

3. COMPLETED PROJECTS



3

INSIDE

3.1

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

3.5

Assessment of therapeutic efficacy of chloroquine in treatment of uncomplicated *P.falciparum* malaria in M.Rampur block of Kalahandi district, Orissa.

3.9

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

3.2

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

3.6

Evaluation of the programme for the insecticide treated bed net and entomological studies for malaria control in three districts (Nawapara, Kandhamal and Kalahandi) of Orissa

3.10

Mid-term evaluation of improving nutritional and health status of children in Umerkote block of Nabarangapur district, Orissa

3.13

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

3.3

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

3.7

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

3.11

Assessment of iron deficiency anaemia among adolescent girls in Orissa

3.4

Role of IgA in Protective Immunity in Human and Experimental Filariasis

3.8

Intervention programme on nutritional anaemia and haemoglobinopathies in some primitive tribal population of India.

3.12

Prevalence of Chlamydia trachomatis infection amongst clinical cases attending OPD—a pilot study



Completed Projects

Status : 3.1 **Immunological characterization of filarial antigens with potential protective response in endemic population.**
Extramural (DBT)

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

Starting date : July 2001

Closing date : July 2004

Objectives:

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

Results:

Earlier we have reported the isolation of an aqueous insoluble, detergent soluble filarial glycoprotein (Dssd1), the antibody response to which is decreased in microfilariae positive individuals but high in microfilariae negative/chronic filarial cases and endemic normals. Surface localization of the Dssd1 antigen in *Wuchereria bancrofti* microfilariae and differential IgG subclass responses in endemic sera were described.

It was also shown that the low or lack of IgG response to Dssd1 is associated with filarial antigenemia (CFA/Og4C3 test) and it is independent of clinical manifestation or parasitological status. Interestingly, depressed IgG levels are noticed in CFA positive vs. CFA negative sera in the same clinical groups, for example endemic normal, hydrocele or elephantiasis patients. The high and low IgG levels to Dssd1 allow a distinction relating to antigenemia in human filariasis. The results in addition show that the antigen assay (Og4C3) primarily detects the carbohydrate determinants of Dssd1. Western blot and periodate oxidation results suggest that antibodies to carbohydrates of Dssd1 are present only in antigen negative sera. Active filarial infection might be linked with the lack of antibody response to the carbohydrate groups. Antigen positive (infected) individuals are devoid of anti-carbohydrate antibodies.

The nature of carbohydrate residues present in Dssd1 antigen was probed through lectin (Concanavaline A, Wheat Germ Agglutinin) coupled to peroxidase. The presence of D-glucose, mannose, N-acetal glucosamine and sialic acid are indicated in the antigen.

Filarial lipid antigens also exhibited higher antibody response similar to Dssd1 antigen, in infection free individuals and diminished antibody response in infected individuals.

Mastomys model for experimental filariae infection was used to evaluate the microfilariae clearance ability of Dssd1 and lipid antigens. Microfilaria appeared in the peripheral blood on day 6 of implantation of gravid adult worm in the peritoneum. Two groups of infected mice with microfilaria in the circulation were immunized with antigens (Dssd1, and lipid antigen). They were immunized twice with each antigen on day 12 and 27 post implantation. It was found that both Dssd1 and lipid immunization drastically reduced the circulating microfilaria from the implanted animals. Antibodies to these two antigens were also detected in the immunized animals.

Further, microfilarial clearance ability of these two antigens were checked in mastomys against challenge infection after pre immunization with the antigens. Significant reduction in microfilariae level was noticed in mastomys immunized with either Dssd1 or lipid antigen compared to control group of mastomys. These experiments indicate anti-microfilariae immunity induced by these antigens.

Conclusion:

Two antigens were isolated from aqueous insoluble residues of filarial parasite *Setaria digitata* – a glycoprotein (designated as Dssd1) and lipid antigens. These antigens although biochemically different exhibited similar immunological responses in *W. bancrofti* exposed people. Diminished antibody level was observed in infected microfilaraemic (antigen positive) compared to uninfected (antigen negative) individuals. In case of Dssd1, the lack or low antibody response in active filarial infection is directed primarily against the carbohydrate determinants of the antigen (Dssd1). Increased antibody response to these antigens in microfilariae negative individuals finds parallel in animal studies. Immunization of the antigen significantly reduced the circulating microfilariae from the implanted Mastomys. The results indicate the ability of these antigens in inducing anti-microfilarial immunity in filarial infection.

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

3.2 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 occurrence of some anti-filarial (Dssd and lipid) antibodies.

Results:

The study was carried out in people living in an apparently low endemic region (Ramchandrapur village, Jajpur districts, Orissa). A total of 550 people are included in this study. The region is characterized by low rate of microfilariae and chronic cases of filariasis. The number of asymptomatic microfilaraemics, hydrocele and elephantiasis cases were 4, 17 and 5 respectively. The corresponding figures for Mf+ve, hydrocele and elephantiasis in the high endemic region (Khurda) were considerably higher i.e. 62, 75 and 45 respectively (n=500). The vector density, vector infectivity rate, infective larval (L3) load and the transmission index of the area was found significantly low compared to high rate in hyper endemic area (Chhatipur village, Khurda district). Breeding sites of mosquitoes are determined. 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%.

Presence of circulating filarial antigen (CFA) level was determined in these sera. A high rate of antigenaemia (50%), in contrast to 25% of antigenaemia in the high endemic zone was observed. About 70% individuals were found positive for IgG antibodies to *Setaria digitata* antigens compared to almost 100% IgG positivity in high endemic Chhatipur village. Dssd1 specific IgG antibodies were present only in 40% of subjects. IgG positivity of 29% to filarial lipid was observed in people living in low endemic area compared to 90% positivity in area of high endemicity. These antibody levels 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. Antibody (IgG) levels to excretory Secretory (ES) antigen, purified from 24 hr culture supernatants of adult female *Setaria digitata*, were measured in sera of individuals of both low and high endemic area. IgG positivity of 88% and 100% were observed in low and high endemic area respectively.

Age dependent prevalence of filarial antibody to Dssd1 and lipid antigens were determined in both low and high endemic areas. IgG prevalence to those antigens was found to be negligible in young children (1-5yrs). The mean antibody level followed a increasing pattern up to the age group 16-20 yrs and stabilized there after.

Conclusion:

A high rate of antigenaemia was observed in the low endemic region, which is characterized by low incidence of microfilariae and chronic cases of filariasis. It indicates that more individuals were actually infected than those in whom microfilariae was detected. Filarial drug distribution could also be directed against such low endemic regions.

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

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

Background:

Human lymphatic filariasis is a spectral disease displaying diverse forms of clinical manifestations. It is assumed that repeated episodes of acute disease could eventually lead to development of chronic forms of disease such as lymphoedema/elephantiasis or hydrocele. The progression from infection to development of disease is a very slow process and takes several years. 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

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

Status :

European Commission

Investigators :

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

International 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



Completed Projects

studied by using crude extracts of different developmental stages of filarial parasites. Several 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 the other developmental stages of the parasites in different clinical categories of human filariasis and thus search for markers of morbidity- the current study makes an effort in this direction. Since acute disease is perceived to lead to chronic forms of disease there is an urgent need to recognize molecular markers, which could be used for monitoring progression of the disease- quantification of plasma levels of pro- as well as anti-inflammatory molecules is expected to result in identification of such markers. The current project addresses this also.

Major Results and conclusions:

- (1) Verification of the dipstick assays as field applicable tools in endemic population affected by *W. bancrofti* and *B. malayi* infections before and after chemotherapy:

About 45 % of sera from Bancroftian filariasis endemic villages react positively with Bm dipstick assay. This was interpreted to be due to either a) possible mixed infections with both *W. bancrofti* and *B. malayi* in patients Or b) that the specific *Brugia* antigen is present only in some strains of *W. bancrofti* and not in others. DNA from purified Mf from carriers were subjected to PCR using specific primers and all but one Mf sample was found to be pure *Wuchereria* infection indicating that Bm dipstick positivity in Bancroftian filariasis cases is not due to mixed infection with *B. malayi*. Further investigations revealed that expression of BmR1 homologs in different field isolates of *W. bancrofti* but were not uniformly antigenic to be detected by BmR1 dipstick assay.

- (2) Identification of immunological markers of morbidity that can identify patients at risk of developing clinical disease

The objectives of this project are to identify molecular markers for assessing i) morbidity, ii) protective immunity as well as iii) immunodiagnostics in human lymphatic filariasis. Since these issues are diverse, three different approaches are being attempted. For identifying morbidity markers to monitor progression of chronic disease, a large panel of pro as well as anti-inflammatory molecules in circulation was monitored. The following molecules were quantified in different clinical categories: 1) IL-6, 2) IL-8, 3) IL-10, 4) TNF- α , 5) TNF- α receptor-55, 6) TNF- α receptor 75, 7) LPS binding protein (LBP), and 8) ICAM-1. The endemic population was categorized into the following groups and for analysis the levels were compared with those of endemic normals: i) Asymptomatic Mf carriers (AS), ii) subjects with cryptic infections (CR), iii) elephantiasis patients, iv) patients with hydrocele, v) acute filariasis cases with filarial antigenemia and vi) acute filariasis cases without antigenemia. Quantitative analysis of circulating levels of the above molecules in the clinical spectrum of lymphatic filariasis offered interesting leads in understanding the clinical manifestations. Acute filariasis were characterized by significantly raised levels of IL-6, IL-8, IL-10, TNF- α and TNFR-55 when compared with endemic normals. The increased level of the above inflammatory molecules in acute disease was not influenced by presence or absence of circulating filarial antigens. The Mf carriers and cryptic cases were also found to display significantly elevated levels of TNF- α although other inflammatory cytokines such as IL-6, IL-8 and TNFR-55 were not elevated in them. The investigations revealed very clear differences between two chronic manifestations of filariasis - patients with elephantiasis were found to have elevated levels of IL-6 and TNFR-75 (Type 2 receptor) while hydrocele cases were displaying enhanced levels of IL-8 and TNFR-55 (Type 1 receptor). Since the two TNF receptors are known to be biologically different the current study has offered a handle to address issues related to pathogenesis of these two diverse forms of chronic disease manifestations. When all the samples were categorized for presence or absence of disease, inflammatory type 1 receptors were significantly elevated in patients with one or the other form of filarial disease and asymptomatic individuals displayed elevated levels of anti-inflammatory type 2 TNF receptor. Interestingly, when the samples were analyzed for presence or absence of only filarial infection (i.e. antigenemia positive cases, regardless of disease status) the type 2 receptors were significantly elevated in antigenemic cases and Type 1 receptors were elevated in non-antigenemic cases. The ratio between the two TNF-a receptors indicated that acute filariasis and hydrocele cases are similar in terms of TNF-s receptor status. None of the studied markers differentiated microfilariae carriers (AS) from subjects with cryptic infections (CR). Both the groups were found to display elevated levels of TNF- α and TNFR-75 and decreased levels of ICAM-1 in comparison to endemic normals. The following are the broad conclusions of the study on morbidity markers:

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

- a) Inflammatory cytokines such as IL-6, IL-8, TNF- α , TNF receptor 55 were consistently elevated in acute disease in comparison to endemic normals.
- b) Hydrocele cases were similar to patients with acute disease in terms of elevated levels of TNF-R55 and decreased levels of TNF-R75, and conversely
- c) Elephantiasis patients and asymptomatic subjects with active filarial infection are similar viz., with elevated TNF-R75 and decreased TNF-R55,
- d) Significantly raised IL-6 levels were observed only in acute cases and elephantiasis patients in comparison to other groups.

3. Identification of novel recombinant antigens for diagnosis of infection and disease:

The availability of the following filarial recombinant proteins has allowed us to address the issue of using antibody responses as a marker of morbidity in different clinical categories of human filariasis. Antibodies to the following recombinant proteins were quantified: 1) Abundant larval transcript-1; ALT-1; 2) ALT-2; 3) Serpin-2 (SPN-2) and 4) Cystein proteinase inhibitor-2 (CPI-2). The first two are molecules produced essentially by infective larval stages, while SPN-2 is synthesized only by microfilarial stages and CPI-2 is present on the surface of adult filarial worms. IgG antibodies to ALT-1 were significantly more in subjects free of patent infection (without circulating filarial antigen, (CFA) as compared to those who were displaying antigenemia. More significantly, an inverse association was observed between filarial antigen units and the IgG antibody levels to ALT-1. These observations clearly indicated a role for IgG antibodies to ALT-1 proteins in restricting the infection load in human Bancroftian Filariasis. This notion is further strengthened by antibody titres in age-stratified endemic population- a progressive increase of anti-ALT-1 IgG was observed with increasing years of exposure to infective larvae in the endemic population. Recombinant proteins ALT-1 and ALT-2 were further used to quantify specific IgG sub-groups reactivity in four different categories of human filariasis. The findings revealed a critical role played by these two dominant larval specific antigens. Enhanced IgG1 to ALT-1 was associated with active infection while enhanced IgG2 to ALT-1 was associated with development of pathology. Interestingly IgG3 ALT-1 was found to be significantly more in subjects with cryptic infections as compared to Mf carriers and levels of IgG4, (considered to be elevated in the infected population when tested using crude filarial antigens) was not found to be significantly different in the four clinical categories. The IgG sub-groups reacting to ALT-2 were different from that of ALT-1 described above. Significantly elevated IgG1 was observed in endemic normals in comparison to Mf carriers and IgG3 to ALT-2 was found to be significantly more in patients with chronic disease and there was no significant difference in IgG4 levels reacting to ALT-2 in various clinical groups.

IgG levels to recombinant CPI-2 (an antigen present on the surface of adult worms) were significantly more in cryptic cases (CR) in comparison to Mf carriers. This indicates a role for this antibody in anti-microfilarial immunity since CR are free of circulating Mf but harbor adult filarial worms. Further, IgG levels to recombinant SPN-2, (an antigen present in microfilarial stages) were significantly more in endemic normals (EN), chronic cases (CH) and in subjects with cryptic infections (CR) in comparison to Mf carriers. This indicates a role for antibodies to SPN-2 in anti-microfilarial as well as anti-adult immunity. More interestingly, the higher levels observed in CH cases as compared to endemic normals indicates that very high antibody response to SPN-2 could be associated with pathology.

All the technical components of the project have been successfully undertaken so far as per the original project proposal. The study has identified markers of morbidity for different clinical categories of human lymphatic filariasis.

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

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.

Background:

Definition and demonstration of protective immunity in human filariasis has been a contentious issue. While several investigators have presumed absence of infection as an indicator

Status :

ICMR Intramural Project

Investigators :

Dr. B. Ravindran, P.I., Dr. A.K. Satapathy,
Dr. P.K. Sahoo, Dr. M.C. Mohanty,
Mr. B.R. Sahoo

Starting date : January 2002

Closing date : December 2004



Completed Projects

of 'protective immunity', genuine immunity needs to be considered as a state associated with absence of infection as well as disease. Endemic normals (EN) are considered putatively immune since they are free of infection as well as disease although clear delineation of EN (asymptomatic, amicrofilaraemic subjects without demonstrable antigenemia) has been possible only in Bancroftian Filariasis since immunoassays for detection of CFA are not currently available for Brugian Filariasis. Immune response phenotype characterized by increased filarial specific T-cell proliferation, IFN- γ , IL-5 production, presence of antibodies to sheath, and raised levels of IgE, IgG1, IgG2 are consistent features observed in patients with chronic disease as well as in Endemic normals. The current study is the first attempt to quantify filarial IgA and characterize protective immunity in clearly delineated clinical groups based on presence or absence of disease as well as infection. Our results suggest a role for IgA in limiting filarial infection as well as in induction of pathology in human filariasis.

Summary of Observations and Conclusions:

The results showed significantly decreased levels of total serum IgA antibodies in asymptomatic carriers as compared to other three groups viz., people with chronic disease, and in subjects with cryptic infections and putatively immune subjects. Since microfilaraemic subjects were found to possess lower levels of total IgA, it was of interest to investigate if this is related to infection load. Since both Mf as well as CFA levels can be quantitatively estimated, an attempt was made to correlate the total IgA levels to infection load. No significant association between the levels of Mf and CFA indicating that the decreased total IgA observed in microfilaraemic subjects is independent of infection intensity.

The sera were tested for reactivity to a solubilized adult worm extract (Fil.Nat) and probed with different second antibody conjugates. There were no significant differences between the two groups when probed with anti-human Ig (polyvalent), anti-human IgG or anti-human IgM conjugates. However filarial IgA was found to be significantly more in EN category in comparison to CH cases. Increased IgA levels were found to be a unique feature of only EN cases since the other three clinical categories viz., mf carriers, patients with chronic disease and subjects with cryptic infection were found to have comparable levels of IgA and the levels in EN were significantly more than the other three groups. Since these observations were novel and have not been recorded earlier, it was of immediate interest to investigate if the IgA are directed towards protein (Fil.Pro) or carbohydrate (Fil.Cho) epitopes of adult stage filarial parasites. Similar reactivity to Fil. Pro and Fil.Cho was observed indicating that filarial IgA are directed towards both protein and carbohydrate epitopes.

When the sera samples were classified according to infection status disregarding presentation of symptoms, subjects with current infection (as shown by presence of Mf and/or CFA) were found to possess significantly less filarial IgA compared to those without active infection. More interestingly, when the sera were classified according to presence/absence of infection and disease, significantly more IgA antibodies were found in females than in males in each of the three categories. A semi-quantitative estimation was done for IgA to sheath in three clinical categories of human Filariasis, namely asymptomatic microfilaraemics, people with chronic disease and putatively immune subjects. The percentage of asymptomatic carriers demonstrating IgA reactivity to sheath was significantly less in comparison to patients with chronic disease and endemic normals.

Analysis of 218 sera collected from all age groups indicated that levels of Filarial IgA appearing in younger age groups (<20 years) are sustained and persist without any significant change in all the higher age groups. A very significant direct association was observed between IgA levels and absolute eosinophil counts. An attempt was also made in to quantify the IgA antibody response against recombinant filarial antigens viz., Bm-ALT-1, ALT-2, CPI-2, SPN-2 and VAL-1 across the clinical spectrum of human filariasis. Significantly higher levels of IgA antibodies to Bm-ALT-1, Bm-ALT-2 and Bm-VAL-1 were observed in subjects with cryptic infections as compared to Mf carriers. The IgA levels were found to be significantly high in cryptic cases as compared to people with chronic disease, when tested against Bm-ALT-2. No significant difference was observed in levels of IgA among the clinical categories to Bm-CPI-2 and SPN-2 antigens. Since, ALT-1, ALT-2 and VAL-1 are proteins largely secreted by third stage larvae of *B. malayi* and not by other

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

developmental stages of parasites, the higher levels of IgA antibodies to these three recombinant antigens in subjects with cryptic infections as compared to Mf carriers suggests a possible role of the isotype of antibodies in mediating anti-larval immunity in filariasis.

3.5 Assessment of therapeutic efficacy of chloroquine in treatment of uncomplicated *P.falciparum* malaria in M.Rampur block of Kalahandi district, Orissa.

Background:

With request from state health department EMCP during February 2005, the pilot project was initiated in M. Rampur CHC of Kalahandi district as identified by state health department.

Objectives:

1. To study the therapeutic efficacy of chloroquine (CQ) in the treatment of uncomplicated *P. falciparum* malaria.

Health facility in the study area:

There is only one CHC located at block headquarters of M Rampur block. Besides this, there are 12 Sub centers with 221 DDCs, 83 FTDs, and one malaria clinic. In addition there is an Ayurvedic health center at Urladani gram panchayat. One mobile health unit regularly visits the selected villages as difficult area of the block at least once in a month. A total of 36 health workers are there in the different villages who take care of the immunization and malaria related work.

Malaria situation of M. Rampur CHC:

The last three years data on the malaria situation of the M. Rampur CHC indicated an increasing trend of the disease. The SPR increased by 6.6% from 2002 to 2004. The Plasmodium falciparum (pf) rate accounts more than 80% in the area. It has gone up from 72.7% to 85% from 2002 to 2004. High SPR (more than 5%) is indicative of high transmission, and ABER is more than 10 indicate needs for proper surveillance in this area. The detail data on malaria situation of the CHC is given in Table-1.

Table 1. Malaria situation according to previous three years CHC data

Year	2002	2003	2004
ABER	28.8	30.9	32.8
SPR	8.3	8.9	14.9
SFR	6.0	7.5	12.7
Pf%	72.7	83.8	85
Total population	63608	63608	68687
BSE	18333	19694	22244

Study Villages:

The study was undertaken in 10 villages under 3 Gram Panchayats (GP) of the CHC. After liaisoning with the ADMO (PH) and with the CHC Medical Officer (in-charge) the high malaria risk villages were selected based on the earlier CHC data and the experience of treatment failure by the CHC staff. The villages are surrounded by the inaccessible mountains in the east, south and north. In the west lies the Rahul river. During monsoon, the area remains cut off from other areas. The villages of other two GPs are in the foothills of hilly mountain but lies beside/nearer to the main road. The population of the study villages under Urladani subcentre is 789 and the of the village under Dhenkenkupa GP is 176 & village under Gocchha-danger GP was 1504. Thus a total of 2,469 population covered during the study period.

Materials and method:

Sample size:

The sample size for study of efficacy of CQ was calculated as per WHO guideline for estimating the population proportion (WHO 2001). The known treatment failure in the study district as per earlier report is 10-12%. With anticipated population proportion of treatment failure (P)

Status :

Extramural (EMCP, Department of Health & Family Welfare, Govt. of Orissa

Investigator :

Dr. A.S. Kerketta

Starting date : February 2005

Closing date : March 2005



Completed Projects

15% at 95% confidence interval and precision (d) 10% & brusing sample size determination table the sample size comes to 49. With the anticipation of drop out of 10%, the total sample of 53 cases were included in the study.

Characteristics of sample:

Study populations are the cases frankly presenting with fever and that showed parasitaemia with *P. falciparum* mono infection and with a density of 1000-100,000/ μ l of blood.

Box-1

Inclusion criteria:

1. All patients more than 5 years age and of both sex
2. All positive for *P. falciparum* mono infection cases with parasite density of 1000-100000-parasite/ μ l of blood
3. History of fever during the present illness
4. Axillary temperature <39.5 degree centigrade
5. Ability to come for the stipulated follow up visits and easy to access to health facility
6. Informed consent by the patient or by parent/guardian for children

Exclusion criteria:

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

Danger signs:

- a. Not able to drink or feed
- b. Repeated vomiting
- c. Convulsions during present illness, lethargic or unconscience, unable to sit or stand up

Drug administration schedule:

Chloroquine-1500mg base (adult dose) was administered under supervision during a period of 3 days as follows

Day 0- First dose-600 mg base =4 tabs of 150 mg base each

Day 1-Second dose-600mg base=4 tabs of 150 mg base each

Day 2-Third dose-300 mg base=2 tabs of 150mg base each

Children received 10mg/kg body weight as first dose, 10mg /kg body weight as 2nd dose and 5mg/kg as 3rd dose.

Study design:

	DAYS							
	0	1	2	3	7	14	21	28
CQ treatment (mg/kg of body weight)	10	10	5					
Clinical examination	Y	Y	Y	Y	Y	Y	Y	Y
Axillary temperature	Y	Y	Y	Y	Y	Y	Y	Y
Parasitaemia	Y		Y	Y	Y	Y	Y	Y
Body weight	Y							
Y= Yes								

Study Procedure:

The therapeutic efficacy of CQ was conducted as per the WHO guideline for Assessment of Therapeutic Efficacy of Anti-malarial Drugs for Uncomplicated Falciparum Malaria (WHO 2001).

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

The health worker of the particular sub centre accompanied the study team. In villages a rapid fever survey was done. A careful and precise registration of address of each case was done. Taking consideration of inclusion and exclusion criteria, the eligible cases were selected for the study. Before enrolment the patient and his/her attendant was briefed in details about the aim, procedure and the benefits of the study. The informed and written consent was obtained from each subject. Thus a total of 53 subjects were enrolled in the study and followed on subsequent days. (The details of these subjects are given in Table 2).

Table 2. Age and Sex distribution of the study population

Age in years	Male	Female	Total
5-14	13(52.0)	12(48.0)	25(47.0)
15 and above	15(53.6)	13(46.4)	28(53.0)
Grand total	28(52.8)	25(47.1)	53

A careful history was taken on consumption of anti malarial drugs during the last three days prior to the present treatment. The axillary temperature was recorded with the help of an electronic thermometer and the body weight was measured with the help of a calibrated scale-weighing machine. Blood smears were collected in duplicate on the days 0, 2, 3, 7, 14, 21 and 28 of initiation of the treatment. Simultaneously the thick and thin films of peripheral smear were prepared and were air dried rapidly. The thin smear was fixed with anhydrous methanol. The smears were stained with Giemsa stain 3% and at pH 7.2 and examined on same day. The parasite count was done against 200 WBC in thick smear. Thin smears were used to confirm the parasite species. The drug Chloroquine 150 mg base (generic name Resochin, manufacturer Bayer Pharma Pvt Ltd) was administered to each patient. The drug was administered on days 0 and 1 and 2 to the patient on the spot, under supervision of the medical team. The study subjects were monitored daily by the physician daily for initial 3 consecutive days and on subsequent follow up days. They were advised not to take any other drug during the study period with out informing investigator.

Follow-up:

The study subjects were followed up with blood smear for parasite count on days 2, 3, 7, 14, 21 and 28. Besides blood smears collection the clinical examination was done on each day for recording the danger signs as shown above.

Dropouts:

Out of total study population 3 (5.6%) dropped out from the study. One case developed concomitant upper respiratory tract infection on day two, one taken treatment from third party during follow- up period and one moved to his relative's house which was outside of reach of active follow-up. Thus a total of 50 (94.3) subjects could be followed for all 28 days of study period.

The therapeutic response classification:

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

- Early treatment failure (ETF): Development of danger signs or severe malaria on day 1, day 2, or 3 in presence of parasitaemia;
- Parasitaemia on day 2 higher than day 0 count irrespective of axillary temperature; Parasitaemia on day 3 with axillary temperature ≥ 37.5 degree C and parasitaemia on day 3 > 25% of count on day 0
- Late treatment failure (LTF)
 - 1. Late Clinical Failure (LCF)
 - 2. Late Parasitological Failure (LPF)



Completed Projects

- LCF - Development of danger signs or severe malaria after day 3 in presence of parasitaemia
- Presence of axillary temperature $\geq 37.5^{\circ}\text{C}$ on any day between day 4 to day 28 without previously meeting any of the criteria of ETF.
- LPF - Presence of parasitaemia on any of the schedule return on day 7, day 14, day 21 and day 28 with axillary temperature less than 37.5°C without previously meeting any of the criteria of ETF or LCF.
- Adequate clinical and parasitological response (ACPR)- Absence of parasitaemia on day 28 irrespective of axillary temperature, without previously meeting any of the criteria of ETF, LCF or LPF.

Results:

A total of 124 fever cases were screened clinically and all suspected malaria cases screened for malaria parasite by the ICT test i.e. SD Pf / Pv malaria kit for quick identification of mixed infection cases. Out of which 64 (51.6%) were *P. falciparum* mono infection, 4(3.2) had *P.vivax* mono infection, 29 (23.4) had mixed infection of *P. falciparum* as well as *P.vivax* infection and 27(21.8) did not have any malaria parasite infection of *P. falciparum* cases, 4 patients were excluded due to pregnancy, 2 patients had very high parasite count and was difficult to count the exact number of parasite and 5 patients had very low parasite count. The PV and mixed infection cases were treated as per the NAMP drug schedule and disposed after wards. Thus 53 cases met the criteria for inclusion was enrolled in the study. The baseline information of the study sample is given in Table 3.

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

	Mean	Range
Age	21.2830	5 - 65
Axillary Temp	37.9321	37.5 – 39.4
ParasiteCount on Day 0	4931.32	1080 - 19980

The data obtained from the study shows that, out of 50 patients who continued till the end of the study, only 3 (6.0%) showed Adequate clinical and parasitological response (ACPR), 8 (16%) showed early treatment failure (ETF), of which one case (2%) developed danger sign on second day and had repeated vomiting, lethargic and unable to sit or stand up and 7 (14.0%) had parasitaemia more than day 0 on day 2. Late clinical failure (LCF) was marked in 1 case (2%) who developed fever on day 28 and had axillary temperature of 39.4 degree centigrade which is more than 37.5 degree centigrade. A total of 38 (76%) had late parasitological failure (LPF) of which 30 (79.0%) had parasitaemia on day 7, 2 (5.3) had parasitaemia on day 14, 5 (13.1%) had parasitaemia on day 21 and 1 (2.6%) had the parasitaemia on day 28 (Fig. 1 and Table 4). Thus around 94.0% patients in the study showed treatment failure with CQ.

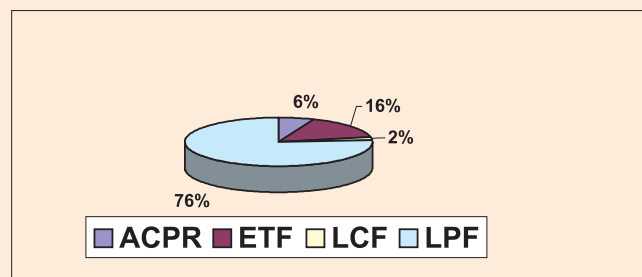


Fig. 1. THERAPEUTIC RESPONSE TO CQ

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

Table 4. Drug response on parasite count

Base level	Nos.	Range	Mean
Day 0/1	50	1080-19980	4931.32
Day 2 ETF	8	1320-22880	6825
Day 7 LTF	30	280-8720	2183.33
Day 14 LTF	2	240-580	410
Day 21 LTF	5	120-1360	644
Day 28 LTF	1	560	560

Conclusion:

The present study has unveiled a starting situation of CQ resistance in M. Rampur block of Kalahandi district. Never before such reports of such a high percentage of resistance has been reported from Orissa. The result of the study pertaining to 10 villages has revealed that, the first line drug CQ is only 6% effective and the treatment failure rate is as high as 94%. This situation warrants a change to the second line of drug Sulfadoxine-Pyrimethamine in this area. However a single report from a small area like this seems insufficient to change over the drug policy. Therefore more such studies should be undertaken intensively in this area as well as other area to identify and delineate such pockets of CQ resistance in the state. This is a continuous process, since the parasite resistance to this drug is a dynamic process and takes place in nature by natural mutation. Therefore the continuous monitoring of CQ resistance in the state can't be over emphasized. This study would be a step towards the promoting evidence based action against the dreaded disease malaria.

3.6 Evaluation of the programme for the insecticide treated bed net and entomological studies for malaria control in three districts (Nawapara, Kandhamal and Kalahandi) of Orissa

Objectives:

a) Impact assessment

1. To assess preparatory activities including survey and measures for the involvement of Panchayat Raj Institutional (PRIs), NGO and self help group (SHGs) for bed net impregnation.
2. To access training and IEC activities.
3. To access the willingness of the community to pay for the insecticide.
4. Operational detail of camp organized for treatment of nets.
5. Action taken for the procurement of insecticide for treatment of the nets.
6. Coverage level achieved.
7. To access the involvement of PRIs/NGOs.

b) Vector assessments

To identify malaria vectors and its species complexes, bionomics, feeding and susceptibility status in 3 identified districts of Orissa.

Results:

Our observations as per terms of references are as follows:

The preparatory activities regarding distribution of insecticide treated mosquito nets were initiated by the District Malaria Control Society under Zilla Swasthya Samity.

Status :

Extramural Project (Enhanced Malaria Control Programme, Department of Health & Family Welfare, Govt. of Orissa)

Investigators :

Dr. R.K. Hazra

Starting date : February 2005

Closing date : March 2005



Completed Projects

Distribution and impregnation of mosquito nets

Kandhamal district:

The district has been provided with 20,000 mosquito nets and 200 litres of Deltamethrine 2.5% flow as 1st lots on 18.2.03 and 250 litres in 2nd lot on 7.6.04 for re-impregnation of the net. As per the decision of Zilla Swasthya Samiti meeting held on 18.8.03, it was proposed to provide the medicated mosquito nets to the boarder of Ashram school hostel at the cost of Rs.10/- per net, to population of below poverty line of three highly malaria prevalent blocks, i.e. Daringbardi, Subarnagiri, Tumudibandha at the cost of Rs.20/- per net and to others at the cost of Rs.30/- per nets. As limited numbers of nets were actually received, it was decided in block level meeting to distribute them in villages with high incidence of malaria, which was identified by Block Chairman and other PRI members. For Ashram School hostels, Project Administrator, ITDA, Phulbani and ITDA, Balliguda informed about their requirement. The required numbers of nets were medicated at District headquarters and then were supplied to the respective ITDAs. The number of nets for the three blocks as decided by the Zilla Swasthya Samiti were provided to the respective Medical Officer-in-charge for impregnation and distribution. Limited number of nets were received, it was distributed in villages of 3 block with high incidence of malaria as identified by Block Chairman and other PRI members. 7000 nets were distributed in Daringbadi CHC, 3000 nets were distributed in Tumudibandh PHC and 4527 nets were distributed in Subarnagiri PHC. The rest nets were distributed in ITDA, Phulbani and ITDA, Balliguda (Table-1). In Kandhamal district 250 litres of Deltamethrine 2.5% was supplied on 7.6.04. Community awareness camp was organized on 20th and 21st of December 2004. In January 2005, 70lts of flow was supplied to Daringibadi, 30 litres to Tumudibandh and 46 litres to Subarnagiri. The re-impregnation work is in progress.

Kalahandi district:

The summary of distribution of treated mosquito nets is given in Table-2. Since the number of nets in Kalahandi district available was limited, and distribution of nets were made to all the blocks hence the average distribution of net per village is only 8 to 9. Kalahandi district CDMO received 25,000 bednet in November 2002. Impregnation of the nets was done in PHCs level. Only in Th. Rampur 6850 nets were distributed and rest were distributed in all other PHCs.

In Kalahandi district, reimpregnation were undertaken from August 2004. Total 251 litres of Deltamethrine 2.5% supplied for treatment. Two NGO namely, Gramvikas and Antodaya were assigned for the reimpregnation.

Nawapara district:

Nawapara district received 25,000 beds net from EMCP. The ZSS meeting held on 19.8.02 decided to distribute the mosquito nets to APL and BPL cardholders at the cost of Rs.30 and Rs.20 respectively. The nets were supplied to PHC medical officer and the PHC medical officer as per instruction of collector handed over the nets to Sarapanch. The Sarapanch redistributed the nets to Ward members, who distributed in the village. The distribution of bed nets is depicted in Table-3.

In Nawapara district, reimpregnation started from 10.3.05. Here two NGOs namely Srusti and Parda are involved. 300 litres of flow received on 8.7.04. The work of reimpregnation is in progress. The team visited to different villages where reimpregnation has just started.

Training:

The district authority organized training for health worker of 3PHCs in Kandhamal, 13 PHC in Kalhandi and 5 PHC in Nawapara. This training was meant for health workers (male and female), Anganwadi workers and Volunteers, who were engaged in impregnation work, doctors and other paramedical staffs. The training programme was also organized for PRIs workers. District authority like ADMO and PHCs doctors organized the training for medical officer and PHC staff and PHC staff trained the other workers. Adequate IEC

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

campaign has been made for motivation of community. In Kandhamal district the district health authority organized community awareness camp in different PHCs. In Kalhandi district by the help of health worker they have conducted a survey for community own net impregnation. They have target of 5000/nets per block.

Evaluation of Bed net distribution and coverage:

In Kandhamal district, in 3 PHCs, bed net was distributed and in Daringbadi where population is 91155 only 7000 bed nets was supplied that comes to 7.6% of the population. In Tumudibandh PHC having population of 49781 received 3000 bed nets (6%) and Subarnagiri PHC received 3000 bed nets where the population is 41650, (7.2%). In all these three PHCs, mosquito bednets were supplied to high malaria endemic villages. Nearly 95% coverage was seen in the villages where the net was supplied. The rest 5% did not received the bednets as they were not available during the time of distribution. The team visited a total 19 villages of three PHC. In each village it was observed that nearly all household received bednets, around 50 % household received one net per family, 43% received two nets, 7.7% received 3 net and rest more than three nets. We observed that while 85% of the population makes regular use of bed nets, 15% kept them for use in rainy season when the mosquito density will increase. People were happy about the distribution of the nets and many have told about collateral benefit.

In Kalahandi district, a rapid sample survey for ascertaining net distribution, re-impregnation, usage and community response was carried out by the evaluation team. This was undertaken in randomly selected villages of in 3 districts and 42 villages where treated nets had been distributed. 241 houses were surveyed in Kalahandi district, which covers 21 villages under 5 PHCs (M. Rampur, Narla, Parla, Pastikudi, and Th. Rampur). Here, the treated nets had not been distributed to every family of the village. Average net distribution per village surveyed was 12. Few families received the nets out of which 89% received one net and 11% received more than two nets. Out of 251 respondent received net, 92.5% were using the mosquito net regularly. Through organizing Focus group discussion (FGD), with the villagers, it was learnt that the community has a fair knowledge about malaria transmission, mosquito-breeding sites, since the number of nets had limited availability and accordingly distribution pattern could not be homogeneous, few people expressed unhappiness for not been distributed nets indicating their eagerness to accept. The persons those received the bed net have told about the collateral benefit of using insecticide treated nets i.e. it also kills the head lice and other insects. They expressed their positive attitude towards procuring nets. None of the respondents using treated mosquito nets has reported any adverse effect.

In Nawapara district, an average of 200-300 net were distributed per Panchayat and 20/30 nets was distributed per ward. Our team visited 12 villages of two PHCs. It was observed that 188 families received the bednet. Nearly 93% of families received one net and rest (7%) received 2 net per family.

Entomological survey:

Vector prevalence: The distribution pattern of Anophelines vector in three districts is depicted in this report (Table 4-6). Ten species of Anophelines have been identified in the present survey. Among ten species of Anophelines, 3 were identified as vectors viz. *An. culicifacies*, *An. fluviatilis* and *An. annularis*. All the species collected both from human dwelling and cattle shed except *An. Fluviatilis*, which were collected only from human dwelling. *An. culicifacies* and *An. annularis* mainly found in the cattle shed. *An.culicifacies* and *An.annularis* were known to be endophilic, endophagous in nature whereas *An. fluviatilis* was endophagus but exophilic in nature. The abdomen of all the species showed half gravid and full fed.

In Kandhamal district mosquitoes were collected from 8 villages of 4 PHCs. A total of 452 mosquitoes were collected, out of which 391 (86.5%) are Anophelines mosquitoes and rest are culex. Out of 391 Anophelines collected 162 (41.4%), 5 (1.3%) and 1 (0.17%) are *An. culicifacies*, *An. fluviatilis* and *An.annularis* respectively. (Table-4)



Completed Projects

In Kalahandi district mosquitoes were collected from 27 villages of 5 PHCs. A total of 1897 mosquitoes were collected from the district out of which 1395 (73.5%) were Anophelines mosquitoes and the rest are culex. Out of 1395 Anophelines collected 33 (2.4%), 221 (15.8%) and 1 (0.07%) were *An. annularis*, *An. culicifacies* and *An. fluviatilis* respectively (Table-5).

In Nawapara district mosquitoes were collected from villages of 3 PHCs. A total of 697 mosquitoes were collected out of which 587 (84.2%) were Anophelines mosquitoes. Out of 587 Anophelines mosquito collected 4 (0.68%) and 113 (19.25%) was *An. annularis* and *An. culicifacies* respectively (Table-6).

In Kandhmal PHC among the vectors *An.culicifacies* was predominating. Average per manhour density of *An.culicifacies* was found to be 19.5 which is higher and it was more than the critical density (PMHD - 3.3). Here only two *An. annularis* were found. Maximum number of mosquitoes collected were half gravid and gravid which denotes that these species are endophilic in nature. In Kalahandi district, the density of *An. culicifacies* was more among the vectors. The average PMHD was found to be 2.78. In Nawapara also similar type of distribution was observed and the PMHD was 4.65.

Anthropophilic index of *An. culicifacies*, *An. annularis*. and *An. Fluviatilis*:

Precipitin test for blood meals of *An. culicifacies* and *An.fluviatilis* was carried out by gel-diffusion technique. The anthropophilic index of *An. culicifacies*, *An. fluviatilis* was found to be 7.5% and 50% in Kandhamal district. In Kalahandi district it was 13.3% and 9.2% for *An. annularis* and *An. culicifacies* respectively in Nawapara district the anthropophilic index of *An. culicifacies* was 8.3% (Table7).

Susceptibility status of *An. culicifacies* in 3 districts of Orissa:

The result reveals that in Kandhamal, Kalahandi and in Nawapara district *An. culicifacies* found to be resistant to D.D.T. where as they are highly susceptible to Deltamethrine (Table-8).

Conclusions:

From our study to evaluate bednet distribution and vector study, it was observed that very limited numbers of bednets were supplied in these three districts, i.e. Kandhamal, Kalahandi and Nawapara. ITMN programme in Kandhamal district has been very well accepted by the community (where it is distributed) as evident from the high rate of mosquito net usage and people's preference to treated mosquito nets. In Kalahandi and Nawapara district the distribution of mosquito nets were made in all the PHCs. The distribution pattern was in a scattered manner; hence the total population of PHC could not be covered. It was distributed in all the PHCs, and each Panchayat received around 200-300 nets in Nawapara and 100 net in Kalahandi. So each ward received only 20-30 bednets in Nawapara and 10-15 in Kalahandi district. Since the distribution of net did not cover all the house holds of Kalahandi and Nawapara districts hence the assessment of efficacy of treated nets at community level is not comparable to that of Kandhamal, where complete coverage was made in the selected villages.

As no base line survey report is available for either of the three districts, periodical entomological and epidemiological surveys should be done to know the effect of bednet on malaria transmission.

Retreatment of net has started in Kandhamal and Nawapara but in Kalahandi due to charges of re-treatment, the community acceptance was very low. The FGDs undertaken indicated that people were eager to buy the nets with subsidized prices and use them. Large proportion of people understands the purpose of nets and its use but awareness is required to get optimal utility of nets. In the present survey three major vectors of malaria viz. *An.fluviatilis*, *An.culicifacies* and *An annularis* were found to be prevalent in Kalahandi, Nawapara and Kandhamal districts. The density of *An.culicifacies* is more in Kandhamal and Nawapara districts.*An.culicifacies* found to be susceptible to synthetic pyrethroid and resistant to DDT. As the collection was done in summer season the number of *An.fluviatilis* collection is very less so the susceptibility status of *An. fluviatilis* could not be done.

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

Table 2. PHC wise distribution of mosquito bednets and 2.5 % deltamethrine flow for 2004-2005 in Kalahandi district

SL. No.	Name of the PHCs	Number of mosquito bed net already supplied	Quantity now supply of Deltamethrine 205 % in litre (one bottle =1 litre)	Remarks
1	Th.rampur	6,850	69	75Km
2	Chilliguda	2,000	20	45
3	Jaipatna	2,000	20	75
4	B.N.Pur	2,000	20	45
5	M.Rampur	2,150	22	45
6	Borda	1,500	15	Impregnation report submitted
7	Parla	1,000	10	
8	Karlakudi	1,000	10	
9	Pastikudi	1,000	10	Impregnation report submitted
10	Chapur	1,000	10	
11	Narla	2,000	20	35
12	kalampur	500	5	Impregnation report submitted
13	Koksara	2000	20	
	Total	25,000	251	

Table 3. Mosquito net distribution in Nawapara district

Sl. No.	Name of PHC	Total number of mosquito nets supplied	Date of supply
1	Sinapali	3000	Sept – 2002
2	Boden	3000	Sept – 2002
3	Kharion	4000	Sept – 2002
4	Komna	7000	Sept – 2002
5	Khariontood	7000	Sept – 2002
6	Reserve for H.Q.	1000	Sept – 2002

Table 4. Entomological report of Kandhamal district during March 2005

Sl. No.	Name of species	PHC									
		Khajuripada (1 Village)		Gumagada (1 village) 1		Daringbadi (4 villages)		Subarnagiri (4 villages)		Tumudibandh (1 village)	
		No.	PMHD	No.	PMHD	No.	PMHD	No.	PMHD	No.	PMHD
1	<i>An. annularis</i>									2	0.17
2	<i>An. culicifacies</i>	4	1.0	69	86	64	4.57	5	1.0	74	6.17
3	<i>An. fluviatilis</i>	-	-			5	0.35	-	-	1	0.08
4	<i>An. subpictus</i>	8	2.0	-	-	33	2.35	51	10.2	38	3.17
5	<i>An. splendidus</i>	-	-	-	-	4	0.28	-	-	16	1.33
6	<i>An. vagus</i>	7	1.75	17	2.1	61	4.35	61	12.2	81	6.75
7	<i>Cx. vishnui</i>	-	-	-	-	2	0.14	13	2.6	28	2.33
8	<i>cx. quinquefasciatus</i>	14	3.5	-	-	5	0.35	27	5.4		



Completed Projects

Table 5. Entomological report of Kalahandi district during March 2005

Sl. No.	Name of species	PHC									
		Pastigudi (6 Village)		Parla (3 village)		Th. Rampur (6 villages)		Narla (4 villages)		M. Rampur (8 village)	
		No.	PMHD	No.	PMHD	No.	PMHD	No.	PMHD	No.	PMHD
1	<i>An. annularis</i>	4	0.4	2	0.33			6	0.35	21	0.91
2	<i>An. aconitus</i>							5	0.29	1	0.04
3	<i>An. culicifacies</i>	11	1.1	10	1.66	16	2.0	76	4.470	108	4.69
4	<i>An. fluviatilis</i>			1	0.16						
5	<i>An. hyrcanus</i>	7	0.7	5	0.83					19	0.82
6	<i>An. jeyporensis</i>	1	0.1	-	-	-	-	-	-		
7	<i>An. subpictus</i>	81	8.1	90	15.0	66	8.25	167	9.82	165	7.17
8	<i>An. tasselatus</i>	1	0.1	2	0.3						
9	<i>An. vagus</i>	65	6.5			102	12.75	199	11.70	162	7.04
10	<i>Cx. quinquefasciatus</i>	33	3.3	19	3.16	47	5.87	7	20.41		
11	<i>Cx. vishnui</i>	29		27	4.5	131	16.37	115	6.76	94	4.08
12	<i>Cx. tritaeniorhynchus</i>	1	0.1								

Table 6. District wise entomological report of Nawapara district

Sl. No.	Name of species	PHC					
		Khariar (3. villages)		Sinapali (2 village)		Khariar road (2 village)	
		No.	PMHD	No.	PMHD	No.	PMHD
1	<i>An. annularis</i>	4	0.44	-			
2	<i>An. culicifacies</i>	12	1.33	101	12.62	-	
3	<i>An. subpictus</i>	76	8.44	115	14.37	-	
4	<i>An. vagus</i>	92	10.22	87	210.87	100	12.5
5	<i>Cx. vishnui</i>	8	0.89	25	3.12	74	9.25
6	<i>Cx. quinquefasciatus</i>	3	0.33	-	-	-	-

Table 7. Anthropophilic index of *An. culicifacies*, *An. fluviatilis* and *An. Annularis*

Area	SP species	No. of tested	Number of positive for Human	% Human
Kandha	An. Culi	53	4	7.5
Kandha	An. fluviatis	4	2	50
Nuapada	An. culi	60	5	8.3
Kalahandi	An. ann	15	2	13.3
Kalahandi	An. cul	65	6	9.2

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

Table 8. Susceptibility Status of *An. culicifacies* (vector of malaria) in three districts of Orissa

Sl. No.	Name of the District	Name of the PHC/Village	Anopheles species tested	Insecticide	Death of Mosquito				% of control mortality	% of test mortality	Corrected mortality
					Control		Test	Dead/Alive			
					1 hr.	24 hrs.	1 hr.	24 hrs.			
1	Kalahandi	NARLA/BURAT,	<i>An. culicifacies</i>	DDT4%	Nil	Nil	Nil	3/15	Nil	20%	20%
		Talapada	<i>An. culicifacies</i>	Deltamethrin 0.05%	Nil	Nil	15/15	-	Nil	100%	100%
2	-DO-	M.RAMPUR/	<i>An. culicifacies</i>	DDT4%	Nil	Nil	Nil	4/15	Nil	26.6%	26.6%
		Ambagan,Tujung	<i>An. culicifacies</i>	Deltamethrin 0.05%	Nil	Nil	14/15	1/15	Nil	100%	100%
3	Kandhamal	Tumudibandh/	<i>An. culicifacies</i>	DDT4%	Nil	Nil	Nil	2/15	Nil	13.3%	13.3%
		Guma,Bilmal	<i>An. culicifacies</i>	Deltamethrin 0.05%	Nil	Nil	14/15	1/15	Nil	100%	100%
4	-DO-	Daringbadi/	<i>An. culicifacies</i>	DDT4%	Nil	Nil	Nil	4/15	Nil	26.6%	26.6%
		Daringbadi,Parthamal	<i>An. culicifacies</i>	Deltamethrin 0.05%	Nil	Nil	15/15	-	Nil	100%	100%
5	-DO-	Gumagada/	<i>An. culicifacies</i>	DDT4%	Nil	Nil	Nil	4/15	Nil	26.6%	26.6%
		Rashmimandi	<i>An. culicifacies</i>	Deltamethrin 0.05%	Nil	Nil	15/15	-	Nil	100%	100%
6	Nawapada	Sinapali/Badagan	<i>An. culicifacies</i>	DDT4%	Nil	Nil	Nil	3/15	Nil	20%	20%
			<i>An. culicifacies</i>	Deltamethrin 0.05%	Nil	Nil	15/15	-	Nil	100%	100%



Completed Projects

Status : 3.7 **Intervention Programme for Cholera, Intestinal Parasitism, Vit A deficiency and Scabies amongst some primitive tribes of Orissa.**
Extramural (ICMR Taskforce)
Investigator : Dr. G.P. Chhotray
Starting date : February 2000
Closing date : January 2005

This community based pilot study involving 4 identified primitive tribes namely, Bondo, Didayi, Kandha and Juanga residing in 4 different geographical regions of the state was conducted in 4 phases 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 tribes such as Bondo, Didayi, Kandha and Juanga out of 13 primitive tribes residing in Koraput, Phulbani and Keonjhar districts of Orissa.
2. Demographic profile studies reflecting the morbidity and mortality patterns arising out of these diseases and their clinical evaluation.
3. To assess the awareness of 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.

Work done:

During the study period a total of 17 Bondo villages, 15 Didayi villages, 25 Kandha villages and 30 Juanga villages out of total 29,37, 68 and 48 villages respectively were selected for the study by using population proportion to size (PPS) sampling procedure and were enumerated. The sample size was estimated by taking the expected prevalence rate of these diseases from the Govt. of Orissa Health Statistics. The absolute number of samples was estimated from a total population of 5565, 5763, 10432 and 6624 from Bondo, Didayi, Kandha and Juanga tribes respectively by using the formula $N = \frac{N_0 p(1-p)}{E^2} \left[1 + t^2 \frac{p(1-p)}{E^2} \right]$ with a confidence level of 95% (where N=population size, t=standard normal deviate correspond to 5% level of significance, P=prevalence rate and E=allowable error of 2%).

Since no base line data were available, a cross sectional study by way of clinical examination, laboratory investigation, enumeration and data collection was performed on 4456 number of individuals belonging to Bondo (n=1012), Didayi (1009), Kandha (1298) and Juanga (1137) (Table 1). The study revealed that majority of the people (52.6% in Bondo, 54.7% in Didayi, 46.5% in Kandha and 48.5% in Juanga) presented with anaemia as a major clinical sign followed by fever in 16.5%, 16.1%, 15.3% and 16% of cases studied in Bondo, Didayi, Kandha and Juanga population respectively. The respiratory diseases such as cough and URTI were found to be prevalent in 9.6% of Bondo, 9.7% of Didayi, 8.6% of Kandha and 9% of Juanga population studied. The diarrhoeal disorders were found to be present in 11%, 10.8%, 10.2% and 10.6% of cases of Bondo, Didayi, Kandha and Juanga study population. Amongst other infectious diseases tuberculosis, leprosy and yaws were observed to be present in only 0.6%, 1.2%, 1.2% and 1.5% of cases studied in Bondo, Didayi, Kandha and Juanga tribes respectively. Hepatitis was found in 0.6% of Bondo and 0.9% of Didayi studied population and was absent in Kandha and Juanga tribes. Non-infectious diseases like cardiovascular diseases, and hypertension was found to be present in 2.3%, 2.9%, 2.6% and 2.7% of cases studied respectively. Assessment of comprehensive health status revealed that at least 34.8%, 36.1%, 37.7% and 37.8% amongst the studied population in the Bondo, Didayi, Kandha and Juanga tribes respectively had no signs and symptoms of any diseases during clinical examination.

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

Table 1. Comprehensive health status of the studied tribes

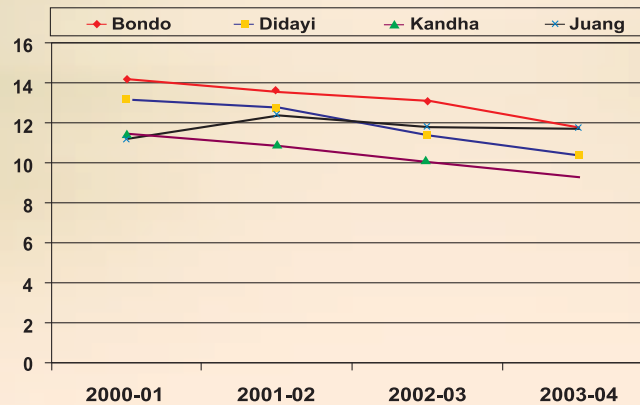
Disease	Bondo n=1012	Didayi n=1009	Kandha n=1298	Juang n=1137
Respiratory diseases, (cough, URTI etc.)	97 (9.6)	98 (9.7)	111 (8.6)	102 (9.0)
Fever	167 (16.5)	162 (16.1)	199 (15.3)	182 (16.0)
Malaria	141 (13.9)	138 (13.7)	169 (13.0)	153 (13.5)
Diarrhoea including cholera	111 (11.0)	109 (10.8)	132 (10.2)	121 (10.6)
Cardiovascular diseases including hypertension	23 (2.3)	29 (2.9)	34 (2.6)	31 (2.7)
Other Infectious diseases	6 (0.6)	12 (1.2)	16 (1.2)	17 (1.5)
Intestinal parasitism	252 (24.9)	251 (24.9)	301 (23.2)	272 (23.9)
Anaemia	532 (52.6)	552 (54.7)	604 (46.5)	552 (48.5)
STDs & HIV	0	0	0	0
Hepatitis	6 (0.6)	9 (0.9)	0	0
Persons with no signs and symptoms of above diseases	343 (34.8)	364 (36.1)	489 (37.7)	429 (37.8)

Diarrhoeal disorders including Cholera:

There is a continuous occurrence of diarrhoeal cases in the community through out the year attaining its peak during July to October (rainy season). To identify the aetiopathogenic agents in diarrhoeal cases during the diarrhoeal episodes, stool samples/rectal swabs were collected and transported to RMRC laboratory for analysis. During the study, a total 222, 332, 276 and 236 rectal swabs / stool samples were collected in successive years (2000-01, 2001-02, 2002-03 and 2003-04) from the study populations. The bacteriological analysis revealed that 91 samples were culture positive during 2000-01, while other samples did not show any growth of enteropathogens. Amongst the culture positive cases *E. coli* was isolated in 24.6%, *Salmonella* in 2.6% & *V.cholerae* in 13.6% of samples. Amongst the *V.cholerae* isolates 10.2% were found to be *V.cholerae* 01 Ogawa and 3.4% were *V.cholerae* 0139 serotype. Amongst the pathogenic *E.coli* isolates, serological and molecular studies revealed that 6.5% were enteropathogenic *E. coli* (EPEC), 4.3% enterotoxigenic *E. coli* (ETEC) and 15.2% enteroaggregative *E. coli* (EaggEC). The incidence of diarrhoeal cases in subsequent years i.e. 2001-02, 2002-03 and 2003-04 remained almost same. But the isolation of *V.cholerae* as enteropathogen from diarrhoea cases decreased to 0% from 4.7% during this period; where as number of *E.coli* isolates has increased from 32.5% to 41.0%.



Completed Projects

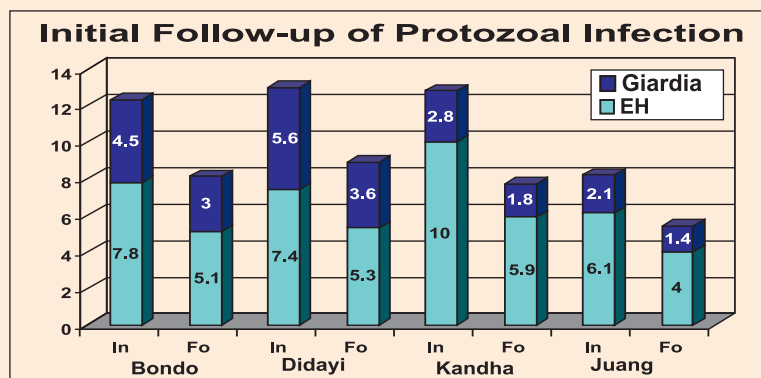


Suitable intervention during diarrhoeal outbreaks with ORS and antibiotics was implemented to cases having mild and moderate clinical signs and symptoms and hospitalization for severe cases. Community based IEC activities were conducted in all the study villages. The rectal swab analysis in follow-up studies showed remarkable decline in the isolation of *V. cholerae* from 4.7% in 2001 to 0% in 2004.

Intestinal Parasitism:

Microscopic examination of 4064 stool samples collected from 4 primitive tribes from all age/sex groups revealed that 41.6% of Bondo, 41% of Didayi, 34.5% of Kandha and 25.6% of Juanga tribe studied had intestinal parasite infection (both protozoa and helminthes). Helminthic infection was observed among 29.2%, 27.3%, 23.6% and 17.5% of Bondo, Didayi, Kondha and Juanga population. Protozoal infection (*E.histolytica* and *Giardia*) was observed in 12.4%, 13.3%, 10.9% and 8.1%, respectively in above communities. Hook worm was found to be the commonest helminthic infection accounting for 17.9% in Bondo, 13.7% in Didayi, 14.8% in Kondha and 10.3% in Juanga population, followed by round worm in 8.6%, 9.5%, 6.8% and 5.8% of cases. Amongst the protozoal infection *E. histolytica* is the commonest infection (7.8% in Bondo, 7.4% in Didayi, 8.8% in Kandha and 6.1% in Juanga), where as *Giardia* was found in 4.5%, 5.6% 2.1% and 3.1% of Bondo, Didayi, Kandha and Juanga tribes respectively. Repeated stool examination after appropriate intervention of antiprotozoal (Metronidazole 400 mg tds for 5-7 days to adult and 200 mg tds to children 5-7days) and antihelminthic treatment (Albendazole 400mg to adult, 200mg to children in single dose) in selected individuals revealed significant decrease in worm burden in the follow-up study. The worm burden has decreased from 41% to 27.2% in Bondo, from 41% to 26.6% in Didayi, from 34.5% to 22.1% in Kandha and from 25.6% to 16.6% in Juanga (Fig. 2 and 3).

Fig.2. Initial follow-up of protozoal infection

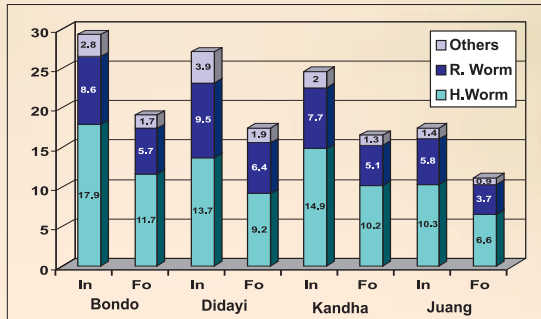


Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

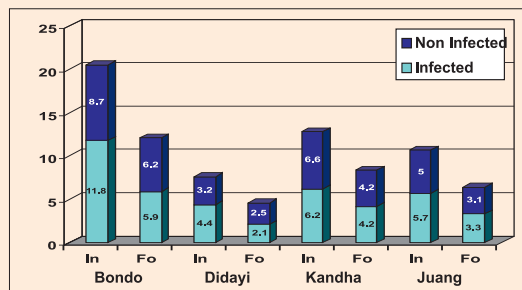
Fig.3. Initial follow-up of heiminthic infection



Scabies:

The clinical examination performed in 4,064 number of tribal population in all age/sex groups among 4 primitive tribes revealed that 20.5% in Bondo, 7.5% in Didayi, 12.9% in Kandha and 10.7% in Juanga tribe had scabies of which 11.8%, 4.3%, 6.5% and 5.5% were infected and 8.2%, 3.2%, 6.7% and 5% were non-infected. The majority of patients showing scabies were in <14 years of age group. With the institution of appropriate intervention (Benzyl Benzoate 12.5% emulsion), personal hygiene and IEC activities, the incidence dropped to 12.1%, 4.6%, 8.5% and 6.4% in Bondo, Didayi, Kandha and Juanga respectively (Fig 4).

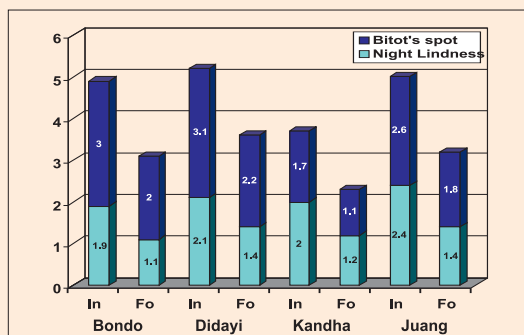
Fig.4. Initial follow-up of scabies



Vitamin A deficiency (VAD):

The prevalence of VAD among pre-school and school going children (6-14 years) by examination of signs/symptoms such as night blindness and bitot's spot during both initial and follow-up after appropriate intervention has been depicted in Fig 5. When reassessed the VAD have reduced from 8.5%, 7.3%, 5.8% and 6.4% to 5.9%, 6.2%, 3.8% and 4.5% in 0-6 years of age group and from 6.1%, 5.1%, 6.1% and 5.5 to 3.8%, 4.0%, 3.5% and 3% in 6-14 years of age group in Bondo, Didayi, Kandha and Juanga respectively.

Fig. 5. Initial follow-up of vitamin-A deficiencies





Completed Projects

Conclusion:

The study revealed that the health status of the studied primitive tribes is poor in comparison to national health status due to isolation, remoteness, lack of health care delivery and ignorance. The diseases studied like cholera, scabies, intestinal parasitism and VAD are preventable in nature after suitable and timely intervention with appropriate therapy. Therefore, a timely intervention, social awareness, health education, IEC activities will promote good health in these primitive tribal communities and reduce morbidity and mortality.

Status : 3.8 Intervention programme on nutritional anaemia and haemoglobinopathies in some primitive tribal population of India.

Extramural (ICMR Task Force)

Investigators :

Dr. G.P Chhotray, Dr. Deepika Mohanty

Starting date :

October 1999

Closing date : September 2004

This is a community based multicentric study undertaken in 4 states (Maharashtra, Gujarat, Tamilnadu and Orissa). In Orissa the study was undertaken in 4 primitive tribes viz. Bondo, Didayi, Kandha and Juanga in 3 phases with the following objectives.

1. To find out the prevalence and aetiology of nutritional anaemia and haemoglobinopathies in 4 primitive tribes viz. Bondo, Didayi, Kandha and 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 investigations have been performed on 962, 1014, 953, and 1065 individuals of all age group and either sex, out of 4010, 2792, 3378 and 5535 population enumerated amongst Bondo, Didayi, Kandha and Juanga primitive tribes during the study period

The overall prevalence of anemia was observed to be 53.1%, 60.5%, 52.1% and 44.6% among Bondo, Didayi, Kondha, and Juanga populations respectively. The severity of anaemia was graded as mild, moderate and severe according to haemoglobin level following the WHO classification, in different age and physiological groups. During the initial study period, it was observed that 48.2% of the studied population had normal Hb (Hb >11g/dl), 1.6% had severe anaemia (Hb <7g/dl), 39.4% had mild anaemia (Hb 9-11g/dl) and 10.6% had moderate anaemia (Hb 7-11g/dl) in Bondo, Didayi, Kandha and Juanga primitive tribes.

The tribe wise distribution of anaemia is shown in Fig 1(a-d). Various laboratory investigation such as Hb, MCV, MCH, MCHC, FeP estimation and peripheral smear examination revealed that 61.4% of the anaemia cases had microcytic hypochromic blood picture indicating iron deficiency anaemia.

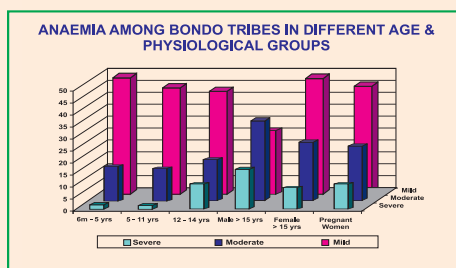


Fig. 1 (a)

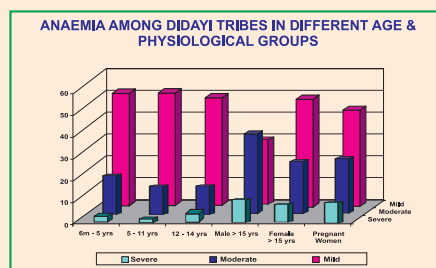


Fig. 1 (b)

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

Rapid diagnostic tests like NESTROFT, Hb electrophoresis, solubility test for sickling, Hb F and A₂ estimation, Hb variant analysis (BIORAD) revealed that 0.7%, 3.2%, 1.2%, 2.7% of the studied population of Bondo, Didayi, Kandha and Juanga primitive tribes respectively had sickle cell disease of which 0.1% are sickle cell anaemia (HbSS) and rest of them are sickle cell trait. Among other haemoglobinopathies, 0.7% of Bondo population, 3.0% of Didayi population, 3.1% of Juanga population and 3.3% of Kandha population studied had α thalassaemia trait, whereas α thalassaemia major was not encountered amongst the studied tribes. The G6PD deficiency was observed to be present in 0.6%, 1.6%, 7.5% and 4.3% of Bondo, Didayi, Kandha and Juanga population respectively. The PCR assay performed in selected G6PD deficiency cases revealed that all of them are G6PD Orissa mutant.

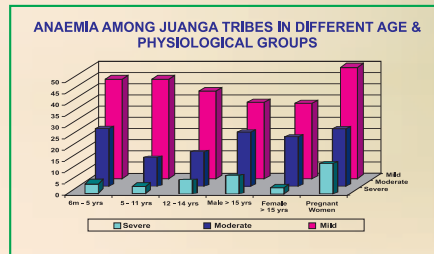


Fig. 1 (d)

Appropriate intervention measures by way of drug supplementation (Tab Fersolate: 60 mg elemental iron / OD) were instituted along with Albendazole (200mg single dose for the children and 400mg for adult). For the community intervention, the IEC activities were undertaken by way of group discussion, interpersonal communication, health awareness and education through posters, pamphlets and audiovisual aids in their respective dialects. A total of 76-discussion sessions was conducted during the study period by the help of selected and trained resource persons from the community.

The follow-up examination and haemoglobin estimation revealed that, there was an improvement of anaemia status in 26.3% of Bondo, 27.2% in Didayi, 24.4% in Juanga and 21.2% in Kandha tribes (Fig 2). The overall repeat stool examination revealed that intestinal parasitic infestation has dropped from 24.6% to 12.4% in Bondo and 24.9% to 13.8% in Didayi, 23.2% to 10.5% in Kandha and 23.9% to 9.8% in Juanga; and the hook worm infestation dropped from 17.9% to 11.7% in Bondo, 13.7% to 9.2% in Didayi, 14.9% to 10.2% in Kandha and 10.3% to 6.6% in Juanga.

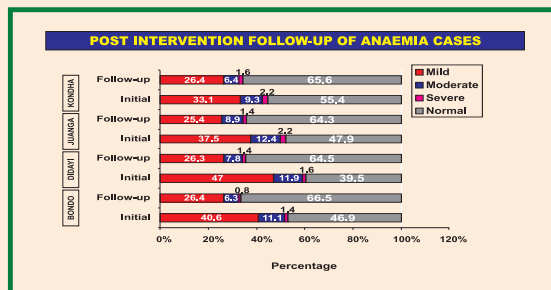


Fig. 2

Table 1. Correlation among the free red cell protoporphyrin with haematological variables (Spearman's Correlation Coefficient)

*p < 0.05; * p < 0.01

Variables	Hbg/ dl	MCV	MCH	MCHC	FEP
Hbg/ dl	1.000	.526**	.488**	.523**	-.478**
MCV	.473**	1.000	.813**	.769**	-.489**
MCH	.623**	.798**	1.000	.569**	-.453**
MCHC	.561**	.698**	.552**	1.000	-.479**
FEP	-.472**	-.431**	-.463**	-.489**	1.000



Completed Projects

One of the important causes attributed towards the prevalence of high degree of microcytic hypochromic anaemia was the high incidence of helminthic infections largely due to hookworm.

Although the study revealed that there is a high prevalence of malaria (SPR: 15.8% in Bonda, 15.7% in Didayi, 12.9% in Kondha and 14.2% in Juanga) and Pf being 92.3% in Bondo, 92.1% in Didayi, 84.2% in Juanga and 91.2% in Kondha. There was no statistical correlation between anaemia and malaria infection.

The FeP estimation was carried out in all the cases and it was observed that there is a negative correlation between the FeP value and other haematological parameters studied (Table 1).

The above data has already been submitted to the Co-Coordinator for central analysis of the multicentric study.

Conclusion:

The study revealed that timely intervention, social awareness, health education and IEC activities have resulted in some improvement by decreasing the worm burden and improving anaemia status in 26.3% of Bondo, 27.2% of Didayi, 24.4% of Juanga and 21.2% of Kandha tribes. This will reduce morbidity and promote good health in the community.

Future strategies:

According to the protocol and MOU the know how is to be transferred to the State Health Authority for conducting NESTROFT and Hb electrophoresis at the P H C level.

- 1) A few technicians have already been trained.
- 2) Intermittent deworming treatment with Albendazole may be introduced in the National Parasite Control Programme.
- 3) IEC activities to be continued in collaboration with National Programmes undertaken in these areas.

Status :

Extra-mural (Ministry of Health & Family Welfare, Govt. of India)

Investigators :

Dr. R.S. Balgir

Starting date : January 2000

Closing date : December 2004

3.9 Intervention for hereditary common haemolytic disorders among the major tribals of Sundargarh district of Orissa

Objectives:

1. Screening and identification of major vulnerable tribals, namely, Bhuyan 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 laboratory 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 syndromes and G-6-PD deficiency are highly prevalent among the tribal populations of India and lead to high degree of anemia, morbidity, mortality and fetal wastage among the vulnerable people. They present increasing challenge to the health care especially to the underprivileged communities. Tribals of Sundargarh district in Orissa, namely Bhuyan and Kharia are highly prone to hemolytic anemia, jaundice, painful crisis, recurrent fever, etc. Since these disorders are hereditary in nature and there is no cure for them, therefore, their prevention is highly essential.

To the best of our knowledge, no intervention programme was undertaken to prevent and control these hereditary disorders in vulnerable tribal communities in Orissa. The undertaken project was designed to fill up this lacuna.

Results:

To achieve the stipulated aims and objectives, sensitisation, motivation and education through pamphlets, holding interactive meetings, discussions, explaining the benefits, purpose and aims and objectives for getting the assured cooperation and help for the implementation of the project was done at district, block and village levels before initiating the study. We adopted biomedical anthropological approach to successfully implement and evolve eco-friendly, tribal-oriented, tribal-friendly, tribal-participatory and, cooperative and health seeking strategy for

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

this project. The success of this strategy was apparent with overwhelming response of tribal people and their participation and cooperation in every stage for improving the health status and quality of life.

The whole village screening of Bhuyan and Kharia tribes for sickle cell disease, β -thalassemia syndrome and other hemoglobinopathies, and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency revealed the high occurrence of hereditary haemolytic disorders among the major scheduled tribes of Sundargarh district of Orissa.

The sickle cell disorders and hemoglobin E disorders were found in high frequency in Dhelki Kharia (12.4% and 3.2%, respectively), whereas, these were not encountered at all in Dudh Kharia. Haemoglobin E was detected for the first time in Dhelki Kharia tribe from Orissa. The frequency of β -thalassemia trait was higher in Dudh Kharia (8.1%) than in Dhelki Kharia (4.0%) in Sundargarh district of Orissa. The G-6-PD enzyme deficiency was considerably high in Dhelki Kharia (30.7%) in comparison to Dudh Kharia (19.2%). Antimalarials needed to be administered carefully in these tribal populations. The Rhesus (D) blood group negativity was very low (1.1%) in Dudh Kharia tribe and in Dhelki Kharia, it was found absent.

The sickle cell trait was confined only to Paraja Bhuyan (0.9%) and Paik Bhuyan (7.3%). A family with hereditary persistence of fetal hemoglobin (HPFH) in Paraja Bhuyan and hemoglobin D trait in Paik Bhuyan family was detected for the first time in a tribal population in Orissa. The frequency of β -thalassemia trait was the highest in Paraja Bhuyan (12.6%), followed by Paik Bhuyan (7.7%) and Paudi Bhuyan (1.7%) in Sundargarh district of Orissa. The G-6-PD deficiency was recorded to be 21.1%, 16.3% and 13.7% in Paraja, Paik and Paudi Bhuyans, respectively in Sundargarh district of Orissa. The use of antimalarials needs a caution in these tribal people. The frequency of Rhesus (D) negative was very low (0.6%) in Bhuyan tribe of Sundargarh district.

Before starting the awareness in the identified tribal people, the knowledge, attitude and practice (KAP) were studied using a pretested proforma as a measure of pre-intervention as well as post-intervention. The impact of present study of bringing awareness, sensitization and education would initially be expected to be slow, but it would be definite in the subsequent generations due to further enlightenment and experience. As we know that health comes by evolution, not by revolution. Health must meet the needs of the people, as they perceive them. Health cannot be imposed from outside against people's will. It cannot be dispensed to the tribal people.

Imparting of relevant training to State's local health authorities such as PHC doctor, laboratory technician, health workers, Anganwadi teachers, Pharmacists, etc. about the simple tests that can be performed at PHC/CHC level had further enhanced the know how and hand on the art of training in the field of study area. The idea behind this training was to motivate the tribal communities to go for carrier detection and then to refer the positive cases to specialized laboratories for further investigations for confirmation of the diagnosis and treatment accordingly.

Each person who had given blood for investigations was provided with investigation report card. The basic idea of intervention was to bring awareness in these tribal communities about the hereditary/genetic disorders, which are silent/hidden killer diseases. Affected individuals were suitably advised for taking follow up action. Both prospective and retrospective genetic/marriage counseling and interventions were imparted to all disease as well as carrier cases of hemoglobinopathies like sickle cell disease and trait, Hb E disease and trait, Hb D trait, β -thalassemia trait and HPFH and the G-6-PD deficiency through local PHC doctor by holding the interactive discussions taking into consideration the confidence and privacy of each person during the course of this project work. This will help in prevention of hereditary hemolytic disorders in the vulnerable tribal communities and improve their health status and quality of life.

The outcome of the present study has generated database, which is useful for prevention, prenatal diagnosis and control of important public health problem of hemoglobinopathies and G-6-PD deficiency in the region. This eventually will help formulate a strategy for improving the health of the affected people and enable the state government to take up intervention programs and integrate them through the PHCs, district headquarters hospitals and medical college hospitals and the people of the state will, substantially, be benefited. An intervention package was developed which could be replicated at other places.

3.10 Mid-term evaluation of improving nutritional and health status of children in Umerkote block of Nabarangapur district, Orissa

A mid-term evaluation has been carried out to evaluate the efficacy of high-energy biscuits (BP-5), along with the ICDS India-Mix ration. The target population was the severely malnourished children in the age group of 6-36 months, in Umerkote ICDS block of Nabarangapur district.

Status :

Extra-mural (World Food Programme)

Investigators :

Dr. A. Mohapatra, Dr. G. Bulliyya

Starting date : February 2004

Closing date : April 2004



Completed Projects

The study was conducted during February to April 2004. Stratified random sampling procedure was adopted and a total of 400 severely malnourished children were evaluated from

Fig 1. Nutritional status of children by IAP classification

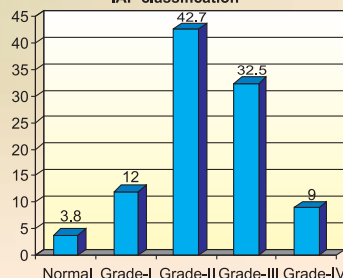
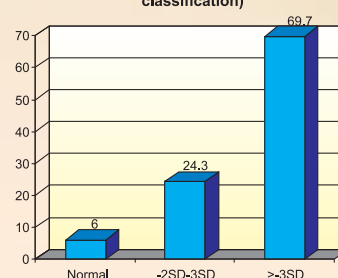


Fig 2. Nutritional status of children (SD-classification)



the list of children selected during baseline. The additional supplement was provided for a period of one year. Growth status (weight-for-age) was evaluated using the IAP cut-off points of Harvard standard and SD classification using NCHS cut-off values.

The results indicate that 3.8% of children were normal, while 12.0%, 42.7%, 32.5%, and 9.0% were grade-I, grade-II, grade-III and grade-IV of malnutrition respectively (Fig. 1). The extent of grade-III and grade-IV malnutrition was more among girls (51.7%) than among boys (30.1%). The proportion of children suffering from underweight was 94%, while 69.7% were severely underweight, as per the SD classification (Fig.2).

The impact of BP-5 biscuits is reflected in terms of improving child growth by using IPA and SD classification in association with quantum of biscuits supplementation. It is suggested to follow the beneficiary children over a period to get the exact impact specifically on each grade of malnourished children separately in terms of improvement, ensuring a systematic cohort follow-up.

Status : 3.11 Assessment of iron deficiency anaemia among adolescent girls in Orissa

EM (Dept. Women & child welfare,
Govt. of Orissa)

Investigator :

Dr. G. Bulliyya, Mrs. G. Mallick,
Mr. R. C. Parida, Dr. S. K. Kar

Starting date : December 2004

Closing date : May 2005



Community awareness campaign on health & nutrition education by RMRC, BBSR

Adolescence (10-19 years of age) is a phase of rapid physical growth and development, the nutrients requirement increase and the risk of nutritional deficiencies more pronounced. Anaemia is of particular concern because anaemia during pregnancy is associated with premature births, low birth weight, and perinatal and maternal mortality. It is estimated that iron deficiency anaemia (IDA) is responsible for one fifth of early neonatal deaths and to about 10% of maternal mortality. In India, anaemia affects an estimated 50% of adolescent girls. The National Nutritional Anaemia Control programme initiated in 1970 based on the seriousness of problem to prevent and control nutritional anaemia with IFA tablets.

Since, there is scanty information about anaemia status of adolescent girls in Orissa, and no data available at districts level. The present survey was carried out to assess the prevalence of anaemia in terms of haemoglobin levels among non-school going adolescent girls covering statistically adequate sample. In addition, household socio-economy, demography and nutritional status of adolescent girls were assessed for evaluating the association with anaemia.

A total of 1937 adolescent girls were covered from Khurda (670), Jajpur (647) and Bargarh (620) using 30 cluster PPS sampling. The quality check of haemoglobin levels between external and internal is in good agreement. The mean haemoglobin level was 9.9 ± 1.4 g/dL among adolescent girls. It was 10.1 ± 1.27 , 9.8 ± 1.37 and 9.2 ± 1.41 g/dL for adolescent girls of the districts Khurda, Jajpur and Bargarh. The mean levels of haemoglobin (9.9 g/dL) were relatively higher in the districts of Khurda in comparison to Jajpur and Bargarh (Fig-1). The mean haemoglobin levels were below the levels reported by NNMB for the state of Orissa (NNMB, 2003).

Overall, 96.5% of adolescent girls had some degree of anaemia, 45.2% were mildly anaemic, 46.9% were moderately anaemic and 4.4% were severely anaemic according to WHO criteria (Fig-2). About 94%, 96% and 99% of adolescent girls in the districts of Khurda, Jajpur and Bargarh respectively were found anaemic (<12 g/dL). While in the districts of Khurda and Jajpur the anaemia was of milder grade, in Bargarh district, moderate anaemia dominated over mild anaemia. The extent of moderate anaemia and severe anaemia was significantly higher in the district of Bargarh (60.8% and 7.5%) as compared to districts of Khurda (52.2% and 2.5%) and Jajpur (51.5% and 3.4%).

Serum ferritin is an indicator of the relative extent of depletion of iron stores. The mean ferritin concentration was 6.5 ng/mL in Khurda district, which was significantly higher ($p < 0.001$) compared to 30.0 ng/mL in Bargarh district. Based on cut-off levels for serum ferritin

Completed Projects



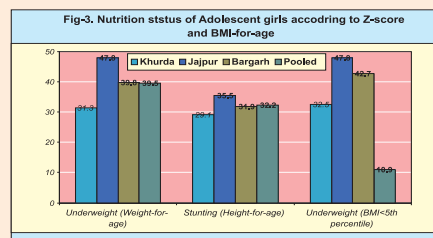
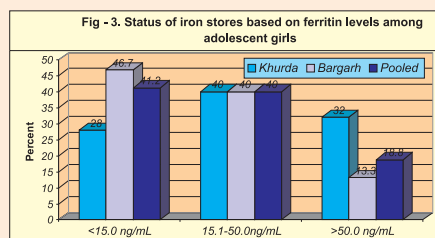
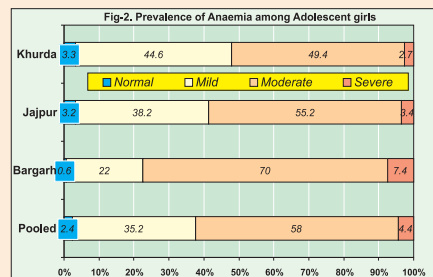
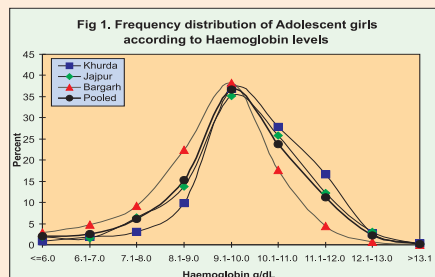
ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

(WHO, 2002), more than forty percent (41.2%) of adolescent girls had ferritin levels below 15 ng/mL reflecting inadequate iron store (Fig-3). The proportion of adolescent girls with inadequate iron stores was 28% and 46.7% in the districts of Khurda and Bargarh respectively. The mean concentrations of haemoglobin increased consistently with increase in cut off levels of ferritin. The variation in concentrations of haemoglobin between cut-off values of ferritin was significant.

About 38% of adolescent girls were underweight (<Median -2SD of NCHS Weight-for-age), ranged between 31.3% in Khurda and 47.8% in Bargarh districts. The prevalence of stunting and underweight (<5th percentile BMI-for-age) was 32% and 41% respectively. The proportion of underweight girls estimated using weight-for-age and BMI-for-age classifications were the same in the districts of Khurda, Jajpur and Bargarh (Fig 3).

The present study has shown the universal prevalence of anaemia among adolescent girls. Hence, the lack of association seen between the anaemia status and socio-economic factors is in the expected direction. The difference between prevalence of anaemia among the adolescent girls by attainment of menarche, and by the working status and nutritional status as assessed by weight-for-age and BMI-for-age show differences in severe forms of anaemia. Inverse relationship observed between prevalence of moderate and severe forms of anaemia among girls with that of their education status, awareness of anaemia, and consumption levels of pulses, green leaf vegetables, eggs and flesh foods is in expected direction.

The study revealed that non-school going adolescent girls were vulnerable in terms of IDA, food intake and nutritional anthropometry. Their nutritional education was inadequate. In conclusion, the results indicated that there is an urgent need to focus the nutritionally vulnerable group of adolescent girls in the community, which is not included in the national intervention programs. It is therefore, really required to have an intervention program with strengthening the nutrition education component for improving the health and nutritional status of adolescent girls, who are entering shortly in to stressful physiological states of pregnancy and lactation.



3.12 Prevalence of Chlamydia trachomatis infection amongst clinical cases attending OPD—a pilot study

Introduction:

Chlamydia trachomatis infection among females is becoming the major bacterial STD in present days. Infections like syphilis and gonorrhoea has been reduced much because of their expression and relatively easier diagnosis, which enable clinicians to provide satisfactory treatment. Although curative antibiotics are available against the agent, difficulty in diagnosing *Chlamydia trachomatis* by conventional tests keeps the infection undiagnosed leading to complications like low backache, PID, infertility, recurrent abortions, intrauterine growth

Status :

Intramural

Investigator :

Dr. B. Dwibedi

Collaborators :

Dr. Jayanti Mania, NIRRH, Mumbai
Dr. S. Pattanaik, Department of Obstetrics and Gynecology SCB Medical College, Cuttack

Starting date : January 2005

Closing date : March 2005



Completed Projects

retardation of foetus, still births and neonatal respiratory tract infections. Such untreated infections ultimately leads to maternal health problems leading to complicated pregnancy and endangering child survival. Presently technology and facility for diagnosis of Chlamydia by PCR method is not available in Orissa. So, an attempt has been made to standardize the diagnostic procedure in a hospital based study. Subsequently community prevalence study can be planned and the technology can be transferred to the State medical college laboratories.

Objectives:

To estimate Chlamydia trachomatis infection in females attending out patients department (OPD) of Gynaecology, SCB Medical College and Hospital, Cuttack.

Methodology:

Selection of patients and sample collection:

Women attending the gynecological OPD of SCB Medical College and Hospital, Cuttack with complains of vaginal discharge (mucopurulent), recurrent abortion, pelvic pain or infertility were taken as the study population. The study subjects were selected based on under mentioned criteria:

Inclusion criteria:

- 1) Women with lower genital tract infection;
- 2) Women with bad obstetric history (> 2 spontaneous abortion);
- 3) Infertile women;
- 4) Women with clinical suspicion of PID or with chronic lower back-ache with vaginal discharge; not having other medical/ orthopedic problems.

Exclusion criteria:

Women treated with antibiotics within one month prior to sample collection.

Collection of cervical specimen:

After patients were enrolled at OPD relevant history was recorded in preformed clinical format. A written consent was obtained from all the patients. The clinical specimen (endocervical swab) was collected under per speculum examination in lying down posture. After removing the excess mucus from the exo-cervix with a sterile cotton ball, the collection swab was rotated in the endo-cervix for 15 to 30 seconds. Then the cervical swabs were kept in separate sterile self-retaining test tubes and transported to the laboratory in icebox and stored in the deep freezer (-140° C).

Diagnosis of Chlamydia trachomatis infection by PCR- Test:

Duplicate coded samples were tested separately at RMRC and NIRRH. DNA extraction was done and Chlamydia infection was identified by PCR using specific primers following the procedure below:

The cervical swabs were dissolved in 1 ml of phosphate buffer saline and the solution was centrifuged after removing the swab stick. DNA was extracted from the cell pellet using DNA extraction solution (DNA Extraction kit, Bangalore Genei) After confirming the presence of DNA (figure 1). DNA segments were amplified by PCR using specific primer (5' GCC GCT TTG AGT TCT GCT TCC 3', 5'GTC GAA AAC AAA GTC ACC ATA GTA 3') in 40 thermal cycles; each thermal cycle set at 94°C for 1 min- 52°C for 1 min.- 72°C for 2 min. Then the amplified product was run in 2% agarose gel with ethidium bromide and visualised

1 2 3 4 5 6 7 8 9 10 11

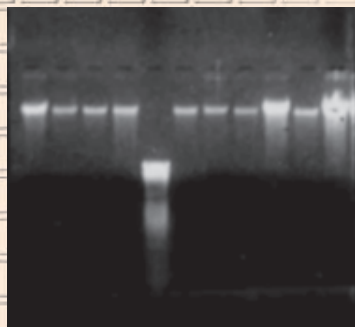
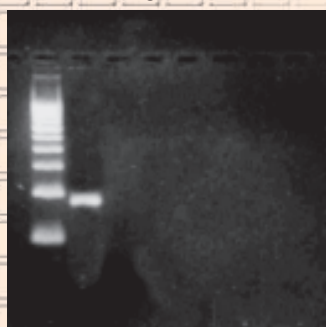


Figure 1.: Genomic DNA on 0.8% agarose gel.
Lane1, 2, 3, 4, 6, 7, 8, 9, 10, 11: Genomic DNA. Lane 5: Ladder

1 2 3



180 bp

Figure 2: PCR product of 180 bp DNA fragment of *C. trachomatis*.
Lane1: 100 bp DNA Ladder,
Lane 2: 180 bp PCR product, Lane 3: Negative control

Completed Projects



ANNUAL REPORT 2004-05
REGIONAL MEDICAL RESEARCH CENTRE, BBSR

under UV transillumination for identification of 180bp DNA strand (figure 2). Then it was subjected to Southern blot test to observe the chemiluminescence (figure 3).

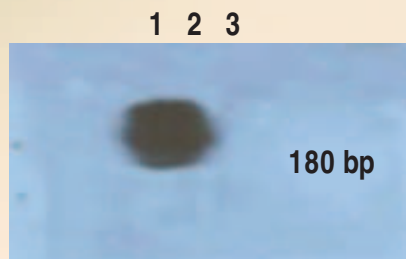


Figure 3: Hybridisation analysis of 180 bp DNA fragment of *C.trachomatis* Lane 1: 100 bp DNA Ladder, Lane 2: 180 bp Hybridisation product, Lane 3: Negative control.

Results:

Patients coming to out patient department of Gynaecology, SCB Medical College, Cuttack were enrolled for study following the criteria of exclusion and inclusion. A total of 108 patients were included in the study and collected specimens subjected to investigation.

The patients were in the age group of 20 to 50years; who presented with single or multiple complains. The observed symptoms were as follows.

Symptoms	Number (n=108)
1. Vaginal discharge	89 (82%)
2. Infertility	21 (19.5%)
3. Recurrent abortion	34 (31.5%)
4. Low backache and PID (suspected)	52 (48%)

All the cervical swabs were subjected to PCR and southern blot technique. Amplification was successful in 71 cases and 7.04% were found positive for presence of *C. trachomatis* infection. One among them had chronic low backache for longer than 10 years and the other was complaining of infertility. Three had history of recurrent abortion. Eighty percent of them had discharge per vagina.

Conclusion:

Symptomatic females attending Gynaecology OPD of SCB Medical College, Cuttack with clinical suspicion of Chlamydia Trachomatis infection were investigated by PCR test and Southern hybridisation, in a pilot mode. The investigation confirmed presence of Chlamydia trachomatis infection in 2.6% of the patients studied. The study documented genital Chlamydia infection confirmed by DNA identification, for the first time in the state of Orissa. Besides, the molecular diagnosis technique of identifying Chlamydia trachomatis has been transferred to RMRC, and the skill can be transferred to Medical colleges of the state.

3.13 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.

Status :

Intramural

Investigators :

Dr. B.B Pal, Dr. G.P. Chhotray,
Mr. H.K Khuntia, Dr. A.S. Acharya.

Starting date : November 2003

Closing date : October 2004



Completed Projects

4. To study the clonality of the clinical strains with the environmental isolates by RADP PCR, Ribotyping and other methods. And the correlation will be studied, if any, between the mid epidemic and epidemic strains along with the previous isolates.

Methodology:

As per the request of the Directorate of Health Services, Govt. of Orissa outbreaks of diarrhoeal disorders were studied from Keonjhar town and its affected villages (July), Malkanagiri town and its affected villages (August); Chitrada village, Mayurbhanj district (August) and Parbatia village, Dhenkanal district (November) during 2003. Rectal swabs were collected in CBT medium, sub cultured in TCBS, MacA, HEA plates. Significant colonies were tested biochemically following standard techniques for different bacterial enteropathogens and confirmed by specific antiserum.

Results:

The biochemical and serological results revealed that the causative organism for these outbreaks was *V.cholerae* O1 Ogawa biotype El Tor .The detailed results have been depicted in the following table.

Table: Outbreaks of cholera in four districts of Orissa

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

Antibiogram:

The general antibiogram of the above isolates revealed that the *V.cholerae* were sensitive to tetracycline, ciprofloxacin, norfloxacin and chloramphenic, and resistant to cotrimoxazole, ampicillin, neomycin and nalidixic acid.

Molecular analysis:

The polymerase chain reaction (PCR) assay on representative isolates of *V.cholerae* for the detection of *ctxA* and *tcpA* genes revealed that all are positive for *ctxA* and *tcpA* genes showing biotype El Tor. Similarly some selected strains of *V.cholerae* were subjected to randomly amplified polymorphic DNA (RAPD) analysis with 1281 primer exhibited similar RAPD pattern like Kolkata stains.