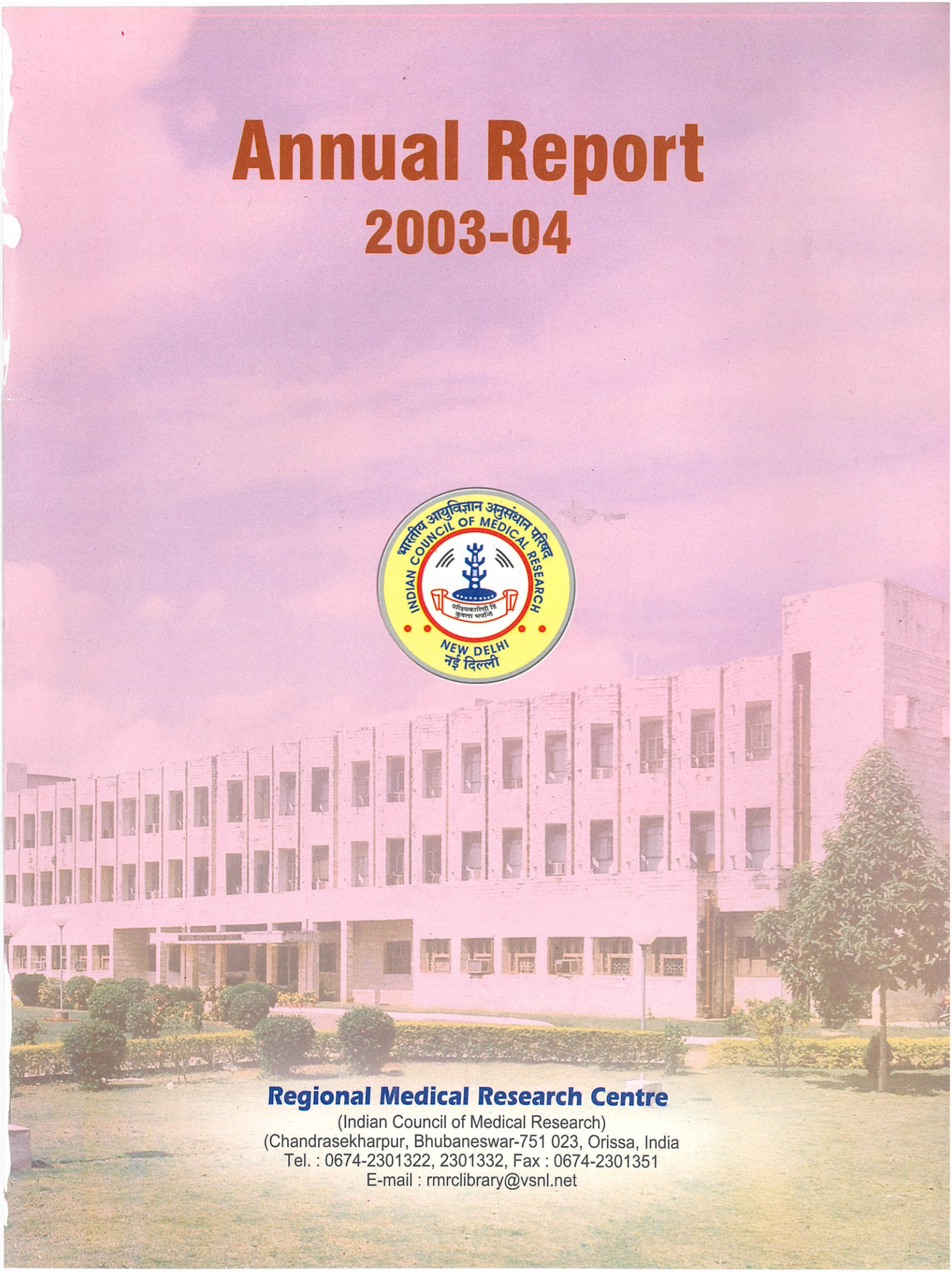

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PREFACE

Carrying out innovative research, human resource development, and infrastructure strengthening have been the major focus of this Centre during the period. Besides, areas like scientific information dissemination, consultancy services towards disease diagnosis and programme evaluation and linkages with State health department were emphasised.

Research activities are addressed to issues pertaining to priority areas of diseases prevalent in this region like filariasis, malaria, and nutritional disorders. New research programmes on tribal health like malaria, hepatitis, STD infections and anaemia have been planned during the year. Research issues are addressed on basic, applied and operational areas intending to develop diagnostic markers for infection, enhancing knowledge towards better understanding of disease or strategy that can find useful application in control programme. Out of 19 research projects in operation, 12 were extramural and seven research projects are completed.

Major focus of research in lymphatic filariasis is development and characterisation of antigens and recombinants that confer protective immune response with vaccine potential. Immunologic markers that can differentiate each clinical spectrum of disease were developed. Studies in animal model indicated existence of innate immunity that play important role against microfilaraemic stage of infection. These studies are crucial in understanding natural protection, regulatory mechanism in host and contribute immensely to R&D activities. Clinical drug trial on morbidity management in chronic filarial lesions emphasised the role of inexpensive regimens with foot care as useful tool in arresting progress and adenolymphangitis attacks with limb size regression. To achieve high and sustainable compliance in Mass Drug Administration (MDA) of filariasis elimination programme in urban population, an innovative strategy has been developed and now being tested in the field. New sensitive tool of IgM assay has been developed that can monitor the infection at very early age amongst young children of paediatric age group to help assessing efficacy of MDA programme. Since side reactions to MDA were reported in this region, the role of endosymbyont Wo/bachia in side reactions is being studied in microfilaraemics. These results will be of immense assistance to filariasis elimination programme.

Malaria is highly endemic in this region that contributes to high mortality and morbidity. Studies on malaria in different geographical regions of Orissa indicate existence of parasite diversity in its distribution and diverse chloroquine resistance pattern. This information is crucial in planning vaccine trial in this region. The information generated in situation analysis of urban malaria, health-seeking behaviour and disease burden in KBK districts of Orissa will immensely help in planning strategy for malaria control. Protective immune response host to malaria has been elicited by using specific recombinants. Studies are now addressed to assess vector infectivity, insecticidal resistance and chloroquine resistance using molecular tools. Nutritional problems are widely prevalent particularly in primitive tribes. Information has been generated in Dongria Kondh and other tribal communities on the extent and pattern of undernutrition including micronutrient deficiency, While assessing risk factors to help developing community based intervention package to combat malnutrition. Loss of Iodine during cooking process was assessed in eastern states of India.

For dissemination of information, the Centre is publishing a six monthly News Bulletin. Besides, first six monthly issue of Newsletter on library services was published during the year, Which is very informative. The pamphlets, booklets and posters on various diseases of public health importance and research achievements have been made and are being used for dissemination of knowledge among public during exhibitions and health me/as.

Efforts were made for strengthening training, networking and infrastructure of informatics. Library provides services like online database search, Internet access, on-line reprint request, ICMR Library network for resource sharing, IEC materials and interlibrary loan facility. Library automation and LAN facility are in progress.

Human resource development activity of this Centre focussed on imparting Ph.D. programme, M.Sc. and M.D. theses work and , teaching of university students on modem biology. During the period, seven students enrolled for Ph.D. programme, two were awarded Ph.D. and nine M.Sc. students from various universities did their dissertation work under scientists of this Centre. Several medical and para- medical staff referred from various organizations were trained on laboratory diagnosis of malaria, haemoglobinopathies and on verbal autopsy.

During the perlod Aprll 2003 to March 2004, the Centre has made 18 publications and 5 are in press, mostly in SCI indexed journals. As per scientometric analysis by ICMR , based on impact factor (IF) by ICMR in year 2002, this Centre with average IF of 4.06 got 1st position among ICMR institutes in average IF. The Centre has made efforts to encourage scientists and staff in pursuing their effort. The scientists have spent considerable time in attending symposia, meetings, conferences and other training activities in India and abroad. They participated in various committees of the Centre and contributed towards organisational and developmental activities of the Centre. Monthly staff council meetings are held with scientists to discuss and streamline administrative issues. They organise journal clubs and seminars and impart training to other researchers.

The Centre organised workshops, symposia and invited guest lectures while observing National Science Day, ICMR Foundation Day and RMRC Foundation Day celebrations. Cultural programmes with staff were organised on RMRC Foundation Day. Scientists and technical staff have participated in Swasthya Melas undertaken in various places in the State and disseminated scientific informations besides rendering diagnostic services and counselling. Regular human and animal ethical committee meetings were organised.

Interaction with State health department has been strengthened in form of providing out-patient service in state headquarters hospital and diagnostic services in diseases like filariasis, malaria, haemoglobinopathy disorders, regular diarrhoea surveillance activity and consultancy in areas like evaluation of programme and outbreak investigations in diarrhoea and hepatitis. The Centre has strengthened linkages with other ICMR institutes like NIN, Hyderabad, NICED, Kolkatta, NIV, Pune, MRC, Delhi, NIE, Chennai, IRR, Mumbai, VCRC, Pondicherry, etc. in terms of collaboration, training of scientists and transfer of technology. Besides, the Centre also rendered assistance to National Vector Borne Disease Control Programme in undertaking training and monitoring. The Centre also collaborated with NII, Delhi, TIFR, Mumbai and other international agencies like WHO, TDR, EC and University of Canada.

There are 99 regular staff in position that included 16 scientists with various expertise who catered to accomplish the output. During the year the Council provided an annual budget of Rs.334.64 lacs. An additional amount of Rs.103.78 lacs was generated by the Centre as extramural research grants received from national and international agencies. Contractual research work was undertaken with request from World Food Programme that generated resources.

Infrastructure strengthening was made by procuring scientific equipment of worth over rupees one crore, and strengthening molecular biology and iodine laboratory. The Centre initiated construction of an auditorium, guest house, trainee hostel and animal house, development of campus horticulture, internal roads and electrical renovation during the year.

The scientists and staff of this Centre made continuous effort and contributed in 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.

S.K.KAR

RESEARCH HIGHLIGHTS (2003-04)

Characterization of filarial antigens like DSSdI derived from S.digitata that induce protective immune responses and thus lead to absence of infection (antimicrofilarial immunity) indicated the role played by carbohydrate epitopes of filarial antigens. Further identification to study the structure of such parasite sugars could lead to development of synthetic vaccines for filariasis. The study on role of endosymbionts, Wolbachia in mediating post-drug reactions in W. bancrofti has been initiated with a view to understand their aetiology .This critical information may be utilized for formulating future control strategies to filariasis control.

For effective morbidity management of filariasis it is essential to understand the pathogenesis of the disease and to develop markers for morbidity .Filariasis presents with acute and chronic manifestations. Towards development of markers the following pro and anti-inflammatory molecules were quantified: TNF-α, TNF-α receptors typel and type2, IL-I0, IL-6, IL-8, LPS binding protein and sICAM-I. The levels of two TNF-α receptors could differentiate elephantiasis and hydrocele; acute disease and chronic filariasis differed in terms of levels of TNF -α. IL-6 and IL- 8 were shown as markers of disease morbidity since subjects with asymptomatic infection were significantly different from those with acute or chronic disease in these two parameters and thus could be differentiated between each other . The role of immunomodulators that decrease pro- inflammatory cytokines such as IL-6, IL-8, TNF-α in clinical management of filariasis need to be studied in future.

Filariaisis is a debilitating disease, characterized by periodical episodes of acute symptoms . Many of these are self - limiting and recovery from the acute episode is achieved within a week. However with repeated episodes, the patient's ability to work for livelihood gets severely compromised. Secondly, chronic manifestations of the disease such as hydrocele and/or elephantiasis also affect significantly the work output of patients. Filariaisis largely affects the poor and the working class of the population significantly, particularly in rural areas. The studies in RMRC, Bhubaneswar has revealed that the economic loss due to acute filariasis was found to be Rs. 222.69 per capita or 3.9% of per capita income and the loss due to chronic filariasis were found to be about Rs. 24.88 or 0.44% of per capita income.

Morbidity management of filariasis has been addressed from the point of view of patients as well as from an etiological angle. The first component was addressed by evaluation of different prophylactic methods such as use of antibiotics such as systemic (penicillin, or local antibiotic oilments with foot care and maintenance of hygiene. It is based on the proposal that secondary bacterial infections could be the primary cause of repeated episodes of acute filarial disease in patients with chronic disease such as hydrocele and/ or elephantiasis and each attack precipitates oedema increased limb size. Three regimens evaluated with Foot- care significantly decresed the lymphedma limb size and decreased the frequency of filarial adenolymphangitis as well. Since most of the economic loss due to filariasis is due to acute episodes of filariasis, the results of the current study is expected to significantly contribute to improved morbidity management of filariasis at community level programme.

Studies were initiated to evaluate the mode of action of albendazole against filarial parasites since the drug is known for poor absorption in the intestine and plasma concentrations do not reach levels conducive for anti-parasitic activity against a systemic helminth such as filarial parasites. The results revealed that albendazole alone was very effective in its anti filarial activity only in patients with concomitant parasitism with intestinal helminths and filariasis than in subjects infected only with filariasis. This indicates that the anti filarial activity of albendazole was mediated through elimination of geohelminths. This observation is expected to assist in designing programme for the ongoing global initiative for control of filariasis in disease endemic countries .

Malaria is one of the major public health problems in the State of Orissa which contributes nearly 45% of mortality due to P .falciparum malaria in India. Apart from Cerebral malaria (one of the commonest cause of mortality associated with P .falciparum malaria globally), it was observed that death was also mediated by other clinical complications such as renal as well as hepatic failure, more so in adult populations. This unusual feature (not widely seen in other geographical areas of the world) required special attention. The RMRC, Bhubaneswar undertook a clinical study that involved adjunct therapy with drugs such as Pentoxifiline, a commonly available drug used for clinical management of crisis in patients with Sickle cell disease etc. It was found that the drug also significantly decreases one of the inflammatory molecules such as TNF-α which contributes significantly to mortality associated with malaria. Patients with cerebral malaria were administered Pentoxyfiline as an adjunct drug along with intravenous quinine. The use of this adjunct drug significantly decreased plasma TNF levels within 24 hrs and also significantly decreased the coma recovery time in patients with cerebral malaria. A double-blind trial to assess its efficacy may offer a new therapeutic regimen for better clinical management of human cerebral malaria.

Protective immunity in malaria has been demonstrated by us to be primarily due to development of antibodies to malarial phospho-proteins designated as PfPo. This has allowed us to develop therapeutic molecules of single chain immunoglobulins reacting with parasite PfPo. Development of such reagents is expected to result in their use for passive immunotherapy, which can be used along with anti-malarial drug for clinical management of severe malaria. Population structure of P .falciparum clinical isolates obtained from various malaria prone districts of Orissa were studied. The frequency of genetic distribution of following genes: GLURP, MSP1, MSP2, and KAHRP were compared in clinical isolates collected from non-complicated and cerebral malaria cases. The observations shows that these can be used as markers in population genetic studies. The association of PfCR T point mutation with severity of the disease indicates the biological advantage enjoyed by strains resistant to Chloroquin.

1. STUDIES ON FILARIASIS

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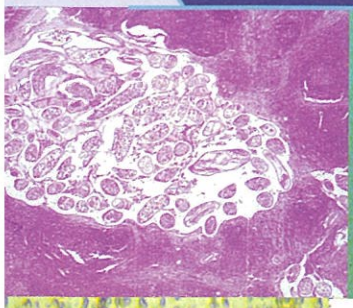
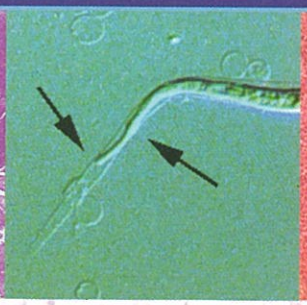
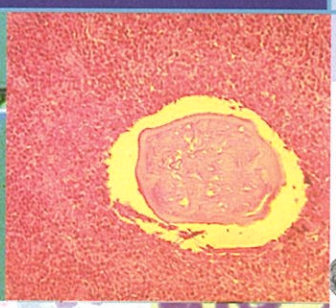
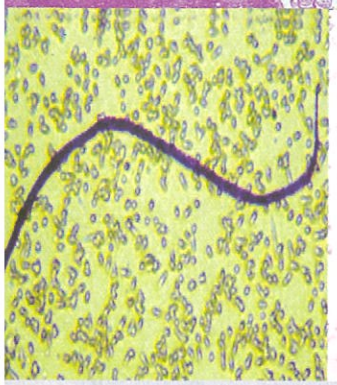
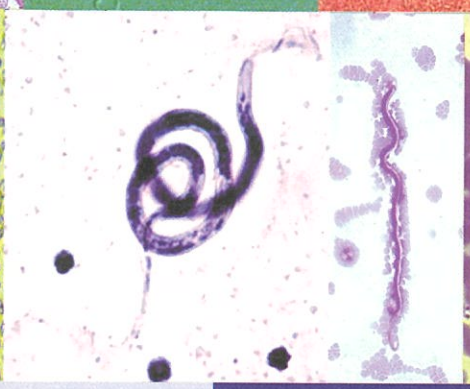
REGIONAL MEDICAL RESEARCH CENTRE, BHUBANESWAR

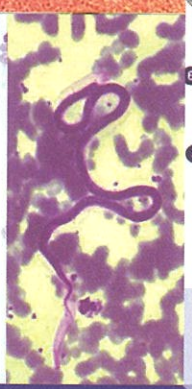


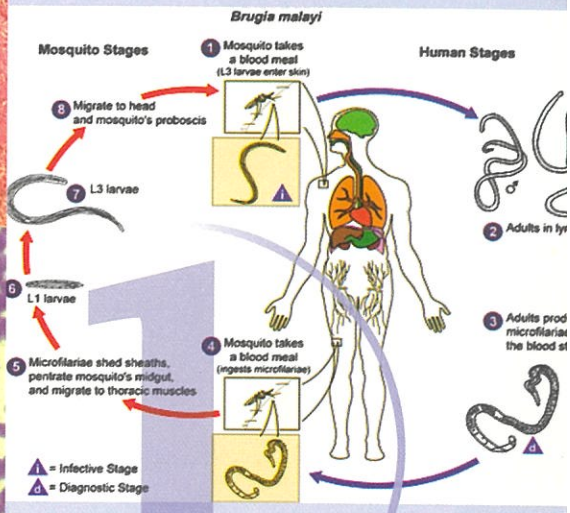












INSIDE

Ongoing Studies
Studies On Filariasis

Status:
Extramural (DBT)

Investigators:
Dr. M. K. Das
Dr. M. S. Bal
Dr. M. K. Beuria
Mr. N. N. Mandal

Starting date: July 2001
Closing date: July 2004

1.1 Immunological characterization of filarial antigens with potential protective response in endemic population

Objectives:

1. Immunochemical characterization of Dssd1 and lipid antigens with potential for microfilarial clearance from infected animals.
2. To study antibody response to these antigens in endemic normal vs. infected population.

Background information:

It was earlier reported that two filarial antigens that are (i) surface glyco-protein (Dssd1) and (ii) lipid exhibited higher antibody response in infection free individuals (circulating filarial antigen negative i.e., CFA -ve) and diminished antibody response in infected individuals irrespective of clinical and parasitological status. Periodate treatment of Dssd1 reduces antibody levels in CFA negative individuals but not in CFA+ve sera. On western blotting analysis CFA–ve sera was found reactive to Dssd1 antigen (molecular weight approximately 210 kDa) but not to CFA +ve sera. Periodate oxidation induced drastic reduction of Dssd1 recognition by CFA –ve sera. CFA +ve sera did not react with oxidized Dssd1. The result indicates the immunogenecity of carbohydrates epitopes of Dssd1 in infection free individuals. Active infection is associated with lack of antibody response to carbohydrates.

The nature of carbohydrate residues present in Dssd1 was probed through lectins (ConA WGA) coupled to peroxidase. The presence of D-glucose, mannose, N-acetyl glucosamine and sialic acid residues are indicated in Dssd1.

Results:

An experimental animal model was used *(Mastomys coucha* implanted with female *Setaria digitata* adult) in order to check the microfilarial clearance ability of Dssd1 and filarial lipid antigens. In this model, MF appears in the circulation around 7 days after implantation. Two groups of infected Mastomys with circulating microfilariae were immunized (i.p.) with antigens (Dssd1 and lipid antigens). Another immunization was given 15 days apart. Microfilariae (MF) numbers were monitored. It was found that both lipid and Dssd1 antigen were able to clear the microfilaria from the circulation. The lipid antigen appears to be more effective in MF clearance ability. Antibodies to these antigens (lipid and Dssd1) were also detected in the immunized animals. These experiments indicate anti-microfilarial immunity induced by these antigens. Experiments are underway to determine if pre immunized Mastomys could be protected against the challenging infection.

1.2 Lymphatic filariasis in young children: an immunological prospective

Status: Intramural

Investigators:
Dr. M. K. Das
Dr. M. K. Beuria
Dr. M.S. Bal
Mr. N. N. Mandal

Starting date: August 2001

Closing date: August 2004

Objectives:

1. To detect pre-patent infection through IgG4 and circulatory filarial antigen assays.
2. Prevalence of anti-filarial antibodies in different age classes in children

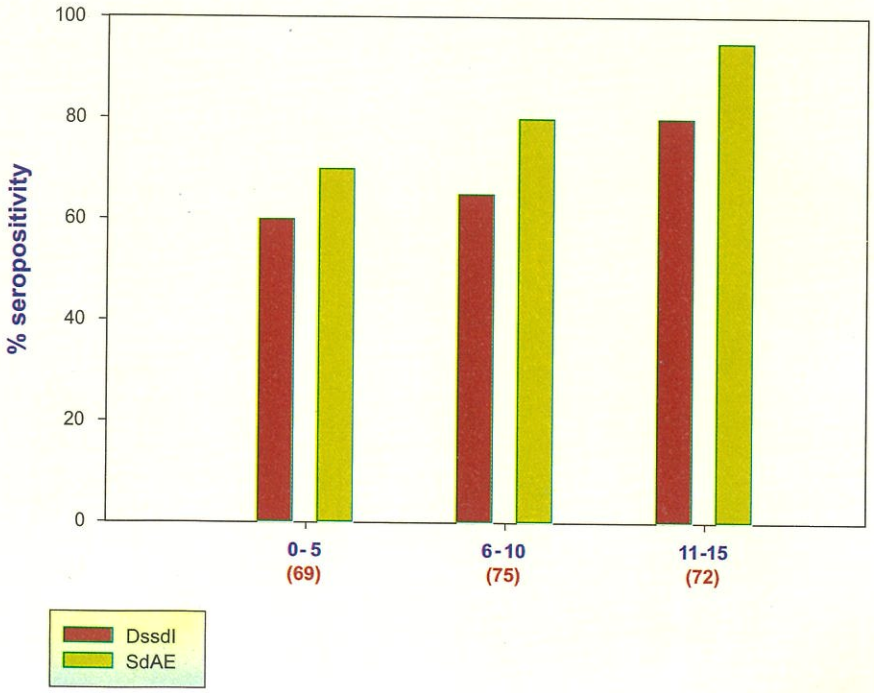
Background information:

Children below 15 years of age were studied in a region endemic for *Wechereria bancrofti* infection (Bajapur village, Khurda district, Orissa). Microfilariae prevalence was 6.54%. Among normal children, a high rate of antigenaemia (25%) was observed indicating the severity of infection. About 95% IgG positivity to *Setaria digitata* adult extract in infection free (MF and CFA –ve) sera was observed indicating that these children are well exposed to filarial infection. IgG4 antibody levels were also determined in children. Out of 40 antigen –ve children followed after one year, one child acquired microfilariae and others remained normal.

Results:

Prevalence of anti filarial antibodies in different age classes of endemic children (n=216) was determined. *Setaria digitata* adult extract antigen and Dssd1 antigen were also used. It was noted that more than 50% of children were IgG positive by age of 5 years. Children between 2 to 5 years of age are being followed up periodically.

Age -wise distribution of IgG levels in endemic children (n=216).



Status:
Intramural

Investigators:
Dr. M. K. Das
Dr. M. K. Beuria
Dr. M. S. Bal
Mr. N. N. Mandal

Starting date: January 2002
Closing date: January 2005

1.3 A comparison of filarial immune response in people living in different (high and low) endemic regions of Orissa, India

Objectives:

1. To estimate the prevalence of anti-filarial antibody isotypes in area of low and high transmission.
2. Age dependent occurance of some anti filarial (Dssd and lipid) antibodies.

Background Information:

Filarial antigenemia were measured in people living in an apparently low endemic region (Ramchandrapur village, Jajpur dist., Orissa). A high rate of antigenaemia (60%) was observed in the people living in this region. The region is characterized by low rate of microfilariae, chronic filariasis and vector density and vector infectivity rate.

Results:

IgG antibodies to *Setaria digitata* antigenic extract were determined in the 153 sera in above population. IgG positivity was observed in 69% of subjects. IgG levels to filarial lipid antigens were checked in sera of CFA+ve and CFA-ve individuals. It was observed that CFA –ve sera have higher antibody response compared to sera of CFA positive subjects. IgG antibody response to excretory secretory(ES)antigens of adult *Setaria digitata* are being determined in these sera.

Breeding sites of mosquitoes are determined. It was observed that almost one cesspit is present adjacent to every households in the region. The practice of use of mosquito nets among the people is around 50%. Number of elephantiasis cases and microfilaraemic individuals are determined. Among 340 individuals, 4 cases of elephantiasis and one micro filaraemic individual is observed.

Status:
Extramural (DST)

Investigators:
Dr M.K. Beuria
Dr. M.K. Das
Dr. M.S.Bal

Starting date: August 2003
Closing date: July 2005

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

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.

Progress:

Glutathione-S-transferase enzymes are purified from the adult cattle parasite *Setaria digitata* using glutathione agarose column. Serum samples are being collected

from endemic area. The project will be resumed after receiving the funds from Department of Science and Technology (DST), Govt. of India. DST approved the project for funding in August 2003.

1.5 Diagnosis of infection and morbidity in lymphatic filariasis: development of field applicable tools

Status:
Extramural (European Commission)

Investigators:
Dr B. Ravindran,
Dr A. K. Satapathy,
Dr P. K. Sahoo,
Dr. M. Mohanty

Collaborators:
Dr. M. Yazdhanbakh, Leiden
Dr. R. M.Maizels Edinburgh
Dr. R.Noordin, Malaysia
Dr. T. Supali, Indonesia

Starting date: November 2001
Closing date: October 2004

Objectives:

1. Identification of novel recombinant antigens for diagnosis of infection and disease.
2. Identification of antibody reactivity patterns and other immunological markers of morbidity that can identify patients at risk of developing clinical disease.
3. Verification of the dipstick assays as field applicable tools in endemic population affected by *W. bancrofti* and *B. malayi* infections before and after chemotherapy.

Introduction and Background:

Human lymphatic filariasis displays diverse forms of clinical manifestations. It has been assumed that repeated episodes of acute disease could eventually lead to development of chronic forms of disease such as lymphoedema/elephantiasis or hydrocele. Findings in our laboratory have raised the possibility of larval exposure driven development of disease. However, it is not clear what factors (parasite as well as host) contribute to this sequence of progression of the disease. Antibody responses in human filariasis have so far been largely studied by using crude extracts of different developmental stages of filarial parasites. However, several of the filarial antigens have recently been cloned, sequenced and the full-length proteins have been expressed. Thus it is now possible to study antibody responses to specific filarial antigens expressed in one or other developmental stages of the parasites- the current study makes an effort in this direction. Quantification of plasma levels of pro- as well as anti-inflammatory molecules is another approach expected to offer markers of morbidity, which can be used for understanding the pathogenesis of disease in human communities.

Results:

Clinical categorization and selection of morbidity makers of filariasis:

The population in endemic areas displays a spectrum of clinical manifestations of filariasis. Based on the status of microfilaraemia, antigenemia, acute or chronic disease manifestations 207 subjects were classified into 7 categories as shown in Table I. Categories 1,2,3, 6and 7 were homogenous and no overlapping features are observed with other categories. About 30% of patients in categories 4 and 5 representing two different chronic manifestations were found to harbour antigenemia. With a view to search for markers of morbidity, eight pro or anti-inflammatory cytokines were quantified in each of the above

seven categories. The first category of subjects who were free of demonstrable infection and were free of acute (current or history) or chronic filarial disease were taken as control and classified as endemic normals. The remaining 6 categories were classified based on infection status (microfilaraemia/antigenemia) or manifestation of acute or chronic forms of the disease.

In the absence of similar investigations in literature with the exception of TNF-α levels studied by us the selection of 8 immunological markers for the current study was based on similar investigations conducted in other parasitic diseases. The selection of markers was also partly dictated by earlier studies on the effect of antifilarial chemotherapy with DEC in microfilaraemic subjects

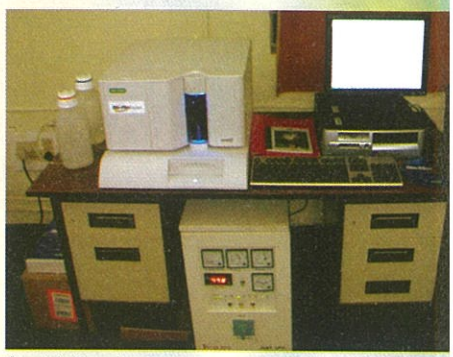
Distribution of different plasma correlates:

Fig. 1 shows box plots superimposed on dot density of raw data (logged values) for 8 different parameters in the 7 chosen categories. It indicates extensive variation in the means (bars with in boxes) with wide scatter between different categories. Fig.2 shows scatter matrix revealing correlations between the 8 variables – this clearly indicate the need for undertaking only multivariate analysis of the data for interpretation of observed values – Fig.2 also shows that the distribution of each the variables is normal with the exception of TNF- α which was found to be partly skewed.

Table II shows the results from a multivariate analysis of variance (MANOVA) of the effects of sex and infection / disease categories on the combined host responses (natural log intensities) for all the 8 parameters. This indicates that when all the seven categories are taken together and analysed, the overall pattern of 8 immunological parameters varied significantly between different categories and that the sex of individuals did not contribute/ influence to the observed variations. The Wilks' Lambda value, which indicates the proportion of variation explained by the factor suggests that categorization of 207 cases may account for approximately 62% of the variation in the 8 chosen parameters in the study. Since categories (as classified in this study) contributed significantly to differences, pair-wise contrasts of the 7 infection/diseases categories were examined based on the combined (multivariate) host responses (natural log intensities). The results of the analysis (Table III) reveal which of the individual categories differed significantly from each other. For e.g., in comparison the Endemic normals (category 1) categories 2,3,4,6 and 7 differed significantly in there combined responses to 8 host response parameters. The table also reveals that the last three categories (5,6 and 7) were similar comparable for the 8 parameters.

The mean and standard error of mean for each of the 8 immune response parameters in all the 7 categories are shown in Table IV and the summary of results of multivariate analysis of pair-wise contrasts of the 7 categories based on the individual immune response parameters (natural log intensities) have been summarized in Fig.3.

The blank boxes in the figure indicate no significant differences; boxes with vertical stripes indicate significant increase and horizontal stripes significant decrease in the levels of a given parameter for each of the comparisons shown. Significantly elevated levels of IL-6 and IL-8 were observed in categories 4,5,6 and 7, all characterized by one or the other forms of clinical filariasis when compared with endemic normals (Category 1); increased TNF-α on the other hand was a feature only in patients with acute disease (categories 6 and 7) and not in patients displaying chronic forms of the disease viz., elephantiasis and hydrocele – TNF-α levels in these patients were comparable to endemic normals. In contrast, the two markers of disease morbidity viz., IL-6 and IL-8 were not significantly elevated in categories 2 and 3 who were asymptomatic but with patent filarial infection – category 2 characterized by presence of circulating Mf and category 3 being amicrofilaraemic but with CFA. On the other hand, these two groups were characterized by significantly decreased levels of circulating sICAM-1 in comparison - sICAM-1 levels in patients displaying clinical symptoms (categories 4,5,6 and 7) were comparable to endemic normals.


(Protein Array)

A clear dichotomy could be observed between the two TNF-a receptors, TNFR-55 and TNFR-75 between different categories as illustrated in Table 5, which shows the statistics of 3 significant canonical variates for the 8 parameters in the study. Canonical varaite is the linear combination of dependant variates that results in the greatest amount of among category to within category variation, and thus provide the greatest power to discriminate between categories. The canonical variate 1 in Table 5 with an Eigen value of 0.7777 accounts for 0.671 of explainable variation and about 0.437 of the total variation in the group of variables studied. Thus for canonical variate 1, the two TNF-α receptors contribute most to 'among category' discrimination. Furthermore, the negative correlation between the two TNF receptors is indicated by the opposite signs viz., standardized canonical coefficient of +0.929 for TNFR 55 and –0.862 for TNFR 75 (Table 5). The inverse relationship of the two TNF-receptors appears to be due to the dichotomy of their levels observed in filarial infection and disease. The TNFR-55 was significantly elevated in patients with disease manifestations, categories 4,5,6&7 (Fig.4a). TNFR-75 on the other hand was significantly elevated in subjects with patent infection, categories 2,3,6 and 30% of patients with antigenemia in categories 4&5(Fig.4b). Thus elevated TNFR-75 emerge as a general marker of patent infection while elevated TNFR-55 appears to be a general marker of filarial disease. While the above conclusions are broadly valid, finer exception could be observed. For e.g., the two forms of chronic filarial diseases were significantly different from each other when the ratio of TNFR-75 and TNFR-55 were analyzed - elephantiasis patients displaying a significantly high ratio in comparison to hydrocele cases (Fig-5). The only host response parameter that failed to discriminate between the 7 categories of human filariasis was the inflammatory molecule, LPS binding protein. Based on the parameters investigated in the present study, the major differentiating markers for infection and different clinical categories of human lymphatic filariasis are summarized in a flow chart (Fig-6).

ONGOING STUDIES

Studies On Filariasis

Table 1: Details of study population*

Categories	EN (1)	AS (2)	CR (3)	EL (4)	HYD (5)	AC+ (6)	AC– (7)
Sample No. (n) Male/Female	27 (15/12)	25 (18/7)	22 (10/12)	24 (8/16)	23 (23/0)	23 (10/13)	63 (32/31)
Age Median (range)	20 (9–	26 (12–65)	35 (10–62)	50 (20–75)	31 (19–65	31 (15–70)	37 (14–82)
Mf status (%)	0	100	0	0	0	ND#	ND#
Mf density /20ul (GMI)	0	6.96	0	0	0	ND#	ND#
CFA Status (%)	0	88	100	29.2	30.43	100	0
CFA density (GMI)	0	26418	974	176	518	1571	0
Clinical symptoms	NO	NO	NO	YES	YES	YES	YES

Fig.1: Box plots of raw data for 8 different parameters in the 7 categories

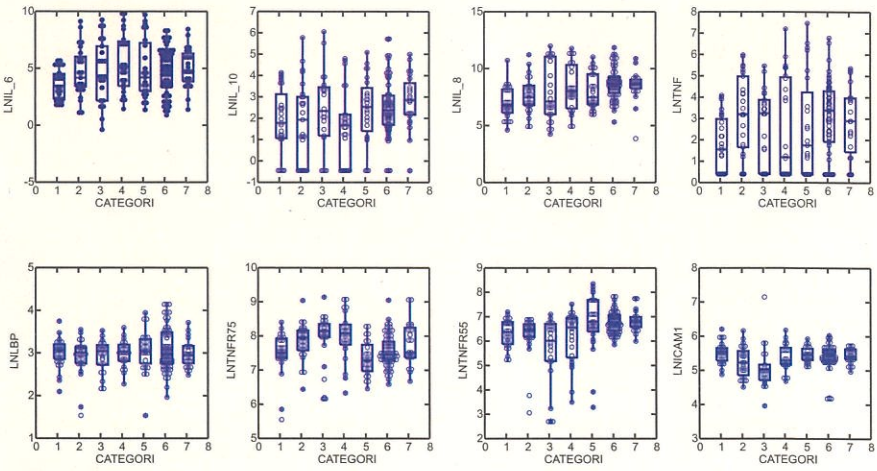


Fig.2: Scatter matrix of raw data revealing correlations between the 8 variables

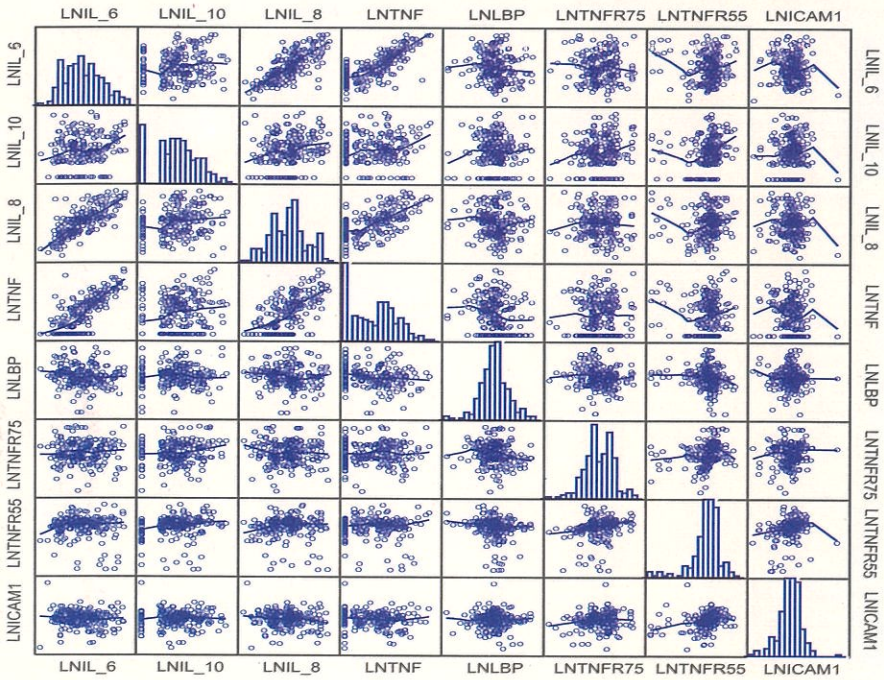


Table II: Multivariate analysis of the effects of sex and infection/disease categories on the combined host filarial immune responses (natural log intensities)

Source	Wilks' Lamda	F	Num Df	Den Df	Pr >F
Sex	0.954	1.12	8	186	0.3529
Category	**0.386**	**4.08**	**48**	**919.26**	**<.0001**
Sex*Category	0.823	0.93	40	813.55	0.5979

Table III: Pair wise contrasts of the 7infection/disease categories based on the combined (multivariate) host filarial immune responses (natural log intensities) examined in the study

Contrast	Wilks' Lamda	F	Num Df	Den DF	Pr >F
1 vrs 2	0.900	2.66	8	192	0.0087
1 vrs 3	0.787	6.49	8	192	<.0001
1 vrs 4	0.851	4.21	8	192	0.0001
1 vrs 5	0.933	1.72	8	192	0.0970
1 vrs 6	0.852	4.16	8	192	0.0001
1 vrs 7	0.916	2.18	8	192	0.0303
2 vrs 3	0.897	2.73	8	192	0.0072
2 vrs 4	0.903	2.59	8	192	0.0104
2 vrs 5	0.842	4.48	8	192	<.0001
2 vrs 6	0.805	5.82	8	192	<.0001
2 vrs 7	0.893	2.83	8	192	0.0047
3 vrs 4	0.893	2.86	8	192	0.0050
3 vrs 5	0.713	9.64	8	192	<.0001
3 vrs 6	0.659	12.4	8	192	<.0001
3 vrs 7	0.790	6.37	8	192	<.0001
4 vrs 5	0.805	5.81	8	192	<.0001
4 vrs 6	0.737	8.57	8	192	<.0001
4 vrs 7	0.869	3.61	8	192	0.0006
5 vrs 6	0.929	1.82	8	192	0.0762
5 vrs 7	0.954	1.15	8	192	0.3310
6 vrs 7	0.959	1.02	8	192	0.4196

Table IV: Mean and Standard Errors of Mean (SEM) of plasma cytokine concentration * in seven categories

CATEGORI		IL-6	IL-10	IL-8	TNF-α	LBP	TNFR 75	TNFR 55	ICAM 1
Endemic	Mean	71.37	15.58	3102.95	12.55	20.36	2012.93	606.97	252.42
normal	SEM	16.21	3.72	1497.49	3.15	1.29	173.09	60.68	15.51
Mf+	Mean	755.90	28.17	6572.81	82.13	19.27	2777.47	594.61	201.59
	SEM	372.36	12.55	2832.29	22.32	1.34	296.71	52.27	18.52
Cryptic	Mean	1216.09	43.53	24911.10	40.10	19.28	3358.53	445.32	200.44
	SEM	521.44	20.14	8423.63	12.11	1.37	395.59	73.40	51.02
Elephantiasis	Mean	2363.93	18.60	19871.05	113.59	20.13	3538.33	658.48	224.42
	SEM	956.67	6.9415	6658.85	53.93	1.22	449.02	94.13	18.67
Hydrocele	Mean	1872.83	27.14	8828.18	163.66	22.76	1714.60	1306.72	237.08
	SEM	776.03	7.84	2861.55	79.19	2.25	198.29	237.11	11.15
Acute CFA -ve	Mean	456.27	28.90	12753.77	65.88	24.27	2095.86	902.84	229.93
	SEM	96.43	6.91	2772.30	17.22	1.68	152.08	53.82	8.14
Acute CFA +ve	Mean	521.82	30.41	8145.44	46.26	20.02	2788.76	951.30	226.05
	SEM	202.18	7.44	2275.38	12.76	1.45	416.01	93.50	10.06

*; All parameters in pg/ml except sICAM1shown as ng/ml

Fig.3

		IL-6	IL-10	IL-8	TNF-α	LBP	TNFr75	TNFr55	ICAM1
EN vs.	Mf+				■		■		▒
	CR						■	▒	▒
	Ele	■		■			■		
	Hyd	■		■					
	AC-ve	■		■	■				
	AC+ve	■	■	■	■			■	
Mf +ve vs.	CR							■	
	Ele								
	Hyd						▒	■	■
	AC-ve			■				■	
	AC+ve		■					■	
CR vs.	Ele							■	■
	Hyd						▒	■	■
	AC-ve						▒	■	■
	AC+ve							■	■
Ele vs.	Hyd						▒	■	
	AC-ve		■				▒	■	
	AC+ve		■					■	
Hyd vs.	AC-ve								
	AC+ve						■		
AC-ve vs.	AC+ve								

▒ Decreased ■ Increased

(Flow cytometre)

Fig.4a: Levels of TNFR-75 and TNFR-55 in Bancroftian Filariasis

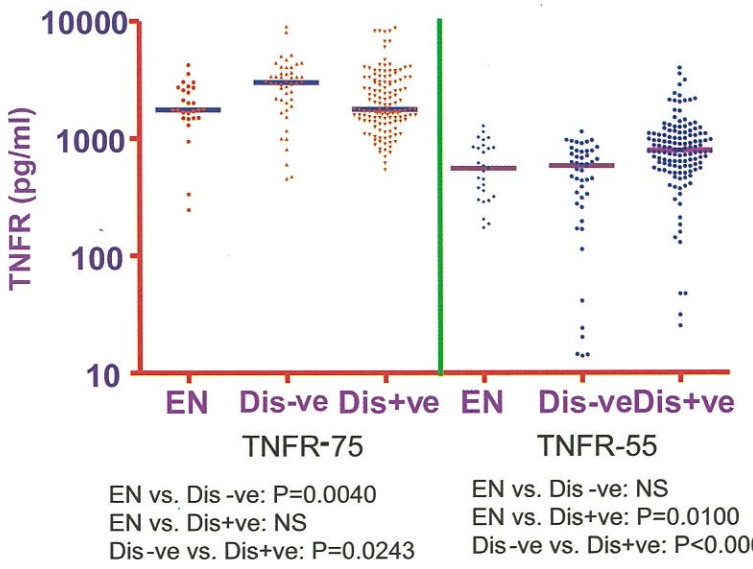


Fig.4b: Bancroftian Filariasis: Levels of TNFR-75 and TNFR-55 in presence or absence of active infection

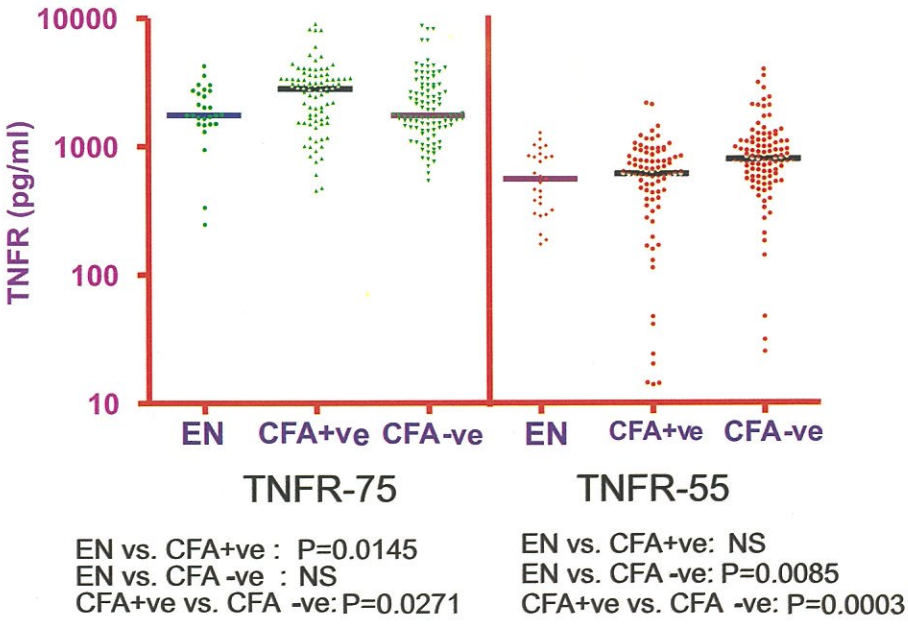


Table V.: Canonical analysis showing the parameters and statistics of the significant 3 canonical variates for the filarial immune response data (natural log intensities)

Standardized canonical coefficients

	Il-6	Il-8	Il-10	TNF-α	TNFR75	TNFR55	LBP	ICAM1
Can1	-0.513	0.147	0.348	0.383	**-0.862**	**0.929**	0.364	0.419
Can2	**-0.796**	0.144	**1.007**	0.474	0.382	0.074	0.203	-0.426
Can3	**1.186**	-0.234	0.309	**-1.087**	0.330	0.166	0.184	0.253

Fig.5:
TNFR-75: TNFR-55 ratio in filarial chronic disease manifestations

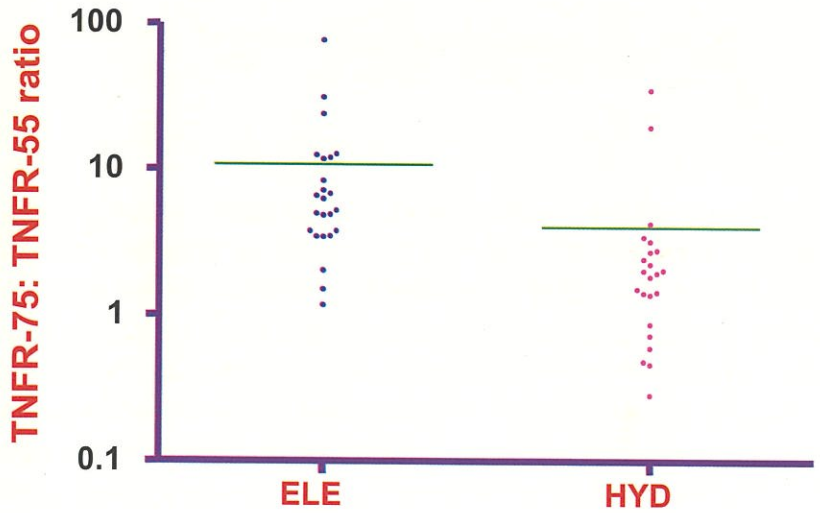
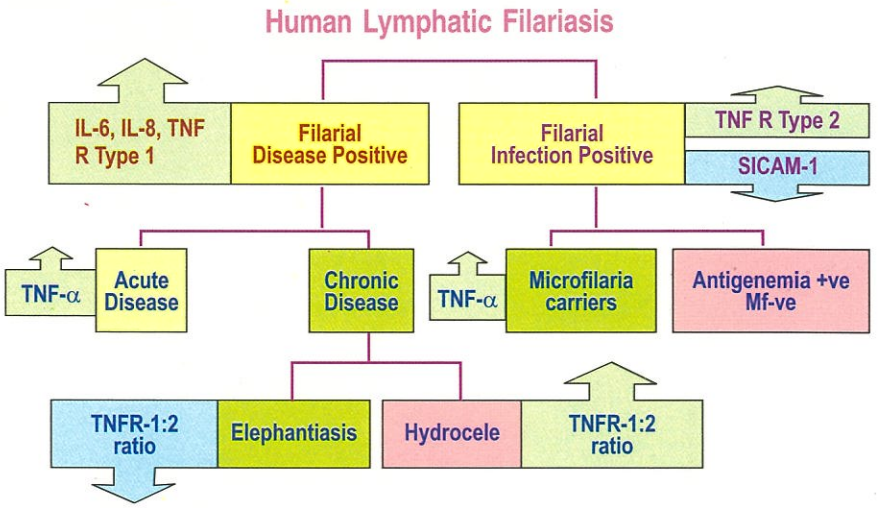


Fig.6:

Summary status of discriminating host immune response parameters in 7 categories of Bancroftian Filariasis – the levels shown are with reference to values in Endemic normals *



Infected subjects in endemic areas can be classified into two categories based on presence and absence of adult worms and /or microfilariae. Mf carriers are those with both adult worms as well as Mf in circulation. They are designated here as AS cases. Detecting circulating filarial antigens, which are metabolic products of adult worms, can identify subjects harboring only adult worms without circulating Mf. They have been designated here as cryptic (CR) cases. In the current set of investigations we have addressed the issue of antimicrofilarial immunity in CR cases in comparison to Mf carriers. The working hypothesis is that Mf produced by the lymphatic dwelling adult parasites could be getting eliminated in CR cases. Since antibodies to Mf sheath was investigated in both the groups since they have been incriminated in anti-microfilarial immunity (Table VI). Although there was a statistically significant difference between the two groups, a large number of sera of CR cases were free of anti-sheath antibodies indicating that other responses could be responsible for absence of Mf in these cases. Levels of IgG antibodies to crude filarial antigen (prepared from *S.digitata* or *B.pahangi*) were significantly more in CR cases as compared to Mf carriers (Figs.7a & 7b). More importantly, the difference between the two groups were significant in IgG 1,2, and 3 sub-class and not in IgG4 levels (Fig.8). When IgG and IgA levels to five different filarial recombinant proteins were analyzed between the two groups, IgG levels to cystein protease inhibitor-2 (CPI-2, an adult surface antigen),

Serpin-2 (SPN-2, an Mf stage specific antigen) and VAL, a larval stage antigen were significantly more in CR cases in comparison to Mf carriers (Fig.9a & 9b). IgA levels to two other larval antigens, ALT-1 and ALT-2 were significantly more in CR cases. These results indicate that IgG responses to some adult and Mf antigens and IgA responses to larval specific antigens could be playing a significant role in maintaining the CR status of subjects with only adult worms. In isotypes analysis shows the IgG3 antibody to ALT-1, ALT-2 and SPN-2 are significantly higher in CR than AS cases whereas IgG-1 antibody to CPI-2 is significantly more in CR cases (Fig.10a, 10b, 10c & 10d). Longitudinal follow-up of CR subjects revealed the stability of these subjects to maintain the CR status without acquiring Mf during the follow-up period of up to three years as shown in Table VII.

Table VI: Anti-sheath antibody between AS and CR cases

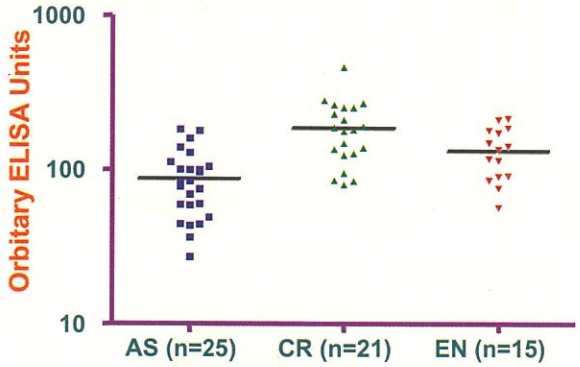
	AS	CR
ASAB+VE	2	10
ASAB-VE	57	52
	P=0.0191; χ^2=5.492	

Table VII: Mf status in cryptic cases on longitudinal follow-up

	Time '0' (%)	18 Months (%)	36 Months (%)	Total (%)
Cohort A	0/24 (0)	1/24 (4.16)	ND	1/24 (4.16)
Cohort B	0/33 (0)	ND	1/33 (3.0)	1/33 (3.0)
Total	**0/57 (0)**	**1/24 (4.16)**	**1/33 (3.0)**	**2/57 (3.508)**

ND → Not Done

Fig.7a: IgG antibody to *S.digitata* in Asymptomatic microfilaria carrier, patients with cryptic infection and EN.



AS vs. CR - P=0.001; t=4.919
AS vs. EN - P=0.0046; t=3.011
CR vs. EN- P=0.0046; t=2.128

Fig.7b: IgG antibody to *B.pahangi* in Asymptomatic microfilaria carrier, patients with cryptic infection and EN.

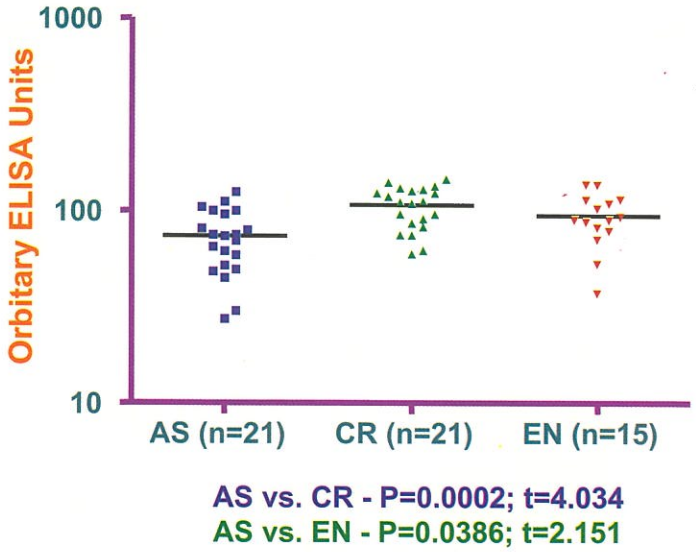


Fig.8: IgG isotypes (IgG1, G2, G3 & G4) antibodies to *Setaria digitata*, in patients with asymptomatic microfilaria and cryptic infection.

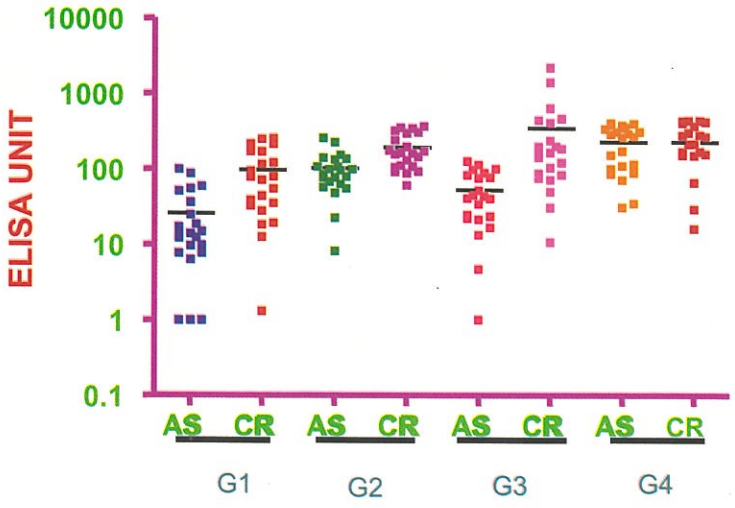
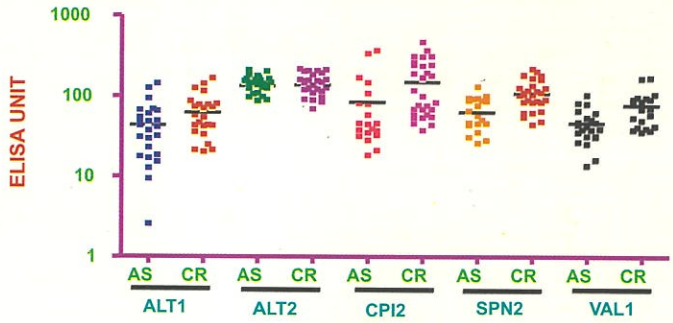
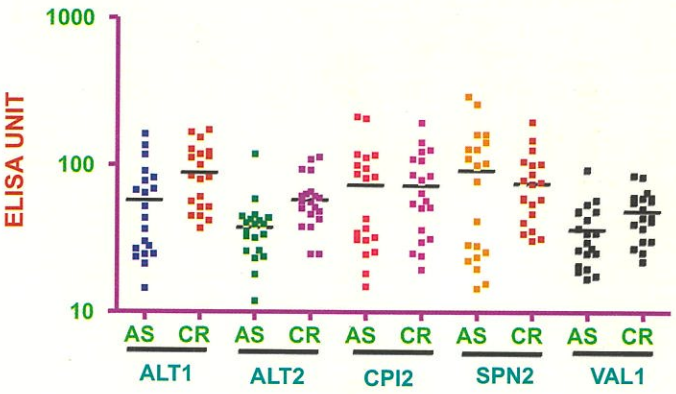


Fig.9a: IgG antibodies to five different recombinant proteins such as ALT 1, ALT 2, CPI 2, SPN-2 and VAL-1 in clinical categories of AS and CR cases



Statistics:
CPI2 - P=0.038, t=2.135
SPN2 - P=0.0003, t=3.932
VAL1 - P=0.0021, t=3.280

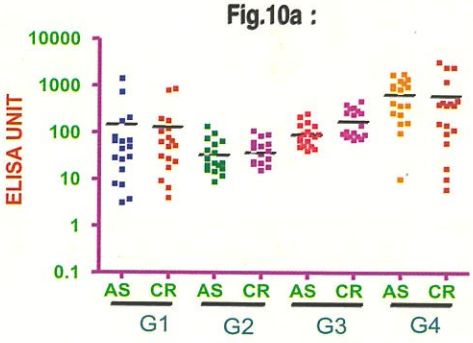
Fig.9b: IgA antibodies to five different recombinant proteins such as ALT-1, ALT-2, CPI-2, SPN-2 and VAL-1 in clinical categories of AS and CR cases



Statistics:
ALT1 - P=0.0225, t=2.379
ALT2 - P=0.0069, t=2.859
VAL1 - P=0.0448, t=2.077

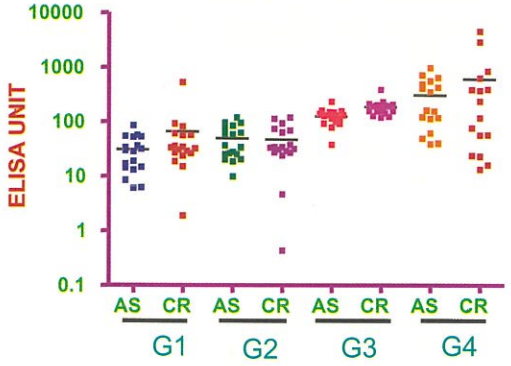
IgG1, IgG2, IgG3 and IgG4 antibodies to different recombinant proteins such as ALT-1(a), ALT-2(b), SPN-2(c), and CPI-2(d) in clinical categories of AS and CR cases

Fig.10a :



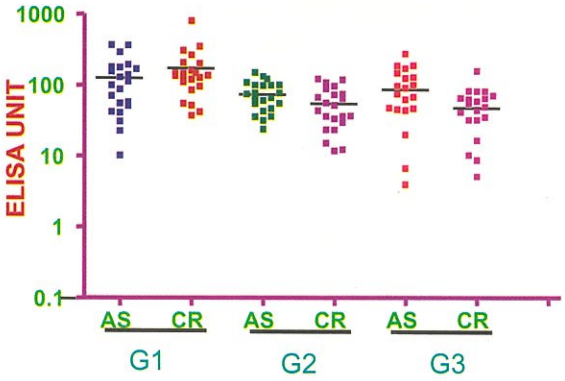
IgG3 - P=0.0085, t=2.793

Fig.10b :



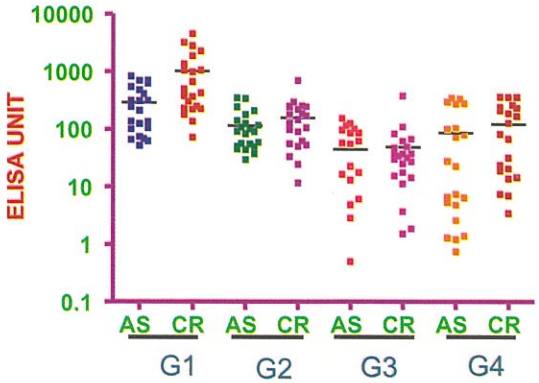
Statistics:
IgG3 - P=0.0011, t=3.558

Fig.10c :



Statistics:
IgG3 - P=0.0266, t=2.298

Fig.10d :



Statistics:
IgG1 - P=0.0060, t=2.895

1.6 Role of IgA in Protective Immunity in Human and Experimental Filariasis

Status:
Intramural

Investigators:
Dr. B. Ravindran
Dr. A. K. Satapathy
Dr. P. K. Sahoo
Dr. M. C. Mohanty
Mr. B. R. Sahoo.

Starting date: January 2002
Closing date: December 2004

Objectives:

1. To correlate filarial IgA levels with clinical spectrum of Filariasis.
2. To correlate Filarial IgA levels with gender and duration of exposure to infection.
3. To identify by immunochemical analysis IgA inducing filarial antigens using as probes sera of putatively immune subjects.

Introduction and Background:

Putatively immune endemic normals (EN) and patients with chronic filarial disease (CH) such as hydrocele and/or lymphedema represent two poles of the clinical spectrum of human lymphatic filariasis. Curiously however, the immune response phenotype in both these two groups have been found to be essentially very similar, the exception being CH patients with active filarial infection (demonstrable by circulating Mf and/or filarial antigens, CFA). Endemic normals and vast majority of CH cases display immunological hyper-reactivity characterized by enhanced filarial specific T-cell proliferation, IFN-γ production, raised levels of parasite specific IgG2, IgE, decreased levels of IgG4 and presence of antibodies to Mf sheath in comparison to infected subjects who display immunological hypo-responsiveness. The above immunological features however fail to differentiate EN cases (asymptomatic, amicrofilaraemic subjects without antigenemia) from patients with chronic pathology and thus these features appear to be essentially a 'result' rather than the 'cause' of EN and CH status in endemic population. Identifying an immunological marker in EN is crucial for understanding the nature of protective immunity and for a rational approach towards development of vaccines for human filariasis. Although serum IgA constitutes nearly 15-20% of total immunoglobulins in humans, no serious effort has been made so far to study the status of IgA in human filariasis in the context of protective immunity.

IgA in generally considered to be an antibody isotype with a role in mucosal immunity. However, the importance of serum IgA in systemic helminthic infections is being increasingly realized since two high affinity receptors for IgA (Fc α) are found on the cell membranes of eosinophils, which is considered as important effector cell in helminth immunity. Earlier using crude filarial antigens we had demonstrated a significantly elevated filarial IgA levels only in putatively immune endemic normals in comparison to infected subjects and patients with chronic disease (Fig. 1). More significantly, the total serum IgA1 levels were found to be significantly decreased in Mf carriers indicating predisposition of such individuals to acquire patent infections (Fig 2).

Fig.1: IgA antibodies to somatic antigen in clinical groups

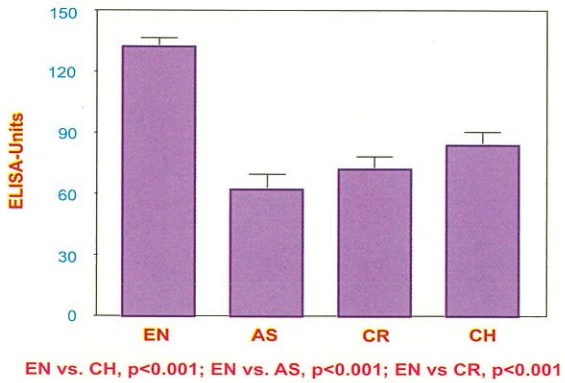
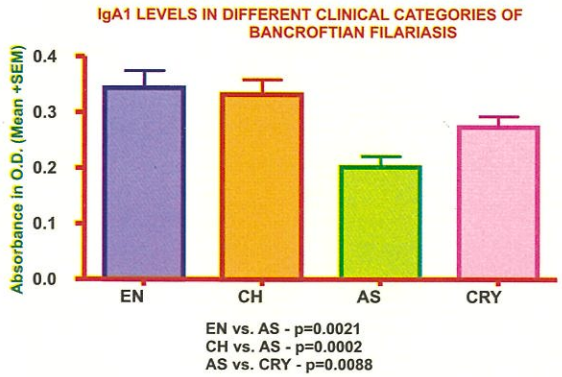


Fig.2: IgA 1 Levels in different clinical categories of bancroftian filariasis.



Status:
Extramural (ICMR Task Force on Parasite Immunity)

Investigator:
Dr. B. Ravindran

Collaborator:
Dr. Vineeta Bal,
National Institute of Immunology,
New Delhi

Starting date: January 2003

Closing date: December 2005

1.7 Innate Immune recognition of filarial parasites by phagocytes

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*.

Introduction and Background:

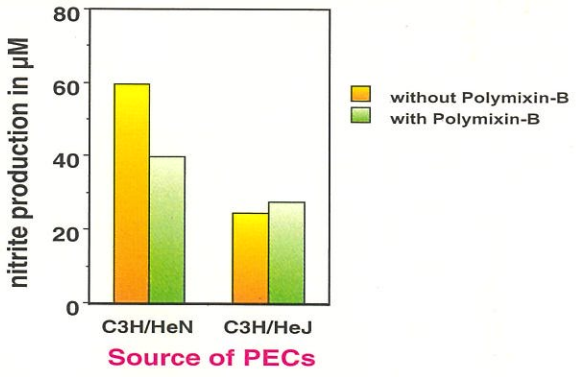
Most of the investigations in both human and animal models of filariasis generally address adaptive immunity and innate mechanisms if any are yet to be investigated. There are instances in animal models of differential susceptibility to filarial infections and it is presumed that innate mechanisms could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

Results:

Initial 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 earlier 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-2^d) 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 preliminary 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].

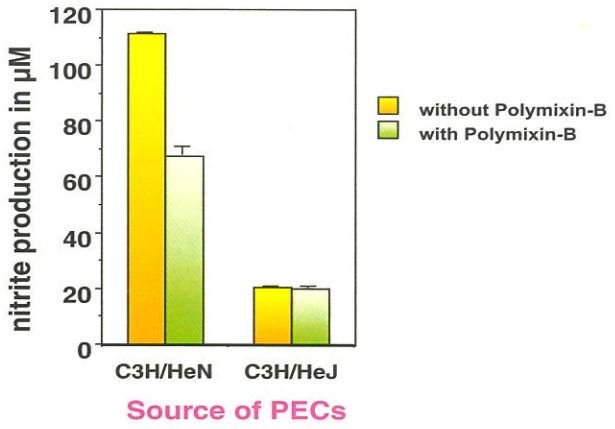
Fig: 1



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.

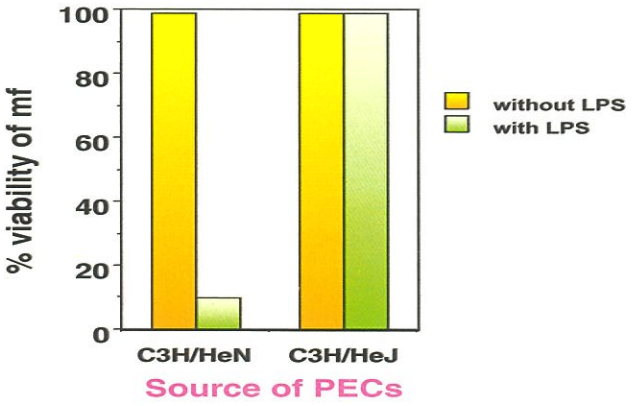
Fig : 2



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. More work needs to be done in this aspect.

Fig : 3



While attempting to characterize these effects of mf extract or AgW on macrophage activation and clearance of mf in LPS-responder and LPS-resistant strains of mice, we are attempting to extend our work to include BALB/c and DBA/2 mice based on preliminary mf clearance data. It appears that there is a potential to explore the differences in macrophages filaricidal activity, NO-producing ability etc. in these strains.

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

Status:
Extramural (Indo-German Project)

Investigators:
Dr.B.Ravindran
Dr. S.K.Kar

Collaborator:
Dr.Achim Hoerauf,
Bernhard Nocht Institute of Tropical Medicine,
Hamburg, Germany

Starting Date: March 2003
Closing Date: February 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.

Introduction and Background:

Single annual dose administration of Diethylcarbamazine to the entire population in filariasis endemic areas has been recommended and being practiced in several countries including India for blocking transmission of infections in human communities. One of the major practical problems faced in this strategy is 'post-drug reactions' observed in a small percentage of subjects. However this small percentage could account for a large number of individuals when the drug is distributed to millions of individuals on a given day. The actual cause of these reactions is yet to be understood – some of the investigators have reported the reactions only in subjects with high Mf density and not in sublects with low or no Mf in circulation. Based on this it has been proposed that the dying Mf release 'toxic molecules' and could trigger such reactions. The discovery of endosymbionts Wolbachia in filarial parasites has offered an opportunity to study their role in post DEC reactions. Wolbachia are Rickettcia like organisms possess lipopolysaccharides, (LPS) generally found in Gram-negative bacterial cell wall and are responsible for bacterial endotoxaemia. LPS are known to mediate inflammation by stimulating a cascade of reactions involving phagocytic cells. Unlike filarial parasites, which are resistant to several antibiotics, Wolbachia are easily eliminated by treatment with Tetracyline/Doxycycline. The current project proposes to study the role Wolbachia in mediating 'post- DEC reactions' observed in Mf carriers.

Results:

An adverse reaction following administration of the anti-filarial drug Diethylcarbamazine (DEC) has been attributed in the past to microfilaricidal activity in the host. The discovery of endosymbiont Wolbachia in filarial parasites has opened up the possibility that post-DEC reactions could be mediated by Wolbachia and this project attempts to address this issue. The work in this project involved identification of Mf carriers in filariasis endemic villages to recruit them for the study and to treat them with Doxycycline, which potentially would free the Mf of endosymbionts. To this end a population of 434 subjects in an endemic village were screened of which 60 were found to harbor Mf in circulation. The detailed results of the survey are shown in Table 1 below:

Table - 1

	Mf +ve(60)		AC Dis(19)		Ele(9)		Hyd(40)		CRY(61)		HYD + Ele(4)		EN(129)	
	+VE	-VE	+VE	-VE	+VE	-VE	+VE	-VE	+VE	-VE	+VE	-VE	+VE	-VE
BP(n=322)	60	0	*0	19	4	5	9	31	61	0	0	4	0	129
BN(n=112)	11	0	**00	11	1	7	11	36	15	0	0	0	0	20

The presence of adult worms in the hosts was tested by quantitation of circulating filarial antigens (CFA) and in about 95 subjects ultrasound examination was undertaken to locate actively motile filarial worms, viz., filarial dance sign (FDS). FDS was performed using a portable ultrasound unit with a 7.5 transponder and fitted with a dopler.

The three parameters viz., Mf, CFA and FDS were compared for their relative sensitivity and specificity since all the three parameters would be utilized to score the effect of treatment with Doxycycline on adult filarial worms. FDS was positive in 56 % of microfialraemic cases and 39.50 % of antigenemic cases. Detailed results of the comparisons are shown in tables 2, 3 and 4 and 5 below:

Comparison of ultrasound (FDS), Mf and CFA in the study samples

Table-2

	Mf	
	+VE	-VE
Ultrasound +VE	22	11
Ultrasound -VE	17	31

* 22/39 =56.41% of Mf +VE individuals were FDS +VE
** 11/42=26.19% of Mf –VE individuals were FDS +VE

Table - 3

	CFA	
	+VE	-VE
Ultrasound +VE	32	2
Ultrasound -VE	49	8

* 32/81 = 39.51% of CFA+VE individuals were FDS +VE

** 2/8= 20% of CFA-VE individuals were FDS +VE

Table - 4

	HYD	
	+VE	-VE
Ultrasound +VE	8	28
Ultrasound -VE	29	26

Statistics: $X^2 = 8.041$ ($P<0.01$)

Table – 4 above indicates that presence of Hydrocele possibly hinders detectability of FDS; however if subjects who were only positive for CFA were tested for ultarsound, presence or absence of hydrocele did not significantly influence sensitivity of FDS as shown in Table – 5 below:

Table - 5

	CFA (n=81)	
	With Hyd	Without Hyd
Ultrasound +VE	9	25
Ultrasound -VE	20	27

Statistics: 9/29 vs. 25/52, $X^2 = 2.216$ (NS)

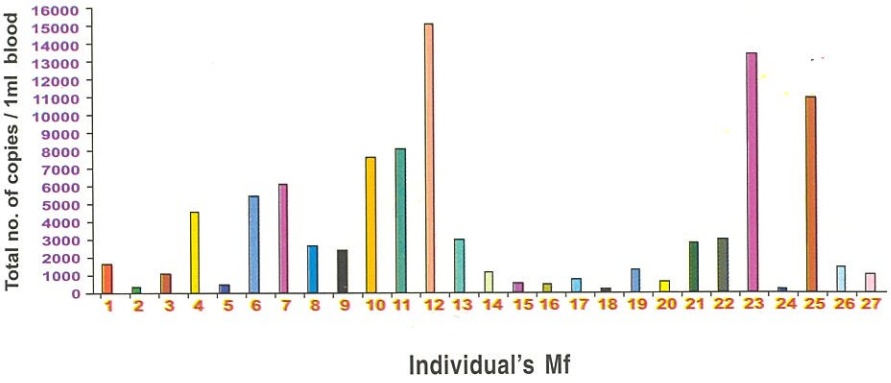
Thirty of the Mf cases have been selected based on inclusion and exclusion criteria and will be treated with Doxycyline to eliminate endobacteria.

ONGOING STUDIES

Studies On Filariasis

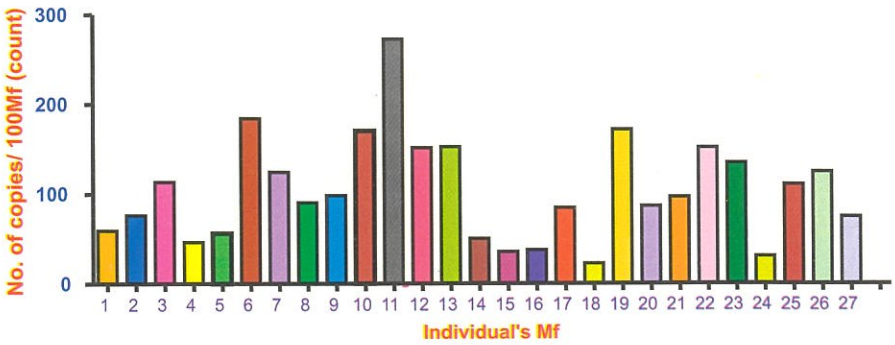
Quantitative PCR has been performed in Mf isolates collected from 27 carriers and the absolute Wolbachia copy number per ml of blood of each of the cases has been calculated with a view to correlate them with post-DEC reactions. By taking into consideration the Mf density in each of the individuals the absolute Wolbachia load in them was calculated and the results are shown in Fig 3 below:

Fig.3: Quantitation of Wolbachia DNA by Real Time PCR



The results of Wolbachia copy number in 100 Mf of each of the cases is shown in Fig-4 below:

Fig.4



The results of treatment with Doxy followed by DEC and the overt and sub-clinical reactions will be monitored in 30 Mf carriers during the next 4 months and the results will be shown in the next year's annual report.

1.9 Identification of serum immunosuppressive factors in human filariasis

Status:

Intramural

Investigators:

Dr A.K.Satapathy

Dr. P.K.Sahoo

Dr. B.Ravindran

Starting date: February 2002

Closing date: January 2005

Objectives:

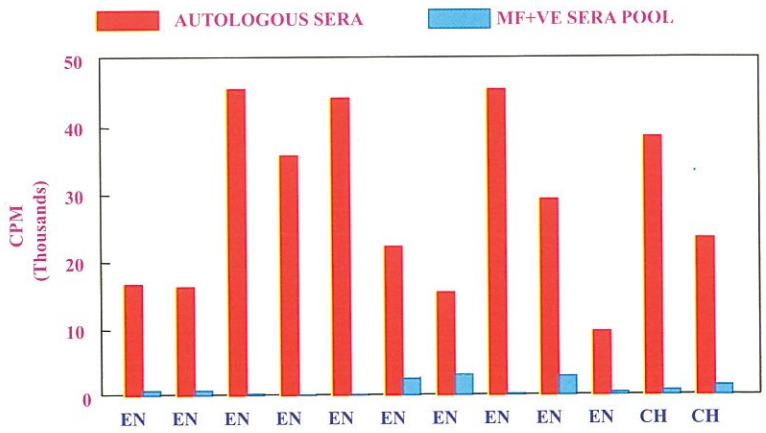
1. To identify the immunosuppressive factors in sera of microfilaraemic subjects.
2. To correlate the degree of immunosuppression with presence/intensity of infection with adult stage parasite.

A hyporesponsive state characterized by low T-cell proliferation and down regulated IFN-α production has been observed in microfilaraemic individuals. The hyporesponsive state in microfilaraemic individuals has been shown to be largely specific for filarial antigens. The proliferative response to non-filarial antigens (PPD) has been found to be similar in both microfilaraemic and amicrofilaraemic individuals. However the mechanism by which hyporesponsive state in microfilariaemic individual maintained is largely unknown. One of the objectives of this study is to study serum-mediated suppression of T-cell proliferative response in filariasis. Phytohaemagglutinin (PHA) mediated proliferation of T-cells was not inhibited by sera of amicrofilariaemic individuals. However individual as well as pooled sera of Mf carrier cases inhibited PHA induced T-cell proliferative response. About 80% of PHA induced T-cell proliferative response. (Fig-1). The mf +ve serum mediated inhibition was demonstrable in PBMC collected from different amicrofilariaemic individuals (Fig-2). The percentage inhibition varied between Mf carriers sera indicating a role of serum factor(s) in mediating suppression of PHA induced T-cell proliferation. The less inhibitory sera were found to behave similarly when cultured with peripheral blood mononuclear cell of different cell donors.

The nature of immunosuppressive factors in microfilariaemic sera is being analyzed. The serum inhibitory factor was resistant to heat treatment at 560C for 30 mins indicating a lack of role for complement components.

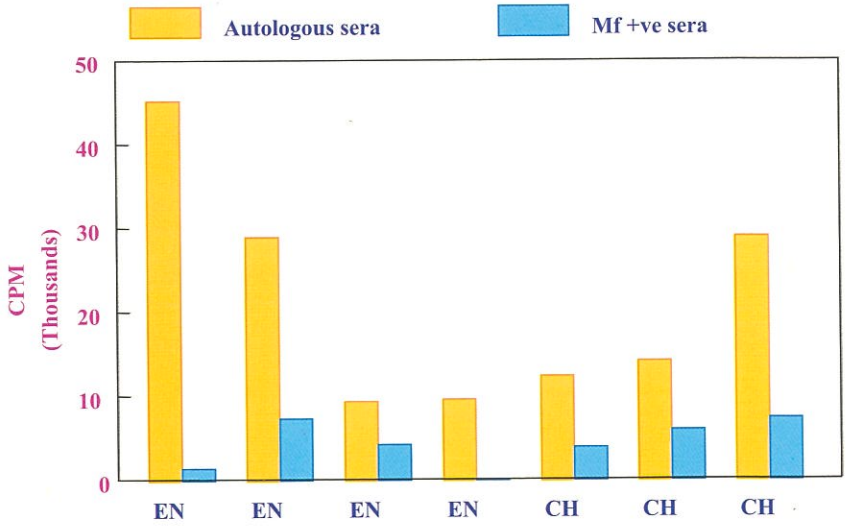
The possibility of the observed inhibition being mediated by induction of nitric oxide (NO) was tested. Aminoguanidine, a known inos inhibitor failed to reverse serum mediated inhibition (Fig-3) indicating that inhibition of proliferation is not mediated by induction of NO *in vitro*.

Fig.1 Inhibition of PHA induced T-cell proliferation by sera pool of Mf carriers



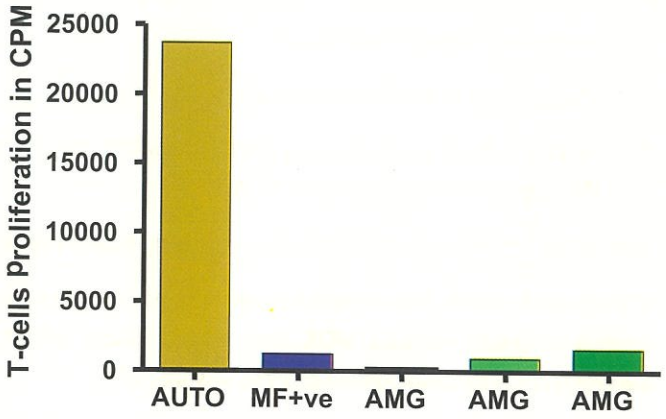
% inhibition	95.9	95.0	99.6	99.8	99.9	89.0	80.47	99.7	90.4	96.2	98.2	93.9

Fig.2 Inhibition of PHA induced T-cell proliferation by sera of different Mf carriers



% inhibition	96.7	74.6	55.0	99.5	68.7	58.0	74.6

Fig.3 Aminoguanidine , inos inhibitor does not reverse serum mediated Inhibition



1.10 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 (WHO/TDR)

Investigators:
Dr. B.V. Babu
Dr. A.S. Kerketta
Dr. A.S. Acharya
Ms. K. Rath
Dr S.K. Kar

Collaborator:
Prof. D.K. Behera,
Sambalpur University

Starting date: July 2003
Closing date: June 2006

Purpose of the study:

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).

Phases of the study:

The study is planned to undertake in two phases. The phase-1 is formative phase and the phase-2 includes intervention, mass drug administration (MDA) and evaluation.

Formative Phase

Objectives of the formative phase:

Broad objective:

Explore and identify opportunities within urban communities, which would help design innovative urban-specific intervention strategy for MDA for elimination of LF.

Specific objectives:

1. Describe the demographic, socio-economic, political and cultural structure and relationships within the community.

a. Study the age/sex/educational/occupational/religious/caste/social networks of community residents.

b. Understand the formal and informal structures of decision making for health and development issues.

c. Study formal and informal channels of communication.

d. Identify subgroups requiring special intervention relevant to the development process towards MDA.

2. Assess the felt needs of the community (including health needs).

3. Identify stakeholders, and understand their roles and assess their perceptions towards lymphatic filariasis, MDA, drug related issues, and their potential involvement in the development process towards MDA.

4. Identify and understand functioning of various ongoing health and development processes / activities (resource mobilization) carried out by GOs/ NGOs/CBOs/private sector in the urban community.

5. Assess the knowledge and perceptions of the community towards LF and their perceptions and experiences (if any) with MDA.

Study site

The study was undertaken in an urban area, Choudwar. Choudwar is a municipality in Cuttack district. Cuttack is one of the coastal districts of Orissa, which are endemic for LF. As per the Census of India (2001), the population of Choudwar town is 52,498 of which 28,243 (53.8%) are male and 24,255 (46.2%) are female.

Study design for formative study:

Sampling frame:

Strata: Information from the sample should be representative of the studied urban area. For sampling purpose, the urban wards are divided into four strata, i.e., high income group (HIG), middle income group (MIG), low income group (LIG-1) and slums (designated as LIG-2). HIG wards are those, which have more than 60% of HIG houses. The same principle is applied for the identification of MIG and LIG-1 wards. The list of slums is obtained from the municipality authorities, to identify LIG-2 areas.

Selection of households from HIG, MIG and LIG-1 strata: After categorizing all the wards into HIG or MIG or LIG-1, two wards are selected from each stratum on a random basis. Having listed all colonies/streets in a ward, five colonies/streets are selected on a random basis. In each colony/street, a random point is selected and from there ten consecutive households are selected for the study.

Selection of households from slums: The list of wards containing slums is prepared on the basis of the list obtained from the municipality. From the compiled list, 10

slums are selected on random basis. From each area, 10 households are selected randomly from five random points.

Quantitative and qualitative approaches:

Both quantitative and qualitative data collection techniques are used in the present study. The quantitative data are obtained through household questionnaire survey. All the sample households from four strata are included for household questionnaire survey. A minimum of 100 households from each stratum is sampled. The qualitative techniques include focus group discussions (FGDs), in-depth interviews and free listing.

A total number of 15 FGDs are conducted, by covering at least 3 from each stratum. FGDs are conducted among members of youth club, members of women group and general community members. In-depth interviews of key-informants are undertaken from all the strata. A total of 37 key-informant interviews are conducted by taking at least 6 key-informants from each stratum. The key-informants are elected representatives (municipality councilors), community leaders, private practitioners, paramedical professionals, media persons, representatives of CBOs and NGOs. The free-listing exercise is done with key-informants from all the strata. Two case studies are recorded in order to narrate the success stories of community participation.

(Community mobilization activity)

Methods of Analysis:

Quantitative data: The quantitative data collected through household survey are processed and analyzed through SPSS. V. 10.

Qualitative data: The FGDs and interviews were transcribed and translated. The analysis was done by using ATLAS/ti for Windows V.4.1. The free listing data were analyzed through Anthropac V. 4.97.

The results of the formative phase are presented under the following heads:

- Communities' Experience – Past and Present
  - Profile of urban communities (attitude & social interaction reflecting the groups in the community)
  - Felt needs and perceptions of problems among different communities and their priorities
  - Community participation and involvement in urban areas (health and development programmes, and cultural activities, social interaction, people's participation, and community involvement)
  - Conflicts and cooperation within social interaction (social interaction, people's participation, and community involvement)
  - Knowledge and perceptions on LF (Felt needs, perception of the problem, awareness of MDA)

- **Role of Health programmes and services to enhance community strategy**
  - Health services for preventive and curative services
  - Community involvement and participation in health programmes
- **Opportunity for linkages and potential for community strategies**
  - Stakeholders' suggestions regarding MDA
  - Stakeholders' suggestions regarding health services and community involvement
  - Motivation of community and communication strategies
  - Stakeholders' involvement, and Enablers and incentives
- **Challenges Ahead: Suggested Strategy for MDA**
  - Master strategy with sub plans for strata
  - Stakeholders' involvement in planning and decision-making
  - Advocacy
  - Initiating CD methods
  - Partnership
  - Drug delivery

Proposed Phase 2: Intervention, Drug Distribution and Evaluation

Objectives:

1. To test an intervention strategy that addresses the challenges for MDA in urban areas, building on an inclusive partnership framework developed on the basis of the research findings on the above objective, and involving in particular the private practitioners and active CBOs.
2. To evaluate the impact of this intervention strategy on perceived need of, and in enhancing support for, MDA amongst all stakeholders including the community, health workers and municipal officials.
3. To describe the preparatory and mobilization process as developed by the stakeholders, and to assess its strengths and weaknesses.
4. To describe the drug distribution process as developed by the government in consultation with the stakeholders, and to assess its strengths and weaknesses.
5. To evaluate the treatment coverage (consumption rate) achieved with the new strategy, and to assess whether after three years of intervention it reaches the desired level of treatment coverage with DEC/Alb that is required for elimination of LF.

6 To determine the feasibility of implementation of the new strategy using existing human resources (health and other sectors) at the municipal and community level.

7 To document the contributions made by various stakeholders and to determine the cost of the new strategy.

Processes of intervention:

Stakeholders' active involvement in planning and decision-making: The results of baseline survey indicate conduciveness of community participation and involvement of partners. Hence, it is proposed to test a health services initiated and driven MDA strategy with inclusive partnership approach and community participation. As it is a government initiated and driven programme, municipality or health department of the municipality will play a key role. The other potential stakeholders to be involved are private practitioners, active community based organizations (CBOs) (like youth clubs, women clubs (self-help groups), residence associations, non-governmental organizations (NGOs), important politicians, health institutions (private, industrial, charitable, etc.), industries, media, religious organizations and heads, school head masters, leaders of ethnic groups, etc. All the stakeholders will be briefed on finding of baseline survey of this project. Also they should be made aware of low priority to the disease, presence of disease in all strata, risk of acquiring disease, concept of MDA, etc.

(Participatory research approach)

This group will form like a steering committee and meet periodically. It will discuss on the issues to be done and issues to be decided. It will take decisions on programme planning, approaches to social mobilization and implementation of the drug distribution. The decisions will be taken under the responsibility of the government health system. It will be attempted to obtain consensus among the stakeholders before initiating the intervention through nominal group technique (NGT) or modified Delphi, as a possible consensus technique.

Advocacy: Advocacy has to be planned and implemented addressing both the population and partners of the programme. Advocacy is to be addressed to identify and include more potential stakeholders, and to further motivate the existing partners. The stakeholders should feel as the managers of the programme. The stakeholders should also involve in advocacy of the programme among the community, particularly, among which the stakeholder has more influence.

Initiating the intervention: The steering committee after developing the plan for the entire urban area, the committee (stakeholders), should identify the local partners (preferably ward-wise) such as CBOs and NGOs. All explorations should be made to identify appropriate groups, suitable to undertake community mobilisation and drug distribution processes. The strengths and weaknesses of these groups should be assessed by the steering committee, by considering the activities to be undertaken. These groups should be motivated to involve in the programme. The ward level groups

should be prepared to undertake community mobilisation and drug distribution with the help of other stakeholders and volunteers. This needs the profiling of the community including the details of population, resources needed, resources available, etc. This can be done in consultation with other community members. The actual activities will be carried out by the group with the assistance of stakeholders.

Partnership: The stakeholders involve in various stages of the intervention as mentioned above. Also these stakeholders involve in mobilization of resources. The micro-level planning (methods of MDA, date, time, duration, possible engagement of DDs, etc.) will be decided by the stakeholders (including ward level CBOs). In addition, these sub-groups (like religious and linguistic minorities, scheduled castes, scheduled tribes, etc.), which will be identified by the stakeholders will be dealt separately with some special strategies.

Special strategies:

Sub-groups approach for more coverage: While dealing the sub groups during community mobilization and drug delivery, special strategies should be employed to achieve higher coverage.

Use of private practitioners: The services of private practitioners can be incorporated in various stages of intervention. The services can be utilized during community mobilization and training. Also their assistance can be taken for management of side reactions along with the government system.

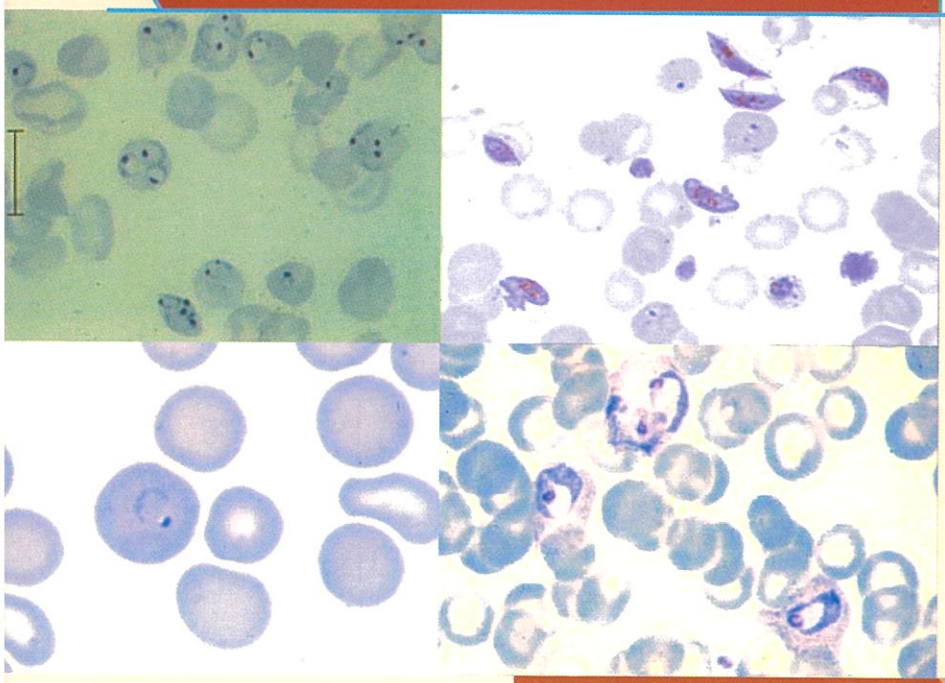
Involvement and sensitization of media personnel: The media, particularly the representatives of local press and prominent journalists, will be a part of the steering committee. This group of stakeholders will be used for mobilization of community and propaganda of the programme. Also, the media should be dealt carefully by other stakeholders while reporting the incidences of side reactions.

Communication and Community mobilization (CCM) activities: Intensive community mobilization activities are to be initiated through environmental building by taking all available resources into consideration.

Drug distribution: The strategy will result in local decision-making with regard to planning and execution of drug distribution. The issues related to methods of distribution, selection of distributors, recording, incentives, etc. will be decided. The health system personnel will be consulted at every stage. The drug distributors (either community volunteers like CBO members, health workers) will undertake the distribution of drugs, preferably in the presence of health personnel. The side reaction surveillance and management are to be integrated with distribution process, as decided by the health system and stakeholders.

Evaluation: The methodology, including sampling, survey methods and instruments, data processing and analysis, and time schedule of evaluation has to be developed by considering the objectives of the study. The detailed methodology of evaluation is being developed.

2. STUDIES ON MALARIA



2

INSIDE

Studies on Malaria

Status:

Intramural

Investigators:

Dr.N. Mahapatra

Dr.S.K. Parida

Mr. N.S. Marai

Starting date: October 2003

Closing date: September 2005

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

Objectives:

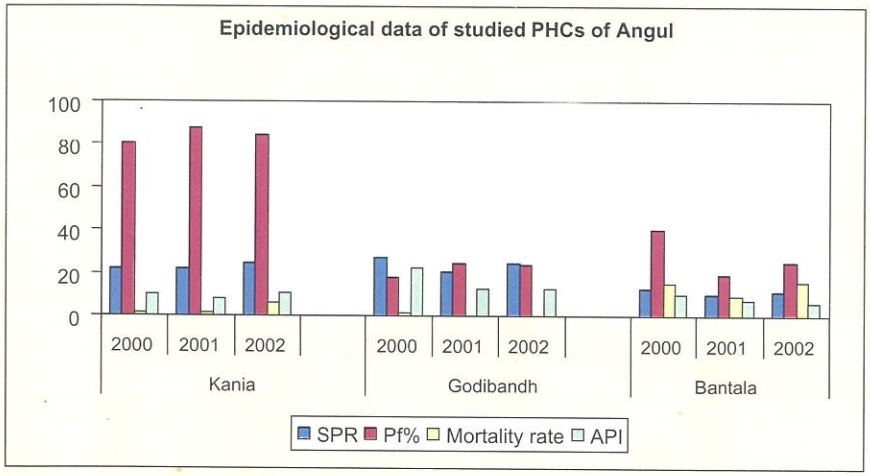
1. To study the prevalence of different sibling species complex of malaria vectors and their susceptibility status to insecticides in Anugul district of Orissa.
2. To study the bionomics of the complex like resting, feeding and biting behaviour, anthropophilic indices, gonotrophic cycle, and preferential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

Results:

Our study from three different geophysiographical regions of the state reveals the presence of *An.culicifacies* B and C and *An.fluviatilis* T (RMRC annual report, 2001). Since the study was fragmentary and point survey was done, an intensive in-depth study was initiated in Anugul district of Orissa, which has reported high malaria deaths during last three years. The stratification of the district based on the sibling species of malaria vector(s) will be made in order to develop an innovative, sustainable, situation specific and cost effective control measure.

Anugul district has eight PHCs having a total population of 11,39,341(census 2001). It has forest, riverine and plain ecotype and it has also developmental dam project areas as well as mining areas. Out of eight PHCs , three viz, Bantala, Godibandh and Kaniha representing each ecotype distribution were selected for entomological studies. From each PHC, 6 representative villages were selected based on different ecotypes (hilly forest 2 , plain 2, riverine 2, construction and development sites 2). Each PHC was visited in two different seasons, winter and summer from November to June. Each village has 100 houses on an average. Mosquitoes were collected from 10% of the households and Cattlesheds from each village. The samplings for all the entomological studies were done as per the WHO procedure (WHO, 1975). After collection, the mosquitoes were identified. Blood meals were collected on Whatmans filter paper for processing by gel diffusion technique. The ovaries were dissected out from semigravid females and were placed in modified Cornoy's fixative. Ovaries were processed in 50% propoionic acid and stained in 2% lacto- aceto-orcein according to the method of Green and Htunt (1980) for making polytene chromosome preparation. The chromosomal preparations were studied under phase contrast microscope.

FIG-7 Epidemiological data of studied PHCs of Anugul district of Orissa.



The epidemiological data of population pertaining to malaria like SPR,API, PF% and mortality rate in the selected PHCs were collected from Directorate of Health Services. The epidemiological situation of the three PHC is depicted in Fig- 7.

The entomological study reveals the presence of 13 species of mosquitoes belonging to four genera *ie Anopheles, Culex, Aedes* and *Armigeres*. Anopheles species were *An.aconitus*, *An.annularis, An.culicifacies, An.fluviatilis, An.hyrcanus, An.maculatus, An.pallidus, An. pseudojamsei, An.subpictus, An.splededus An.tessellatus, An.vagus* and *An.varuna* in the district. The prevalence of anophelines in different PHC during winter and summer is depicted in Fig. 8-12. In Bantala PHC, three known malaria vectors viz., *An.culicifacies*, *An.fluviatilis* and *An. annularis* were prevalent. In Kania PHC, *An. culicifacies* and *An. annularis* were found while in Godibandh PHC, only *An. annularis* was found during winter (Fig 8 -10). During summer only An *culicifacies* was found in Bantala PHC while in Kania both *An. culicifacies* and *An. annularis* were found (Fig 11and 12). In summer, survey could not be done in Godibandh PHC.

The density of the anophelines collected from three different ecotypes (foothill, riverine and plain villages) of three PHCs together are presented in fig 13-15. In foothill villages *An. culicifacies, An. fluviatilis* and *An. annularis* were prevalent. In riverine and plain villages both *An.culicifacies An.annularis* were found. *An.culicifacies* B, and C were found in all the ecotypes and *An. fluviatils* S was found in foothill villages

Detection of sporozoite by molecular method:

Anopheline mosquitoes (96) were processed for detection of sporozoite using PCR and dissection was done in 103 mosquitoes. One specimen of *An. annularis* of Bantala PHC was found positive for *P.falciparum* sporozoite by PCR method. The work is in progress.

Precipitin test for the blood meals of anophelines was conducted by using gel-diffusion technique. The anthropophilic index of *An.annularis*, *An.vagus*, *Cx.quinquefasciatus* and *Cx.vishnui* were 35.7% ,57.9%,72.7% and 22.2%.

Figure 8: Per man hour density (PMHD) of Anophelines of Bantala PHC during winter (Nov. 03-Feb. 04)

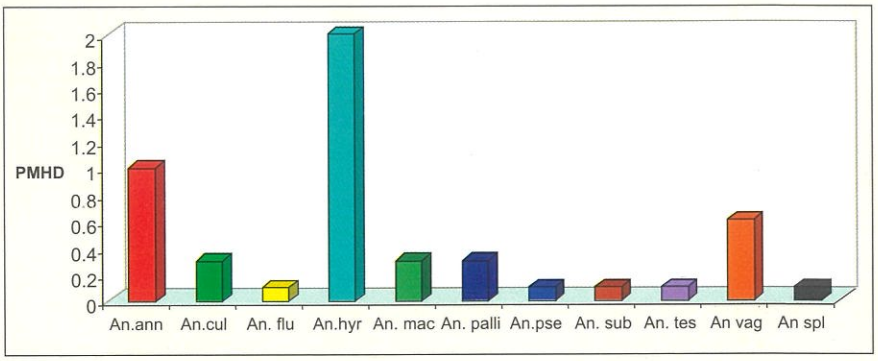


Figure 9: Per man hour density (PMHD) of Anophelines of Kania PHC during winter (Nov. 03-Feb. 04)

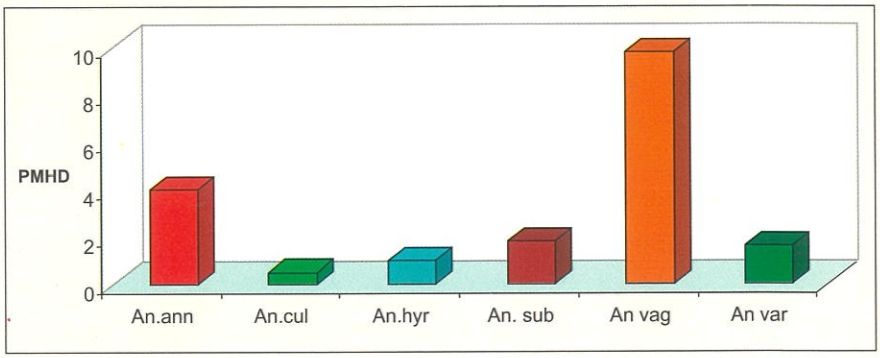


Figure 10: Per man hour density (PMHD) of Anophelines of Godibandha PHC during winter (Nov. 03-Feb. 04)

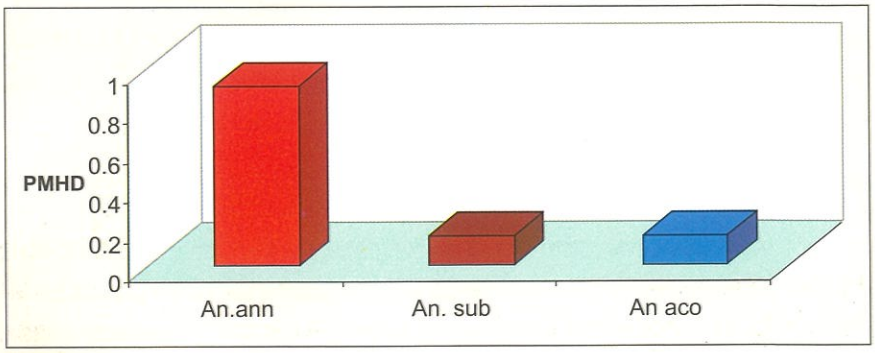


Figure 11: Per man hour density (PMHD) of Anophelines of Bantala PHC during summer (Mar. 04-June 04)

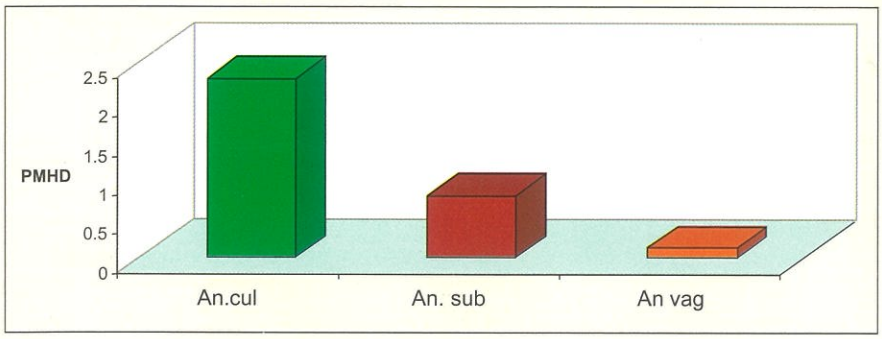


Figure 12: Per man hour density (PMHD) of Anophelines of Kaniha PHC during summer (Mar. 04-June 04)

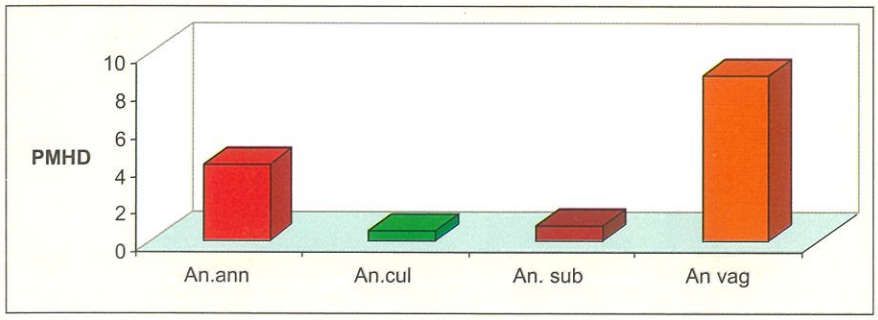


Figure 13: Anopheline density of foothill villages of three selected PHCs of Angul district (Nov. 03-June 04)

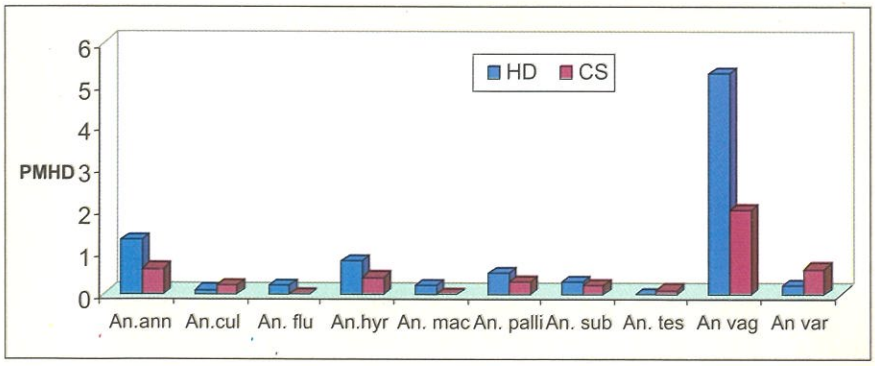


Figure 14: Anopheline density of riverine villages of three selected PHCs of Angul district (Nov. 03-June 04)

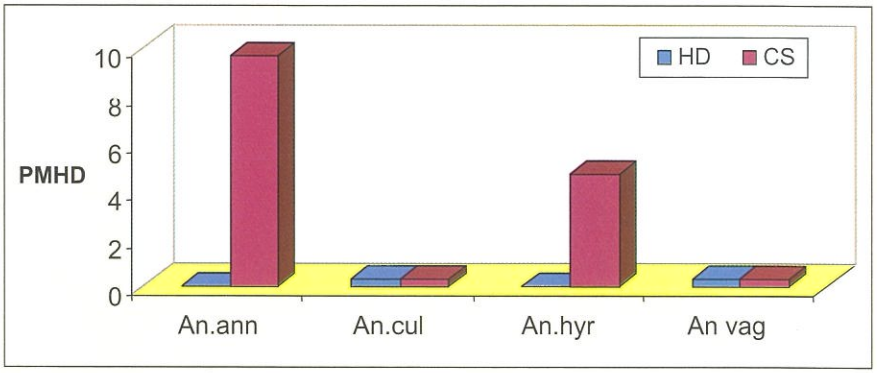
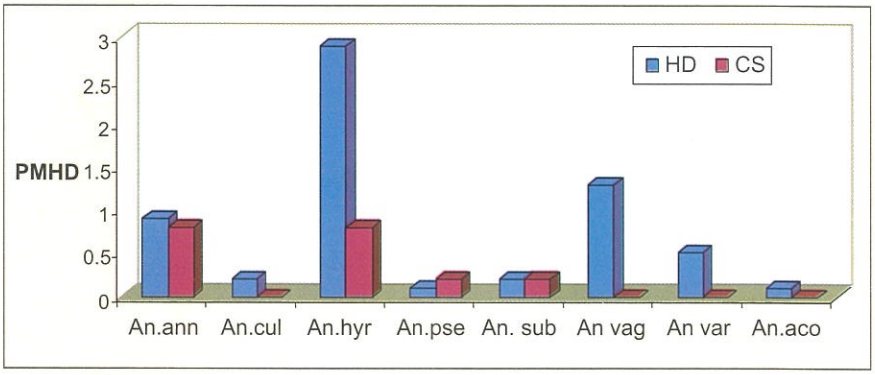
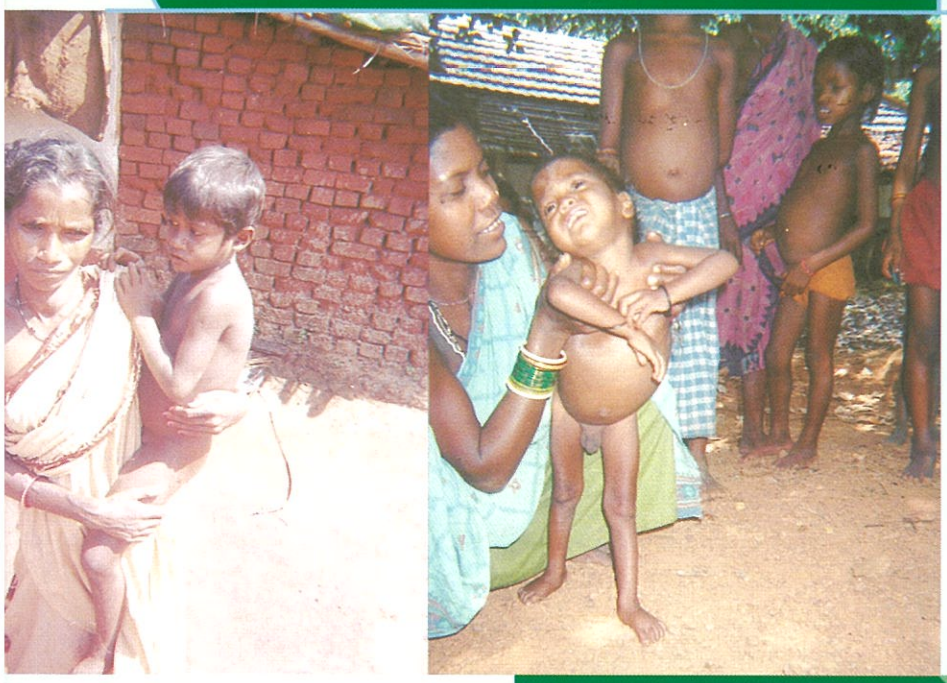


Figure 15: Anopheline density of plain villages of three selected PHCs of Angul district (Nov. 03-June 04)



3. STUDIES ON NUTRITION



3

INSIDE

Studies on Nutrition

Status:
Extramural (ICMR Task Force)

Investigator:
Dr. G.P. Chhotray

Starting Date: October 1999

Closing Date: September 2004

3.1 Intervention programme on Nutritional Anaemia and Haemoglobinopathies in some primitive tribal population of India

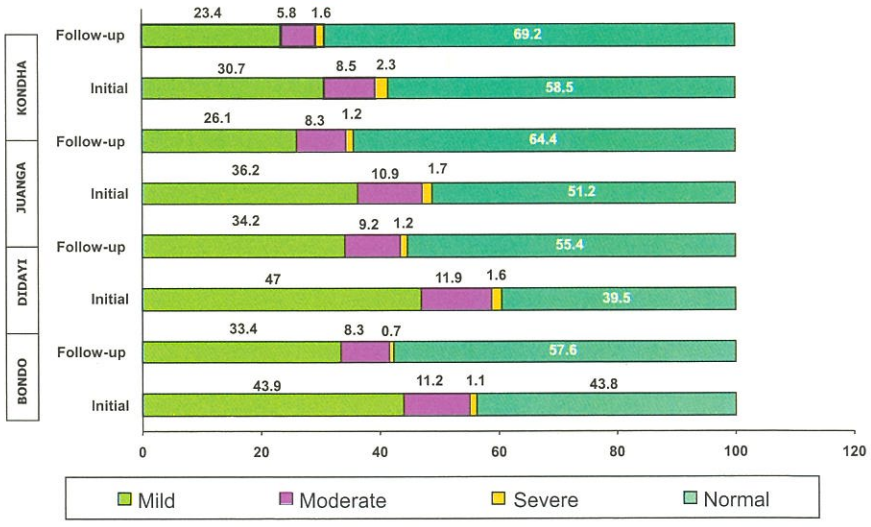
Objectives:

The multicentric study was under taken in 4 primitive tribes of Orissa namely Bondo, Didayi, Kondha and Juanga with the following alms and objectives.

1. To find out the prevalence and aetiology of nutritional anaemia and haemoglobinopathies in 4 primitive tribes Bondo, Didayi, Kondha & Juanga.
2. Clinical evaluation, management and monitoring of detected cases of anaemia and haemoglobinopathies.
3. To provide necessary supplementary intervention programme for formulating the future strategies of education and training to the doctors at the PHC level.

Detailed clinical examination and laboratory investigation has been performed in total 3377 individuals of all age groups in both sex of Bondo (839), Didayi (1014), Kondha (645) and Juanga (879) primitive tribes of Orissa from October 1999 till date. Anaemia status has been graded based on Hb level.

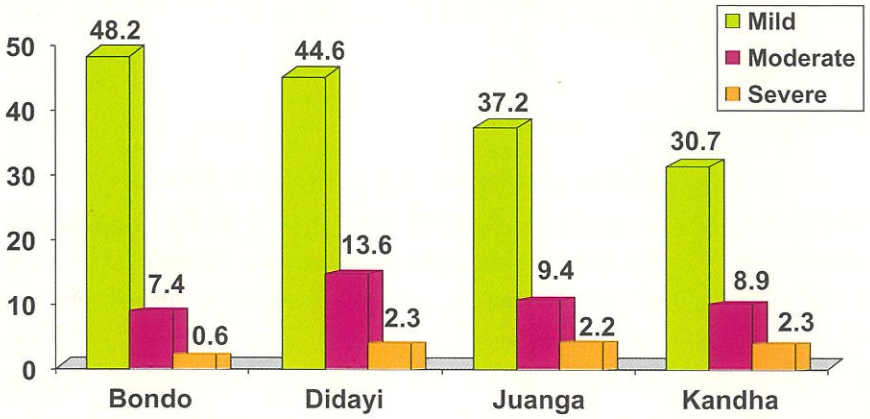
Fig 1: Prevalence of anaemia among studied tribes



Only 47.3% of the studied populations were found to be normal (Hb > 11 g / dl), 1.6% had severe anaemia (Hb < 7 g /dl), 40.3% had mild anaemia (Hb 9 – 11 g /dl) and 10.8% had moderate anaemia (Hb 7 – 9 g/dl). The tribe wise distribution of different grades of anaemia is shown in Fig 4. Various laboratory investigations such as Hb estimation, MCV,

MCH & FEP estimation and peripheral blood smear examinations revealed that 61.4% of the anaemia cases had microcytic hypochromic blood picture indicating iron deficiency aneamia. The hereditary haematological disorder analysed by Hb variant analyzer (BioRAD variant II) revealed that sickle cell anaemia (AS) was 0.72% in Bondo, 3.6% in Didayi, 1.8% in Juanga and 1.6% in Kandha; while β thal was 0.7% in Bondo, 3.2% in Didayi, 2.7% in Juanga and 3.2% in Kandha. The G6PD deficiency was found to be 0.4% in Bondo, 1.7% in Didayi, 13.1% in Juanga and 7.9% in Kandha tribes studied.

Fig 2 : Prevalence of different grades of anaemia in different tribes before intervention



Appropriate intervention measures and IEC activities was instituted at individual and community level as stipulated in study protocol. The follow up examination revealed improvement in the heamoglobin status in 26.3% Bondo, 27.2% Didayi, 24.1% of Juanga and 21.7% of Kondha tribes respectively (Fig 1). The study indicates that community intervention with intensive IEC activities may reduce the incidence of anaemia. The data of the study has been submitted to co-ordinator of study central analysis of the multicentric study.

3.2 Study on nutritional status of Dongria Kondh primitive tribe and Domb scheduled caste populations of Orissa.

Investigators :

Dr.G.Bulliyya
Dr.B.Dwivedi
Mrs.G.Mallick
Mr. P.K.Jangid

Starting date : 1.7.2003
closing date : 30.6.2005

Funding : Extramural ICMR-Taskforce

Objectives:

- To study demography, socio-economy and morbidity status;
- To assess the nutritional status of all age groups;
- To study the household food and nutrient consumption patterns and seasonal variation;
- To evaluate the availability and utilization of health care and nutritional programmes;

ONGOING STUDIES

Studies On Nutrition

Background:

The study is proposed to evaluate nutritional status of on Dongria Kondh primitive tribal group (PTG) and Domb scheduled caste (SC) populations inhabited in Niamgiri hills in Rayagada district. These are the groups confined to the remote areas that are socially isolated from mainstream. Dongria Kondh is one among 12 PTGs on the basis of extremely low literacy, primitive level of agro-technology, stagnant or diminished population growth. Domb lives in many Dongria villages as neighbours and mediators who are having symbiotic relation. The Dongria community is divided into several exogenous clans and women are considered as economic asset of the family.

Progress:

Field surveys have been conducted in Muniguda, Bissamcuttack and Kalyansighpur revenue blocks of Rayagada district. Half of the total villages were selected for the study adopting block-wise stratification and probability proportionate to size sampling method. The sampling interval calculated by dividing the total population to half of the total number of villages. Random start number selected between 1 and sampling interval for choosing the first village and by adding the same interval to the random start, remaining 65 villages were selected. All the households belonged to Dongria Kondh PTG and Domb SC population are included. Sample size for each of study variable was estimated based on prevalence of nutritional deficiencies at 10% relative precision of estimate and 95% confidence interval.

Household data collected from 210 households that include 165 Dongria PTG and 45 Domb SC. The data comprised of household demography, socio-economy, utilization of healthcare services, knowledge-attitude practices about health and nutrition. Diet survey carried out on 50-households and computation for nutrients is in progress. Anthropometric data collected on 861 Dongria and 293 Domb individuals. The distribution of age, sex and physiological groups covered is presented in Table 1. The distribution of preschool children assessed for nutritional status according to weight-for-age (underweight), height-for-age (stunting) and weight-for-height (wasting) using Standard Deviation classification and NCHS standards is presented in Table 2. Nearly 90%, 75% and 80% of Dongria children were malnourished (<median-2SD) respectively in terms of underweight, stunting and wasting. The proportion of children with underweight (71.7%) and wasting (60.4%) was relatively low among Domb children. Though the overall prevalence of undernutrition between both sexes were comparable for mild and moderate degrees, the proportion of severe grade underweight and stunting was observed to be greater for girls. However, statistical sex variation observed was observed for wasting among Domb children. The proportions of grades of children with wasting were in the order of mild, moderate and severe grades in both sexes and

populations. Children of underweight and wasting were greater significantly in the Dongria Kondh than in the Domb.

The mean anthropometric values of adult men and women of study populations measured using standard equipment and procedure (Table 3). Mean values of body weight and BMI were almost similar for both study groups, however, Dongrias males and females were shorter than their counter part Domb. Overall, anthropometric values (circumferences of mid-arm, waist and hip and skinfolds thickness) reveal that Domb were better in their nutritional status in comparison to Dongria Kondh. The distribution of adult men and women according to their body-mass index (BMI) grades are shown in Table 4. Overall, prevalence of chronic energy deficiency (CED) was about 57% for Dongria Kondh and Dombs and about 40% were normal as per their BMI. A negligible proportion of males (2.2%) and females (2.2%) observed to be over-weight among the Domb. The prevalence of grade III CED is greater in Dongria (16.7%) that that in Domb (11%). The variations between sexes were significant for both the populations. The difference in nutritional grades between Dongria and Domb population groups were found to be significant.

The micronutrient status of both the communities was assessed for iodine, vitamin A and iron. A total of 242 salt samples collected from the households on the day of survey tested for iodine using rapid test kits in the field and iodometric titration in the laboratory. The proportions of household salt samples contain adequate levels of iodine (>30ppm) estimated by spot test and titration were significantly lower for the Dongria Kondh (7.7% and 3.0%) than for the Domb (16.2% and 26.7%). School children in the age group of 6-12 years were examined for their goitre status by palpation method and graded according to WHO classification (Table 6). Prevalence of total goiter rate (TGR) ranged between 24.0% in the Dongria Kondh and 24.5% in the Domb children reflecting the problem of iodine deficiency disorders. Urinary iodine excretion levels (UIEL) reveal that proportions of children free from IDD (>100ug/L) were less than the children with different grades of iodine deficiency for Dongria, while it was reverse for Domb children. Children with mild, moderate and severe UIEL are 22.6%, 13.9% and 22.6% respectively for Kondh, while it was 18.9%, 20.7% and 9.5% for Domb children.

Finger prick blood samples (20.0ul) were collected from a total of 143 individuals on filter papers and tested for haemoglobin (Table 7). The sample include 105 (47 males and 58 females) Dongria Kondh and 38 (22 males and 16 females) Domb SC population groups classified using WHO cut-off values into different nutritional grades for age, sex and physiological groups separately. Only 27.6% of Dongria Kondh and 23.7% of Domb had normal levels of haemoglobin. The proportion of individuals suffering from mild, moderate and severe anaemia were 32.4%, 22.9% and 17.1%

respectively for Dongria Kondh, while it was 31.6%, 26.3% and 18.4% for Domb. Vitamin A deficiency was assessed in terms of night blindness and Bitot spots among preschool children (1-6 years) and school-age children and results were not compiled due to small sample size and the study is continuing.

Table 1. Age and sex distribution of study populations

Age & physiological group	Dongria Kondh PTG			Domb SC population		
	Male	Female	Total	Male	Female	Total
0-1 years	15	11	26	6	7	13
1-5 years	47	64	111	19	21	40
5-10 years	64	57	121	21	25	46
10-20 years	49	63	112	25	30	55
20-60 years	145	212	357	48	48	96
60< years	7	36	43	5	8	13
Pregnant women	-	26	26	-	6	6
Lactating mothers	-	65	65	-	24	24
Total	327	534	861	124	169	293

Table 2. Nutritional status of preschool children (0-5years) according to SD classification

Nutritional index as per NCHS standard	Dongria Kondh (137)			Domb SC (53)		
	Boys (62)	Girls (75)	Total (137)	Boys (25)	Girls (28)	Total (53)
Weight-for-age	(Underweight)					
≥-1SD (Normal)	17.7 (11)	5.3 (4)	10.9 (15)	24.0 (6)	28.6 (8)	26.4(14)
-1SD-2SD (Mild)	24.2 (15)	25.3 (19)	24.8 (34)	24.0 (6)	17.8 (5)	20.8(11)
-2SD-3SD(Moderate)	25.8 (16)	30.7 (23)	28.5 (39)	40.0 (10)	28.6 (8)	33.9(18)
<-3SD (Severe)	32.3 (20)	38.7 (29)	35.8 (49)	12.0 (3)	25.0 (7)	18.9(10)*
Height-for-age	(Stunting)					
≥-1SD (Normal)	22.6 (14)	22.7 (17)	22.6 (31)	24.0 (6)	25.0 (7)	24.5(13)
-1SD-2SD (Mild)	21.0 (13)	16.0 (12)	18.2 (25)	24.0 (6)	28.6 (8)	26.4(14)
-2SD-3SD(Moderate)	27.4 (17)	20.0 (15)	23.4 (32)	40.0 (10)	17.8 (5)	28.3(15)
<-3SD (Severe)	29.0 (18)	41.3 (31)	35.8 (49)	12.0 (3)	28.6 (8)	20.8(11)
Weight-for-height	(Wasting)					
≥-1SD (Normal)	17.7 (11)	20.0 (15)	19.0 (26)	32.0 (8)	46.4 (13)	39.6(21)
-1SD-2SD (Mild)	46.8 (29)	40.0 (30)	43.1 (59)	32.0 (8)	32.2 (9)	32.1(17)
-2SD-3SD(Moderate)	22.6 (14)	30.7 (23)	27.0 (37)	36.0 (9)	7.1 (2)	20.8(11)
<-3SD (Severe)	12.9 (8)	9.3 (7)	10.9 (15)	0.0 (0)	14.3 (4)*	7.5 (4)*

Figures in parentheses denote number. $\chi^2_{(3)}$ *p<0.05

Table 3. Mean anthropometric measurements of adult populations

Anthropometric variable	Dongria Kondh (288)			Domb SC (184)		
	Boys (138)	Girls (150)	Total (288)	Boys (88)	Girls (96)	Total (184)
Age years	36.1±10.6	38.4±12.6	37.5± 11.9	33.0±8.7	32.4±8.4	32.2±6.5
Body weight kg	46.7±5.9	37.6±8.72	42.1±8.7	46.7±9.1	39.4±6.6	43.0±8.7
Height cm	158±7.5	145.±14.9	151.1±13.4	159.3±10	148.5±9.9	153.8±11.3
B.M.I kg/m^2	18.7±3.0	17.6±2.9	18.28±3.3	18.9±3.0	17.7±2.0	18.3±2.14
Mid-arm girth cm	20.8±2.1	21.2±2.7	22.0±2.60	22.9±1.7	20.9±2.6	21.8±2.4
Waist girth cm	69.1±4.6	67.5±6.9	68.3±5.86	70.5±6.3	71.4±6.5	70.8±6.4
Hip girth cm	78.9±5.1	76.8±8.9	77.9±7.23	79.6±4.1	77.9±7.5	79.0±5.7
Biceps sft mm	2.57±0.6	3.15±1.1	2.8±0.89	3.1±1.4	3.9±1.8	3.5±1.6
Triceps sft mm	4.5±1.2	7.1±2.6	5.7±2.34	5.2±2.0	8.2±2.3	6.7±2.6
Sub-scapular mm	6.7±1.8	7.7±2.7	7.2±2.34	7.4±2.4	8.3±2.3	7.8±2.4
Suprailiac sft mm	7.0±2.4	8.3±3.6	7.7±3.12	6.9±3.4	9.8±3.9	7.9±3.8

Table 4. Distribution of adults (>20 years) by BMI grades

BMI (kg/m^2)	Nutrition grade (CED)	Dongria Kondhs PTG (288)			Dombs SC (184)		
		Male (138)	Female (150)	Total (288)	Male (88)	Female (96)	Total (184)
< 16.0	Grade III	7.2(10)	25.3(38)	16.7(48)	6.8(6)	14.6(14)	10.9(20)
16.0 - 17.0	Grade II	13.1(18)	18.7(28)	16.0(46)	11.4(10)	25.0(24)	18.5(34)
17.0 - 18.5	Grade I	20.3(28)	28.0(42)	24.3(70)	36.4(32)	20.8(20)	28.3(52)
18.5 - 20.0	Low-normal	39.1(54)	17.3(26)	27.8(80)	31.8(28)	25.0(24)	28.3(52)
20.0 - 25.0	Normal	20.3(28)	10.7(16)	15.2(44)	11.4(10)	12.5(12)	11.9(22)
25.0 <	Obesity	0.0(0)	0.0(0)*	0.0(0)	2.2(2)	2.1(2)*	2.1(4)*

Figures in parentheses denote number. $\chi^2_{(5)}$ *p<0.05

Table 5. Households according to iodine levels in salt samples

Spot test			Titration		
Iodine(ppm)	Dongria(168)	Domb(74)	Iodine(ppm)	Dongria(168)	Domb(74)
0 ppm	50.6 (85)	25.7 (19)	0-7	51.2 (86)	18.9 (14)
7 ppm	41.7 (70)	58.1 (43)	7.1-15.0	45.8 (77)	55.4 (41)
15 ppm	7.7 (13)	8.1 (6)	15.1-30.0	2.4 (4)	14.9 (11)
30 ppm	-	8.1 (6)*	30.0<	0.6 (1)	10.8 (8)*

Figures in parentheses denote number. $\chi^2_{(5)}$ *p<0.05

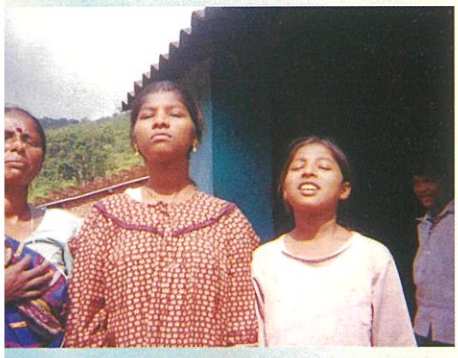
Table 6. Distribution of urinary iodine excretion levels in school-age children

Goitre grade	Dongria (137)	Domb SC (53)	Urinary iodine excretion (ug/L)	Dongria (137)	Domb SC (53)
Grade 0	75.9 (104)	75.5 (40)	Normal (>100.1)	40.9 (56)	50.9 (27)
Grade I	15.3 (21)	13.2 (7)	Mild (50-100)	22.6 (31)	18.9 (10)
Grade II	8.8 (12)	11.3 (6)	Moderate(20-50)	13.9 (19)	20.7 (11)
TGR(%)	24.1 (33)	24.5 (13)	Severe (<20)	22.6 (31)	9.5 (5)
Figures in parentheses denote number.					

Table 7. Prevalence of anaemia by haemoglobin levels among study populations (g/dL)

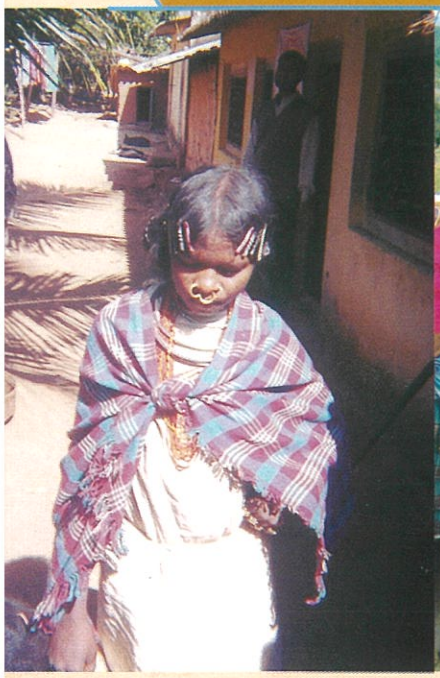
Anaemia grade by Hb level	Dongria Kondh PTG			Domb SC		
	Male (47)	Female (58)	Total (105)	Male (22)	Female (16)	Total (38)
Normal	25.5 (12)	29.3 (17)	27.6 (29)	27.3 (6)	18.8 (3)	23.7 (9)
Mild	31.9 (15)	32.8 (19)	32.4 (34)	31.8 (7)	31.2(5)	31.6 (12)
Moderate	25.5 (12)	20.7 (12)	22.9 (24)	22.7 (5)	31.2(5)	26.3 (10)
Severe	17.1 (8)	17.2 (10)	17.1 (18)	18.2 (4)	18.8 (3)	18.4 (7)

Anaemia gradation by haemoglobin levels of ≤11g% for preschoolers and pregnant women, ≤12% for school-age children, adolescent girls and adult females, and≤13% for adolescent boys and adult males. Figures in parentheses are number. Figures in parentheses denote numbers.



(Siblings with goitre)

4. STUDIES ON TRIBAL HEALTH



4

INSIDE

Studies on Tribal Health

Status:

Extra-mural (ICMR Funded)

Investigators:

Dr. R.S.Balgir

Dr. B.P. Dash

Starting Date: January 2000

Closing date: December 2004

4.1 Intervention for hereditary common hemolytic disorders among the major tribals of Sundargarh district of Orissa

Objectives:

1. Screening and identification of major vulnerable tribals, namely, Bhuyan, Munda and Kharia for hemoglobinopathy, thalassemia and G-6-PD deficiency.
2. Sensitisation, motivation and education through audio-visual aids like posters, charts, pamphlets for carrier detection of above genetic conditions.
3. To provide information for prospective and retrospective genetic/marriage counselling to the affected persons.
4. Imparting of relevant training to the state's local health authorities, like lab. technician, health workers, etc.
5. Periodic follow up for evaluation, intervention and clinical management of affected cases through local PHC/hospital.
6. To develop a suitable intervention package for prevention and control of hereditary disorders like hemoglobinopathy, thalassemia, G-6-PD deficiency and Rhesus blood group incompatibility.

Background of the study:

Hereditary hemolytic disorders like sickle cell disease, thalassemia and G-6-PD deficiency are highly prevalent among the tribal populations and lead to high degree of anemia, morbidity, mortality and fetal wastage. Tribals of Sundargarh district of Orissa, namely Bhuyan, Munda and Kharia are highly prone to hemolytic anemia, jaundice, painful crisis, etc. Since these disorders are hereditary in nature and there is no cure for them, therefore, their prevention in the vulnerable people is highly essential.

Progress of work done:

Last year, we studied Bhuyan tribal community and found that hemoglobinopathies (7.9%) and G-6-PD deficiency (19.0%) were major public health problems in Bhuyan tribe of Hemgiri block in Sundargarh district of Orissa. Both beta-thalassemia trait (10.2%) and sickle cell disorders (4.1%) were common in the community. For the first time, hemoglobin D was encountered in trait form in Khandyat Bhuyan (1.2%) community in the district, which is quite common in North-Western states of India namely, Punjab, Gujarat, Maharashtra and Uttar Pradesh. Further, for the first time in Orissa, a tribal (Paraja Bhuyan) family was detected having hereditary persistence of fetal hemoglobin (HPFH).

This year, the work for this project was initiated in Lahunipara block of Sundargarh district after holding the interactive meetings with the villagers and village leaders (*Sarpanch*, ward members, etc), BDO, CDPO, Anganwadi workers and PHC

doctor. We selected three villages, namely, Badjal, Budhabhuin and Kuliposh Colony belonging to Paudi Bhuyan primitive tribe in consultation with above authorites. A house-to-house census was taken and all the households in the villages were numbered and pedigrees were drawn. Clinical examination of subjects was done, medicines were distributed to the ailing persons and blood samples were collected for laboratory investigations.

Out of coverage target of 500 population of Paudi Bhuyan primitive tribe, 381 were covered and blood samples were collected. The samples were analysed for total hemoglobin, HbA_2, fetal hemoglobin, HbS, G-6-PD deficiency, β-thalassemia, other hemoblobinopathies like HbD and HbE and ABO and Rh blood groups, by standard methods.

Results:

The study carried out in three villages, namely Badjal, Budhabhuin and Kuliposh Colony in Lahunipara block of Sundargarh district in Northern Orissa, it is shown that G-6-PD deficiency (13.6%) was the major genetic and public health problem in Paudi Bhuyan primitive tribe. The G-6-PD deficiency was found to be common in males (7.9%) and female heterozygous (5.0%) and homozygous (0.8%) forms. Although β-thalassemia was prevalent among the Paudi Bhuyan tribe, the frequency is very low (2.1%). Higher prevalence of β-thalassemia among other major scheduled tribes in Orissa like, Paraja (8.5%), Santhal (8.0%), Lodha (6.6%), Bhatra (6.6%), Kondh (6.3%), Saora (6.2%) and Munda (5.2%) was shown by us earlier. The usual clinical signs and symptoms like pallor, joint pains and abdominal pains were observed in these subjects.

The frequency of Rhesus negative blood group was very low (0.3%) among the Paudi Bhuyan primitive tribe of Lahunipara Block in Sundargarh district of Orissa. In general, among the tribal populations of Orissa, the frequency of Rhesus negative blood group is low as compared to general populations of India. The preponderance of blood group B over A was found among the scheduled tribes of Orissa. The frequency of blood group O (23.0%) is lower as compared to A (31.0%) and B (33.1%) in Paudi Bhuyan.

The knowledge, attitude and practices (KAP) studies among the Paudi Bhuyan primitive tribals showed that the people are unaware of the hereditary health problems. As per their perception, the common health problems were: malaria, cough, cold and asthma (breathlessness). Among the other health problems were: weakness, body ache or joint pains, but they were not aware of the cause of these symptoms. They do not feel any necessity of treatment. However, if the illness persists, they go to local quack and occasionally to PHC for treatment. Further intervention along with sensitisation, motivation for carrier detection and bringing awareness in Paudi Bhuyan tribe is in progress.

Status:
Extramural (I.C.M.R.)

Investigators:
Dr. G.P. Chhotray
Dr. B.B. Pal

Starting date:
February 2000

Closing date:
January 2005

4.2 Intervention programme for Cholera, Intestinal Parasitism, Vit A deficiency and Scabies amongst some primitive tribes of Orissa

Objectives:

This community based pilot study have been undertaken in 4 primitive tribes viz., Bondo, Didayi, Juanga and Kondha with the following objectives.

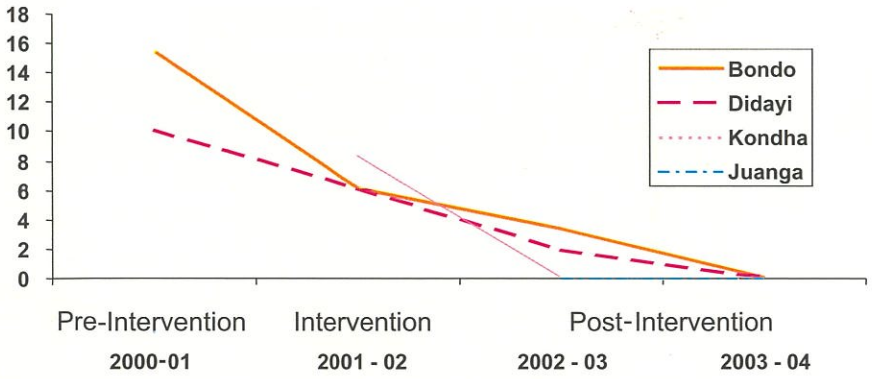
1. A comprehensive assessment of health status and epidemiological profile in respect of cholera, intestinal parasitism, Vit-A deficiency and scabies will be performed in 4 identified primitive tribes such as Didayi, Bondo, Kondha and Juanga out of 13 primitive tribes residing Koraput, Dhenkanal, Phulbani and Keonjhar districts of Orissa.
2. Demographic profile studies reflecting the morbidity and mortality patterns arising out of these disease and their clinical evaluation.
3. To assess the awareness of health, health culture and related behaviour to carry out intervention programme with a view to enhance their acceptability.
4. To plan and execute various intervention programmes among these beneficiaries with a view to augment the existing health care delivery system in those areas.
5. To formulate and develop a module with aim of educating and training the medical and paramedical workers at PHC level in health care delivery system – a future strategy for timely detection and management of these diseases.

Progress:

During the period under report total 1528 (Bondo : 313, Didayi : 331, Juanga : 518 and Kondha : 366) individuals belonging to all age and sex groups in the selected study villages were clinically examined to assess the morbidity pattern arising out of cholera, intestinal parasitism, vitamin A deficiency and scabies. Laboratory investigations were performed to identify the aetiological agents.

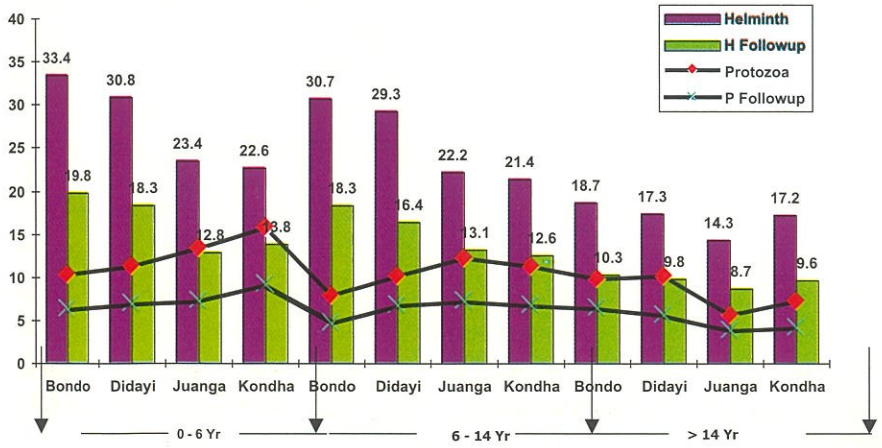
Diarrhoea including cholera: Total 204 rectal swabs (Bondo 83, Didayi 16, Juanga 11 & D / K.Kondha 94) were collected from diarrhoeal patients admitted in the hospital as well as from the community. Of the total rectal swabs analysed 93 (45.6%) samples were found to be culture positive for various enteropathogens like *E.coli* (45.1%) and *Shigella* sps. (0.5% *Shigella flexneri* type – 6). Of the total *E.coli* isolates 74% were non pathogenic and 26% were pathogenic (6.52% Entero Pathogenic *E.Coli* (EPEC), 4.3% Entero Toxigenic *E.Coli* (ETEC) and 15.2% Entero Aggregative *E.Coli* (EaggEC). Suitable intervention during diarrhoeal outbreak (ORS and antibiotics) were administered to cases having mild to moderate signs / symptoms. Community based IEC activities were undertaken in all the study villages. The rectal swab analysis shows a remarkable decline in the isolation of *V.cholerae* from 2001 to 2004 (Fig 1). This may be due to timely intervention and IEC activities under taken in the community.

Fig 1 Yearwise situation of rectal swab analysis of V.cholerae in different studied tribes (Study in. Kondha tribe was initiated in 2001 – 02 and Juanga in 2002-03.



Intestinal Parasitism: Microscopic examinatin of 1528 stool samples collected among the 4 primitive tribes from all age and sex groups revealed that 41.6% of Bondo, 36.7% of Didayi, 36.4% of Kondha and 34.8% of Juanga tribe had intestinal parasites infestations belonging to both protozoa and helminthes. Helminthic infestion was observed among 34.7%, 25.4% and 21.8% of Bondo, Didayi, Kondha and Juanga respectively in age group of children less than 14 years. Amongst adults of more than 14 years these were found in 20.2%, 19.8%, 21.3% and 20.6% respectively in above communities. Hook worm (19.4%) was observed to be the commonest helminthic infection followed by round worm (8.8%) and trichuris (1.4%). Amongst the protozoal infection, *E.histolytica* was the commonest infection (10.8%) followed by *Giardia* (3.2%). Repeat stool examination conducted after 4 months of administration of appropriate antiprotozoal / antihelminthic treatment in selected individuals revealed a significant decrease in the worm burden (Fig 2).

Fig 2 : Prevalence of Intestinal Parasitic infections before and after intervention.



Vitamin A deficiency: The prevalence of Vitamin A deficiency (VAD) among preschool (0-5 years) and school going children (6 – 14years) was studied by examining the clinical signs / symptoms of VAD such as night blindness, bitot's Spot and conjuctival xerosis. The results of the VAD for the period under report is depicted in Table 1.

Table 1: Vit A deficiencies among studied tribes

Tribe	Age Group	Night Blindness	Conjuctival Xerosis	Bitot's Spot
Bondo	0 – 5 (n = 158)	18 (11.4%)	41 (25.9%)	14 (8.4%)
	6 – 14 (n = 133)	10 (7.5%)	29 (21.8%)	10 (7.5%)
Didayi	0 – 5 (n = 182)	5 (2.7%)	44 (24.2%)	25 (13.7%)
	6 – 14 (n = 220)	6 (2.7%)	47 (21.4%)	24 (10.9%)
Juang	0 – 5 (n = 67)	6 (6.9%)	11 (12.4%)	3 (3.4%)
	6 – 12 (n = 181)	7 (3.7%)	32 (17.0%)	8 (4.3%)
Kandha	0 – 5 (n = 67)	5 (7.4%)	12 (17.9%)	10 (14.9%)
	6 – 12 (n = 430)	6 (4.0%)	16 (10.8%)	9 (4.8%)

When reassessed among the preschool children, the prevalence of night blindness decreased from 11.2% to 7.4% in Bondo, 2.6% to 2.3% in Didayi, 7.8% to 5.7% in Juanga and 7.6% to 5.2% in Kandha; conjuctival xerosis was reduced from 24.8% to 17.2% in Bondo, 24.1% to 18.3% in Didayi, 18.4% to 13.2% in Juanga and 14.6% to 9.8% in Kandha and bitot's spot was reduced from 9.2% to 6.4% in Bondo, 13.8% to 9.2% in Didayi, 13.9% to 9.4% in Juanga and 4.8% to 2.9% in Kandha; while amongst the school going children the night blindness was reduced from 7.6% to 5.4% in Bondo, 2.7% to 2.1% in Didayi, 3.6% to 2.1% in Juanga and 4.2% to 2.6% in Kandha, conjuctival xerosis was reduced from 21.6% to 15.2% in Bondo, 19.3% to 13.8% in Didayi, 12.3% to 8.8% in Juanga and 16.8% to 11.4% in Kandha and Bitot's spot reduced from 7.1% to 4.3% in Bondo, 9.8% to 5.2% in Didayi, 7.8% to 5.1% in Juanga and 5.9% to 3.8% in Kandha studied tribes.

Scabies: The clinical examination revealed that 21.3% of Bondo 12.2% of Didayi, 17.3% of Kondha and 12.8% of Juanga children (<14 years) were having infected / non – infected scabies, while among the adults (>14 years) 20.2% of Bondo, 3.4% of Didayi, 12.7% of Kondha and 9.3% of Juanga were having infected / non – infected scabies. A follow up examination of the patients after appropriate intervention (both drug intervention and IEC) revealed that the disease has been reduced from 20.3% to 9.5% in Bondo, 12.5% to 6.8% in Didayi, 14.8% to 8.6% in Juanga and 14.2% to 7.8% in Kandha population.

5. SURVEILLANCE AND OTHER SCIENTIFIC STUDIES

5

INSIDE

Studies on Diarrhoeal Disorder

Status:
Intramural

Investigators:
Dr.B.B Pal
Dr.G.P. Chhotray
H.K Khuntia
Dr.A.S Acharya

Starting date: November 2003
Closing date: October 2004

5.1 Molecular characterization of *V.cholerae*: Strain typing pattern associated with diarrhoeal outbreaks in Orissa

Objectives:

1. To isolate different strains of *V.cholerae* and other diarrhoeagenic vibrios from diarrhoea patients and environmental samples during outbreaks.
2. To identify and type the various isolates of *V.cholerae* for their antibiogram.
3. To detect the various virulence genes like ctxA and tcpA by polymerase chain reaction (PCR) assay.
4. To study the clonality of the clinical strains with the environmental isolates by RADP PCR, ribotyping and other methods. And to study the correlation between the mid epidemic and epidemic strains along with the previous isolates.

Methods:

Outbreaks of diarrhoeal disorders were studied from Keonjhar town, Malkanagiri town; Chitrada village, Mayurbhanj district and, Parbatia village Dhenkanal district between August to November 2004. Rectal swabs were collected in CBT media, sub cultured in TCBS plates. Significant colonies were tested biochemically following standard techniques and confirmed by specific antisera of *V.cholerae* O1 and O139 sero groups. Selected strains were subjected to PCR assay for the detection of ctxA and tcpA genes for *V.cholerae.*

Results:

Area of Outbreak	Name of villages affected	Period	Total rectal swab scollected	No. +ve for *V.cholerae* (%)	Sero group
Keonjhar town	Tikarguma, Badahal, Raisua, Satsingh, Durgabahal etc.	July 2003	36	20(74.1)	O1
Malkanagiritown	Reglamisin, Katamita, MV8, Latiaguda, Rangamunda etc.	August 2003	30	24(96.0)	O1
Chitrada Mayurbhanj district	Chitrada	August 2003	13	10(100)	O1
Parbatia, Dhenkanal district	Parbatia	November 2003	6	4(100)	O1

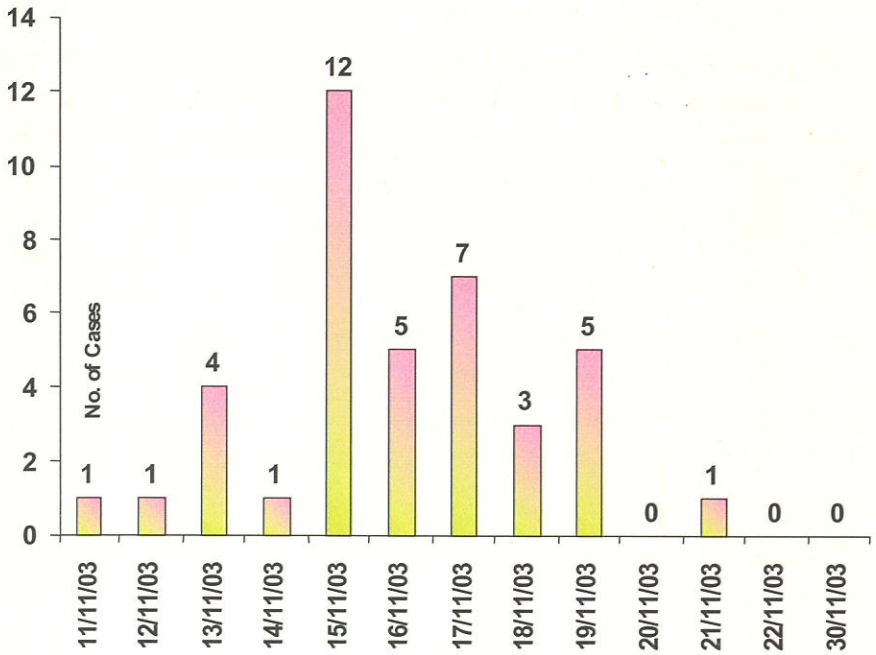
Antibiogram:

The general antibiogram of the above isolates revealed that the *V.cholerae* are sensitive to tetracycline, ciprofloxacin, norfloxacin, chloramphenicol, and resistant to co-trimaxazole, ampicillin, neomycin and nalidixic acid.

PCR Assay:

The PCR assay for the detection of ctxA and tcpA genes on selected strains of *V.cholerae* revealed that all are positive for ctxA and tcpA genes showing biotype ElTor.

Fig.2: Incidence of Diarrhoea Cases in Parbatia Village
(11th November to 30th November 2003)



Surveillance and other scientific studies

Status:

Intramural

Investigators:

Dr. B.B. Pal

Mr. H.K. Khuntia

Dr. A.S. Acharya

Mr. C.R. Samantara

Starting date: April 2003

Closing date: March 2004

5.2 Diarrhoeal Surveilance

Bacteriological analysis of enteropathogens causing diarrhoeal disorders reported from selected coastal districts of Orissa

Objectives:

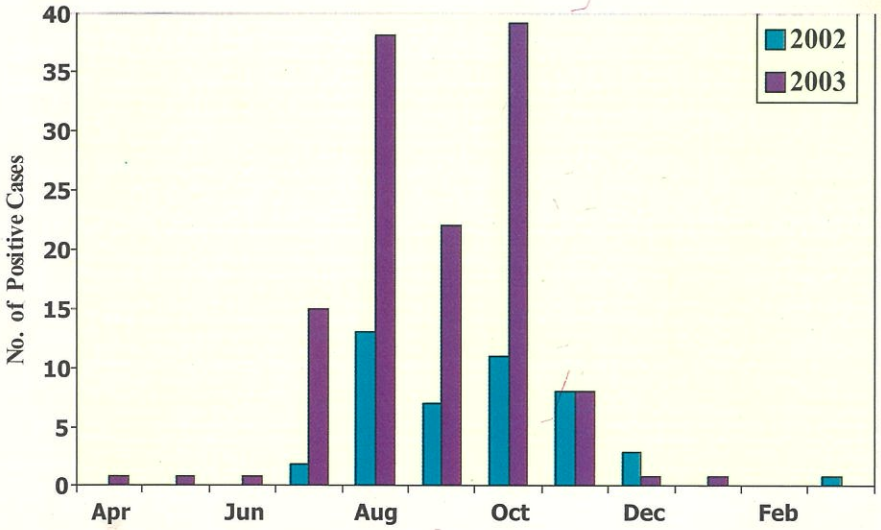
1. Isolation and identification of various bacterial enteropathogens like *V. cholerae, E.coli,Salmonella* and *Shigella* from acute diarrhoeal cases of all age groups from identified hospitals of Puri and Khurda districts of Orissa.
2. Strains typing and antibiogram of various bacterial enteropathogens isolated from above cases.
3. To study the seasonal variation in incidence of diarrhoeal disorders.

Sporadic outbreaks of diarrhoeal disorders were reported from Puri / Khurda district of Orissa. Further *V.cholerae* was isolated in repetitive occasions from diarrhoeal cases reported from this region in the past. Hence to augment the early warning system, to observe the trend of incriminating pathogens and to obtain information of any change in strain pattern, diarrhoeal surveillance was undertaken in diarrhoeal cases attend to selected PHCs and hospitals of Puri and Khurda districts of Orissa.

The surveillance was made through regular fortnightly visits to these hospitals where all admitted cases in diarrhoea ward were registered for their identification, history of diarrhoea and clinical examination.While collecting sample of faecal specimens using standard procedures the stool samples were kept in CBT media and transported to laboratory and tested for enteropathogens namely *E.coli, Shigella, Salmonella, V.cholerae* commonly associated with diarrhoeal outbreaks. Areas reporting large number of cases were visited and surveyed to estimate the total number of cases. Water samples were tested to identify the source.

During this period 718 stool/rectal swabs were collected from Capital Hospital, Bhubaneswar (145) ; Pipili CHC(30); Bolanga CHC((40); Satyabadi hospital(25) and I.D.Hospital(477), Puri and bacteriologically analysed to isolate different bacterial enteropathogens. Out of 718 stool/rectal swabs studied 60.0% were found culture positives. Of these *E.coli* were 61.9%, *V.cholerae* 37.1%(*V.cholerae* 01 ogawa- 36.7% and *V.cholerae* 0139- 0.4%) followed by *Shigella* species 1.0%(*Shigella flexneri* type-6) rest 40.0% were found culture negative.

Month wise Isolation of *V.cholerae* from ID Hospital, Puri (June 2002 – March 2003)



Sixty-one *V.cholerae* (23.1%)were isolated during 2002 and 160 during 2003 from different hospitals. There is a decline of isolation of *V. cholerae* 0139 strain during this year in comparison to the previous year isolation observed. (2002-3.5% and 2003-0.4%). The dominance of *V. cholerae O1* sero group over 0139 was also seen. It was noted that highest number of *V. cholerae* were isolated from indoor diarrhoea patients of ID hospital Puri (127), which serve as referral hospital followed by Capital hospital BBSR (18); Pipili CHC (9); Balanga CHC (7) and Satyabadi hospital (6). More numbers of *V.cholerae* O1 ogawa were isolated during July to October 2003 from ID hospital, Puri. There was heavy rainfall and flood during July to September 2003, which might be the reason for increasing the number of cholera cases due to contamination of water. The cases reported were scattered in several villages of Puri district. Last two years data of ID hospital Puri indicated isolation of more number of *V.cholerae* during monsoon and post monsoon season (Fig-1). Secondly, the isolation rate of *V.cholerae* from ID hospital, Puri increased three fold during August to October 03 in comparison to the same period of 2002, which is mainly due to torrential rainfall and flood.

5.3 HEPATITIS EPIDEMIC: Hepatitis epidemic in Kuamara P.H.C. of Mayurbhanj district (September 2003)

As per the request of the CDMO, and DHS, Orissa a investigating team consisting of Dr. S.K. Kar, Director, Dr. G.P. Chhotray, Deputy Director, Mr. B.N. Sethi, Lab. Tech., Mr. K. Dhal, Census Taker, Mr. R.N. Nayak, Census Taker, Mr. K.C. Dalai, Lab Asst. visited the affected villages and house-to-house for detection of symptoms and signs of viral hepatitis. During the visit both verbal autopsy and clinical examination was done in the affected communities. Hospital records were examined. The attack rate of jaundice was found to be 4.56 per 1000 population and 2.8% of the household in the village was attacked. Total 63 blood samples were collected for further biochemical and immunological

(BioELISA) analysis to confirm the diagnosis of effected cases in the epidemic. The Clinical Examination (100% Icterus positive) along with Liver Function Test (Alk Phos : 239.90 – 566.60 IU / L, SGOT : 35.10 – 180.1 IU / L and SGPT : 61.80 – 146.20 IU / L) and Urine Examination (100% Bile Salt & Bile Pigment positive) confirmed that the cases are of Hepatitis. Out of total samples tested by BioELISA, 35 were found to be positive for HEV IgM antibody and 2 were for HAV IgM antibody while 1 sample was positive for both HEV and HAV IgM antibody. The report has been sent to the concerned health authority for necessary action.

Investigators:

Dr. A.S. Kerketta

Mr. R.N. Nayak

Mr. K. Dhal

Mr. T. Moharana

5.4 OPD Services for Filariasis Patients:

RMRC provided OPD care to the filariasis patients, twice a week at Capital Hospital, Bhubaneswar. During the period of reporting a total of 622 new cases were examined clinically, diagnosed and treated. The infection was confirmed by OG4C3 assay (table -1). Adenolymphangitis, funiculitis and orchitis representing acute features were present in 136 (21.9%) case and rest 445 (71.5%) had chronic clinical features either lymphoedema, hydrocele, chyrulia, TPE or nodule. Other 41 cases (6.6%) presented with complaint of myalgia, arthritis, neuralgia and urticaria. Adenolymphangitis cases presented with acute lymphangitis and adenities of limb in (77.2%) and 22.8% cases had were with different grades of chronic lymphoedema (Table-2). A total of 564-blood samples were tested for circulating filarial antigen and 62 (11.0%) were found to be positive. The detail of CFA positive cases is given in (Table-3). A total of 27 patients with different grades of lymphoedema were given intermittent decompression therapy weekly for one hour. The Special care for maintenance of hygiene of the limb for morbidity management was demonstrated and advised to the lymphoedema patients. The IEC material on foot hygiene through the booklet and leaflet were also distributed to make them aware on foot care practices.

Table-1. Clinical presentation of filariasis in OPD cases (April 2003-March 2004)

CLINICAL DIAGNOSIS	N %	Male	Female
Acute cases			
Adenolymphangitis	127 (20.4)	87 (14.0)	40 (6.4)
Funiculitis	3 (0.5)	3 (0.5)	-
Orchiitis	6 (1.0)	6 (1.0)	-
Chonic cases			
Lymphoedema Gr I	324 (52.0)	207 (33.2)	117 (18.8)
Lymphoedema Gr II	50 (8.0)	26 (4.2)	24 (3.8)
Lymphoedema Gr III	31 (5.0)	17 (2.7)	14 (2.2)
Hydrocele	21 (3.4)	21 (3.4)	-
TPE	3 (0.5)	2 (0.3)	-
Chyluria	2 (0.3)	1 (0.2)	2 (0.3)
Filarial Nodule	14 (2.2)	9 (1.4)	5 (0.8)
Others*	41 (6.6)	17 (2.7)	24 (3.8)
Total	***622***	***387 (62.2)***	***226 (36.3)***

*Others include myalgia, arthritis, cellulitis, urticaria and neuralgia

Table-2 Clinical features of cases presenting with adenolymphangitis

Adenolymphangitis	N	Male	Female
Affecting limb as (LNG and LND)	98 (77.2)	65 (51.2)	33 (26.0)
Lymphoedema Gr.I	9 (7.1)	6 (4.7)	3 (2.4)
Lymphoedema Gr.II	6 (4.7)	3 (2.4)	3 (2.4)
Lymphoedema Gr.III	14 (11.0)	8 (6.3)	7 (5.5)
Total	127	81 (63.8)	46 (36.2)

Table-3 Presence of circulating filaria antigen in Different disease spectrum

	N =62(10.9)
Acute cases	
Adenolymphangitis	7 (11.2)
Funiculitis and orchitis	2 (3.2)
Chonic cases	
Lymphoedema grade I	37 (60.0)
Lymphoedema grade II	3 (4.8)
Lymphoedema grade III	1 (1.6)
Hydrocele	5 (8.0)
TPE	3 (4.8)
Chyluria	1 (1.6)
Filarial nodule	2 (3.2)
Myalgia	1 (1.6)

5.5 Referral Services for Hemoglobinopathies :

Referral Services were rendered for diagnosis to the cases referred from local PHCs, hospitals and Medical colleges and Hospitals in Orissa. Diagnostic services were provided to 77 families referred during the period from April 2003 to March 2004. Out of 200 cases screened by electrophoresis, 17 (8.5%)were diagnosed as homozygous sickle cell disease, 3 (1.5%) sickle cell-â-thalassemia, 35 (17.5%) sickle cell trait; 12 (6.0%) â -thalassemia Major, 49 (24.5%) â -thalassemia trait;

Investigators:
Dr. R.S. Balgir
Dr. G.P.Chhotray
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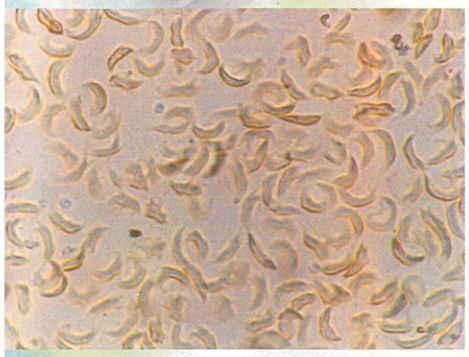
Mr. B.K.Kahnar

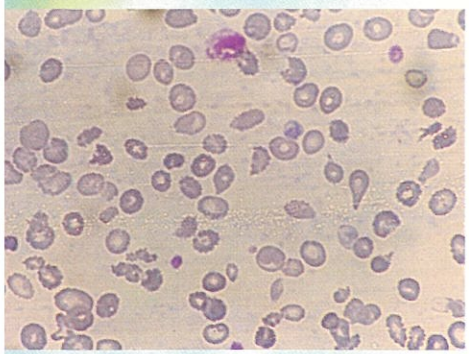
6 (3.0%) Hb AE, 2 (1.0%) E- â -thalassemia, 1 (0.5%) δâ -thalassemia and 75 (37.5%) cases were found normal. Genetic/marriage counselings were given to affected families.

In another series, a total number of 136 cases including 73 male were referred. Most of the cases had complaints of refractory anaemia, progressive weakness and jaundice. Out of 136 cases, 94 belong to General Category, 28 to Scheduled Caste, 3 to Scheduled Tribe and 11 to other minority communities. A detailed clinical examination and laboratory investigation such as haematological profile by automated cell counter (MS9), quantitative analysis of Hb, HbA_2, HbF and its electrophoresis was carried out by established methods. Out of total 136 cases, 31.6% (43) were found to be electrophoretically normal (HbAA), 17.6% (24) sickle trait (HbAS), 8.8% (12) Sickle disease (HbSS), 2.2% (3) HbE- â thal, 0.7% (I) HbAE, 7.4% (10), 3 thalassaemia major and 30.8% (42), B-thalassaemia trait. Community wise distribution revealed that hereditary haematological disorder was more reported from general population as compared to SC and ST.

RMRC, Bhubaneswar monitored the malaria situation in Kalahandi and Nuapada districts of Orissa during November 2003 as requested by NAMP, Govt of India.

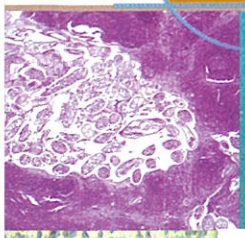
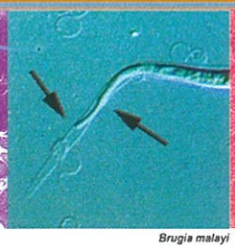
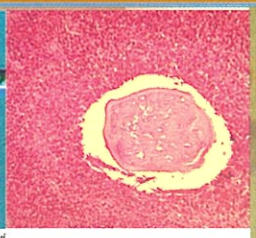
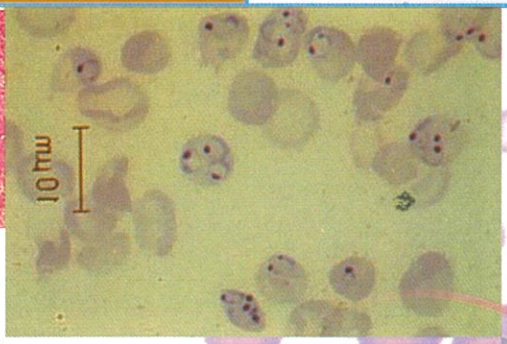


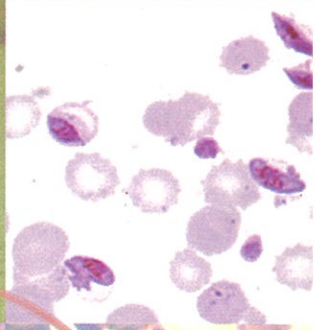
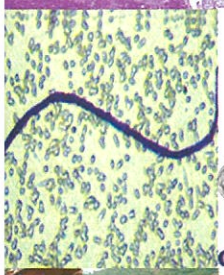
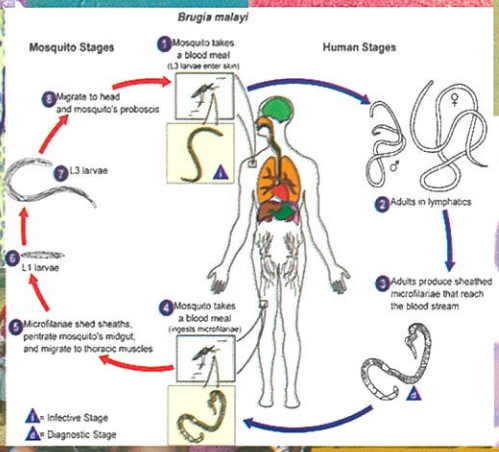
(Sickling of Red Cells)

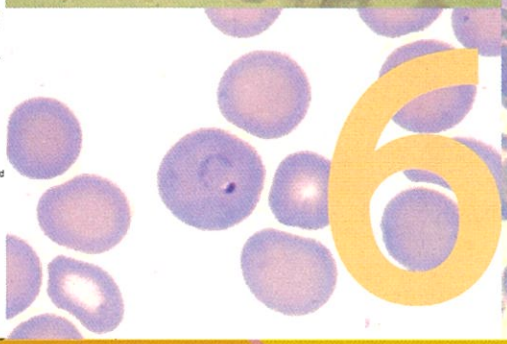
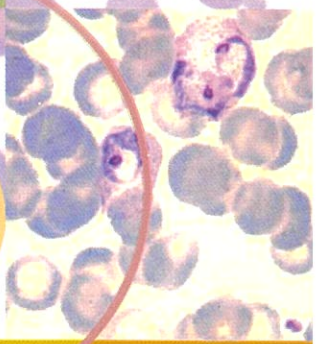


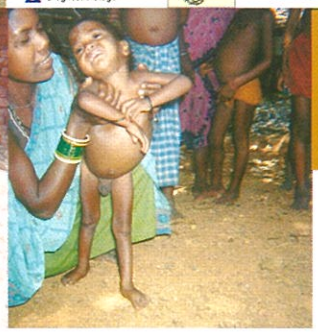
(Red cell Morphology of B-thalassemia major case.)

6. COMPLETED PROJECTS

INSIDE

Completed Projects

Status:
Intramural

Investigators:

Dr. A.S. Kerketta
Dr. B.V. Babu
Mr. P.K. Jangid
Ms. K Rath

Starting date: January 2001
Closing date: December 2003

6.1 Morbidity control in filarial lymphoedema: Controlled clinical trial and treatment seeking behaviour and utilization of health care services in endemic population of Khurda district of Orissa

Objectives:

1. To evaluate the efficacy of long term use of oral antibiotic with foot care in the management of various grades of filarial lymphoedema.
2. To report the treatment seeking behaviour for morbidity control and to understand various practices of morbidity control (like foot care, massaging) among lymphoedema patients.
3. To assess the knowledge and attitude of service providers, particularly the peripheral health workers, with regard to lymphoedema care and to report the facilities and other resources available at health services.

Methodology:

This study was conducted in eight filarial endemic villages of Khurda district of Orissa. Home to home visits were done to identify the cases with different grades of unilateral filarial lymphoedema. The lymphoedema grade was determined by the WHO's classification of lymphoedema (WHO, 1992). Detailed clinical examination has been carried out to check the eligibility as per the inclusion and exclusion criteria. The history of adenolymphangitis (ADL) was elicited by confirming the ADL criteria i.e. presence of local signs and symptoms such as pain, tenderness, local swelling and warmth in the groin or limb with associated constitutional symptoms such as fever, nausea or vomiting (WHO, 1992). Using exclusion and inclusion criteria a total of 300 cases were enrolled for the study. In similar fashion, 50 such cases were enrolled as controls to record the incidence of ADL episode. A written informed consent was obtained from each individual. The three different drug regimens were allocated randomly among the study cases using the random number table, following permuted block design method. All the 300 study cases were demonstrated on the foot care methods. After the allocation of drug regimens, initially all cases were followed daily for one week and closely monitored for side effects of drugs. There after, the cases were assessed once a month for 12 months. Compliance was ascertained by pill count method. The parameters studied were, reduction in the limb circumference and reduction in the frequency of ADL episodes. The circumferences of both normal and affected limb were measured at three different fixed points (1) lower part of the limb i.e., on foot, 10 cm from the tip of the toe, (2) on distal part of lower limb, 15cm from the ground and (3) on proximal part of lower limb, 30cm from the ground by using a standard measuring tape on four occasions, i.e. on 0, 90, 180 and 360 days after the initiation of the drug regimen.

Inclusion criteria:

1. All cases of unilateral filarial lymphoedema (found otherwise healthy in physical examination)
2. Aged between 14 – 65 years
2. Willing to participate in the study and give informed consent
3. History of ADL episodes in past two years

Exclusion criteria:

1. Patient complicated lymphoedema like discharge sinus, nodularity or ulcers
2. Patient with the history of multiple allergies or drug intolerance
3. Patient with the history of renal or hepatic diseases
4. Patient with the history of convulsions or other diseases of central nervous system
5. Patient uses drug or herbal medications (including alcohol, plant derived intoxicants, etc.) that may interfere with the evaluation of the efficacy of the drugs
6. Patient has any situation or condition, which may interfere with participation in study as judged by clinical investigator
7. Old debilitated and mentally deranged patients
8. Pregnant and lactating women

Treatment regimens:

Regimen I- Oral Penicillin (Penicillin "G" 8,00,000 units twice daily for twelve days and is repeated in every 3 months for 1 year) and foot care.

Regimen II- DEC standard dose (6 mg/ kg body weight for 12 days, repeated in every 3 months for 1 year) and foot care.

Regimen III-Topical antibiotic (Bitadine ointment) and foot care.

Foot care includes:

a. Regular cleaning of foot with soap water and keeping the foot dry

b. Regular application of white field ointment between the webs and toes (prevent fungal infection and smoothen the skin)

c. Regular clipping of toe and finger nails (to prevent from injuring the skin by scratching affected limbs due to itching)

d. Prompt attention to all injuries in the body (including minor ones elsewhere in the body)

During the study, 34 cases recruited were dropped out from the study for varied reasons, Thus a total of 266 cases completed one-year treatment. After one year of treatment, the cases were left without treatment and kept under observation for another one year. Till the end of second year, all the cases were followed up for ADL frequency and the limb circumference. The data collected were computerized through Microsoft Excel and analysis was done by SPSS V.9.

Simultaneously, various socio-behavioral surveys have been undertaken among lymphoedema patients, as well as primary health care providers in the study area. The knowledge and practices of various foot care measures are assessed among various groups of patients, including those involved in the study. The other group includes patients who attended filariasis clinic at state headquarters hospital and a group of patients from community other than those participated in the trial. In addition, the acceptability and perceived benefits of the treatment regimens are assessed among the patients of the study. From the lymphoedema patients the data were collected by in-depth interviews. To know the knowledge, practices and attitude regarding lymphoedema care of peripheral health workers and to the facilities available at peripheral levels, in-depth interviews were undertaken. For this purpose 10 primary health centers (PHCs) from 10 blocks of the districts are sampled. From each PHC at least four health workers (two stationed at PHC and two attached to health sub center) are selected. The data obtained from all these qualitative survey were computerized and analysis was done by Text Base Beta software.

Results:

Effect on lymphoedema, in terms of reduction of oedema:

The data on limb circumference at three different points on both normal and affected limb are analyzed to assess the extent of lymphoedema reduction across the three regimens. These results show that around 50% of patients in all the regimens shown reduction of edema since the 90 days of treatment. The proportion of people shown reduction is slightly low in regimen-II. The result shows the details of reduction of limb circumference in terms of ranges of percentage of reduction. In all regimens, about 20% of those shown reduction experienced the reduction of lymphoedema of about 75-100% and major proportion of the remaining relieved with the reduction of less than 25%. No remarkable differences are noticed across the regimens. Around 15% people continued the reduction of oedema about 75-100% even after one-year

follow up period (without treatment). Figure-1 reveals means of oedema, i.e. difference in circumference between affected and normal limbs at different time periods in all the three regimens. In all the three regimens reduction of oedema is noticed since three months of treatment. The reduction of lymphoedema is recorded significantly in all the three regimens and it appears that all regimens are efficacious in reducing lymphoedema. It may be due to having the components of foot care uniformly in all the three regimens. Regimen-III consists only use of a topical antibiotic (Betadin) externally in addition to foot care, and it shown result similar to the other regimens.

Effect on frequency of ADL episodes:

The ADL frequencies were assessed for three time periods, i.e., one year prior to treatment, one year of treatment period and one year of follow-up after treatment, and these results are shown as Fig.-2. In all the three regimens, there is significant reduction of ADL frequency after treatment. In Regimen-II, the initial ADL frequency is slightly lower than remaining two regimens. After one year of treatment, it is noticed that the frequencies are significantly lower in Regimen I and III, which may be due to practice of footcare, as well as use of antibiotics.

Differences in efficacy of drug regimens across lymphoedema grades

To assess the impact of lymphoedema grading on efficacy of regimens, ANOVA was conducted for mean percentage reduction between different lymphoedema grades for measurements at different points in all the regimens. The reduction is high and significant among patients with grade-1 lymphoedema followed by grade-2, and this variation is conspicuous at point-1 measurement i.e. on foot. However, there is remarkable reduction at points-2 and 3 (lower limb) in grade-2 and 3 lymphoedema patients. The reduction of ADL frequency during the treatment period was significant among all the three grades of lymphoedema (Fig-2). The mean ADL frequency is higher among grade-3 patients than grade-2 and grade-1 and the reduction is also more visible in that sequence.

Footcare practices among lymphoedema patients in the community and patients attended filariasis clinic:

The surveys to investigate the footcare practices among filarial lymphoedema patients, which may be relevant to strengthen the intervention programmes on morbidity management, were undertaken in two groups of lymphoedema patients. First group is drawn from filariasis clinic of state head quarter hospital, Bhubaneswar and the second group is from the community (patients other than those participated in the trial). The results of these surveys are presented in previous Annual Report. Most of the patients are practicing one or two methods. Substantial numbers of patients are practicing the washing of the affected limb regularly. Though majority use normal footwear, none of

the patients use specially made footwear. Other footcare practices like, massaging, elevation and exercising of affected limb, and use of bandage are practiced by a few patients in both the groups. The multiple regression analysis indicated that patients' education and lymphoedema grade had significant influence on number of footcare practices adopted.

Peripheral health workers' knowledge, attitude and practices related to lymphoedema care and facilities available at peripheral health centers:

Majority of health workers agreed that many lymphoedema patients visited PHC/HSC and mostly got treatment for acute ADL episodes and a few patients visited for treatment of swelling of limb. Similarly, the health workers are consulted by lymphoedema patients (Table-1). Mostly health workers advised the patients to go to doctor. Also they advised to get their blood tested and take DEC tablets or flourocid injection. Some time they give antepyretic drugs like paracetamol. None of the health workers received any training on lymphoedema care, but a small proportion got instructions from higher authorities to refer these cases to hospital. Around two-thirds of respondents said that there are no facilities or drug to take care of lymphoedema patients. Only three of the ten PHCs had the facility for blood testing. When health workers were asked whether some thing can be done for minimizing the suffering of lymphoedema patients, many of them said it can be minimized by medication. All health workers know that lymphoedema patients suffer frequently from acute episodes of ADL and it should be treated by DEC and antipyretics. Around 17% respondents said that it can be prevented by avoiding mosquitoes. Simultaneously the health workers were probed to know whether they know the concept of foot care or foot hygiene. Only 37% of health workers know little about footcare. However, when they were probed about various methods of footcare individually, some health workers responded positively. These details are given in table-1. Around 68% of health workers advise patients to wash their affected limb regularly. But some know the benefit of washing but they do not advise. Massaging is advised by around half of the health workers. Similarly bandage is also advised by around 68% of health workers. They know that massaging and use of bandage reduces the swelling. Use of proper footwear, elevating the affected limb and exercising the limb are the methods advised by considerable proportion of health workers. But many of them do not know the reasons for these methods. Around 30% of health workers suggest home remedies like using herbal medicines. Interestingly around three-fourths of health workers gave some suggestions regarding food. Many of them prescribed various food taboos.

Practice, acceptability and perceived benefits of various components of drug regimens among participants of clinical trial:

An in-depth questionnaire survey is undertaken among the patients, who participated in the trial. This survey is aimed to understand the acceptability, practice

and perceived benefits of various components of trial. The acceptability of drugs including oral antibiotic, DEC and local antibiotic is very high. Foot cleaning and drying (after cleaning) are practiced regularly by around three fourths of participants. Of the participants who are not practicing occasionally, 20% in regimen-I said that they stopped as lymphoedema/fever incidence is reduced. In regimen-II, the reasons are they did not get any relief (20%) or negligence (30%). In regimen-III, it is due to negligence.

The participants of the study are probed whether they perceived any benefits after taking this treatment (Table-2). Majority of the participants perceived benefits of treatment and felt relief after the treatment. More than 60% of participants in all the regimens said that they got relief from fever and 30% of participants got relief from pain. The reduction of limb swelling is perceived by 23% of participants of Regimen-1, 46% of participants of Regimen-II and 47% of participants of Regimen-III. With regard to expectations of patients, around 50% of patients each expected that fever as well as limb swelling will be reduced due to treatment. The participants in the study felt beneficial and they are willing to continue this treatment including footcare, if material and drugs are available. It is observed that many patients continued various foot care components, even after the study period. That may be the reason for reporting reduction of lymphoedema as well as ADL frequency even after the one year of treatment.

Conclusion:

The results on efficacy of drug regimens among the patients in terms of reduction of oedema and frequency of ADL episodes clearly indicate that in all the three regimens for which footcare is common are efficacious in reducing oedema and frequency of ADL episode. Though the efficacy is slightly high in regimen-I, the differences are not so apparent as footcare is common in all the regimens and it seems the impact is due to footcare. The mainstay in the treatment of lymphoedema and prevention of acute ADL episodes is local care of the affected limbs. The filarial patients visit to the institutions of primary health centers in rural areas as well as urban health centers, for treatment of various forms of LF. The foot care practices in lymphoedema patients (who have not involved in the trial) are upsetting. The medical and paramedical staff of the peripheral institutions should be educated about recent developments in the clinical management of LF. The peripheral health workers should advocate for the promotion of footcare practices, such as regular cleaning of affected parts with soap and water, the use of antibiotics/antiseptics, limb elevation, exercising, etc. Though these people are living in endemic areas and aware of elephantiasis, many people fail to recognize early stages of lymphoedema and subsequent treatment. Appropriate care at early stage can help in prevention or reversal of the progression of the disease. The peripheral level health institutions should take lead in encouraging the patients to modify their behaviour with footcare practices.

Table -1 responses of health workers with regard to lymphoedema care

Responses of health workers	*Per cent*
Seen that lymphoedema patients visit PHC/HSC	92.7
Consulted by lymphoedema patients	95.1
Informed by higher authority on lymphoedema care	17.1
Agreed that PHC/HSC has no specific facilities/drugs for lymphoedema care	61.0
Think that some thing should be done for lymphoedema patients	80.5
Know that these patients suffer from acute ADAL episodes	100.0
Know the concept of foot care	36.6
Advice lymphoedema patient to wash the affected limb regularly	68.3
Advice lymphoedema patient to keep the affected limb dry	56.1
Advice lymphoedema patient to massage the affected area	48.8
Advice lymphoedema patient to use bandage	68.3
Advice lymphoedema patient with regard to wounds and blisters	70.7
Advice lymphoedema patient to use some footwear	85.4
Advice lymphoedema patient to elevate the affected limb during sleep/rest	65.9
Advice lymphoedema patient to exercise (move) the affected limb	56.1
Advice other methods like home remedies	29.3
Advice them with regard to food	75.6

Table -2 perceived benefits due to treatment among patients

Benefit after treatment	*Regimen-1*	*Regimen-2*	*Regimen-3*
Felt relief after treatment	86.4%	83.8%	85.0%
Relief from fever	61.7%	62.5%	62.5%
Relief from pain	29.6%	33.8%	27.5%
Swelling reduced	23.5%	46.3%	47.5%
Just felt well	17.3%	10.0%	12.5%

Fig.-1 CHANGE IN MEAN DIFFERENCE (IN CENTIMETER) OF LIMB CIRCUMFERENCE

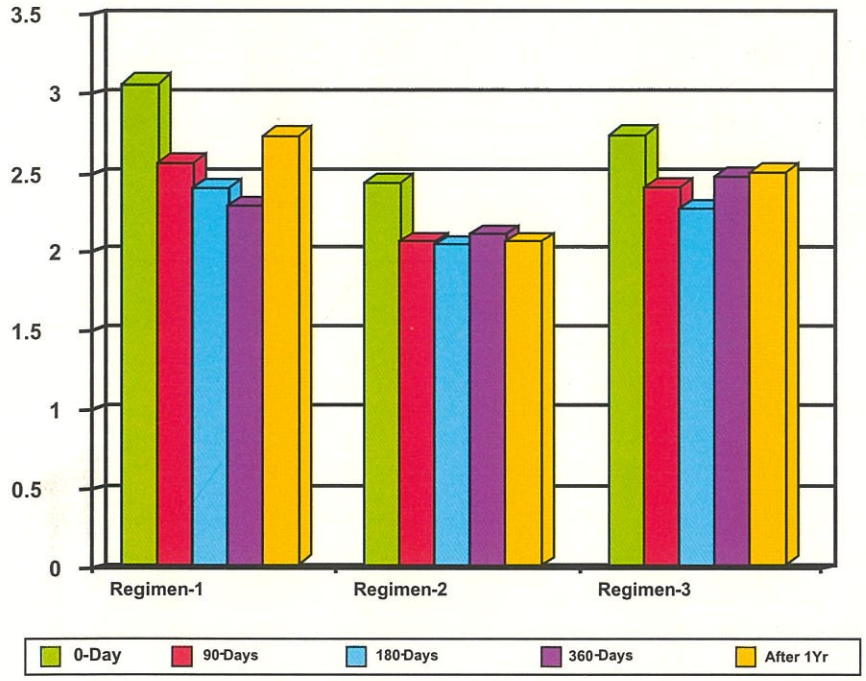
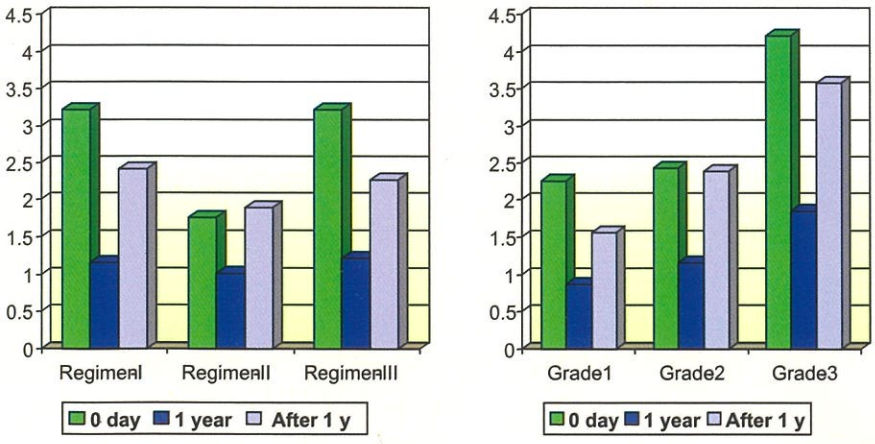


Fig.- 2 CHANGE IN MEAN ADL FREQUENCY AT DIFFERENT TIME PERIODS (1 YEA R PRIOR, DURING TREATMENT AND 1 YEAR AFTER TREATMENT) ACROSS THE REGIMENS AND GRADES



Status:

Extramural (ICMR Task Force on New Molecules through Genomic Research)

Investigators:

Dr. B. Ravindran

Collaborators:

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Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai.

Dr. S. Ramachandran,

Centre for Biotechnology, Delhi.

Starting date: November 2001

Closing date: October 2003

6.2 Towards the development of therapeutic agents using information from malaria immune persons from endemic areas of Orissa

Objectives:

1. To develop therapeutic agents through construction of a phage display antibody library from B-cells of persons clinically immune to malaria.
2. Specifically the drug targets of P0, Pf9 and Pf2 and other novel targets identified by the TIFR laboratory will be used for the development of therapeutic products.

Results:

Since the original immunoscreen was performed with λPf2 cDNA expression clone, it was decided to check the malaria immune sera against the Pf2-peptide. The reactivity of the Pf2-peptide with the malaria immune adult sera samples from Orissa (India), and Ivory Coast (Africa), respectively. The cut-off value used was the mean OD_{405} + 3SD (Standard Deviation) observed with the sera samples from Orissa children' and healthy adults from Mumbai, India. The Y-axis shows the ratio of the OD_{405} value to M+3SD value for each adult sample, and any value >1 was scored as positive reactivity with Pf2 epitope. For Orissa adults this was 86%, while for Ivory Coast this was seen to be 80%. The Pf2-peptide specific antibodies were affinity-purified from five samples and the antibodies were then tested by immunofluorescence. The reactivity on the IFA was specific for Pf2-peptide epitope, as this reactivity could be competed out in the presence of 0.5 mg/ml of Pf2-peptide solution. These affinity-purified antibodies were then used on GIA in two different sets of experiments, and the percentage inhibitions were observed to be 62% and 58% as compared to cultures without any antibody.

It was decided to use various synthetic peptides to assess the antibody reactivity of the samples. PfP0 was represented with four peptides; N1 and N2 representing the amino terminal domain, and C1 and C0 representing the carboxy-terminal domains. Pf2 and Pf9 peptides were used for these two antigens. The carboxy-terminal 250 amino acid domain of AMA1 was used as a HIS-tag fusion protein. It is clear that the response of adults is much higher as compared to that of children for each of the peptide antigens used. This is consistent with our differential screen, which had used immune versus susceptible sera for screening. These results also show that the reactivity of these peptide antigens with sera from immune adults is also observed in Kenya, Africa, and is not a peculiarity of Orissa, India. Thus, the reactivity of these samples showed that immune response is generated against these epitopes in Africa

as well as in India. It was observed that the same immune sera samples reacted with the peptides, although the cut off parameters predicted different frequencies of reactivities for different epitopes. It has been shown that none of these peptides are cross-reactive and that these are distinct antigens on *P. falciparum.* Such data suggests that the immune responsiveness is a state attained by the immune adults, and that there is a general polyclonal activation achieved against several parasite determinants in the immune adults.

6.3 Situation analysis of malaria transmission in Bhubaneswar.

Status:

Intramural

Investigators:

Dr. N. Mahapatra

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Dr. A. Mahapatra

Dr. A .S. Kerketa

Dr. R.K. Hazra

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Mr. D.P.Hansdah

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Collaborators:

State Health Department, RH & FW, BDA, ORSAC, BMC

Starting date: March 2001

Closing date: March 2004

The study envisages situation analysis of malaria in urban set up of Bhubaneswar city in first phase and developing module for malaria control in urban slum and non-slum areas in the 2nd Phase.

Objectives;

1. To assess the prevalence of malaria: disease and infection in the city.
2. To assess the vector population, and its incrimination for malaria transmission.
3. To assess the KAP and the health seeking behavior pattern of urban population in relation to malaria.
4. To make appropriate recommendation for the implementation of comprehensive vector control (CVC) programme for Bhubaneswar.

Methodology:

Administrative data, demographic data, housing pattern and health infrastructure and geographical map of the city were collected. Monthly malaria epidemiological information such as, total fever cases reported at the Hospital, blood slides collected from suspected malaria cases, slide positivity rate(SPR) , Pf % death due to confirmed malaria were collected from all the possible health infrastructure such as , Capital Hospital, Bhubaneswar Municipality Hospital and 8 dispensaries, Central Malaria Laboratory (Govt. of Orissa), Regional Office of Health and Family Welfare and were compiled. The city has been divided into five ecozones each having six wards .In each zone the survey was conducted having both slum and urban locality. Zone -1 comprises of plain lands with irrigation facility, zone 2,4 and 5 are completely plain land where as zone -3 is plain land with riverine belt.

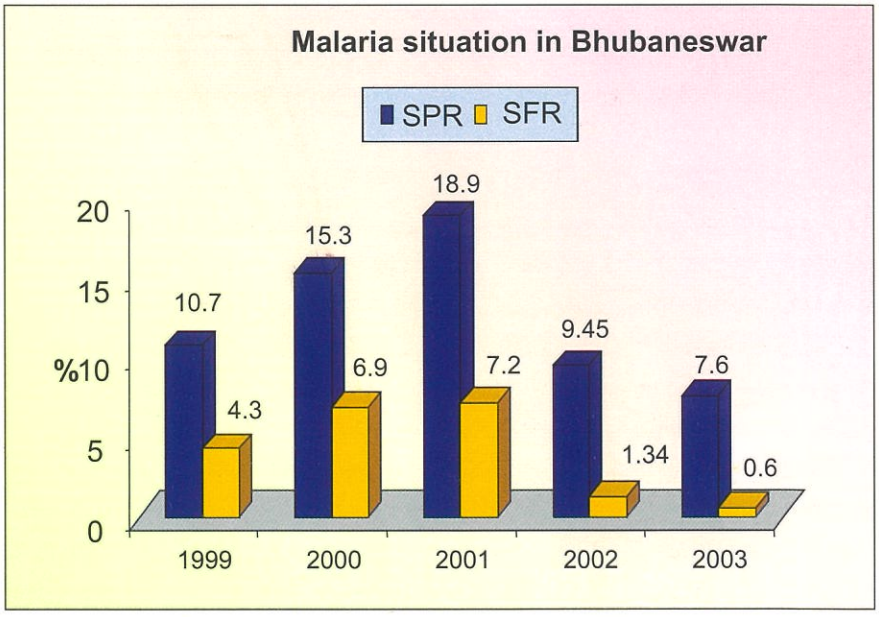
Rapid fever survey was carried out from randomly selected localities of both urban and slum areas. Spleen survey was conducted in 10% of school children of 6-9 years of age in each ecozones were graded as per WHO grading in each region. Slides were brought to the laboratory, dehaemoglobinised and were stained by Giemsa stain and were examined for malaria parasite.

Adult mosquito and larval collection were done once in a week from each zone following the standard entomological procedures(WHO,1975). Mosquitoes were collected during morning and evening hours by using Sucking tube and Mechanical aspirator. All anophelines routinely collected were identified and were dissected for the presence of sporozoites and oocysts. Larval surveys were carried out in different breeding places like rice fields, cess pits, cess pools, ornamental fountain, overhead tanks, domestic water reservoirs, unused pots, water logged areas etc. KAP study was done with the help of a pre-tested structured questionnaire.

Results:

The demographic data available from Municipal corporation reveals the population of the city is 6,47,302 (2001 census) .There are 59 authorised and 131 unauthorised slum areas with a population of 2, 91,285 which is about 45% of the total population of the city. The morbidity data on malaria of Bhubaneswar City collected from different health infrastructure reveals an increasing trend of malaria incidence from 1999 to 2001 which started declining in2002 and2003. (Fig 1).

Fig. 1: Malaria incidence of Bhubaneswar city from 1999 to 2003



Morbidity data on malaria was obtained from five ecotypes having both slum and non-slum areas by making weekly visit to these areas and collecting blood slides from fever cases. Number of blood slides collected from fever cases of zone -1 to zone 5 are 285,166,149,177 and174 respectively.

SPR was found to range from 6.2 % to17.4 % during 2001 to 2002 in different zones (Fig-2).SPR was highest (17.4 %) in Zone-3 followed by zone-1 and least in zone-4.The month wise epidemiclogical situation of malaria prevalence is assessed from passive case detection units of different health infrastructure .The data shows that malaria is prevalent round the year (Fig:6). Fever survey was carried out among the school children revealed 4.2% of malaria infection(Table-1).Spleen survey in primary school children was done by palpation method. The grade 1 spleen was found only in 3 out of 701 children examined. Thus spleen rate was found to be 0.4% only.(Table 1) which shows the urban locality of Bhubaneswar is hypoendemic for malaria.

Table -1 Results of fever survey among school children of Bhubaneswar.

Locality	No of school children examined	No with palpable enlarge spleen	G-1	G-2	G-3	G-4	G-5	No of fever cases examined	Nos +ve	Result
Zone-1	143	0	0	0	0	0	0	12	0	0
Zone-2	289	1	1	0	0	0	0	18	0	0
Zone-3	129	0	0	0	0	0	0	10	0	0
Zone-4	48	0	0	0	0	0	0	1	0	0
Zone-5	92	2	2	0	0	0	0	7	2	Pf

Indoor resting collection could be done in slum areas only. In non-slum areas cooperation was not obtained from many houses for indoor collection. Larval surveys were carried out both in slum and non-slum localities. Adult collections were done in morning and evening hours. Adult mosquitoes belonging to five genera viz. *Aedes*, *Anopheles*, *Culex, Mansonioides* and *Armigeris* were collected. Three known malaria vector species viz., *An.annularis, An. culicifacies* and *An. stephensi* were found(Figure 3-5) in five different ecozones of the city. All the three vectors are prevalent in zone 1 and zone 3 and An stephensi was prevalent in all the zones except in zone 5.

Figure 2: Malaria incidence in different zones of Bhubaneswar during 2002-2003.

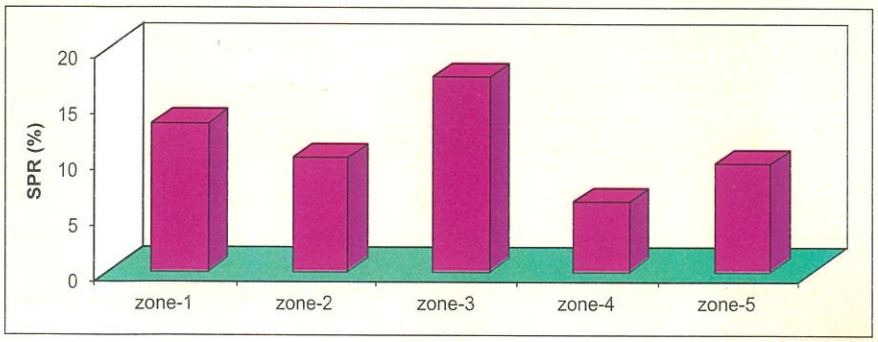


Fig 3: Per man hour density of *An culicifacies* in different ecozones in Bhubaneswar (2001-2003).

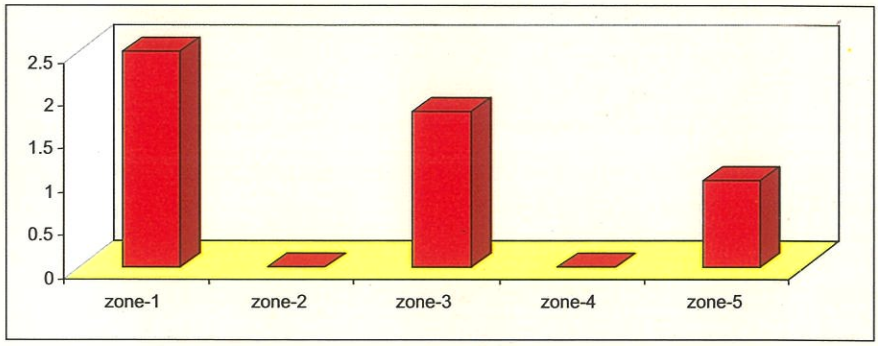


Fig 4: Per man hour density of *An.annularis* in different ecozones in Bhubaneswar (2001-2003).

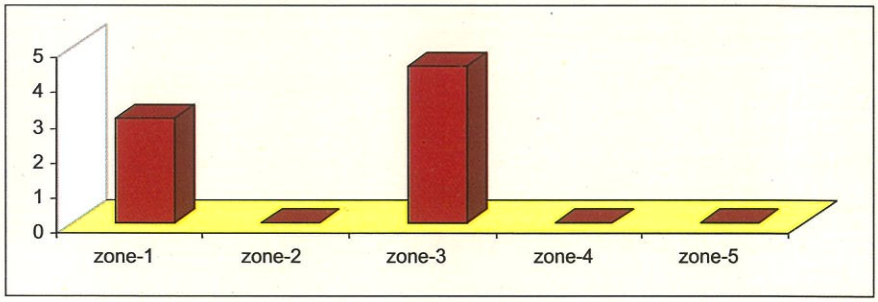


Fig.5: Per man hour density of *An.stephensi* in different ecozones in Bhubaneswar (2001-2003).

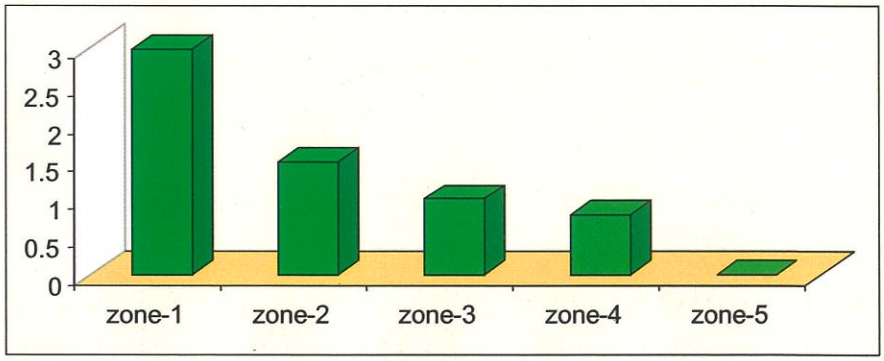
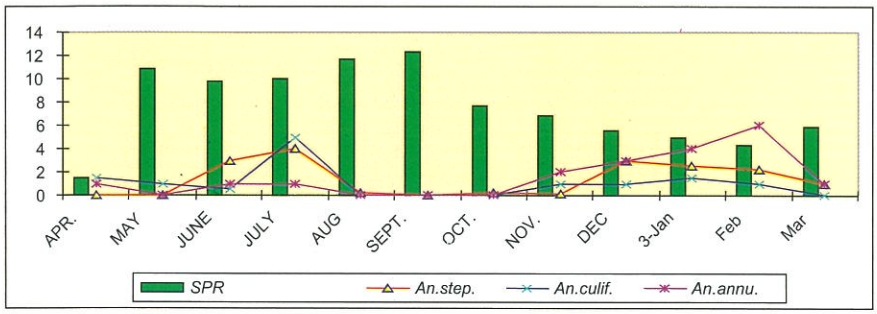


Fig 6: Month wise per man hour density of *Anopheline* vectors in Bhubaneswar City (Year 2002-2003).



Studies on vector incrimination was carried out by detection of sporozoites by PCR method as well as by dissection method from *An stephensi, An culicifacies* and *An annularis* were collected from all the five ecotypes .So far no sporozoite was detected by both the methods.

KAP findings :-

Knowledge, Attitude and Practices (KAP) related to malaria revealed (**Knowledge about malaria)** 65% of the populations consider malaria as a disease while 23.4% consider malaria as a feverish condition. 20% of the respondents know about normal malaria, 42% know about brain malaria and 37% know about the both. (Knowledge about transmission) 46% people know that mosquito bite is responsible for malaria. 22.8% consider malaria as a communicable disease. . **Personal protection methods**: Almost all the households used some protection methods against mosquito bite. 40. % Use mosquito repellents, 44% use mosquito net and 16% use both. **Treatment procedures adopted by people during fever**: Around 55% of the population had good knowledge about malaria treatment. Maximum number (51.6%) of people followed homeopathic treatment while suffering from malaria, as it is much cheaper.28.4% took medicines from shops (Quacks) and only 20% consulted doctors. **Population movement and malaria:** 45.6% of people visited their native place once in every three months. 31.3% visited every month, 11.9% visited once in six months and 10.4% visited yearly once. 56 % of the people got malaria within one month of return from their native place.

The essence of the findings as discussed earlier indicates that month wise malaria incidence is not correlated with the vector density (Fig -6).The prevalence of vector does not seem to correlate with prevalence of malaria .The month with high vector density shows low malaria prevalence and vice versa. Therefore the role of the other influencing factor such as migration can not be overruled. Our KAP study have

supplemented this hypothesis by revealing that that 45.6% of the slum population visited their native places once in every three months and 31.3% visited their native places in every month which are highly endemic for malaria.

Conclusion: Malaria is prevalent through out the year. There is no difference in -malaria incidence between slum(SPR 11.7 %) and non-slum area (SPR 11.9 %)of Bhubaneswar. Though the Govt. data showed SPR 9.5 % and declining trend of Pf % (14 %) during 2002, our survey reveal SPR rate 11.8 % and Pf % (62 %). Three known malaria vectors namely., *An.annularis, An. culicifacies* and *An. stephensi* were prevalent in the city. Malaria prevalence does not correlate with vector prevalence. There was to and fro population movement between Bhubaneswar and known high malaria endemic areas. There is no surveillance mechanism to asses the infection load in migratory population. Our data reveals that 85% of the confirmed malaria positive cases had contracted malaria from endemic areas as per the corelation of the incubation period and their sajourn in the endemic area.The rest 15% of the positive cases acquired the infection localy. Therefore , the possibility of indigenous local transmission can not be ruled out. Therefore, malaria infection in Bhubaneswar city occurred throughout the year due to migratory population from endemic malaria areas and a few indigenous cases of malaria transmission occurred twice in a year viz, one in January and February and other in July. Hence these findings necessates further indepth study with appropriate regular active surveillance round the year with prompt detection and treatment facility in order to control urban malaria situation effectively.

6.4 Population structure of Plasmodium falciparum clinical isolates of Orissa.

Status:
Intramural

Investigators:
Dr. M.R. Ranjit
Dr. G.P. Chhotray
Ms. Anamika Das

Starting Date: March 2002
Closing date: February 2004

Objectives:

1. To study the extent and / or frequency of genetic diversity in natural population of *P.falciparum* in Orissa.
2. To observe the difference in parasite carrier sequence between complicated and non-complicated malaria cases.

Around 200 *P.falciparum* isolates collected from different geographical regions of the state (Keonjhar, Malkangiri, Kondhamala and Cuttack) were genotyped taking the GLURP, MSP1 (K1, MAD 20 & RO 33), MSP2 (3D7 and FC27) and KAHRP genes as markers. Parasite DNA was isolated from 100 µl of blood by phenol-chloroform and ethanol precipitation. The polymorphic repeat regions of block2 of MSP1, block 3 of MSP2, RII region of GLURP and C-terminal repeat domain of KAHRP were amplified by nested PCR using the primers as described by Snounon *et al* (1999) and Kant and Sharma (1996). A 157 µl aliqurt of each PCR was separated by electrophoresis on 2% agarose gel and visualized by UV Tranilluminator of the ethidium bromide staining. Analysis revealed the presence of 8 allelic variants (450 bp to 1100 bp) in GLURP; 5

(150 bp to 350 bp) in K1, 4 (150 bp to 260 bp) in MAD 20 and 1 (150 bp) in RO33 family of MSP1 gene; 6 (450 bp – 750 bp) in 3D7 and 6 (320 bp to 750 bp) in FC27 family of MSP 2 gene; and 3 allelic variants (340 bp, 370 bp and 400 bp) in KAHRP gene in all the study sites. When the prevalence of genotypes of *P.falciparum* was compared between the uncomplicated (n=40) and complicated malaria cases (n=36) collected from same geographical area, the frequency of certain alleles of GLURP (900 bp), K1 (220 bp) / MAD20 (200bp) family of MSP1 and 3D7 (550bp) of MSP2 gene were significantly higher ($p<0.01$) in severe cases than in uncomplicated case (Fig 1 to 3). Complexity of infection with respect to MSP 2 alleles was significantly high ($p<0.001$) in severe cases than uncomplicated ones and (iii) The incidence of PfCRT (K76 T) point mutation associated with high parasite density and clone multiplicity was significantly high in severe malaria patients than in uncomplicated cases.

Conclusion

(i) Extensive polymorphism in the vaccine candidate antigen genes indicates the high transmission of malaria in this part of the country. It also indicates that in future malaria vaccine programme, this factor need important consideration.

(ii) Association of PFCRT (K76T) point mutation with the severity of the disease indicates biological advantage of the CQ resistant parasites, which may be either due to a progression of uncomplicated to severe disease following treatment failure or to increased virulence of the CQ resistant parasite, which needs further indepth study.

Fig1: Frequency distribution of GLURP alleles

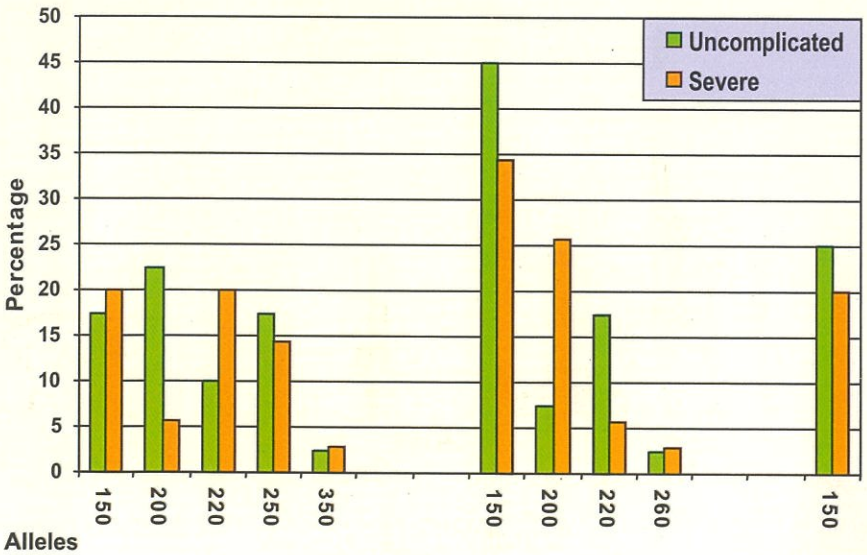


Fig2: Frequency distribution of MSP1 gene

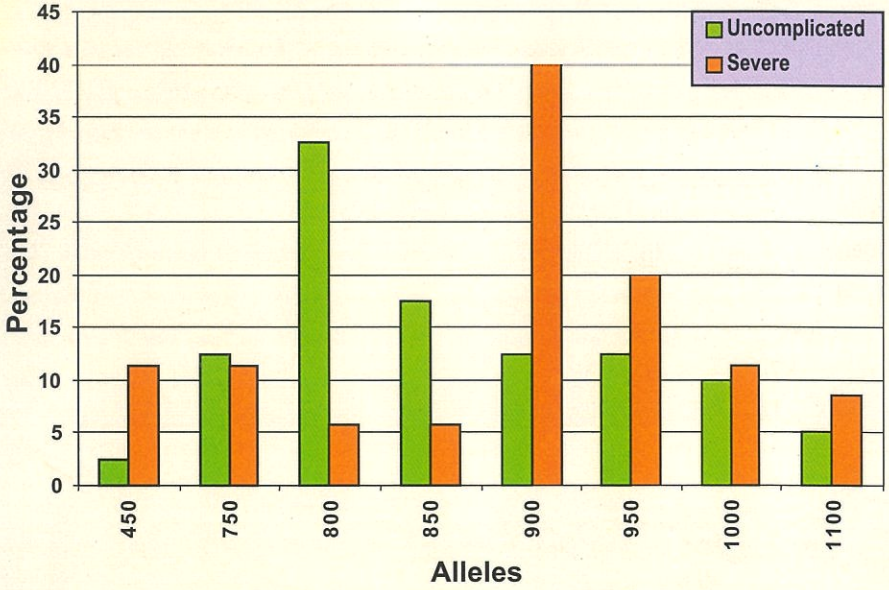
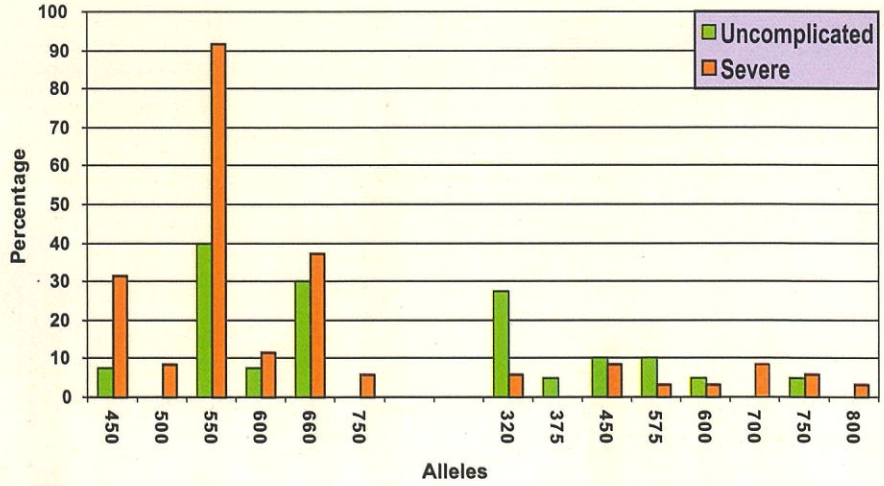


Fig 3: Frequency distribution of MSP2 gene



6.5 Anthropological perspective of malaria in KBK districts of Orissa

Status:

Intramural

Investigators:

Dr. A. Mohapatra

Mr. M. Panda

Mr. D. P. Hansdah.

Starting date: March 2002

Closing Date: February 2004

Objectives:

1. To assess the morbidity and mortality pattern of Malaria in KBK region and to map out malaria prone areas in KBK region using epidemiological parameters.
2. To assess the knowledge, attitude, belief and practices (KABP) on malaria along with the health seeking behavior and health culture of the population in KBK districts.
3. To develop a suitable social mobilisation package, for malaria control in the KBK region.

Background Information:

Orissa records the highest deaths due to malaria, in India, since a decade or so. In Orissa state the KBK region, namely Koraput, Bolangir and Kalahandi (KBK) wrest while districts, shares almost one third (33%) of the malaria burden of the State. The hilly terrain, tribal pockets, seasonal rivers, water bodies and recent insurgence of canal system alongwith bordering forest areas with other malaria endemic areas in the states of Andhra Pradesh, Madhya Pradesh, Jharkhand and Chattisgarh, etc. were presumed to be the main factors contributing towards the malaria situation in KBK region of Orissa. Out of the total population of KBK region, Scheduled Caste and Scheduled Tribes constitute 20% and 35% respectively. The socio-cultural aspects including the health seeking behaviour have emerged as the crucial factors in malaria control programme. It is a well recognised that malaria exerts a heavy economic and social toll; especially tends to strike the most economically productive age group (26-49 years) and then the infants (1-5 years), as evident from the available data. From the study areas it has been evident that the working males (63%) bears the heavy burden due to malaria as they meet most of the household and agricultural needs. Besides this, the females suffering from malaria (37%) also cost the household, with a low quality household care and less agricultural contribution too.

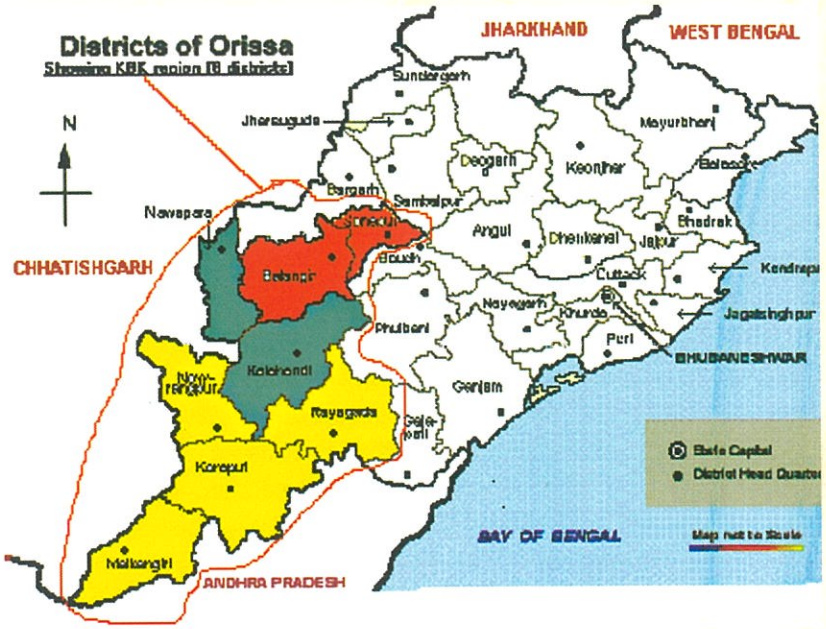
Inadequate management of health care units of the region in terms of non-utilisation of modern health care facilities in time, inadequate knowledge of drug, its availability and use etc. were some of the main reasons of malaria problem in KBK region. The poor socio-economic status of the population increases the problem of the infant and under five-year-old mortality, due to lack of timely treatment (14.6%) and lack of money (Rs. >1000/pm 65.0%) in the KBK regions of Orissa. In an epidemiological analysis of the malaria related data revealed that, during the years 1990 to 1999 there was an increasing trend of malaria related mortality (22.0% to 42.3%); however, from 2000 to 2002 this trend has started decreasing (32.76% to 17.85%), and again showing a up-surge in the year 2003 (22.5%).

Methods:

The KBK region consists of eight districts namely, Kalahandi, Nuapada, Bolangir, Sonepur, Koraput, Nabarangapur, Malkangiri and Rayagada. These districts

were surveyed, during each visit, interactions were held with the district administration, district health authorities along with the peripheral health staff at community level. Minimum two high risk PHC/ CHCs from each district were selected for the purpose of the present study. A total of 39 high-risk sub-centres were sampled from the selected 19 PHC/CHCs of eight districts on the basis of last five-years of malaria morbidity and mortality data, concentration of tribal population and location / topography and climatic parameters etc. of the area following NAMP-WHO protocol. A total of 59 villages were thus sampled- two big (>1000 population) or three/four small (<500 population) high-risk villages from each of sub-centre.

MAP SHOWING THE KBK Districts of ORISSA



Results:

A total 879 respondents were sampled from KBK region. Around 52.2 % of the respondants belongs to different tribal groups. The respondents were further classified as Fever cases (624) and non fever cases (255). Among the fever cases 228 had malaria infection as tested. Of the respondents, 63.0% were males and 73.0% of married. Almost half (50.5%) of the population was illiterate. Among the literates 26.2% were literate upto primary level of schooling. The major occupations of the respondants were cultivation (35.6%), agricultural field labour work (31.2%), and collection of forest produce/ at home (24.0%). Collection of forest produce was observed to be an important source of livelihood. The house types were mostly Kuccha (60.3%) and houses were surrounded by paddy fields (38.1%), forest (15.4%) or bushes/shrubs (34.6%), which contribute substantially towards different malariogenic situations in the surroundings. Around 63.1% of the respondants confirmed that, their family members had experienced malaria during last three years. Mostly (38.3%) respondants ranked malaria as 1st and 44.5% gave 2nd rank as per the severity according to their perception of disease (Tab-2a, 2b).

The response on **treatment seeking behaviour** among the respondants indicated that, more than half of the respondants (61.9%) mentioned that during illness, they consult neighbours / family members first; and go by their treatment advise / schedule first. Around one fourth (24.9%) of the respondents go to quacks; the reasons ascribed for this are the easy accessibility, installment payment facility of fees and use of injections that cures a patient fast (a perception). Only 14.6% of the respondants take proper treatment of malaria on same day and another 85.4% of the fever patients wait 2 to 3 days or more; they belief that by waiting some days the fever will ripe / mature in their body, and then only the treatment will be effective; & recurrence of malaria may not occur –(as perceived). **Utilisation of Health Facilities:** The study revealed that, the types of health care facilities available in the villages or nearby was sub-centres (34.7%) and private practitioners- quacks –(25.1%). These quacks play a major role in managing the health care problem in these villages. Around one fourth (25.7%) of the respondents didn't have any health facility in the village. The effective places of malaria treatment as opined were government hospital 36.2%, private practitioner 22.7%, local health personal and others 22.0%. **Preventive practices** were observed to be poor among the population of KBK region; Fumigation was observed to be the popular preventive practice in the region. Lakh- Jhuna (Sap of Sal tree), dried Neem / Turmeric / Sal leaves were used as the common fumigation materials alongwith this cow dung cakes/ wood (20.3%). Kerosene / Karanj oil massage (12.3%) was practised while going out to jungles, to prevent malaria, germs of the area. It may be noted that all these prophylatic measures were taken to keep away the malaria germs, but not meant to repelll mosquito vector. **Use of mosquito net** was reported by 14.7% of the respondents only. These were little well off families and had an impact of education and an urban exposure to the family. Use of MMN is not observed during the survey period in the survey population. However, the villagers appreciated the Medicated Mosquito Net (MMN) concept, during the FGDs in general. They were even ready to bear the social marketing price, to avail the net at subsidized rates. **The sleeping habit** pattern of the people reveal that, people sleep inside the houses (44.3%) and half of them prefer to sleep outside (21.8%). Sleeping habits of (33.9%) people changes seasonally. The practice of sleeping habit alongwith the domestic animals (33.2%) in the same room may affect the Zoophilic and Anthropophilic feeding habit of the vector. **In the ethno-medicinal Practices,** it was observed that, consumption of leaves of - Oleaceae *'Gangaseuli' (Nyctanthes arbortristis L)* for prevention of malaria was growing popular (46.9%) among the people of the KBK region of Orissa. The qualitative findings also reveal that, the juice of Gangaseuli leaves is working fast as the most useful preventive medicines for those who are suffering malarial regularly. The people have a mis-conception; that consumption of pure country liquor (*Mahuli*) (13.3%) may avert malarial fever. The mothers of young children believe that the children fall sick because of some evil spirits; Hence majority of them approach the Disari / Sira. From the findings it is also clear that, the attitude of the people and the practices related to malaria prevention need to be re-framed, so as to modulate the health seeking behaviour of the population of KBK region.

Table. 2 (a) : Information on background characteristics of respondants of KBK region

Category of information	No. of respondants (n=879)	%
1. Category of respondant interviewed		
Fever cases	624	71.0%
Non-fever cases (villagers elites etc)	255	29.0
2. Sex of the respondant		
Male	554	63.0
Female	325	37.0
3. Educational Status		
Illiterate	444	50.5
Primary	230	26.2
Middle	139	15.8
Secondary	30	3.4
Senior Secondary	36	4.1
4. Occupation of the respondant		
Cultivation	313	35.6
Labour work	274	31.2
Petty business	48	5.5
Service	33	3.7
Other work (At Home / Collection of forest Produce)	211	24.0
5. Marital status		
Married	642	73.0
Unmarried	231	26.3
Widow	6	0.7
6. Ethnicity		
Tribal group	459	52.2
Non-Tribals	420	47.8
7. House type		
Pucca house	87	9.9
Semi-Pucca	262	29.8
Kuccha	530	60.3
8. House surrounding		
Paddy field	335	38.0
Forest	135	15.4
Bushes/ Serbs	304	34.6
River/ Channel/Stream etc	13	1.5
Others (Broken house, barren land etc)	92	10.5
9. Sleeping habits		
Inside house	389	44.3
Out side house/Open places	192	21.8
Both insides and some times outside open places	298	33.9

Table. 2 (b): Information on malaria by respondants in KBK region, Orissa

Category of information	No. of respondants	%
1. Ranking of Malaria (n=879)		
1st Rank	337	38.3
2nd Rank	390	44.5
3rd Rank	127	14.4
Can't Say	25	2.8
2. Consultancy pattern during malaria among experienced cases (n=506)		
Kith and kin	313	61.9%
Village Quacks – private practitioners	126	24.9%
Anganwadi Centre	31	6.1%
Other local health facilities	36	7.1%
3. Days between fever & treatment effort (n=506)		
Same days		14.6%
2 to 3 days & more		85.4%
4. Village DDC, whether providing medicines for cure of malaria (n=879)		
Yes	279	31.7%
5. Types of health care centres nearby the villages (n–879)		
Sub-Centre	305	34.7%
Pvt. Practicenor	221	25.1%
Other means of treatment	127	14.5%
No health facility	226	25.7%
6. Effective places of malaria treatment (n=506)		
Govt. hospital	183	36.2%
Pvt. Practioner	115	22.7%
Local health personnel	101	20.0%
Can't say	107	21.1%
7. Personnel protection practices followed by respondents to avoid malaria (n=879)		
Prevent mosquito bite	184	20.9%
Fumigation sleeping inside house	178	20.3%
Use Kerosene/ Karanja oil while jungle visit	108	12.3%
8. Bed-net practices followed by the respondant or any family member (n=879)		
Yes	129	14.7%
9. Ethno-medical practices followed by respondents to prevent malaria (n=879)		
Consumption of Gangaseuli leaves	412	46.9%
Use of pure country liquor	117	13.3%
Consumption of bhuin-neem root	70	8.0%
Non users of any traditional methods	280	31.8%

Status:
Intramural

Investigators:
Dr. M.R. Ranjit,
Dr. G.P. Chhotray,
Dr. B.P. Dash

Starting date: April 2001.
Closing date: March 2004.

6.6 Studies on molecular variants of G6PD deficiency with special reference to G6PD Orissa (44 Ala – Gly) among malaria endemic population of Orissa.

Objectives:

1. Molecular characterization the G6PD deficiency variants prevalent amongst the male tribal population of malaria endemic areas.
2. To study the clinical profile, morbidity and mortality pattern in G6PD deficiency population with special reference to G6PD Orissa.

During the study, total 3657 male individuals, belonging to 11 tribes (Bhuyan, Gonda, Bathudi, Kandha, Halva, Paraja, Kolha, Munda, Santal, Koya and Orang) and 5 primitive tribes (Kutia Kondha, Dongria Kondha, Bondo, Didayi and Juanga) were screened for G6PD deficiency by DCIP decolourisation test using 20 µl of finger prick blood. The molecular analysis was done by extracting high molecular weight genomic DNA from 300 µl of blood samples. Amplication of exons were performed with 100 ng of genomic DNA in a 25 µl of PCR reaction mixture using the primers as depicted in the Table 1. PCR products were digested by HaeIII for G6PD Orissa and MboII for Mediterranean with appropriate buffer for overnight incubation at 37^{0}C temperature. The PCR digested products were run in 3.5% Nu – sieve agarose gel, stained with ethidium bromide and visualized with an UV transilluminator. Of the total cases, 241 (6.6%) individuals were found to be G6PD deficient. The tribe wise distribution of G6PD deficiency is depicted in Table 2. Molecular analysis revealed that 61% of the deficient samples were having "G6PD Orissa (44 Ala – Gly)" mutation and 35.3% with the G6PD Mediterranean (188 Ser→Phe)" mutation. Clinically 80% of the subjects with G6PD Med and 60% of G6PD Orissa variants were found to be anaemic. Signs of icterus and hepatomegaly was not found in any of the cases examined. The haemotological picture of the G6PD deficient individuals and biochemical characteristics of the G6PD enzymes has been shown in Table 3. It was evident that the Hb level in G6PD Med individuals was significantly lower compared to G6PD B (normal) individuals, while G6PD Orissa individuals did not show any significant difference. Only 2 of the G6PD deficient individuals (G6PD Orissa) were found to be slide positive for P.falciparum infection. From the verbal autopsy both of them were found to have consumed antimalarial drugs (chloroquine), but no adverse effect was reported.

Table - I : Oligonucleotide and Restriction Enzymes used in the analysis of G6PD Alleles

Oligonucleotide	Variant	Restriction Enzymes	Sizes in Normal	Sizes in Variants
5'ACTCCCCGAAGAGGGGTTCAAGG3' 5'CCAGCCTCCCAGGAGAGAGGAAG3'	G6PD Med	MboII	377, 119	277, 119, 100
5'CAGCCACTTCTAACCACACACCT3' 5'CCGAAGTTGGCCATGCTGGG3'	G6PD Orissa	HaeIII	107, 75, 66, 48, 45, 11	123, 107, 66, 45, 11

Table 2: Prevalence and molecular variants of G6PD deficiency gene among different tribal communities

Ethno – linguistic group	No. Examined	No. (%) deficiency	Variant
Indo – European			
Keonjhar			
1. Bhuyan	176	25 (14.2)	25 Orissa
2.Gonda	98	7 (7.1)	7 Orissa
3.Bathudi	212	24 (11.3)	24 Orissa
4.Munda	131	16 (12.2)	16 Orissa
5.Santal	106	13 (12.3)	13 Orissa
6.Juanga*	427	56 (13.1)	51 Orissa / 5 Uncharacterised
7.Kolha	283	44 (15.5)	36 Med / 8 Orissa
Kandhamal			
8.Kandha	188	15 (7.9)	12 Med / 3 Orissa
9.Kutia Kandha*	388	5 (1.3)	4 Med / 1 Uncharacterised
10.Dongria Kandha*	98	4 (4.1)	4 Med
Malkangiri			
11.Holva	197	0 (0.0)	-
12.Paroja	204	11 (5.4)	11 Med
13.Bonda*	449	2 (0.4)	2 Med
14.Didayi*	478	8 (1.7)	6 Med / 2 Uncharacterised
15.Koya	101	0	
16.Oraon	121	11 (9.1)	10 Med / 1 Uncharacterised
Total	3657	241 (6.6)	85 Med / 147 Orissa / 9 Uncharacterised

* Primitive tribes

Table 3: Haematological and biochemical features of G6PD variants investigated

Haematological biochemical features	Hb	PCV	TRBC	TWBC	MCV	MCH	MCHC	Enzyme activity in RBC % of normal	Electrophoretic mobility % of normal	G6P Km µm
G6PD Med (n=65)	9.8 ± 2.0	37.4 ± 5.8	4.7 ± 0.62	8.6 ± 1.9	72.6 ± 6.4	19.6 ± 3.2	26.3 ± 3.9	0-7	100	23.0 ± 3.7
G6PD Orissa (n=147)	10.7 ±1.9	39.1 ±5.6	5.3±0.7	8.4± 2.0	73.5 ±6.8	20.1 ±3.01	27.5 ±3.7	13-28	100	135± 19.0
G6PD normal (n =171)	11.8± 1.3	39.9± 6.3	5.4±0.8	8.3± 1.8	73.9± 8.7	22.1± 3.5	30.0± 4.6	100	100	69.3± 3.0

Conclusion:

I. There are only two different variants of G6PD deficiency were found to be prevalent amongst the tribals of Orissa.

II. The G6PD Med individuals were found to be moderately anaemic compared to the normal subjects while individuals having G6PD Orissa mutation are mildly anaemic.

6.7 Multicentric study on determination of loss of iodine in different types of cooking process

Investigator:

Dr. G.Bulliyya

Mrs.G.Mallick,

Starting date : 1.7.2003;

closing date: 31.4.2004

Funding :

Extramural-WHO (through ICMR)

Objectives:

- The general objective is to study the effect of various cooking methods on the iodine content of foods commonly used by different population groups;
- Identification of commonly used food groups;
- Determination of iodine content in different foods items;
- Determination of iodine in drinking water sources;
- Determination of iodine content in iodised salt to be used in cooking;
- Preparation of various food items in laboratory commonly consumed by populations;
- Assessment of iodine content in cooked foods by different methods of cooking.

Background:

Iodine deficiency disorders is a public health problem in the country and it is the most preventable cause of mental retardation. It is estimated that daily requirement of iodine is 100-150ug 60-70% of the total body iodine, deficiency results from geological and environmental rather than social and economic factors. Iodine is present in soil as iodide imbibed through foods grown on the soil. Humans critically dependent on an external supply of iodine through the soil and about 90% of daily needs are met from food and 10% from water. However, soil iodine has been leached out continuously that makes vulnerable to low iodine and low nutritional iodine intake by inhabitants. The fortification of edible salt with iodine is continued in the country since 1962 as a corrective measure of iodine deficiency and the degree of iodine loss from food and soil is a matter of concern to scientific community. The content of iodine of food consumed is not necessarily equivalent to that of raw foods since some iodine is lost during cooking and washing. The loss of iodine is reported upto 70% in different cooking methods. Much data is not available indicating the loss of iodine in different cooking process adopted in different regions of the country.

Progress

This study is one among the four multi-centres in the country, which covered eastern region. The eastern region includes seven states namely Orissa (Cuttack, Puri, Keonjhar and Sudargarh), Jharkhand (Ranch, Gumla and Palamu), Bihar (Patna, Mujafarpur, West Champaram and Goplalgunj), West Bengal (Malda, New Jalpaiguri, Cooch Behar and Darjeeling), Sikkim (East Gangtok), Assam (Debrugarh and Jorhat)

and Meghalaya (Shillong). A total of 19 districts have been selected on the basis of endemicity of goiter prevalence of school children surveyed by the DGHS (1964-2000). Villages selected in each district randomly from the Census lists of blocks. As per objectives of the study the households were informed and requested for cooking the selected recipe. The required ingredients supplied to the housewife that were purchased from local market along with three portions of same ingredients. All the ingredients were weighed including water prior to cooking the recipe. As per common protocol household salt was tested for iodine using rapid spot test kits then iodized salt provided for cooking, if the household salt was not adequately iodized (>15ppm). Half of the cooked recipe blended thoroughly and brought to the laboratory in ice flask. Three portions of ingredients brought to laboratory cooked the same recipe twice again in the laboratory using the same proportion of ingredients and cooking procedure. An uncooked raw recipe prepared using same proportion of ingredients without cooking. Each of the food recipes blended thoroughly 3-4 times and aliquots were dried in air oven at 60^0C for 2-3 days. The dried samples powdered after grinding and sieving preserved in pre-labeled airtight plastic bottles until analysis. A total of 46 recipes have been collected from the households of eastern region (Table 1).

(Recipe preparation in Coochbehar Dist, W.B.)

Household salt samples tested in laboratory for iodine content using iodometric titration method. Household water samples used for cooking were analyzed in the laboratory for iodine content using standard spectrophotometric methods. Iodine content of dry food samples was analyzed by the dry-ash spectrophotometric method employing Sandell-Kolthoff reaction. For each of the food recipe, 4-sets of dry samples (one field, two laboratory and one raw recipes) were analyzed for iodine content. In each set of assay, blank, standards, casein-quality-control, casein-recovery and food recipe samples were analyzed in duplicate with deionised-triple glass distilled water.

The iodine contents of salts used in recipes were well above the recommended levels of market (>30ppm) and consumer (>15ppm). The iodine content of salt ranged from as low as 38.1ppm to as high as 53.5ppm. However, household salt had relatively low iodine content in Puri (9.5-30.7ppm), Cuttack (12.7-41.02ppm), Keonjhar (11.6-12.97ppm) and Sundargarh (12.97-15.07ppm) districts of Orissa. Household salt samples from other states had adequate levels of iodine with an exception of West Champaram district of Bihar, where iodine content was much below the recommended levels (3.45-4.2ppm). The iodine content of household water is relatively high in Puri (1.11-1.58ng/dl) followed by Cuttack (0.845ug/dl), Keonjhar (0.56-1.64ug/dl), and Sundergarh (0.33-0.35ug/dl) districts of Orissa. The levels of water iodine were higher in Ranchi (4.4-4.64ug/dl) in comparison to Gumla (1.19-1.22) and Palamu (3.20-3.68ug/dl) districts of Jharkhand. Out of four districts in Bihar, water iodine was much better in Patna and Gopalgunj than in Mujafarpur and West Champaram. In Newjalpaiguri, where water iodine is negligible ((0.02-0.07ug/dl) when compared to neighbouring districts of Malda, Darjeeling (0.30-0.34ug/dl) and Coochbehar (0.6ug/dl) of West Bengal.

The concentrations of iodine (per 100g of dry recipe) in uncooked raw recipes were consistently higher than the recipe cooked either in field or in laboratory without

any exception. In Orissa, iodine content of raw recipes was ranged from as high as 871ug in khanga-fish-curry, 611ug in kobi-alu-bhaja, 541ug in mula-bhaja and 430ug in Chanra-fish-curry to as low as 199ug in alu-bharta and 136ug in tomato-khatta. The levels of iodine was less in cooked field sample than their respective recipes cooked in laboratory on the same day of collection from Puri and Cuttack districts, it was reverse from the recipes cooked in the laboratory more than 3-days after cooking. The loss of iodine varied from recipe to recipe, it was ranged from 7.69% from field and lab cooked kosala-sag to 63% from filed Kanga-fish-curry and 67.38% from rohi-fish-curry. The levels of iodine from recipes cooked in the field were commonly higher than the respective recipes cooked in the laboratory for the states of Jharkhand, Bihar, West Bengal and Sikkim. The loss of iodine for cooked field recipe from raw recipe varied in Jharkhnd (16.3-55.8%), Bihar (18.0-48.1%), West Bengal (9.8-57.8%)and Sikkim (9.1-35.4%). The loss of iodine for cooked laboratory recipe from raw recipes for Jharkhnd (16.5-66.3%), Bihar (14.8-40.6%), West Bengal (16.3-54.5%)and Sikkim (9.8-50.6%) states. The recipes of states from Assam and Meghalaya are to be analyzed. However, iodine content of these recipes contributed through the ingredients as well as iodized salt used in the cooking recipes.

Table 1. Distribution of food recipes collected from eastern sates of India

State	District	No	Food recipe
Orissa	Puri (19.3)!	6	1.Ghanta,2.Alu-bharta, 3.Tomato-khatta,4.Saga-santula 5.Khanga-fish, 6.Chanra-fish
	Cuttack (21.6)!!	2	7. Kobi-alu-bhaja, 8.Dalma
	Keonjhar (15.8)!!	2	9. Rohi-fish-curry, 10. Bhendi-bhaja
	Sundargarh (30.3)!!!	2	11.Kosala-saga-bhaj, 12. Mula-Bhaja
Jharkhand	Ranchi (10.2)!	2	13. Sem-alu-sabji, 14.Chicken-curry
	Gumla (10.2)!	2	15. Chhole-curry, 16. Egg-curry
	Palamu (20.9)!!	2	17. Chana-dal, 18. Fish-curry
Bihar	Patna (26.5)!!	2	19. Alu-dum-gobi, 20. Tadka
	Mujafarpur (41.7)!!!	2	21. Aludum, 22. Murga-crry
	W.champaram(64.3)!!!	2	23. Meat (mutton), 24. Palank
	Gopalganj (24.4)!!	2	25.Chemica-subji, 26. Baigan-curry
West Bengal	Malda (10.3)!	2	27. Khichdi, 28. Palank-sag
	N.Jalpaiguri (33.2)!!!	2	29. Guguni, 30. Fish-curry
	Coochbehar (21.7)!!	2	31. Sukto, 32 Dim-curry
	Darjeeling (35.2) !!!	2	33.Neutella-kopta, 34. Momo-veg
Sikkim	East Gangtok (37.8)!!!	4	35. Momo-veg, 36. Massam 37. Chhole-mattar, 38. Raya-sag
Assam	Debrugarh (65.8)!!!	2	39.Goroi-mas, 40. Alu-surua
	Jorhat (13.2)!	2	41.Alubilahitarakari, 42. Mati-dal
Meghalaya	Shillong (2.3)	4	43. Matar-suti, 44. Dalna 45. Soya-tarkari, 46.Puti-mas
7	19	46	

Severity of IDD among school-age children based on total goiter rate (TGR) by the DGHS Surveys (Normal <4.9%, !Mild 5.0-19.9%, !!Moderate 20.0-29.9, !!!severe >30.0%)

7. GENERAL INFORMATIONS & PUBLICATIONS

7

INSIDE

Library, Information & Publications

7.1 Library & Information

During the year the library & information division has published its first issue of Library News letter. Half yearly RMRC News Bulletin is regularly published. Like last year, the library has renewed its subscription for all 70 journals. Now the Library is subscribing total 40 foreign, 34 Indian Journals for its users. Besides these more than 20 Newsletters are being received from various research organizations as gratis. Library is also getting 7 daily Newspapers and 3 magazines for readers. The library caters library, Information and reprographic support to its users. Recently library has procured a Digital copier cum LAN Printer for reprographic activities.

Prof. L.N.Mohapatra, Ex- Director of this Centre has donated many books (125 books) and reprints to this library. Prof. Mohapatra's collections have been kept in a separate section. These rare collections are very useful for researchers.

Library Automation: This year Library has procured " Libsys- IV (Web OPAC)" software for library computerization. The software has installed and initial data entry work has started. LAN installation in RMRC building has already initiated by HCL infosys Ltd. as per ICMR's recommendation. As soon as LAN is completed and data entry work of library collections is completed the RMRC library can be accessed through LAN and Web.

Services: The library & Information division continued its information services to the readers. The services like On-Line Database search, INTERNET access, E- mail facility to the scientists, On- line Reprint request, ICMR library Network for resource sharing are being rendered to the scientists regularly. Information services like local library network for interlibrary loan, photocopying services, Off-line MEDLINE database search (CD-ROM), News clipping and maintaining scientific publications of the Institute are being rendered by the division. IEC materials like Filariasis, Malaria and IDD leaflets and booklets distributed to DHS, Govt. of Orissa and all CDMOs, District Collectors of all 30 districts of Orissa and distributed to public during field studies.

The library and information division is doing publication activities of the centre. RMRC News bulletin is being published bi-annually from this division. Besides, the division looks after the publication of centre's Annual Report every year.

7.2 Publications (April 2003- March 2004)

1. Babu BV and Kar SK. Coverage, compliance and some operational issues of mass drug administration during the programme to eliminate lymphatic filariasis in Orissa state of India. *Tropical Medicine and International Health* 2004; 9 (6): 702-709. **(*Impact factor=2.156*).**
2. Babu BV and Nayak, AN. Treatment costs and work time loss due to episodic adenolymphangitis in lymphatic filariasis patients in rural communities of Orissa, India. *Tropical Medicine and International Health* 2003; 8 (12): 1102-1109. **(*Impact factor=2.156*)**
3. Babu BV, Hazra RK, Chhotray GP and Satyanarayana K. Knowledge and beliefs about elephantiasis and hydrocele of lymphatic filariasis and some socio-demographic determinants in an endemic community of Eastern India. *Public Health* 2004; 118 (2): 121-127. **(*Impact factor=0.697*)**

4. Bal MS, Beuria MK, Mandal NN and Das MK. Parasite antigenemia and IgG4 antibodies to a filarial protease in an endemic population in India. *Journal of Helminthology* 2003; 77: 287-290. **(*Impact factor=0.939*).**

5. Balgir RS, Dash BP and Murmu B. Blood groups, hemoglobinopathy and G-6-PD deficiency investigations among fifteen major scheduled tribes of Orissa, India. *The Anthropologist* 2004; 6 (1): 69-75.

6. Balgir RS, Mishra RK and Murmu B. Clinical and Hematological Profile of Hemoglobinopathies in Two Tribal Communities of Sundargarh District in Orissa, India. *International Journal of Human Genetics* 2003; 3 (4): 209-216.

7. Balgir RS. Morphological and Regional Variations in Body Dimensions of the Gujjars of Different Localities in North-Western India. *Anthropologischer Anzeiger* 2003; 61 (3): 275-285.

8. Balgir RS. Prevalence of abnormal Hemoglobin E gene in the Dhelki Kharia tribal population. *Current Science* 2003; 85 (11): 1604-1608. **(*Impact factor=0.694*).**

9. Beuria MK, Bal MS, Mandal NN and Das MK. Age dependent prevalence of asymptomatic amicrofilaraemic individuals in a *Wuchereria bancrofti* endemic region of India . *Transactions of Royal Society of Tropical Medicine and Hygiene.* 2003;97:297-298.**(*Impact factor=2.114*)**

10. Bulliyya G. Secular deterioration in nutritional status of young children, an alarming menace for the state of Orissa. *Man in India.* 2003; 83(1&2): 49-71 **(*Impact factor (SSCI)=0.035*)**

11. Bulliyya G. Anthropoecological dimensions of the Easter Ghats section of Orissa: an overview. *South Asian Anthropologist* 2004; 4(1): 73-88.

12. Bulliyya G. Atherogenic aspects of tobacco smoking in a coastal population of Andhra Pradesh, India. *Journal of Human Ecology* 2003; 14(5): 349-353.

13. Chhotray GP, Dash BP, Ranjit MR. Spectrum of Haemolglobinopatheis in Orissa, India. *Hemoglobin.*2004;28:69-75. **(*Impact factor=0.505*).**

14. Mannan BA, Patel K, Malhotra I, Ravindran B and Sharma S. How specific is the immune response to malaria in adults living in endemic areas? *Vector Borne Dis.* 2003 Sep-Dec; 40 (3-4): 84-91.

15. Nayak AN and Babu BV. Tribal health problems: an anthropological appraisal. *Man in India* 2003; 83 (3 & 4): 301-313. **(*Impact factor (SSCI)=0.035*)**

16. Pal BB, Khuntia HK, Acharya AS and Chhotray GP. Drug abusers – a new high risk population for HIV infection in Orissa. *Indian Journal of Medical Microbiology* 2003; 21 (3): 262-264.

17. Panda M and Mohapatra A. Malaria Control : An over view in India. J Human Ecology. 2004; 15(2): 101-104.

18. Vathsala PG, Pramanik A, Dhanasekharan S, Ushadevi C, Pillai CR, Subbarao SK, Ghosh SK, Tiwari SN, Sathyanarayan TS, Deshpande PR, Mishra GC, Ranjit MR, Dash AP, Rangarajan PN and Padmanavan G. Widespread

occurrence of the Plasmodium falciparum chloroquine resistance transporter (PFCRT) gene haplotype SVMNT in P.falciparum malaria in India. *American Journal of Tropical Medicine and Hygiene* 2004; 70 (3): 256 – 259. **(*Impact factor=2.105*).**

Publications in press:

1. Bulliyya G. Coronary artery disease risk profile with reference to alcohol use. *Indian Journal of Public Health.*
2. Mangla A, Khare A, Vineeth V, Pandey NN, Mukhopadhyay A, Ravindran B, Bal V, George A and Rath S. Pleiotropic consequences of Bruton's tyrosine kinase deficiency in myeloid lineages lead to poor inflammatory responses. Blood. 2004 Apr 29 ***(Impact Factor = 10.120)***
3. Ranjit M R, Das A, Chhotray G P, Dash B P, Das B N. The Pfcrt (K76T) point mutation favours clone multiplicity in P.falciparum infection. *Tropical Medicine International Health.* ***(Impact Factor =2.156)***
4. Ranjit M R, Das A, Chhotray G P, Roth R N, Kar S K. The Pfcrt (K76T) point mutation and its usefulness for monitoring chloriquine resistance. *Annals Trop Med Parasitol .**(Impact Factor= 1.01)***
5. Rath K, Swain BK, Mishra S, Patasahani T, Kerketta AS and Babu BV. Peripheral health workers' knowledge and practices related to filarial lymphoedema care: a study in an endemic district of Orissa, India. *American Journal of Tropical Medicine and Hygiene.* **(*Impact factor=2.105*).**

Chapter in Book:

1. Bulliyya G. Habitat, health and nutritional problems of Kondhs: a major scheduled tribal community of Orissa. In: *Dimensions of Researches in Indian Anthropology.* Oriental Anthropologist Series-1. V.S.Sahay and P.K.Singh (Eds). Serials Publications, New Delhi. 2003; pp.326-356.

7.3 Meetings / Seminars/ Symposium organized

1. **Summer Training Programme** : RMRC Organised two days summer traing programme for M.Sc. Biotechnology students of Trident Academy of Creative Technology, Bhubaneswar from 29-30 May 2003 at RMRC, Bhubaneswar.
2. **Training on Verbal Autopsy:**Two days training programme on verbal autopsy (VA) was organized at RMRC Bhubaneswar on 9th and 10th December 2003.Training was imparted by Dr. A.S. Kerketa , R.O (clinical) to 50 numbers of SRS surveyors. Dr.Prabhat Jha, Director Center for Global Health and Research, Toronto and Dr.R.C Sethi, DRGI, (SRS) of Registrar General of India and Dr. S.K.Kar, Director. RMRC delivered the lecture on Evolution and Importance of verbal autopsy.

7.4 Human Resource Devlopment

Ph.D Programme:

(Enrolled)

1. Mr. H.K.Khuntia, R.A has registered for award of Ph.D. degree to Utkal University, Bhubaneswar on the topic "Molecular epidemiological analysis of

Vibrio cholerae associated with epidemic and endemic cholera in coastal and tribal districts of Orissa" under the guidance of Dr. G.P. Chhotray.

2. Mr. N.S.Marai, R.A has registered for award of Ph.D. degree to Utkal University, Bhubaneswar on the topic "Current trends in Malaria transmission in Orissa, India" under the guidance of Prof. A.P.Dash, Director, MRC, Newdelhi and co-guide Dr. N. Mohapatra.

3. Mr. N.N.Mandal, R.A has registered for award of Ph.D. degree to Utkal University, Bhubaneswar on the topic "Studies on the immuno protective potential of detergent soluble and lipid antigen of filarial parasite in lymphatic filariasis" under the guidance of Dr. M. K. Das.

4. Ms. Anamika Das, S.R.F. has registered for award of Ph.D. degree to Kalyani University, Kalyani, West Bengal on the topic "Clinical malaria: association of CD36 gene polymorphism and P.falciparum genotypes" under the guidance of Dr. M.R.Ranjit.

5. Mr. B. R. Sahu, SRF has registered for award of Ph.D. degree under Utkal University on the topic " Role of antibodies in protective immunity in human and experimental filariasis " under the guidance of Dr. B. Ravindran.

6. Mr. Alok Das Mohapatra has registered for award of Ph.D. degree under Utkal University under the guidance of Dr. B. Ravindran.

7. Mr. Mahendra Panda, SRF has registered for Ph.D degree under Utkal University on the topic " Problem of Endemic Malaria among tribal and Non-tribal; population in KBK region of Orissa" under the guidance of Dr. A. Mohapatra.

(Awarded)

1. Dr. A.S Acharya has awarded Ph.D in Science (Statistics) under Utkal University on the topic "*Sequential estimation of genetic parameters and its applications*" under the guidance of Prof K.Dutta, Head, Dept. of Statistics, SambalPur University and Dr. G.P.Chhotray, D D.

2. Dr. Bidut Kumar Das, Asso. Professor, SCB Medical College, Cuttak has awarded Ph.D under Utkal University on the topic " Immunological correlates in plasmodium falciparum infection with special reference to cerebral Malaria" under the guidance of Dr. B. Ravindran., DD (SG).

M.Sc. Dissertation Work:

1. Ms. Sagarika Pradhan of Post Graduate Dept. of Applied and Industrial Microbiology, Utkal University, Vanivihar did her M.Sc. dissertation on "Isolation and identification of Escherichia coli" under the guidance of Dr. G.P. Chhotray and Dr. B.B.Pal,

2. Mr. Himadri Bhusan Bal of Post Graduate Dept. of Applied and Industrial Microbiology, Utkal University, Vanivihar did his M.Sc. dissertation on "Assessment of Geohelminth worm burden by percoll gradient method" under the guidance of Dr. G.P. Chhotray.

(Foundation Day Celebration)

3. Ms. Gitanjali Mohanty of Centre of Post Graduate Studies in Microbiology, Orissa University of Agriculture and Technology, Bhubaneswar did her M.Sc. dissertation on "Chloroquine resistant gene polymorphism in complicated and non – complicated malaria cases" under the guidance of Dr. G.P. Chhotray.
4. Ms. Sikha Mohanty of Dept. of Biotechnology, Ravenshaw College, Cuttack did her M.Sc. dissertation on "Molecular characterization of Plasmodium falciparum" under the guidance of Dr. M.R. Ranjit.
5. Ms. Pragyan Priyadarsini Mohanty of Dept. of Biotechnology, Ravenshaw College, Cuttack did her M.Sc. dissertation on "Isolation and characterization of Human G6PD enzyme" under the guidance of Dr. M.R. Ranjit.
6. Ms Binduprabha Pati of Revenshaw College, Cuttack did her M.Sc. dissertation on " Polymerase Chain Reaction(PCR) detection of sporozoite in mosquito vector" under the guidance of Dr .N. Mahapatra.
7. Ms Paramita Panda M.Sc. student from Dept. of Biotechnology, Ravenshaw college underwent training in laboratory techniques in Immunology (Nov. 2003 – Jan 2004) under the guidance of Dr M.K. Das.
8. Mr. S.R Nayak student from Allahabad Agricultural Institute (Deemed University) worked on "Shigellosis in some urban areas of coastal districts of Orissa" under the guidance of Dr. B.B.Pal.
9. Mr. Ashok Kumar Sarangi student of Microbiology from Trident Academy of Creative Technology , Bhubaneswar worked on " Shigellosis from hospitalized diarrhea patients under the guidance of Dr. B. B. Pal.

7.5 Events & activities:

National Technology Day: RMRC, Bhubaneswar observed National Technology Day on 12th May 2003. Prof. G.B.N. Chainy , Prof & Head , Dept. of Zoology, Utkal University, Bhubaneswar delivered a talk on *" Technology development in the field of Endocrinology".*

(Common protocol development meeting on "Tribal Health".)

Meeting on Common Protocol development on Tribal Health: A meeting on development of common Protocol on Tribal health was held at RMRC, Bhubaneswar on 18-19 Sept. 2003. Scientists from RMRC, Bhubaneswar, RMRC, Jabalpur, NIN Hyderabad, NICED, Kolkata, MRC, New Delhi, VCRC, pondichery, Tribal Wlfare Dept. Govt. of Orissa and other local experts on Tribal Health participated in the meeting. The Meeting was conducted under the chairmanship of Prof. K.Ramachandran, Ex-Prof . of Biostatistics AIIMS & advisor to ICMR, Dr. A. Sarangi, Commissioner- cum-Secretary, Tribal health, Govt. of Orissa addressed the group.

ICMR Foundation Day Celebration: Regional Medical Research Centre (ICMR), Bhubaneswar observed ICMR foundation day on 17th November 2003. On this occasion eminent scientist Prof. Basudev Kar, Ex- Principal, SCB Medical College, Cuttack and President Orissa Vingyana Academy delivered a talk on " Diabetes and human Health".

Malaria co-ordination meeting: RMRC conducted " Malaria Co-ordination Meeting" on 21st July 2003. Director, Health Services, Govt. of Orissa, Joint Director

(H &FW), Regional Director Health & Family Welfare, Director, EMCP, experts from UPDP and Ex-DHS, Govt. of Orissa participated the meeting. The current status was discussed and proposals made for better co-ordination and implementations.

Protocol development meeting on " Nutrition" was organised on 5-6th Dec 2003 in the Centre.

Animal Ethical Committee meeting was held on 27th August 2003 for review of various ethical issues in scientific projects dealing with animals and addressed the interim progress of projects on ethical issues.

Swasthya Mela:

1. RMRC participated in the Parivar Kalyan Swasthya Mela organized by District Swasthya Samiti, Puri at Satyabadi Hospital from 15th to 17th Februray 2004 and conducted a mass screening of Haemoglobinopathies.
2. RMRC participated in the International Thalassemia Day Celebration for screening of Thalassemia cases by performing NESTROFT in Shishu Bhawan, Bhubaneswar on 8th May 2003.
3. RMRC participated in Swasthya Mela Organized by Family Planning Association of India (Bhubaneswar Chapter) during 17-19th October 2003 at Sundergarh and during 12-14th February at Nayagarh organised by Zilla Swasthya Samitee and Information International, Bhubaneswar. Mass screening of Haemoglobinopathies has conducted and Genetic/marriage counseling were provided to the affected people.

(Scientific Talk on Foundation Day Celebration)

Award: Dr. G.P.Chhotray, has been awarded **"Sir Shriram Memorial Award for 2003-2004"** by National Academy of Medical Sciences(India), New Delhi.

7.6 Other Facility:

Insectariums

The insectorium facility is maintained at the Centre under Entomology Department by rearing of various stages of vectors used for lab studies. Cyclic colony of the following mosquito species are being maintained in our Insectary.

1. Aedes *aegypti* (black eyed Liverpool strain).
2. *Anopheles stephensi*
3. *Culex quinquefasciatus*

Cyclic colonies of different mosquitoes were maintained for conducting different experiments, such as development of different strains and species of filarial worms which will help in the selection of proper animal model, conducting bio-assays of different plant products for observation of its insecticidal properties.

Animal House

Animal facility in the Centre continues to be used under Immunology Department catering to all on-going research projects requiring animal experimentation. Currently *M. couch,* BALB/C mice, Guinea pigs are available for experimentation. Animal facility has been registered with CPCEA. All the projects concerning animal use/

experimentation are discussed in duly constituted Animal Ethical Committee of the Centre and work progress review periodically by committee. The facility is well maintained with animal house attendant and other trained researcher. Animal house is maintained regularly with periodic inspection and health monitoring by veterinarian. Now a new animal house is being constructed to cater to large number of animals and better facilities. Staff maintains the periodic records.

Budget and Extramural Grants:

The total sanctioned Budget in respect of the Centre (Non-Plan & Plan) for the year 2003-04 is Rs. 334.64 lakhs .

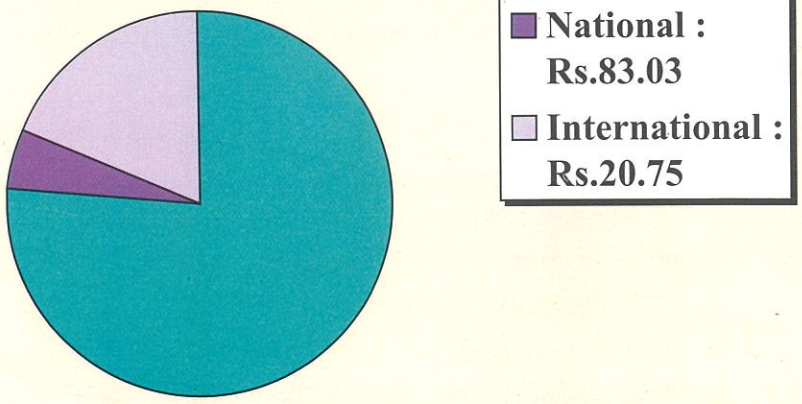
The total expenditure made by the Centre for the year 2203-04 Rs.323.26 lakhs.

In total 18 nos of extramural projects were under taken at RMRC, Bhubaneswar in the year (2003-04) with a total extramural grant received during the year was Rs.103.78 lakhs.

(Auditorium Under Construction)

Intramural :Rs. 334.64 (76%) **(In Rs. Lakh)**

Extramural : Rs.103.78 (24%)



Resource Generation 2003-2004

7.7 Meetings Seminars /Symposium/Workshop attended

(April 2003-March 2004)

1. Dr. S.K.Kar delivered talk on "Geriatic Problems- Future Perspectives" as Chief Speaker in a Workshop organized by WHO at Dr. Abhinna Chandra Homeopathic Medical College & Hospital, Bhubaneswar on 22nd April, 2003.
2. Dr. S.K.Kar Participated as an Invitee by the Dept. of Health & Family Welfare, Government of Orissa in "Gramsat Programme" at Biju Pattnayak Film & TV Institute, Cuttack on 26th April, 2003.
3. Dr B Ravindran attended a meeting as a Member of the Sub-Committee on "Molecular aspects of Host-Parasite relationship and genetic diversity of filarial parasite" and presented a pre-proposal on "Human Lymphatic Filariasis: Genetic polymorphism of host molecules involved in immunity and immuno-regulation" on 4th April 2003.
4. Dr. S.K.Kar delivered talk as Chief Speaker on "Thalassemia and Current Perspective" on the occasion of "Observance of International Thalassemia Day" organized by TASWEL, Orissa on 8th May 2003 at Shisu Bhawan, Bhubaneswar.
5. Dr. S.K.Kar delivered Guest lecturer on " Biotechnology" at Seminar organized by Dept. of Botany, Ravenshaw College, Cuttack on 10th May 2003 .
6. Dr. R.S.Balgir participated as Expertise on the request of TASWELS, ORISSA, in the International Thalassemia Day Celebration for screening of Thalassemia cases by performing NESTROFT in Shishu Bhawan, Bhubaneswar on 8th May 2003.
7. Dr. S.K.Kar delivered Guest lecturer on "Methods in Biotechnology" at Biotechnology Seminar, organized by Dept. of Zoology, Utkal University as Chief Speaker on 12th May 2003.
8. Dr. S.K.Kar delivered talk as invited speaker on "IDD Status in Orissa" at State Institute of Health & Family Welfare, Bhubaneswar on 22nd May 2003
9. Dr. S.K.Kar participated in workshop on "Finalisation of protocol and development of standarsized tools for the use in the multi-centre study on drug delivery strategies in lymphatic filariasis elimination in urban areas" organized by UNDP/World Bank/WHO's Special Programme for Research and Training in Tropical Diseases (TDR) at Gurgaon, during 27-29, May 2003.
10. Dr. B. V Babu attended a workshop on "Finalisation of protocol and development of standardised tools for the use in the multi-centre study on drug delivery strategies in lymphatic filariasis elimination in urban areas" organised by UNDP/World Bank/WHO's Special Programme for Research and Training in Tropical Diseases (TDR) at Gurgaon, during 27-29, May 2003.
11. Dr. G.Bulliyya attended Dissemination Workshop on GOI-UNICEF-IDD-IEC Project 2002-2003 held at Conference Hall of State Institute of Health & Family Welfare, Bhubaneswar and presented a paper entitled Community study on

(Training in Verbal Autopsy)

knowledge-attitude-practice activities of iodine deficiency disorders in Orissa on 22nd May, 2003.

12. Dr. G.Bulliyya attended a Taskforce Meeting on Multicentric study on Determination of loss of iodine in different types of cooking processes with financial support of WHO held at ICMR Headquarters, New Delhi on 11th June 2003.
13. Dr. S.K.Kar Participated in Symposia on "Multi Drug Resistant & Complicated Malaria and Management" and delivered talk on "Drug Resistant Malaria" at Hotel Swosti, Bhubaneswar on 1st July 2003, organized by THEMIS MEDICARE.
14. Dr. B. Sahoo attended one day " Library Connect Seminar" on Science Direct, BMN Reviews and Online Journals at Kolkata on 26th August 2003 organized by Elsevier, Singapore
15. Dr. S.K.Kar presented Scientific report on "MDA in Orissa" at a Meeting on "Evaluation of MDA Project" at VCRC, Pondicherry on 3-4th September, 2003.
16. Dr. R.S.Balgir participated in Tribal Task Force Meeting for Project Protocol Development on Tribal Studies held at RMRC, Bhubaneswar during September18-19th 2003.
17. Dr. G.P.Chhotray Participated in the tribal task force project development meeting and initiated the process of development of new project proposals on Diarrhoeal and Viral Diseases and Haemoglobinopathies along with the other member of different institutes from 15th to 17th September 2003.
18. Dr. B. V Babu attended the meeting for development of protocol for research on tribal health, at RMRC, Bhubaneswar, during 18-19 September 2003.
19. Dr. B. V Babu attended a workshop on "Qualitative data analysis" organised by UNDP/World Bank/WHO's Special Programme for Research and Training in Tropical Diseases (TDR) at KEM Hospital and Research Centre, Pune, during 20-21, September 2003.
20. Dr. G.Bulliyya attended Tribal Task force Meeting of Protocol Development on Tribal Studies held at RMRC, Bhubneswar and presented a paper on nutritional profile of the Indian tribal communities during 18th –19th September 2003.
21. Dr. M.R.Ranjit participated in the tribal task force project development meeting and initiated the process of development of new project proposals on Malaria along with the other member of different institutes from 15th to 17th September 2003.
22. Dr. A.S.Kerketa Participated on the meeting on common protocol development for research on Tribal Health at RMRC, Bhubaneswar from 18th-19th September 2003
23. Dr. A.S.Kerketa attended training on Verbal Autopsy methodology at Hyderabad, during 7th –9th November 2003.

24. Dr. B. V Babu attended a workshop on "Interim workshop on qualitative data analysis" organised by UNDP/World Bank/WHO's Special Programme for Research and Training in Tropical Diseases (TDR) at KEM Hospital and Research Centre, Pune, during 10-11, November 2003.

25. Dr. R.S.Balgir attended "National Thalassemia Day" organized by Taswels, Orissa on at Bhubaneswar 14th November 2003.

26. Dr. R.S.Balgir participated and presented a paper entitled "Spectrum of Hemoglobinopathies in Orissa (India): A Nine Years Cohort Study" in the 12th Asia Pacific Conference of International Society of Blood Transfusion held at New Delhi during 15-18th November 2003.

27. Dr. S.K.Kar delivered Guest lecture on " Psychiatric Problems and Future perspective" at State Branch Conference of Psychiatrists of India held at ICMR at Hotel, May Fair, Puri on 14th December 2003.

28. Dr. S.K.Kar participated in "Protocol Development Meeting" for Tribal health at ICMR Headquarters on 16-17th December 2003 and presented the protocol on "Formative Research in Nutrition".

29. Dr. G.P.Chhotray Participated in Independent Assessment of the Operaional feasibility of the introduction of Rapid Diagnostic Kits (RDK) and Blister Packs for strengthening the early diagnosis and prompt treatment under the Enhanced Malaria Control Project at M.R.C., New Delhi from 26th to 29th December 2003

30. Dr. G.P.Chhotray attended the 10th Asian Conference on Diarrhoeal Diseases and Nutrition held at Dhaka, Bangladesh and presented scientific paper "Health status in some primitive tribes of Orissa – an eastern state of India with special reference to Diarrhoeal disorders including cholera" from 7th December to 9th December 2003.

31. Dr. G.P.Chhotray attended 52nd Annual Conference of Indian Association of Pathologist and Microbiologists (IAPM) and presented the paper "Haemoglobinopathy in Orissa" held on 19th to 21st December 2003.

32. Dr. G.Bulliyya participated in Meeting to Develop a Common Protocol on Development and Evaluation of IEC Material (an Intervention Module) for Nutritional Deficiencies held at RMRC, Bhubaneswar and presented a paper entitled Development of IEC Intervention strategies in combating nutritional deficiencies among tribal communities of India during 5th –6th December 2003.

33. Dr. G.Bulliyya attended XXXV Annual Conference of Nutrition Society of India held at Hyderabad and presented a paper entitled Prevalence of anaemia among the elderly in three primitive tribal groups of Orissa during 12th and 13th December 2003.

34. Dr. G.Bulliyya participated as Chief-resource Person at the district level Sensitization workshop on Iodine Deficiency Disorders control for NGO activists Organised by Directorate of Field Publicity (Bhubaneswar) held at Wilkinson Nagar (Khurda) and given talk on Universal Iodization Programme on 31st December, 2003.

(Seminar in progress)

35. Dr. A.S.Kerketa attended workshop on Verbal Autopsy at New Delhi from on 17th December 2003.

36. Dr. P.K.Sahoo attended workshop on "ABI PRISM 7000 Sequence Detection System" held at Genetic Analysis and Technical Training Centre of Lab India Instruments Pvt. Ltd., New Delhi on 4th & 5th December 2003.

37. Dr. B. Sahoo attended meeting on " E- Database for biomedical journals" held at ICMR hqd. On 4th Dec. 2003.

38. Dr. S.K.Kar participated 48th All India Annual Conference – 2004 & Indian Public Health Association at KIIT, Bhubaneswar, organized by State Institute of Health & Family Welfare on 24th January 2004.

39. Dr. R.S.Balgir attended the Governing Council Meeting as Office Bearer (Treasurer) of the Indian Society of Human Genetics held at Bangalore on 9th January 2004.

40. Dr. R.S.Balgir participated in 91st Indian Science Congress at Chandigarh and presented papers entitled " Genetic Variations in Three Breeding Isolates of Bhuyan Tribe in Sundargarh District of Orissa" and in Symposia as Oral presentation entitled "Biological Endowment of Anthropology to Human Betterment, Development and Public Welfare" during 3-7th January 2004.

41. Dr. R.S.Balgir participated in 29th Annual Conference of the Indian Society of Human Genetics at Bangalore during 8-11th January 2004.

42. Dr. N. Mohapatra attended 3rd Global meet at Bangalore and presented a paper entitled " Sibling species distribution in Orissa" from 12 th to 18th January 2004.

43. Dr. G.Bulliyya participated as Chief-resource Person at the Block level Sensitization workshop on Iodine Deficiency Disorders control' for NGO activists Organised by Directorate of Field Publicity (Bhubaneswar) held at Barimund village and given talk on 'Status of IDD in Orissa on 3rd January, 2004

44. Dr. M.R.Ranjit attended the 3rd Global meet on parasitic diseases organized by Indian society of Parasitology and Bangalore university, Bangalore and presented paper entitled "Distribution of P.falciparum genotypes in clinical isolates of Orissa, India" from 12th to 16th January 2004.

45. Dr. A.S.Kerketa attended meeting on progress of Verbal Autopsy implementation and issues of quality and coverage at Trivandrum from 7th –9th Jan 2004.

46. Dr. S.K.Kar participated in the Task Force Meeting on Filariasis held at ICMR Hqrs. On 28th February 2004.

47. Dr. S.K.Kar participated in Technical Committee Meeting on " Use of Rapid Diagnostic Test Kit (RDT) in combating epidemic" at Disease Surveillance Cell, Govt. of Orissa on 25th February 2004 and presented the Standard Operational Procedure (SOP) on malaria.

48. Dr. B. Ravindran visited University of Leiden, Netherlands in connection with EU partners' meeting for 6 days from 20th February 2004.

49. Dr. M.K.Beuria and Dr. A.K.Satapathy attended WHO – ICMR Workshop on "IPR and WTO issues" at RMRC for Tribals at Jabalpur during 16 – 17th February 2004.

50. Dr. S.K.Kar participated in State Level Workshop on "Home Based Management of Young Infants" at RMRC, Bhubaneswar organized by NIAHRD, Cuttack on 1st March 2004.

51. Dr. S.K.Kar attended Workshop on National Dissemination of Impact Evaluation of Iodine Deficiency Disorders in the Country at NIN, Hyderabad on 17th March 2004.

52. Dr. R.S.Balgir attended the National Seminar on "Tribal Health in India: Issues and Challenges" held at Bhubaneswar during 30-31st March 2004.

53. Dr. G.P.Chhotray Invited as a resource person in a District level workshop on HIV / AIDS held at Nayagarh organized by Gania Unnayan Committee on 23rd March 2004.

54. Dr. G.P.Chhotray invited as guest speaker to the National Seminar on "Tribal Development In India : Issues and Challenges" Organized by Department of Anthropology, Utkal University, Vanivihar from 30th – 31st March, 2004.

55. Dr. N. Mohapatra attended 2nd and 3rd steering committee on malaria at Bhubaneswar held on 12th December 2003 and 23rd March 2004.

56. Dr. B. V Babu presented a paper entitled, "An anthropological appraisal of tribal health in India" in National Seminar on Tribal Development: Issues and Challenges, held at Utkal University, Bhubaneswar, during 30 - 31 March 2004.

57. Dr. B.B.Pal attended the workshop on "Bio diversity for Generating Wealth and Biotechnological Intervention" organized by the Institute of Life Sciences, Bhubaneswar from 11th to 13th March'2004.

58. Dr. B.B.Pal participated in a meeting on HIV / AIDS at Nayagarh organized by Gania Unnayan Committee on 23rd March 2004

59. Dr. A.S.Kerketa attended workshop on Verbal Autopsy implementation at New Delhi from 15th –16th March 2004.

60. Mr. N.S.Marai attended national workshop on bioinformatics tools in Genomics and Proteomics at Institute0f Life science, Bhubaneswar from 16th to18th March 2004.

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Dear, Prof. & Head, Dept. of Clinical Pharmacology
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Dr Dipali Mukherjee DDG(SG) & Chief, ECD Indian Council of Medical Research Ansari Nagar, New Delhi 110 029	ICMR Representative
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7.	Dr. B. Ravindran DD (Sr. Gr.), RMRC, Bhubaneswar	I/C Animal facility

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Mr.K.C.Parichha	Insect Collector
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