ANNUAL REPORT

1994 - 95

REGIONAL MEDICAL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

BHUBANESWAR, ORISSA.

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PREFACE

Filariasis has been the major thrust area of research at this Centre from the very beginning. Basic studies were continued on the immunology of filariasis using various haptens, putative antigens and enzymes of cattle parasite *S.digitata* to understand their antigenicity and/or immunogenicity with protective influence in human filariasis. Many imponderables have to be crossed to devise immuno-prophylactic agents for multicellular nematode parasite *W.bancrofti* which lives in deep lymphatic channels. This Centre had achieved considerable progress in the experimental animal models of filariasis using *Mastomys councha*. Clinical trial with Ivermectin to document its role in reducing sickness due to filariasis is in the final stage. The biocide *B.sphaericus* is being field tested to document its efficacy in controlling vector population and their vectorial capacities to transmit filariasis. Newer plant products are also being tested for their efficacy in changing the biology of the vector mosquitoes and their reproductive capabilities.

Documentation of the extent and severity of health and nutritional problems in this region, covering Orissa and its neighbouring states has been viewed as the major task of this Centre. Studies on pre-school children, mothers and school children were carried out in different segments of the population. The study of haemoglobinopathies is proposed to be taken up at community level, instead of hospital based studies. Cluster sampling and selection of clusters on proportionate basis of population (PPS) from the villages or sub-centres listed will be used for health surveys in this region.

The Joint RMRCs Meeting (June, 1995) has identified filariasis and micro-nutrient deficiency disorders as the thrust areas for this Centre. The VCRC, Pondicherry and the NIN, Hyderabad have been identified as the networking institutions for collaborative work. Dialogue was established with medical colleges of Orissa and Andhra Pradesh to support research programmes by interested faculty members, by extending technical support and by providing training facilities to young research workers in their group. This aspect was also emphasized and approved by the Joint RMRCs Meeting.

Necessary training for middle level workers and interaction with sister institutions have been the strengths of this Centre. I take this opportunity to thank all our SAC members and staff of this Centre for helping me to steer this Centre with greater confidence and with a sense of fulfilment. We are grateful to Dr.G.V.Satyavati, Director General, ICMR and to Prof.L.N.Mohapatra, SAC Chairman and Ex-Director of this Centre for their valuable guidance and encouragement during the year.

Place : Bhubaneswar,
Date : 15.07.1995.

Dr.K.Satyanarayana
Director

STAFF POSITION AS ON JULY 1995

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Dr.Dasarathi Das	M.Sc.,Ph.D.	Research Assistant
Mr.N.Mandal	M.Sc.	Research Assistant
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H.S.Naik	Dip.MLT	Lab.Asst.

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CONTENTS

I. PARASITE IMMUNOLOGY DIVISION

1.1. IMMUNOLOGICAL INVESTIGATIONS IN HUMAN FILARIASIS USING PURIFIED ANTIGENS

Scientists	:	Dr. M.K. Das Ms. M.S. Bal
Technical staff	:	Mr. H.S. Naik

1.1.1. Detergent-soluble fraction of *Setaria digitata*

This laboratory has earlier described the low IgG levels to a detergent-soluble antigenic fraction of *Setaria* in microfilaraemic individuals compared to amicrofilaraemic people (J. Bioscience, 19:201-206, 1994). In a similar search, a high molecular weight (Approx $1X10^5$ KDa) antigen was obtained which exhibits reduced IgM levels in Mf positive individuals and even in chronic filarial patients. The highest IgM response was observed in endemic normals (disease free adult residents). Dynamics of this antibody response in endemic communities are being studied.

1.1.2 The filarial allergen, Sd30

A low molecular weight (Fraction-III, 30 KDa) fraction with allergenic activity in endemic population was earlier isolated from *Setaria* and biochemically identified to be a proteolytic enzyme.

A hyperimmune rabbit antiserum to Sd30 was prepared and the extent of cross-reactivity with other (crude and purified) filarial antigens was determined (Table 1). The antisera cross-reacted maximum (55%) with the adult extract (AE) of *Setaria*. Infective-larval antigens of setaria (20%) or human parasite of *B.malayi* (22%) reacted to a lesser extent. The filarial proteases SdP_{I} and SdP_{III} (vide project 1.2.2) also reacted with the antiserum (30-45%).

Table - 1 Reactivity of Rabbit antisera to Sd30 with filarial antigens

Antigens	ELISA reactivity (%)
Sd30	100
SdAE	55
Sd L_3	20
Sd Mf	32
Sd egg	26
B.malayi L_3	22
Sd P_I	45
Sd P_{II}	30

It is of interest to note that although the allergen (Sd 30) was isolated from adult stage of parasite, all the other stages do possess structural homology akin to this allergen.

1.2. ANTIGENICITY OF FILARIAL ENZYMES IN ENDEMIC POPULATION

Scientists : Dr.M.K. Das
Ms. M.S. Bal
Mr. N.N. Mandal

Technical staff : Mr. H.S. Naik.

1.2.1. Superoxide dismutase

Filarial parasites have evolved sophisticated systems of survival strategies to evade host immune responses. Antioxidant enzymes, Superoxide dismutase (SOD) one of these, contribute to parasite survival. We have earlier quantified SOD activities in filarial parasites. Further studies were carried out in this direction.

Biochemical nature: Extract of *Setaria digitata* was treated with organic solvents (chloroform/ethanol) and assayed for SOD activity. There was no loss of enzyme activities following treatment with organic solvents indicating the presence of Cu/Zn SOD. On SDS-PAGE, a prominent band was observed at 32 KDa. The absence of high molecular wt (86 KDa) band is also an evidence against the presence of Mn-SOD. Filarial enzyme is thus shown to be a Cu/Zn-SOD with 32KDa molecular wt.

Presence of SOD specific antibodies in human filarial sera: IgG purified by Protein A-sepharose column from the sera of chronic filarial patients was found to inhibit the SOD activities in filarial parasites in contrast to IgG from non-endemic normal (Koraput) sera (Table - 2).

Table - 2 Filarial IgG mediated inhibition of SOD activity in the parasites

Parasites	Inhibition(%)
B.malayi L_3	60.3
S.digitata AE	64.9
ES of adult *Setaria*	80.9

A relatively higher extent of enzyme inhibition was observed in excretory-secretory (E-S) products. The inhibition by different amounts of filarial IgG was also studied.

1.2.2. Proteases

Two proteolytic enzymes with high activities (SdP_I and SdP_{II}) were isolated from the somatic stages of *Setaria* adults.

IgM antibodies to SdP_I were significantly elevated in asymptomatic Mf carriers (or in chronic patients) compared to endemic normals. This is similar to IgG response studied earlier (Current Science, 67, 1018-20, 1994). Preliminary studies indicate that antibody response to SdP_{II} however is not greatly different between endemic normals and Mf-carriers.

Both SdP_I and SdP_{II} were metal dependent cysteine proteases, active at neutral pH.

Considering the diagnostic potential of SdP_I, IgG subclass response is being analyzed in filarial sera. Work is also continuing to study the immunological reactivity of proteases present in E-S products of filarial parasites.

1.2.3. Glutathione-s-trasferase (GST)

These are a group of enzymes which play an important role in the detoxification of toxic compounds (derived exogenously and endogenously).

GST activity was detected only in the adult stages of filarial parasite. It was not detected in E-S products. The activity was enhanced considerably (twenty-five fold) following affinity purification on glutathione-agarose column. IgG levels to the affinity purified (GST-binding) proteins were detected predominantly in *Wuchereria bancrofti* infected individuals, however IgM levels could be detected in as much as 50% of endemic normals and in all filarial patients (n=30, each case).

1.3. IMMUNE RESPONSE TO PARASITIC LIPIDS IN HUMAN FILARIASIS

Scientists : Dr M.K. Das
Ms. M.S.Bal

Technical staff : Mr. H.S.Naik

This project will attempt to determine the antigenic reactivity of filarial lipids in lymphatic filariasis. A lipidic preparation has been isolated from adult *Setaria*, antibodies to which are detected in human filarial sera only. This is interesting since it suggests the specificity of antibody response to filarial infection. Antigenicity of lipids is being evaluated in different categories of endemic population.

OTHER ACTIVITIES

1. Dr M.K.Das was invited to deliver a lecture "Immunochemical approaches to the control of human filariasis" in Annual Seminar of Chemistry Dept. (11.4.94) Utkal University, Bhubaneswar.

2. Dr M.K.Das was invited to participate in All India Congress of Zoology, April 21-23, 1994, Utkal University, Bhubaneswar.

3. Dr M.K.Das attended the XVI International Congress of Biochemistry and Molecular Biology, 19-22nd September 1994, New Delhi.

4. Dr M.K.Das was the Chairman for IV International Symposium on Vectors and Vector Borne Diseases, 12-15 November 1994, RMRC, Bhubaneswar.

5. Dr. M.K. Das was appointed as examiner of M.Phil.(Immunology) in Dept. of Biosciences, Ravi Shanker Shukla University, Raipur, He also delivered a lecture entitled "Immunoregulatory mechanisms in filarial skin tests," in the Biosciences Dept. on 23rd November, 1994.

Papers accepted/presented in scientific seminars

1. Immunologic role of filarial superoxide dismutase in *Wuchereria bancrofti* infection. MS Bal and MK Das. 8th All India Congress of Zoology, April 21-23, 1994, Dept. of Zoology, Utkal University, Bhubaneswar.
2. An allergen from *Setaria digitata*: IgE response, skin test reactivity and 'blocking antibodies' in filaria endemic population. MK Beuria and MK Das, International Symposium on Vectors and Vector Borne Diseases, 12-15th November 1994, Bhubaneswar.
3. Biochemical characterization and antigenicity of filarial proteases. MS Bal and MK Das. International Symposium on Vectors and Vector Borne Diseases, 12-15th November 1994, Bhubaneswar.

List of Publications

1. A.K. Praharaj and M.K. Das. Enhanced antibody response to a detergent soluble antigen in human filariasis after treatment with diethylcarbamazine. J. Biosciences **19**, 201-206 (1994).
2. M.S. Bal and M.K. Das. Immunodiagnostic potential of a filarial protease. Current Science **67**, 1018-20 (1994).
3. M.S. Bal and M.K. Das. Antigenicity of filarial superoxide dismutase in human bancroftian filariasis. J. Biosciences **20**, 157-166 (1995).
4. M.K. Beuria, M. Bal, A.P. Dash and M. K. Das. Age-related prevalence of antibodies to infective - larvae of *Wuchereria bancrofti* in normal individuals from a filaria-endemic region. J. Biosciences **20**, 167-174 (1995).

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II. APPLIED IMMUNOLOGY DIVISION

2.1. IMMUNOLOGICAL STUDIES ON ANTIBODIES TO DIETHYL CARBAMAZINE - AN APPROACH TO DEVELOPING AN IMMUNOPROPHYLACTIC AGENT AGAINST FILARIAL PARASITE

Scientists : Dr.B.Ravindran
Dr.A.K.Satapathy
Ms.S.Mukhopadhyay
Mr.P.K.Sahoo

Starting date : November 1990

INTRODUCTION

Immunoprophylaxis in experimental filariasis has been the subject of intense investigations in recent years. Some of the protective antigens from infective larvae and/or microfilarial stages have been identified. Recombinant proteins of filarial parasites have been produced in reasonable quantities in a few laboratories (not in India) and attempts have been made to test them for immunodiagnosis of particular stage and strain of filarial parasites and as putative candidate vaccines in experimental animals. Identification of a suitable antigen molecule or haptens which can safely and effectively induce protective immunity is a Herculean task. This laboratory has been working with haptens and also antigens of *S.digitata*, (a bovine filarial parasite) and *B.malayi* to evaluate them for their protective efficacy in terms of microfilaricidal action in animal models.

Previous investigations performed in our laboratory have indicated that antibodies to diethyl carbamazine (DEC) raised using MPCA (methyl piperazine carboxylic acid, a derivative of DEC) as hapten react with Mf sheath of *W.bancrofti*, *B.malayi* as well as

S.digitata and subsequently we had demonstrated their reactivity to soluble antigens of infective larvae also. These findings of serological cross-reactivity have raised the possibility of utilizing the hapten, MPCA in conjunction with carrier molecules for eliciting a protective immune response in experimental filariasis.

PROGRESS

During in the last one year further progress was made with reference to the identity of the filarial antigen that reacts with anti-DEC. A large molecular weight, heat stable (100°C-15 min) glycoprotein was identified by its reactivity to the lectin, Wheat germ agglutinin. The antigen could be affinity purified using WGA-agarose and monospecific polyclonal antibodies were raised to this WGA binding filarial antigen which has been designated as AgW. Using the specific antibodies and WGA-peroxidase as probes AgW was found to be present on Mf surface. The antigen was quantified by lectin-ELISA and was found to be present in solubilised antigens prepared from infective larvae, adult stages and also in the excretory-secretory products of adult stage parasites. Human filarial sera collected from patients displaying different clinical manifestations viz, acute or chronic filariasis, asymptomatic Mf carriers as well as endemic normals, were found to contain naturally occurring antibodies to affinity purified AgW. The relationship between these antibodies and anti-DEC that we had demonstrated in human filarial sera a few years ago is under investigation. Preliminary studies have indicated that the antibody reactivity to AgW in human filarial sera could be inhibited by MPCA-BSA and not by BSA. Further studies with affinity purified anti-DEC from human filarial sera is expected to confirm the reactivity of human anti-DEC to AgW.

The *in vivo* biological activity of anti-DEC was investigated in *B.malayi* or *Setaria digitata* infected *Mastomys coucha*. Animals with on-going microfilaraemia with either of the above parasites were used to investigate the microfilaricidal activity *in vivo*. Immunization of a group of *Mastomys* with MPCA-BSA resulted in statistically significant decrease of microfilaraemia of *S.digitata* in comparison to control animals which were mock immunized only with the carrier molecule. In a second set of experiment, groups of animals with on-going *S.digitata* microfilaraemia were injected passively with antibodies purified from immune sera of *Mastomys* earlier immunized with MPCA-BSA or BSA. In comparison to the control

group injected with anti-BSA, animals injected with anti-MPCA showed a lower microfilaraemia. More significantly, when oral DEC was administered at sub-curative doses (10 mg/kg instead of the curative dose traditional 250 mg/kg), the animals injected with anti-DEC cleared circulating microfilariae to a significant degree. Similarly, in *Mastomys* with on-going *B.malayi* microfilaraemia, MPCA-BSA immunized animals cleared very rapidly the microfilaraemia after administration of sub-curative doses of DEC while the control group of *Mastomys* immunized with only the carrier molecule and administered sub-curative doses of DEC continued to show microfilaraemia of high magnitude. These findings indicate the potential role of anti-DEC in controlling circulating microfilaraemia particularly in the presence of very small quantities of DEC. Whether a similar mechanism is operational in Mf clearance observed in human populations after long term administration of small quantities of DEC is an aspect that is currently under investigation. Although the use of DEC-medicated salt in decreasing microfilaraemia in human population has been practised for many decades the precise mechanism of action has not been understood so far.

2.2. STUDIES ON SERUM CYTOTOXIC FACTORS

Scientists : Dr. B. Ravindran
Mr. P.K. Sahoo

Starting date : July 1991

INTRODUCTION

Cytotoxic factors presumably non-antibodies in nature have been detected in human and experimental malaria. They have been termed as 'crisis form factors' since they were demonstrated to induce 'crisis forms' of intra erythrocytic stage parasites. While merozoite inhibitory antibodies have been demonstrated to be largely species specific, the soluble serum cytotoxic factors have been found to be non species specific. Malarial sera have been demonstrated to effectively induce crisis forms of intraerythrocytic stages of other species of plasmodia also. These observations prompted us to investigate if such malarial sera could act on other parasites found in circulation viz. microfilariae. We had demonstrated

earlier that human malarial sera, particularly those collected from cerebral malaria cases could mediate cytotoxicity to microfilariae also *in vitro*. Sera collected from patients with different clinical manifestations of lymphatic filariasis were not however found to mediate Mf cytotoxicity. We had also reported evidence to indicate that the observed effect was not mediated by chloroquine or its metabolites likely to be present in human malarial sera. The Mf cytotoxic factor was found to be heat stable (56°C for 30 min) and non-dialysable using a 10,000 kd cut-off membrane. The cytotoxic activity was found to be not due to TNF-α since a) the levels of TNF-α in malarial sera did not correlate with the observed cytotoxicity and b) none of the sera of 50 cases of acute filariasis which were found to contain varying levels of TNF-α could mediate Mf cytotoxicity. Lipid peroxidation products associated with LDL were found to be marginally involved with Mf cytotoxicity. However, removal of LDL with fumed silica failed to absorb the cytotoxic factor in many cytotoxic sera.

Since phospholipase A_2 from porcine pancreas was shown to be cytotoxic to Mf *in vitro*, attempts were made to inhibit PLA_2 activity with specific inhibitors. While nordihydroguaiaretic acid (NDGA) at high concentration appeared to inhibit the cytotoxic activity, manoalide a very potent PLA_2 inhibitor could not eliminate the cytotoxic activity. It is proposed to use retinal, another PLA_2 inhibitor to establish unequivocally the role played by PLA_2 in malarial serum mediated *in vitro* cytotoxicity to microfilariae.

2.3.IMMUNOBIOLOGICAL STUDIES IN ACUTE BANCROFTIAN FILARIASIS

Scientists : Dr. B. Ravindran
Dr. Bidyut K. Das
Mr. C. C. Rath
Mr. Prakash K. Sahoo

Starting date : November 1994

INTRODUCTION

The immunological changes associated with acute filariasis which occurs at infrequent intervals over a period of time have not been studied in detail so far. The asymptomatic

microfilaraemic state is associated with immunological anergy to filarial antigens while patients with chronic pathology demonstrate high immunological responses to filarial antigens. It is generally believed that the switch over from the anergic state to hyper-reactive state results in acute and chronic manifestations of filariasis. Conclusive evidence to this effect is yet to be produced since the cause-effect relationship between disease expression and immune response to filarial antigens has not yet been established.

PROGRESS

Inflammatory cytokines such as Tumour necrosis factor-α,(TNF-α), Interleukin 1α(IL-1α), Interleukin-6(IL-6), and Interferon-γ (IFN-γ) have been demonstrated to play a central role in other parasitic diseases, particularly malaria and we decided to study the role of TNF-α in acute filarial disease. The TNF-α levels were quantified by a sandwich ELISA in a)asymptomatic Mf carriers (AS) b) patients with acute filarial symptoms and in c)patients with chronic manifestations such as elephantiasis and hydrocele (CP).

About 50% of patients with acute episodes were found to have significant levels of circulating TNF-α (> 20pg/ml) while less than 5% of AS and CP cases were found to have raised levels of TNF-α. When the fifty patients with acute disease were further sub-divided into three groups as mild, moderate and severe cases depending on the intensity of acute manifestations, there was a significant association between TNF-α and severity of acute disease. While only 6.2% of mild and 50% of moderate cases were found to have raised levels of TNF-α, 90% of cases with severe acute manifestations had significantly raised levels of TNF-α. These observations indicate the possible use of human TNF-α inhibitors such as pentoxyfiline or chloroquine in clinical management of acute filariasis in human communities. The nature of stimulus for TNF-α is not known. Two lines of investigations were undertaken to study this aspect. Lipopolysaccharides (LPS) from gram negative bacteria and lipotechoic acid from gram positive cocci are known to be TNF-α inducers. Blood cultures were performed in cases of acute filariasis. The detailed results are shown in Table - 1. There was no association between culture positivity with severity of acute disease. The association if any between culture positivity and TNF-α levels is currently under investigation.

Table - 1 Organisms identified tentatively based on biochemical characters

Patient	Growth	Organisms
1.	+ 1d	*S.paratyphi A,Klebsiella sp,St.sp.*
2.	+ 7d	*S.paratyphi A*
3.	+ 7d	*St. sp.*
4.	NG	-
5.	NG	-
6.	NG	-
7.	+ 1d	*St.sp., Bacillus sp.*
8.	+ 1d	*Pseudomonas ar.Bacillus sp.*
9.	+ 1d	*P. malei, St.sp.*
10.	+ 1d	*Sal. sp., St.sp*
11.	NG	-
12.	NG	-
13.	+ 7d	*St. sp.*
14.	+ 1d	*St. sp*
15.	+ 1d	*P. malei, St. sp.*
16.	NG	-
17.	NG	-
18.	+ 1d	*St. sp.*
19.	+ 1d	*St. sp.*
20.	+ 1d	*Sal. sp., Bacillus sp.*
21.	NG	-
22.	NG	-
23.	NG	-
24.	NG	-
25.	NG	-
26.	NG	-
27.	NG	-
28.	+ 1d	*Sal. sp.*
29.	NG	-
30.	+ 1d	*Bacillus sp.*
31.	NG	-
32.	+ 1d	*St. sp.*
33.	+	*St. sp.*

The possibility of a filarial antigen mediating TNF-α induction was also investigated. A mouse model made hypersensitive to TNF-α by administration of D-galactosamine was used to investigate TNF-α induction by excretory-secretory antigens of adult stage parasites. Preliminary investigations have identified the presence of a heat stable (100°C-15 min) filarial antigen in E-S products, which is capable of inducing TNF-α in mice. Further studies are now in progress to demonstrate TNF-α induction by such antigens *in vitro* in macrophage cultures.

2.4. DEVELOPMENT OF A LABORATORY ANIMAL MODEL FOR *W.BANCROFTI*

Scientists : Dr. B. Ravindran
Dr. A.P. Dash
Ms. S.Mukhopadhyay

INTRODUCTION

W.bancrofti, the helminth responsible for nearly 90% of global incidence of lymphatic filariasis has not been successfully adopted in laboratory animal models. Even though the complete development of infective larvae of *W.bancrofti* to mature adult stages in small animals has not been achieved so far, induction of microfilaraemia has been successfully attempted. Passive transfer of microfilariae purified from asymptomatic Mf carriers have been found to induce microfilaraemia of shorter duration in mice. However, the number of Mf required per mouse has been found to be very high (nearly 0.2 million) and it is impractical since it will require very large quantities of blood from infected people to induce microfilaraemia in a very small number of animals.

Establishment of a microfilaraemic model for *W.bancrofti* (without the development of adult worms) in small laboratory animals is in itself will be useful and is an essential requirement for a) understanding the biology of the Mf clearance, b) analysing potential filarial antigens and above all for c) screening newer antifilarial drugs against *W.bancrofti* (currently human trials in infected individuals is the only method of testing the efficacy of newer drugs against *W.bancrofti*).

Clearance of circulating microfilarae have been shown to be dependent on immune response to antigens present on the Mf sheath which are largely made up of carbohydrate determinants. Since immune response to such sugar antigens have been earlier demonstrated to be T-independent in nature, we have attempted to undertake studies on CBA/N strain of mice which are known to be genetically deficient on immune response to T-independent antigens. Since the scarce availability of parasite material of *W.bancrofti* from infected human subjects would not allow different combinations (viz. doses, routes, induction of immunosuppression with different agents, etc.) it was decided to use the animal filarial parasite *S.digitata* which is available in large quantities.

Microfilarae of *S.digitata* released *in vitro* from gravid adult female were intraperitoneally injected ($1X10^5$ per mouse) into groups of BALB/C (immuno competent strain) and CBA/N (deficient in T-independent response) mice. In BACB/C mice there was a transient microfilaraemia (about 25 days) of low magnitude with the peak Mf count reaching 15 per 100 µl of blood on the 10th day post injection. While in CBA/N strain the microfilaraemia lasted for about 70 days with peak Mf count of 40 per 100 µl of blood on the 21st day. Studies are currently in progress to achieve microfilaraemia of this magnitude in CBA/N mice using smaller number of Mf (about 10,000) per mouse by direct intravenous inoculation of *S.digitata* and *W.bancrofti* Mf.

The microfilaraemia model induced by intraperitoneal implantation of gravid adult female worms of *S.digitata* in *Mastomys coucha* reported in the previous years was found to be useful for studying the *in vivo* effect of antifilarial drugs, for e.g., oral administration of DEC-citrate (50 mg/kg for five consecutive days) resulted in 80% decrease in microfilaraemia by 6th day and total elimination of Mf by 40 days while a dose of (250 mg/kg) resulted in 90% decrease in microfilaraemia by 6th day and total elimination by 25 days. These results are very significant since in other common laboratory animal models such as *B.malayi* or *B.pahangi* infected jirds or cats, the commonly used drug DEC does not show any micro filaricidal activity and for these reasons the above animal models have not been found to be useful for screening potential antifilarial compounds. The *S.digitata-Mastomys coucha* model thus offers a readily available system for evaluating antifilarial drugs before undertaking clinical trials in infected human subjects.

2.5. IMMUNOLOGICAL COMPONENTS OF ANTI-DISEASE RESPONSE IN HUMAN AND EXPERIMENTAL MALARIA

Scientists : Dr.B.Ravindran
Dr.Bidyut Ku. Das
Mr.P.K.Sahoo

Starting date : March 1995

INTRODUCTION

Acquired immunity in malaria has been perceived to have two components namely, anti-parasitic component and anti-disease component. Certain individuals, after experiencing repeated attacks of malaria continue to harbour parasites in the absence of clinical manifestations. In endemic and hyper-endemic areas asymptomatic parasitemia of 30-60% have been reported. Understanding the mechanisms behind such an immune response which enables tolerance of asymptomatic parasitemia is crucially important for decreasing the morbidity and mortality associated with malaria in human communities. Investigations in human malaria in the last two decades have addressed mostly the anti-parasitic immunity and very little has been understood with reference to anti-disease immunity.

PROGRESS

Heat stable toxic malarial antigens released *in vitro* by erythrocytic stage parasites were found to induce TNF-α in mice. The production of TNF-α *in vivo* was detected by prior administration of D-galactosamine hydrochloride rendering the mice hypersensitive to action of TNF-α. Even very small quantities of TNF-α become lethal to mice in this system. Mice could be effectively immunized against TNF-α releasing endotoxin like malarial antigens. Since the active component of this antigen was reported to be phospholipids, we attempted to quantify anti-phospholipid antibodies (anti-PL) in human cerebral malaria. Anti-PL can be expected to neutralize the toxic malarial antigens that induce TNF-α which is one of the most important inflammatory cytokine responsible for disease manifestations in malaria. We found significantly elevated levels of IgG anti-PL in the sera of cerebral malaria(CM) patients who recovered completely after quinine therapy. In comparison, the anti-PL titres

were significantly low in those cases of cerebral malaria who succumbed to the disease in spite of adequate quinine therapy. When IgG subgroups within anti-PL activity were analyzed IgG_1 titres were found to be significantly more in cerebral malaria survivors, in comparison with those who expired with CM. There was also a significant inverse correlation between IgG_1 anti-PL levels and circulating TNF-α levels in cerebral malaria patients indicating the protective role of anti-PL in anti-disease immunity in human cerebral malaria.

Apart from anti-PL another naturally occurring auto antibody namely anti-gal was analyzed to investigate its role if any in cerebral malaria. When anti-gal were quantified by cell-ELISA, IgG_1 was found to be the predominant class followed by IgG_3, IgG_2 and IgG_4 in malarial sera. IgG_1 and IgG_4 were significantly elevated in cerebral malaria survivors in comparison to CM fatal cases. The proportion of anti-gal in circulating immune complexes (CIC) was found to be very high in uncomplicated *P.falciparum* malaria in comparison to cerebral malaria cases. The protective role if any of the CIC associated anti-gal in human malaria is currently under investigation.

Other Activities

1. Dr.B.Ravindran was the Convenor and organized the III Brain Storming Session on Molecular Immunology at RMRC, Bhubaneswar on Dec 9-11, 1994. The meeting was funded by ICMR, CSIR and the Dept. of Biotechnology, Delhi.

2. Dr.B.Ravindran was examiner for adjudication of doctoral theses of scholars from AIIMS, Delhi, Jawaharlal Nehru University, Delhi and Tamil Nadu Medical University, Madras.

3. Dr.B.Ravindran was elected as a member of the New York Academy of Sciences, New York.

4. Dr.B.Ravindran was invited by Dept. of Zoology, Utkal University, Bhubaneswar to be a resource person for the refresher course for college teachers.

5. Dr.B.Ravindran delivered a series of six lectures on basic and applied immunology to the final year M.Sc. students at the Dept. of Zoology, Utkal University, Bhubaneswar.

6. Dr.B.Ravindran attended the annual meeting of the Indian Society of Parasitology, held at Panaji, Goa and presented a paper entitled "Malarial sera mediate cytotoxicity to filarial parasites", Jan 23-25, 1995.

PUBLICATIONS

1. B.Ravindran, A.K.Satapathy and P.K.Sahoo (1994) Bancroftian filariasis - Differential reactivity of anti-sheath antibodies in microfilariae carriers. Parasite Immunology, 16: 321-323.

2. C.L.Labo, Santosh K.Kar, B.Ravindran *et al.* (1994) Noval proteins of *P.falciparum* identified by differential immuno-screening using immune and patient sera. Infection and Immunity, 62 : 651.

3. Bidyut K.Das, S.Parida and B.Ravindran (1995) A prognostic role for anti-phospholipid antibodies in human cerebral malaria. Clinical Experimental Immunology. (in press).

III. CLINICAL DIVISION

3.1. COMPARATIVE STUDY ON THE EFFICACY OF IVERMECTIN AND DEC IN THE TREATMENT OF BANCROFTIAN FILARIAL ADENOLYMPHANGITIS (WHO/TDR SUPPORTED)

Scientists	:	Dr.S.K.Kar* Principal Investigator of the Project
		Dr.Sumitra Patnaik Project Medical Officer
Technical staff	:	Mr.T.Moharana
		Mr.K.Dhal
		Mr.R.N.Nayak
Starting date	:	1991
Completion date	:	July, 1996

OBJECTIVE

A double blind study was undertaken to compare the efficacy of multiple doses of Ivermectin (MK-933) or diethylcarbamazine citrate (DEC-C) in controlling adenolymphangitic (ADL) attacks in patients with *W.bancrofti* lymphatic filariasis.

A total of 120 patients were enrolled into this field based study. These patients were registered from 4 villages in Khurda district of Orissa based on the criteria laid down in the W.H.O. protocol. All these patients suffered from at least two attacks of adenolymphangitis during the year proceeding to their enrollment into this study. These attacks were taken as

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*Currently Director, RMRIMS (ICMR), Patna.

their baseline ADL incidence before therapy programme. One third of the 120 patients were to receive 12 doses of Ivermectin (400 µg/kg body weight) at monthly intervals, another one third were to receive 12 doses of DEC (10 mg/kg body weight) at monthly intervals, and the remaining one third were to receive placebo at similar intervals. This allocation was done randomly by pre-assigned codes which were not known to the patients or to the investigators (double blind). The total therapy period in each patient would be for one year. All these patients would be followed up at fortnightly intervals for another one year after completion of one year therapy phase to detect the incidence of adenolymphangitis during the post therapy period. At the end of the study the baseline ADL incidence/year figures were to be compared with the post-therapy ADL incidence/year to assess the efficacy of the drug in controlling the adenolymphangitic attacks. Regular measurements of the affected parts at fixed intervals was undertaken to assess any reduction in the size of the lymphoedematous part.

SAFETY ANALYSIS

Like any other drug trial, safety measures were taken care of by carrying out blood analysis and thorough clinical examination. Pregnancy tests were conducted at regular intervals and four patients were excluded from the study because of pregnancy, as per the protocol.

PROGRESS OF THE WORK TILL DATE

The work is progressing satisfactorily with coverage of 120 cases. All 12 doses of drug therapy have been completed in 111 cases by February 1995. Rest of the 9 cases will be completing 12 months therapy period in the month of April, 1995. The first batch of 55 patients who were registered much earlier completed one year therapy as well as one year post therapy observation period by January, 1995 (Table-1). Thus this batch of 55 patients who were registered much earlier have completed one year therapy as well as one year post therapy observation period also. Another 20 cases who were registered in June'93, will be completing one year therapy and one year post-therapy observation by June'95. The exact position of drug therapy and follow up period in various groups of patients who were registered at different time points is given in Table 1. There were five batches of patients and the last batch of 9 patients, who were registered in March'94 will be completing the 2

Table - 1 No. of doses and months of follow-up completed and yet to be completed by various groups of patients registered at different time points

No. of months of follow-up & no.of doses completed	No.of patients	Period needed to complete follow-up (Month of completion)
24th month (12 doses by Jan, 94)	55	Nil (Jan,95)
20th month (12 doses by June, 94)	20	4 months (June,95)
16th month (12 doses by Octo, 94)	06	8 months (Octo,95)
12th month (12 doses by Feb, 95)	30	12 months (Feb,96)
10th month (10 doses only by Feb, 95)	09	14 months (April,96)
Total	120	

years study by April'96. The project will be closed by July 1996 after analysis of data and submission of report during the period between May 96 and July 96.

RESULTS

Comparison of data on the incidence of ADL attacks in the 55 patients who have completed 24 months of the study period showed some encouraging results (Table-2).These 55 patients experienced 200 ADL attacks in the base line year before the initiation of therapy (Table-2), at the rate of 3.6 attacks/person/yr. During the therapy period of one year 36 of

Table - 2 Number of persons and number of ADL attacks (total and average attacks per sufferer) in the three periods (baseline, therapy and post therapy) of one year each

	Baseline period	Therapy period	Post therapy period
No. of patients under follow-up	55	55	55
No.with ADL attacks (as % of total)	55 (100%)	36 (65%)	26 (47%)
Total ADL attacks/yr (attacks/person/year)	200 (3.6/Yr)	75 (2.1/Yr.)	46 (1.8/Yr.)
Total ADL attacks as % of baseline.	100%	37.5%	23.0%

these 55 patients experienced only 75 attacks constituting 37.5% of the expected attacks/ one year. During the post therapy follow-up period, only 26 of 55 patients had a total of 46 attacks in one year, constituting 23% of expected attacks. The 77% reduction, 46 attacks in post therapy period as compared to 200 attacks experienced by them in the pre-therapy baseline year was contributed by two elements (Table - 2). 1.The number of persons suffering from ADL attacks was 36 during therapy period of one year and 26 only during post-therapy one year follow-up period, as against all 55 patients having experienced 200 ADL attacks in the baseline year. 2. Besides more than 50% reduction in number of people suffering from ADL attacks the mean attack rate per sufferer came down by half from 3.6 attacks/person/ yr. during pre-therapy period to 1.8 attacks/person/yr. during post therapy period. Thus these two elements i.e., reduction in the number of sufferers (26 verses 55) and also reduction in the number of attacks per sufferer per year (3.6 versus 1.8) contributed to 3/4th reduction of total suffering in the total community. However, we can not comment on the nature of drugs bringing about this change, because of double blind nature of the study. The

benefit, if any, due to Ivermectin or DEC will be known only after decoding of the secret codes.

REASONS FOR CONTINUATION

1. Sixty five cases are to be followed up at fortnightly interval for the 2nd year of the study for post therapy observation to detect any changes in their ADL incidence. Majority (56) will complete the study by February'96 and the remaining (9) by April'96.

2. Nine patients are yet to complete all the 12 doses of the trial drug. This batch will complete therapy in April'95 and post therapy observation by April, 96.

3. At the end of the study the data will be analyzed to assess the efficacy of Ivermectin, DEC and placebo in bringing down ADL attacks in filarial patients. These steps will be undertaken between May 96 and July 96 and the project will come to an end by July 1996.

The 9th SAC of this centre approved continuation of this project upto July, 1996 in order to complete the study in all its aspects and to prepare the final report. The SAC also approved preliminary analysis of data in 1995, on 55 patients, who have completed post therapy observation, without jeopardizing the double blind nature of the study.

3.2. EXISTENCE OF AN ENDEMIC GOITRE BELT IN WESTERN ORISSA

Scientist : Dr. S.S.S. Mohapatra

Technical staff : Mr. K. Dhal
Mr. S.C. Rout

Starting date : March, 1995.

The prevalence of goitre in Orissa has not been well documented. The only reference available was the mere mention of two districts of Orissa identified to be endemic for goitre

by a survey conducted by the Central goitre survey team of D.G.H.S. (NFI, 15,2 April 1994, the National programme-current status). The NIN, Hyderabad surveyed ICDS programme and reported goitre from Sundergarh district. Dr.R.K.Satpathy, one of our SAC members presented some clinical findings from Paikamal block during the SAC meeting. A preliminary survey was carried out with a view to identify and demarcate the endemic areas/zones in Western Orissa.

The newly formed Baragarh district (a part of the undivided Sambalpur district) was visited. The two blocks namely, Paikamal and Bhatli which were suspected to have endemic goitre at a higher level of prevalence were surveyed by random selection of two villages and two schools. The results of the survey are presented below.

a) In village Sukuda of Bhatli block, 11 families comprising of 80 members were examined clinically for thyroid enlargement by palpation method and the results were recorded as per WHO grading. Six cases of thyroid enlargement of different grades constituting a prevalence of 7.5% was observed. Similarly, in Khandijhar village of Paikamal block the prevalence was found to be 8.2%. Survey in a school in the same block showed a prevalence of 9.2%. Interestingly an orphanage named Kasturiba Matru Niketan having a total of 118 inmates showed a very high prevalence of 22.8% for endemic goitre. In this orphanage 40 children (34%) had sign/symptoms of Vitamin-A deficiency in the form of night blindness, xerosis and Bitot's spots. The prevalence of Bitot's spots was found to be very high being 15.3% (18 out of 118).

Thus a total of 445 persons examined in Baragarh district showed a goitre prevalence of 12.1%. This prevalence is only slightly lower than that found in Assam which was 15.3% (Annual Report, RMRC, Dibrugarh, 1993). In that survey in Assam also about one-fourth of school children had endemic goitre.

This report probably is the second or third of its kind in revealing the possible existence of an endemic goitre belt in Western Orissa which requires further detailed study. In an earlier study on ICDS programme evaluation, a report from NIN, Hyderabad documented the existence of endemic goitre in Sundergarh district.

b) **Geographical location of the endemic area :** The area extends from Paikamal block, which is bounded by Kalahandi in west and Bolangir in south, to Ambhabona block, which has a common border with Madhya Pradesh on the west. Both are hilly areas with high concentration of SC/ST population varying from 47% to 51%.

c) **Use of Iodised salt :** In both areas people used the rock salt or other non-iodised salt. They never used iodised salt at any time and the female folk were particularly ignorant about the health benefits of iodised salt.

d) **Source of drinking water :** In spite of the fact that tube wells are available in the villages, people in this region prefer to use stream water for its taste.

Systematic studies are being planned to document the prevalence of endemic goitre in about 30 sub-centres and schools (clusters) by examining adequate number of school children in this area. This methodology is similar to the procedure outlined by WHO/UNICEF/ICCIDD group, 1993 and GOI/UNICEF/ICCIDD documents for district surveys. Intervention studies are also planned by using fortified salt with iron and iodine (double fortified salt).

SCIENTIFIC CONFERENCES AND COURSES ATTENDED

Dr.S.S.S. Mohapatra successfully completed the annual certificate course in nutrition at the N.I.N., Hyderabad during 01.12..94 to 28.02.95.

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IV. MICROBIOLOGY DIVISION

4.1. HIV/AIDS in the country and in this region

Scientists : Dr.V.R.Subramanyam
Dr.B.B.Pal
Dr. K. Satyanarayana

Starting date : 1987 ongoing

The HIV/AIDS sero-positivity for 24.8 lakh samples tested in the country stood at 7.25/1000 samples screened at the end of March, 1995 (NACO Report, March 1995). Five top most states tested 3.7 lakh samples and reported 10,498 confirmed sera positive samples with a very high sero-positivity rate (28.01/1000 samples). These five states : Manipur (97.27), Nagaland (76.40), Maharashtra (23.51), Pondicherry (22.85) and Goa (10.14) with higher rates tested 15.1% of total sera samples in the country and contributed a disproportionate share of 58.3% of confirmed HIV/AIDS sero-positive samples recorded in the country according to NACO report (March, 1995). The remaining 19 states and U.T.s tested 21 lakh samples (84.9% of the country's total) and reported 7,521 sero-positive samples (41.7% of country's total) and had a low sero-positivity rate of 3.58/1000 samples tested. The figure for Orissa state was 0.99/1000 and its place was third from bottom after Assam (0.63) and Bihar (0.36). The rate for the state of Andhra Pradesh was in the middle of the ranks (11th rank) with 4.34/1000 samples. The state of Orissa, which was hitherto believed to be nearly free from the onslaught of HIV/AIDS, had now come into its grip, though with low sero-positivity rate and in the last three states with lower sero-positivity rates.

The figures available for repeatedly ELISA positive samples from different centres of the region indicate a disturbing picture. The port city Visakhapatnam (5.08/1000) and pilgrim town Tirupati (7.06/1000) have been consistently reporting higher prevalences in this coastal

region. It is disturbing to note that Berhampur city blood bank with Ganjam district samples, would appear to be catching up with the rate of Visakhapatnam (RMRC, Annual Report 1993-94). In addition to return of migrants from higher endemic areas, jail inmates from neighbouring Thailand straying into our waters is a cause for concern. The findings from this Centre call for greater alertness in this regard. The following sections outline sero-surveillance activities in different groups.

4.1.1. Sero-surveillance activities

The sero-surveillance for HIV/AIDS infection was initiated at this Centre in 1987. At the beginning the number of samples was very small and the samples screened per year increased gradually. During the year 93-94 the number of samples screened came down due to the closure of sentinel surveillance in March, 1993. In the current year (94-95) only referred samples were screened. This year a total of 305 (Indian-211, Foreigner-94) sera were tested for HIV antibodies from different risk groups, like blood donors, foreign tourists, jail inmates (Indians and foreigners), etc. (Tables - 1 & 2). Out of 305 sera, 35 were repeatedly ELISA positive and 33 were found to be WB positive. Majority of the confirmed sera (27 81.8%) for HIV antibodies were from foreigners (27) and rest were from donors (6; 18.2%). The sero positivity rate/1000 samples for Indian subjects rose from 0.00 in 1991-92 to 0.30 in 92-93; to 3.55 by 93-94 and to 28.44 by 94-95 (Table 1). The cumulative sero-positivity rate (which includes all categories from earlier years as well) was found to be lower than the above figure. It was found to be 0.00 in 1991; 0.18 in 1992; 0.98 in 1993 and 1.78 in 1994 (94-95). This rate is applicable only to samples tested from all types of Indian subjects (mostly Oriyas) at this Centre and is slightly higher than the figure of 0.99/1000 samples as per NACO report for the entire state, including data from other testing centres.

(i) **Blood donors:** For the current year blood donors contributed highest number of sero-positivity for HIV antibodies and the cumulative figure for five years (8/3392) showed a sero-positive rate of 2.36/1000 samples screened. Most of the confirmed sera were referred from Red Cross Blood Bank, Berhampur and were from migration labourers and other business groups. Higher sero-positivity rate for Berhampur Blood Bank, which is nearly catching up with the 5.08/1000 rate of Visakhapatnam could be due to two reasons. Firstly, the migratory nature of manual labourers to Western India and secondly due to business activities with neighbouring towns with higher endemicity.

Table - 1 HIV/AIDS sero-prevalence in Indian subjects tested at RMRC, Bhubaneswar

Year	Blood donor	Sentinel	Tribals	Jail inmates	TB patients	Others	For that year	Cumu-lative
Upto 1990	0/7	-	-	-	-	0/709	0/716	0/716
1991	0/989	0/30	0/340	-	-	0/13	0/1372	0/2088
1992	0/1517	0/971	0/834	-	-	1/1	1/3323 (0.30)	1/5411 (0.18)
1993	2/868	0/547	-	0/26	2/227	2/24	6/1692 (3.55)	7/7103 (0.98)
1994 (Jan'94 to Mar'95)	6/11	-	-	0/146	0/1	0/53	6/211 (28.44)	13/7314 (1.78)
Cumulative	8/3392	0/1548	0/1174	0/172	2/228	3/800	-	13/7314 (1.78)
(Sero-positivity/1000 samples)	(2.36)	(0.00)	(0.00)	(0.00)	(8.77)	(3.75)	-	(1.78)

Figures in parentheses are sero-positive rates per 1000 samples screened.

Table - 2 HIV/AIDS sero-prevalence among samples tested from foreigners at RMRC, Bhubaneswar

Year	Tourists	Jail inmates	Total	
			For that year	Cumulative since inception
Upto 1990	0/24	-	0/24	0/24
1991	-	-	-	0/24
1992	0/4	-	0/4	0/28
1993	0/14	-	0/14	0/42
1994	1/15	26/77	27/92	27/134
Seropositivity/ 1000 samples	66.7	337.7	for all the year	201.5

(ii) **Sentinel surveillance:** This study was carried out in the OPDs of Capital Hospital, Bhubaneswar. The cumulative figure was 1548 and not a single sample was found positive for HIV antibodies. This type of screening came to a halt in April'93. However, this activity is being revived this year from May 95 after receipt of grant for surveillance activities.

(iii) **Tribals:** A total of 1174 samples were collected from tribals of Phulbani district during the years 1991 and 1992 and all these samples were found to be negative for HIV antibodies. Though the heterosexual promiscuity was supposed to be common due to sexual freedom before marriage, not a single sample was found positive in this survey in tribal population. This showed that the HIV/AIDS epidemic did not penetrate into that community at the time of that survey in 1992.

(iv) **TB patients:** HIV infected persons are more prone to opportunistic infections, tuberculosis (TB) is one of them. A surveillance study was conducted last year to document the prevalence of HIV infected persons in the indoor patients of the three TB Hospitals of Orissa, from Puri, Chandpur (Khurda) and Bhabanipatna. Out of 228 sera only two were confirmed for HIV antibodies showing a rate of sero-positivity of 8.8/1000 samples screened. Whereas, in the other states it was 29/1000 in Bombay (Mohanty *et al.*, 1993), 7/1000 in Madras (Anuradha *et al.*, 1993) and 34/1000 in Pondicherry (Arora *et al.*, 1993). The sero-positivity rate of HIV/AIDS in inpatients of TB hospitals of Orissa was thus found to be lower than the rates observed in the leading five endemic states. This observation is in line with the lower sero-positivity rate for overall samples screened, when compared to other states of the country. However, the entry of infection into Orissa and into TB infected population is now a reality and has to be faced with intense IEC activities for its containment.

(v) **Others:** The sera which were not having any details like age, sex and details on risk factors and which did not fit into the above defined categories were presented under this category. This group which probably includes undeclared sexual promiscuity segment, had 3 positive samples out of 800 samples tested. The sero-positivity rate in this undeclared group was 3.75/1000 and was greater than that recorded in blood donors (2.36/1000), the latter group had many positive samples from Berhampur blood bank. Therefore the so called other group has to be watched much more critically.

(vii) **Foreigners:** It was alarming to note that majority of the confirmed sera (27 out of 40) were from foreigners and it was for the first time that such a large number of persons showed sero-positivity for HIV antibodies on the soil of Orissa (Table-2). Out of 27 sero-positives from foreign subjects, 26 samples were from jail inmates (discussed in detail in the following chapter) and one was a tourist. The cumulative figure to all the years showed a sero-positivity rate of 201.5/1000 samples (Tourists 66.7/1000 and Jail inmates 337.7/1000) screened.

Such heavy influx of (importation of) HIV/AIDS infection into this region should be closely watched and the native population should be guarded carefuiiy. The authorities were aware of the risks and have made arrangements to keep foreign inmates of jail in a separate section. Majority of these 26 young men (61.5%) with HIV infection were aged between 21-30 years and about 20% each were aged less than 20 years and 30-40 years. The National HIV Reference Centre at NICED, Calcutta, had reported that 11 of 26 young men carried HIV-1 virus; 5 had HIV-1 and HIV-2 and the remaining 10 had HIV-1 and indeterminate status for HIV-2. Unless special precautions were taken, the HIV-2 virus may make an entry from this source and route.

Information on such a large scale sero-positivity was passed on to the concerned authorities like Superintendent, Circle Jail; NACO, New Delhi; DHS, Govt. of Orissa and ICMR, New Delhi. The local Government in consultation with central authorities made arrangements for deportation of infected foreigners. Regular screening of prisoners from foreign countries housed in jails in this region will be taken up by this Centre. Also the DHS, Government of Orissa refers, foreign tourists needing HIV/AIDS certificate, to this centre for ELISA testing. The 9th SAC of RMRC recommended to carry out sero-surveillance work at Paradeep (Port) town and Puri town among high risk groups.

4.2. IEC ACTIVITIES (HIV/AIDS)

Scientists : Dr.B.B.Pal
Mr.A.Mohapatra
Dr.D.Das
Mr.C.C.Rath

Starting date : 1992, ongoing

We have been actively involved in IEC activities on HIV/AIDS both at technical and non-technical levels. We have procured video cassettes and posters etc. both in English and Hindi on HIV/AIDS and distributed them to NGOs, representatives from high risk groups and institutions. We have also prepared Oriya posters and audio cassette in Oriya on HIV/AIDS which were frequently used in providing training to different representatives of NGOs and for IEC activities organised at different places.

This year three IEC programmes were conducted at different places.

(1) **At RMRC, Bhubaneswar:** Three volunteers from Gania Unayan Committee (GUC) from Gania attended the training programme on HIV/AIDS. Theoretical lectures were given on HIV/AIDS followed by poster presentation. Video and audio cassettes were also played on HIV/AIDS. Demonstration of ELISA testing was carried out explaining the procedure for the detection of HIV antibodies in blood samples.

(2) **At Gania, Nayagarh district:** Another IEC programme on HIV/AIDS was conducted with GUC at Gania, Nayagarh district. More than 200 participants from different walks of life especially youth (including female volunteers) participated in this workshop. They were explained through oral presentation in Oriya language followed by posters, video show, audio cassette playing in simple Oriya language. At the end, there was a question-answer session. A task force discussion was held and the future modalities were discussed for the prevention and control of spreading HIV/AIDS in that area. The GUC organisers proposed a screening programme for HIV amongst the volunteers and keeping a list ready with blood grouping for emergency blood donation. In this context, screening of blood samples for HIV antibodies was requested by this NGO.

(3) **At RMRC, Bhubaneswar :** Demonstration of ELISA for HIV antibodies was arranged for M.Sc. (Microbiology) students, O.U.A.T., Bhubaneswar.

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4.3. MICROBIOLOGY OF LOCAL HOT SPRINGS

Scientists : Dr. V. R. Subramanyam
: Mr. C.C. Rath

Starting date : Sept. 1992

Hot springs offer a unique opportunity to study thermophilic microbes. The objective of this project was to study the microbes in the local hot springs. The temperature of the hot springs ranged from 45°C to 60°C. We have isolated 35 strains of bacteria and 26 strains of fungi from the three hot springs of Orissa (RMRC, Annual Report 93-94).

Microbes from hot springs offer potential sources for industrially important bio-active substances such as enzymes. Thermophilic bacteria have been screened for various enzymatic activities such as amylase, protease, lipase, glutaminase, cellulase, DNase and B lactamase at different temperatures. Thirteen strains of bacteria showed amylase activity at 60°C. Two strains SRT-12 and SRT-41 showed highest amylase activity in comparison to other strains. The optimum activity for both the strains was observed within 55-60°C.

Eight and 13 strains showed proteolytic activity both at 60°C and 45°C respectively. Eleven (7 *Bacillus sp.* and 4 *Pseudomonas sp.*) and nine (5 *Bacillus* and 4 *Pseudomonas*) strains showed lipase activity using Tween -20 and Tween-80 at 60°C respectively. Three strains SRT-16, SRT-17 and SRT-18 showed optimal glutaminase activity at 55°C. SRT-16 and SRT-17 showed two pH optima at pH 6 and pH 9 whereas SRT 18 showed only one pH optima at pH 6. Thirteen isolates shared DNase activity even at 60°C. Except SRT-13 and SRT-39 all the isolates were found positive for B.lactamase activity.

All 26 fungal isolates were screened for amylase, protease, lipase, glutaminase and cellulase activities. Sixteen strains showed amylolytic activity at 60°C. Out of these 16 strains, only 2 strains showed activity at 80°C and the amylase activity was observed within a very wide range of pH (pH 4-11). Eight strains were found positive for cellulase activity. All the isolates were negative for protease, lipase and glutaminase activities under test conditions.

4.4. HELP RENDERED TO BLOOD BANK, CUTTACK

Some disposable syringes (different trade marks) of 2 ml and 5 ml capacity were sent by Red Cross Blood Bank, Cuttack for checking the sterility. They were tested and none was found to be contaminated. These results were used by the blood bank authority to convince their purchase committee to purchase quality syringes at a lower cost.

PUBLICATIONS

1. A note on the growth and viability of *Vibrio cholerae* and other bacteria in Ragi (*Eleusine coracana*) gruel.V.R. Subramanyam and C.C. Rath. Biomedical letters (1994) **50**: 147-150.

2. Characterisation of resistance to essential oils in a strain of *Pseudomonas aeruginosa* (VR-6).S. Pattnaik, C.C. Rath and V.R. Subramanyam. Microbes (in press).

3. Effect of essential oils on the viability and morphology of *Escherichia coli* (SP-11). S. Pattnaik, V.R. Subramanyam and C.C. Rath. Microbes (in press).

4. Thermostable amylase activity of fungi isolated from a hot spring.C.C. Rath and V.R. Subramanyam. Proceedings of National Conference on Fungal Biotechnology, Bhopal.

5. B.B.Pal and S.P.Bhunya. Mutagenicity testing of Zinctox, a rodenticide in mammalian *in vivo* system. *In Vivo* (1994), accepted.

6. V.R.Subramanyam, B.B.Pal and K.Satyanarayana. HIV sero-surveillance in Orissa. CARC Calling (1994), communicated.

ABSTRACTS PUBLISHED

1. Enzyme activity of Bacteria from hot springs. MICON-International'94 and 35th Annual Conference of AMI, Mysore Nov.12-14 (1994).

CONFERENCES ATTENDED

B. B. Pal

1. Indian Association of Medical Microbiologists, 18th Conference, AFMC, Pune. Presented a paper entitled "HIV seropositivity in tuberculosis patients from Orissa: a hospital based study".

2. Nutritional Society of India, Annual meet of Orissa Chapters at RMRC, Bhubaneswar. 23-24 February (1994).

OTHER ACTIVITIES

Conducted ICE programme on HIV/AIDs at Gania Unnayana Samiti, Gania 23rd Jan (1994).

V. CLINICAL PATHOLOGY DIVISION

5.1. STUDY OF ERYTHROCYTIC G-6-PD DEFICIENCY AND ITS VARIANTS AT MOLECULAR LEVEL IN A MALARIA ENDEMIC TRIBAL POPULATION OF ORISSA

Scientists	:	Dr. G.P. Chhotray Dr. M.R. Ranjit
Co-investigator	:	Prof. Lucio Luzzatto
Technical staff	:	Mr. B.N. Sethi Mr. H.K. Khuntia
Starting date	:	April, 1986.

Red cell glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) in man is an X-linked enzyme. Mutations within the coding region of the G6PD gene lead to it's deficiency. The most typical clinical manifestation of the G6PD deficiency is haemolytic crisis, precipitated by exogenous factors. In terms of their clinical features WHO has grouped G6PD abnormalities into 3 classes; viz. 1) which do not manifest any clinical disorders (Example G6PD Baltimore-Austin, Ibadan-Austin), 2) which cause haemolytic reaction when administered with fava bean and certain drugs (Example G6PD Negroes, G6PD Chinese, G6PD Mediterranean) and 3) which induce congenital non-sphaerocytic haemolytic disease (CNSHD). Based on biochemical characterisation, which include the level of enzyme activity, the electrophoretic mobility and the enzyme kinetics, approximately 400 variants of G6PD enzyme have been described. Studies on G6PD variants at the molecular level during the last few years have already revealed more than 50 different mutations scattered throughout the entire coding region of the gene. The common African variants (Gd^A and Gd^{A-}) have

arisen because of the substitution of A to G at nt 376 and G to A at nt 202, whereas Mediterranean variant of G6PD deficiency ($G6PD^{B-}$) has a C ->T substitution at nt 563 and has been found to be prevalent in several Mediterranean and Asian populations. Here, we report the results of a study of the molecular characterisation of G6PD in deficient population of both tribal and non-tribals of Keonjhar district of Orissa.

Total 677 tribal males belonging to Bhuyan, Bathudi, Ganda, Koda, Munda, Santal Saunti and Juanga communities and 216 non-tribal males belonging to Pana community were screened randomly for G6PD deficiency. The deficiency ranged from 3.7%-14.7%, the highest being among Bhuyans (Table-1).

Table - 1 G6PD deficiency and its variant in Keonjhar district, Orissa

Populations	No. of subjects	No.with G6PD deficiency (% prevalence)	Molecular characterisation No. of samples tested	O^+/O^-
A) TRIBALS				
1. Bhuyan	204	30(14.7)	7	6/1
2. Bathudi	106	12(11.3)	7	6/1
3. Others(Ganda, Koda, Munda, Santal, Saunti & Juanga)	367	29(7.9)	6	3/3
Total	677	71(10.5)	20	15/5
B) NON TRIBALS				
1. Pana	216	8 (3.7)	4	4/0
Grand Total	893	79 (8.9)	24	19/5

O^+ : Numbers of persons showing Orissa mutation

O^- : Numbers of persons not showing Orissa mutation

Blood samples were collected from 24 volunteers belonging to different communities for both biochemical and molecular characterisation of G6PD enzyme. The G6PD activity in the G6PD deficient samples ranged from 10 to 20% of the normal (G6PD B). The Km for G6P revealed 5 fold higher than that of normal enzyme and normal electrophoretic mobility. DNA was extracted and transported to co-investigator laboratory in U.K. The coding regions of the G6PD gene were amplified and labelled (α 32 pdCTP). The labelled PCR products were then run on a SSCP gel. The α 32 pdCTP labelled PCR fragments from the coding exons of 3 and 4 in 19 samples showed abnormal migration compared to normal control (G6PD B). Sequencing of these two exons (3 & 4) revealed a base substitution in nt 131 (Codon 44) of exon 3, whereby C has been replaced by G i.e. GCC ->GGC. This base change predicts the substitution of amino acid Alanine to Glycine in position 44. The community wise distribution of this G6PD Orissa variant has been shown in Table - 1. Out of 20 samples collected from tribals, 15 (75.0%) showed the G6PD-Orissa mutation, while all the four samples (100%) from non-tribals exhibited G6PD-Orissa mutation.

Since this amino acid is within the sequence of amino-acids (residues 37 to 44), which is suggested to be co-enzyme (NADP) binding site and it is believed that this mutation may be responsible for favism. Further it is of interest to note that this mutation has not been previously seen in non-tribals within India in whom by far the most common G6PD variant encountered was G6PD Mediterranean.

5.2. DIAGNOSTIC PERSPECTIVE OF FILARIAL SKIN TEST BY *BRUGIA MALAYI* INFECTIVE LARVAL ANTIGEN

Scientists	:	Dr. G.P. Chhotray Dr.(Miss) M. Mohapatra
Technical staff	:	Mr. B.N. Sethi Mr. H.K. Khuntia
Starting date	:	August, 1993.

The present study was undertaken to assess the specificity and sensitivity of filarial skin test (FST) in the diagnosis of human lymphatic filariasis by using *B. malayi* infective larval antigen.

A total of 80 volunteers belonging to endemic normal (those, who do not show any clinical and parasitological evidence of filariasis); asymptomatic microfilarae carriers (people who do not show any signs/symptoms of filariasis, but have microfilariae in their blood); acute manifestation (individuals who manifest recurrent episodes of filarial fever accompanied by lymphadenitis and lymphangitis); past history of filariasis (those who had acute manifestation quite long back (>3 years) but presently they are not having clinical symptoms) and chronic manifestations (subjects with obstructive lymphatics manifested by elephantiasis, hydrocele or chyluria) groups, were selected from 4 villages (Ahamadpur, Panchukera, Badahata and Birabalabhadrapur) of Sakhigopal block of Puri district. FST was conducted by injecting (intradermal) 0.05 ml of reconstituted *B.malayi* antigen solution, containing 2 µg of protein on the volar aspect of forearm. The lyophilised antigen was supplied by CDRI, Lucknow. The development of reaction was noted 15-20 mins after the antigen injection. Reaction was considered to be positive, if the ratio of the final reaction area (measured in sq mm) to the initial reaction area was found to be 1.5 or >1.5.

It was observed that, majority of the (20 out of 25; 80%) Mf +ve cases and nearly three-forths (40 out of 55; 72.7%) of the Mf -ve cases showed positive skin reaction to larval antigen. Taking this into consideration the overall sensitivity and specificity of FST was computed to be 80.0% and 27.3% respectively (Table-2). Thus many persons showed positive skin test reaction but did not show Mf in circulation. If Mf in circulation is to be taken as the confirmatory parameter the specificity of skin test is very poor (27.3%) as the false positive component was found to be very high. Majority of persons showed positive skin test from Mf positive group and Mf negative group. About one-third of skin test positive persons (20 out of 60; 33.3%) and one-fourth of skin test negative persons (5 out of 20; 25%) had Mf in the circulation.

Table - 2 FST reaction according to Mf status

Result	Mf +ve	Mf -ve	Total
FST +ve	20	40	60
FST -ve	5	15	20
Total	25	55	80

Considering the sensitivity and specificity of the FST, it would appear that this may not serve useful purpose in field studies.

5.3. INTESTINAL PARASITISM IN KHURDA DISTRICT, ORISSA.

Scientists : Dr. M.R. Ranjit
Mr. A. Mohapatra

Technical staff : Mr. B.N. Sethi
Mr. H.K. Khuntia

Starting date : July, 1994

Intestinal parasitic infections of mankind are of considerable medical importance throughout the developing world. In India, the overall prevalence is about 50% in the urban areas and 68% in rural areas. However, the available information on the prevalence of intestinal parasites does not adequately bring out the differences between various groups like urban, semiurban and rural areas. Therefore in the present study, a survey was conducted in three ecologically different areas of Khurdha district of Orissa to get a baseline data on the prevalence of parasites transmitted mostly by faeco-oral route.

A total of 1114 school children with reference to urban/ semiurban/rural areas and Hindu/Muslim communities were screened. From the survey it was observed that the

protozoan infections were *E.histolytica*, *Giardia lamblia* and *Entamoeba coli* and helminthic infections were *Ascaris lumbricoides*, hookworms, *T. trichiura*, *S.vermicularis*. The multiple infections include various combinations of either protozoan or helminthic or both infections. The prevalence of *E.histolytica* in both urban (16.3%) and rural (11.3%) localities were generally higher compared to the prevalence in semi urban school children (6.0%). But the *G.lamblia* infection was higher in semiurban (16.0%) than urban (8.0%) and rural (7.8%) localities. However, the prevalence of total protozoan infection did not show any ecological variation (Table - 3).

The prevalence of total helminthic infections was higher in semi-urban localities (24.5%) than the urban (8.6%) and rural (10.8%) localities. *A.lumbricoides* prevalence was more in semiurban (17.8%) than the urban (2.2%) and rural (6.9%) areas. The prevalence of other helminthic parasites range from 0.5% to 3.6% in the various groups of the studied population.

Semi urban population had higher prevalence of (20.3%) multiple infections than urban (5.7%) and rural (7.4%) population. Amongst the multiple infections, the *E.coli* and *E.histolytica* combination was most common in protozoan group, the *A. lumbricoides* and *T.trichiura* in helminthic group and *E.histolytica* and *A.lumbricoides* in mixed protozoan and helminthic group.

Table - 3 Prevalence of intestinal parasites by localities and species

Parasites	Urban No. (%)	Semi-urban No. (%)	Rural No. (%)	Total No. (%)
(A) SINGLE				
(i) Protozoa	134 (26.3)	94 (23.5)	45 (22.1)	273 (24.5)
E.histolytica	83 (16.3)	24 (6.0)	23 (11.3)	130 (11.7)
G.lamblia	41 (8.0)	64 (16.0)	16 (7.8)	121 (10.9)
E.coli	10 (2.0)	6 (1.5)	(6 (3.0)	22 (2.0)
(ii) Helminths	44 (8.6)	98 (24.5)	22 (10.8)	164 (14.7)
A.lumbricoides	11 (2.2)	71 (17.8)	14 (6.9)	96 (8.6)
Hook worm	19 (3.6)	6 (1.5)	5 (2.4)	30 (2.7)
T.trichiura	1 (0.2)	11 (2.8)	1 (0.5)	13 (1.2)
S.stercoralis	5 (1.0)	5 (1.2)	–	10 (0.9)
H.nana	5 (1.0)	–	–	5 (0.5)
H.dimunita	–	–	1 (0.5)	1 (0.1)
E.vermicularis	3 (0.6)	5 (1.2)	1 (0.5)	9 (0.8)
(B) MULTIPLE	29 (5.7)	81 (20.3)	15 (7.4)	125 (11.2)
Mixed Protozoa	11 (2.2)	12 (3.0)	3 (1.5)	26 (2.3)
Mixed Helminths	2 (0.4)	20 (5.0)	2 (1.0)	24 (2.2)
Mixed Protozoa and Helminths	16 (3.1)	49 (12.3)	10 (4.9)	75 (6.7)
Total infected	207 (40.6)	273 (68.3)	82 (40.3)	562 (50.4)
Total cases studied	510	400	204	1114

Table - 4 shows the distribution of single and multiple parasite infections according to sex of the population irrespective of their age. There was no significant difference between the two sexes for the overall infection rates with various intestinal parasites.

Table - 4 Single and multiple parasite infestations in different sexes

Sex (n)	Infected No. (%)	Single		Multiple		
		Protozoa No. (%)	Helminths No. (%)	Protozoa No. (%)	Helminths No. (%)	Protozoa+ Helminths No. (%)
Male (579)	289 (49.9)	137 (23.7)	90 (15.5)	16 (2.8)	18 (3.1)	28 (4.8)
Female (535)	273 (51.1)	136 (25.4)	74 (13.8)	14 (2.6)	15 (2.8)	34 (6.4)
Total (1114)	562 (50.5)	273 (24.5)	164 (14.7)	30 (2.7)	33 (3.0)	62 (5.6)

Table - 5 represents the religion wise distribution of single and multiple parasite infestations, which reveals that Hindu children had six fold higher rate of protozoan infestations (26.6% versus 3.9%), while Muslim children had five fold higher (54.4% versus 10.7%) helminthic infestations. Multiple helminthic infestations and mixed infestations were higher in than that of Hindu children.

School children from semi urban schools would appear to suffer heavily, as compared to urban school children and rural school children. Peculiar socio-economic features and semi-permanent housing with lesser access to sanitary disposal of human wastes and potable water could be partly responsible for the dismal picture seen in semi-urban school children. The pattern of intestinal parasitism in Muslim children was found to be distinctly different. More than three-fourth of 103 children showed manifestations and the bulk was constituted by helminths and more than half children had this group of parasite. Health education

measures and mass treatment programme would greatly benefit the school going children of all regions.

Table - 5 Single and multiple infestations in different communities

Community (n)	Single		Multiple		
	Protozoa No. (%)	Helminths No. (%)	Protozoa No. (%)	Helminths No. (%)	Protozoa + Helminths No. (%)
Hindu (1011)	269 (26.6)	108 (10.7)	30 (3.0)	18 (1.8)	56 (5.5)
Muslim (103)	4 (3.9)	56 (54.4)	–	15 (14.6)	6 (5.8)

5.4. FLUOROSIS IN BOLGARH BLOCK

Scientists : Dr. Anasuya Das, DD,NIN, Hyderabad (Co-investigator)
Dr. G. P. Chhotray
Dr. S. S. S. Mohapatra
Mr. Anil Kumar
Mr. A. Mohapatra
Dr. M. Mohapatra

Technical staff : Mr. B. N. Sethi
Mr. H. K. Khuntia

Starting date : June 1993

5.4.1. Clinical studies

This Centre has screened about 70% of total population of 2 villages by examining all the available volunteers from all the families of both the villages. A total of 322 individuals were covered for the clinical examination by a team of two clinicians. All the families cooperated with the team. The composition of total sample revealed that 11-20 years age

Table - 6 Fluoride content of different water sources

Water source	F level in ppm	Position from stream
Hot spring	19.19	Stream
Well No.4	8.36	Near
Well No.3	7.15	Near
Well No.2	2.38	Away
Well No.1	0.57	Away
Tube well No.1	0.75	Away
Tube well No.2	3.80	Near
Govt.supplied water	1.08	From adjacent village

group constituted 23% of the sample, where as age groups 6-10, 21-30 and 31-40 constituted between 12-16% each. Only 6 infants were examined constituting about 2% and 28 pre-school children could be examined, constituting about 9% of the sample (Table-7). The age group 51-60 constituted 6.8% and from the next age group (61+ yrs) only 3 persons presented themselves for examination and therefore were clubbed with 51-60 years age group and the group was designated as 51+ years with a total sample of 25 members. Thus all groups had more than 25 individuals, excepting infants. Higher representation in the 11-20 years age group could be due to the awareness of the villagers for the manifestations of the fluorosis in this age group. Therefore, almost all adolescents must have taken advantage of the survey. The composition of men and women was nearly equal in all the age groups, excepting for infants, which was dominated by girls (5 out of 6).

Table - 7 Prevalence of mottling in males and females in the affected villages

Age (yrs)	Males		Females		Both sexes	
	Sample size	Mottling	Sample size	Mottling	Sample size	Mottling
	No	%	No	%	No.	%
< 1	1	-	5	-	6	-
1 - 5	13	7.7	15	-	28	3.6
6 - 10	25	40.0	23	73.9	48	56.3
11 - 20	42	76.2	32	56.3	74	67.6
21 - 30	25	60.0	28	21.4	53	39.6
31 - 40	21	28.6	28	10.7	49	18.4
41 - 50	24	20.8	15	6.7	39	15.4
51 +	13	15.4	12	8.3	25	12.0
All ages	164	43.3	158	29.1	322	36.3

Out of 322 persons 181 persons did not exhibit any dental lesions constituting 56.2% with nil dental abnormalities in the sample. Out of the 141 individuals who exhibited dental abnormalities, 117 had mottling (either alone or in combination with the other dental problems). Another group of 24 individuals had shown missing teeth, caries, gingivitis, etc. Missing teeth was mostly seen in elderly individuals and caries was seen at about 10% prevalence level in 6-10 years age group and 5% prevalence level in 11-20 years age group. Thus more than 1/3rd (36.3%) of the total population examined had mottling, as the dental lesions in the study population (Table-7).

Mottling appeared at very low level by 5th year (3.6%) and reached a higher level of 56.3% in 6-10 years age group (Table-7). At this age group, girls dominated and had 73.9% prevalence. The next age group (11-20 years) recorded the highest prevalence of

Table - 8 Prevalence of genuvalgum in males and females in the affected villages

Age (yrs)	Males		Females		Both sexes	
	Sample size	Mottling	Sample size	Mottling	Sample size	Mottling
	No	%	No	%	No.	%
< 1	1	-	5	-	6	-
1 - 5	13	7.7	15	-	28	3.6
6 - 10	25	12.0	23	4.3	48	8.3
11 - 20	42	28.6	32	9.4	74	20.3
21 - 30	25	4.0	28	-	53	1.9
31 - 40	21	4.8	28	3.6	49	4.1
41 - 50	24	16.7	15	-	39	10.3
51+	13	7.7	14	21.4	25	16.0
All ages	164	14.0	158	5.1	322	9.6

(67.6%) mottling among the age groups studied. In this age group boys dominated with 76.2% prevalence as against 56.3% observed in girls. From the age 21-30 onwards the prevalence of mottling was seen at a lower level, declining from 40% in 21-30 years age group to about 12% in the 51+ years age group. Women had a very low prevalence compared to men in the four higher age groups. The prevalence in women varied between 1/2 to 1/ 3rd of that seen for men in these higher age groups. Thus women in the higher age groups had comparatively lower prevalence of mottling unlike school age and adolescent girls who suffered equally and registered equal prevalences as those of opposite sex.

Genuvalgum was the presenting symptom that caused alarm mostly in the adolescent groups in these two villages. There were 31 individuals who had genuvalgum either alone (25) or in combination with other skeletal abnormalities (6). There were 7 cases of

genuvalgum in the higher age groups. A total of 49 individuals constituting 15.2% of the population had shown skeletal abnormalities related to fluorosis and other disorders. The overall prevalence of genuvalgum in the various age and sex groups was shown in Table-8. The prevalence in males was found to be 14.0% as against 5.1% seen in females. The prevalence of genuvalgum was found to be always on the higher side in the male sex excepting in the 51+ years age group. The highest prevalence was seen in 11-20 years age group, with 28.6% of boys and 9.4% of girls showing this skeletal abnormality. The preponderance of lesions in male sex was seen in the younger age group (6-10 years) as well as in the higher age groups (21-30 and 41-50). There was a second peak with 16.7% for men in the 41-50 years age group and with 21.4% for women in the 51+ years age group. The nearer absence of skeletal abnormalities in the 21-50 years active reproductive age group for women may have physiological and endocrinological connotations.

Male sex would appear to have greater prevalence of both dental abnormalities and skeletal abnormalities in the higher age groups. There was no second peak for dental abnormalities signifying different turnover rates and susceptibilities between the two tissues. During adolescence dental abnormalities did not exhibit particular sex preference, whereas skeletal abnormalities did exhibit nearly 3 times preference to male sex. This could be partly due to greater growth of skeletal tissues, greater physical exertion, greater necessity for out door life and probably greater consumption of fluids with higher quantities of fluorides. The peak that was seen for skeletal abnormalities in the older age groups may be related to the disturbed balance between osteoporosis and osteosclerosis.

B-complex deficiency was seen in 6.2 % of individuals and Vitamin-A deficiency was seen in 2.5% of the sample studied.

5.4.2. Estimation of Fluoride content of water sources in affected villages

The villages have two functioning tube wells, four dug wells and two ponds as drinking water sources. However, there is also a perennial stream which runs east to west from the nearby hot spring, which is situated about 300 meters away from the village.

Water samples were collected from the affected villages from all the existing water sources including the recent government supplied water to the village which is being pumped from the nearby village.

Since fluorosis is known to be endemic in a given geographical pocket, water samples from the adjacent five villages within a radius of 3-5 km were also collected and analysed for fluoride content at the NIN, Hyderabad.

The results showed that, three out of four dug wells had fluoride content more than the permissible limits showing 2.38 ppm, 7.15 ppm and 8.36 ppm respectively.

Out of the two functioning tube wells, one tube well which is adjacent to the stream showed high fluoride content, 3.8 ppm. However, both the ponds had less than the permissible limit of fluoride being 0.67 ppm and 0.17 ppm respectively. The villagers were using all the above mentioned water sources for drinking, cooking and bathing purposes since at least three decades or more. Only since last three years they have switched over to the Govt. supplied water containing 1.08 ppm fluoride. The hot spring stream which flows surrounding the village showed the highest content of fluoride, being 19.19 ppm.

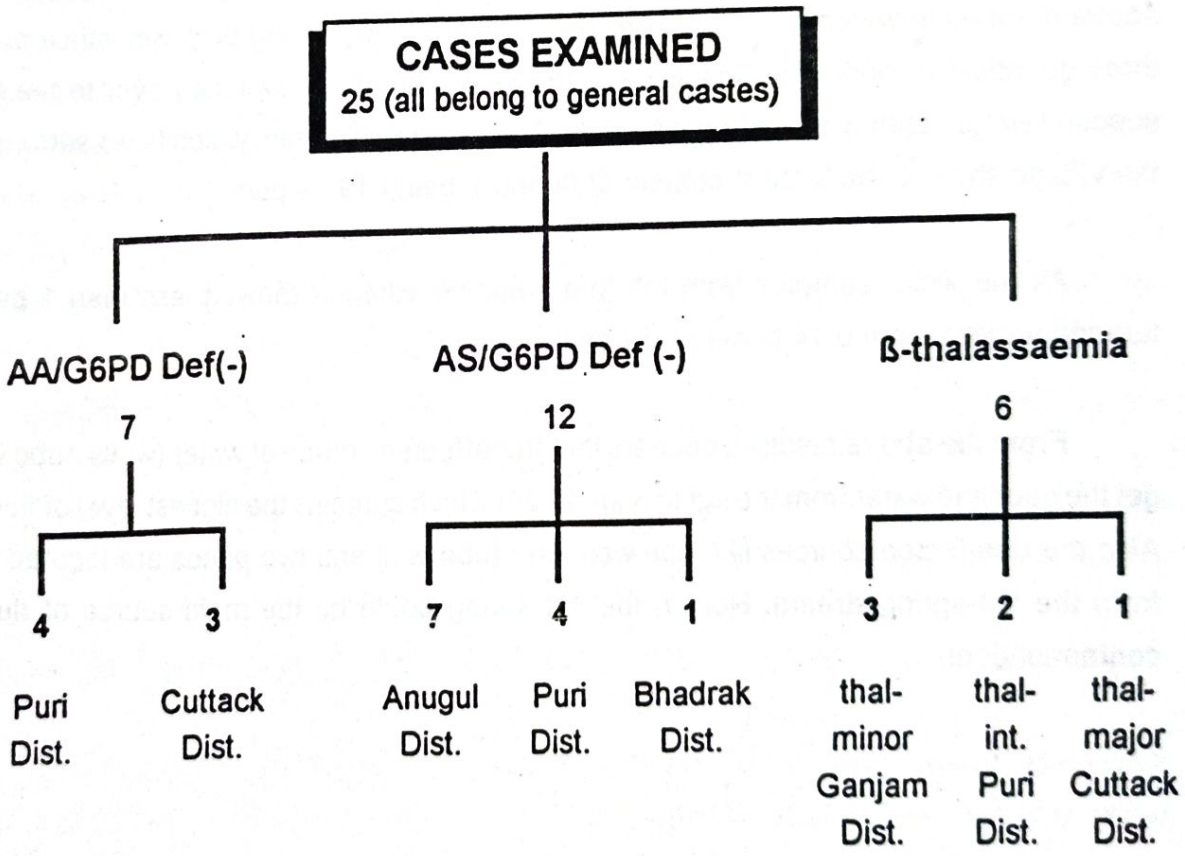
All the water samples from the five adjacent villages showed less than 1 ppm of fluoride varying from 0.14 ppm to 0.67 ppm.

From the above results it appears that the affected source of water (wells, tube wells) get the seepage water from the hot spring-stream which contains the highest level of fluoride. Also the unaffected sources like one well, one tube well and two ponds are located away from the hot-spring-stream. Hence, the hot spring could be the main source of fluoride contamination.

5.5. DIAGNOSTIC SERVICES

The clinical pathology division of RMRC, Bhubaneswar has rendered its diagnostic services to patients referred from different hospitals of the state for the diagnosis of haemoglobinopathies, thalassaemia and G6PD deficiency.

From the figure, it is evident that the sickle cell and ß thalassaemia genes are not uncommon amongst the general castes of Orissa population. Out of 25 person referred and screened 12 persons had sickle cell trait (AS) and 6 persons had β - thalassaemia. Therefore extensive studies are needed to locate the exact foci and prevalence of sickle cell disorder and thalassaemia.



SEMINARS/WORKSHOPS ATTENDED

1) Dr. M.R. Ranjit on behalf of S. Mukhopadhyay, A.P. Dash and B. Ravindran. attended the INDO-US workshop on New animal models for parasitic diseases held at CDRI, Lucknow from 6.12.94 to 8.12.94 and presented the poster on the paper entitled "*Setaria digitata* in *Mastomys coucha* : A filarial model for chemotherapeutic and immunological studies".

2) Dr. M.R. Ranjit attended the V National Network meeting on haemoglobinopathies and allied disorders held at Institute of Immunohaematology, Bombay on 20.3.95 and discussed about the present knowledge on prevalence of sickle cell disease and G6PD deficiency in Orissa.

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1) Ranjit, M.R. and Dash, A.P. (1994) Aphid extracts and juvenoids influence glycogen metabolism in mosquitoes. *Tropical Biomedicine* **11**: 1-5.

2) Mohanty, U.L., Dash, A.P., Mohapatra, N. and Ranjit, M.R. (1994). Bioefficacy of *Anacardium occidentale* L. against mosquitoes. *Biol. control. Pest.* **4**: 139-142.

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VI. MEDICAL ENTOMOLOGY AND PARASITOLOGY

6.1. PRESENT STATUS OF *BRUGIA MALAYI* AND *MANSONIOIDES* MOSQUITOES IN ORISSA

Scientists : Dr A.P.Dash
Mr. R. K. Hazra

Starting date : September, 1989

Closing date : September, 1994

6.1.1. Susceptibility status of *Ma.annulifera* and *Ma.uniformis*

Table-1 Mean mortality of *Ma.annulifera* and *Ma.uniformis* for different insecticides

Adults/Larvae	Insecticide	Concentration	*Ma.annulifera*	*Ma.uniformis*
Adult	DDT	4 %	95 %	85 %
Adult	BHC	0.4 %	100 %	100 %
Adult	Malathion	5 %	100 %	100 %
Adult	K-otherine	0.025 %	100 %	100 %
Larvae	Fenthion	0.025 ppm	100 %	100 %
Larvae	Fenitrothion	0.125	100 %	100 %
Larvae	Temephos	0.025	100 %	100 %
Larvae	Malathion	0.02	100 %	100 %

Periodic monitoring of susceptibility status of mosquito vectors to insecticides, has become an essential component of mosquito borne disease control programme. This study was undertaken to study the susceptibility status of adults and larvae of *Ma.annulifera* and *Ma.uniformis* for different insecticides.

Susceptibility status of adults and larvae of *Ma.annulifera* and *Ma.uniformis* to various insecticides like D.D.T., B.H.C., K-othrine, malathion, fenitrothion, fenthion and temephos was studied following standard WHO procedure. The results revealed that DDT at its diagnostic concentration for culicines produced 85% mortality for *Ma.uniformis* and 95% for *Ma.annulifera*. BHC, malathion and K-othrine showed 100% mortalities. In larval stage, fenthion, fenitrothion, temephos and malathion at a concentration of 0.025 ppm, 0.125 ppm, 0.025 ppm and 0.02 ppm showed 100% mortality of both the species of *Mansonioides* (Table - 1).

6.2. TRANSMISSION OF *BRUGIA MALAYI* IN ORISSA

6.2.1. Nature of Disease

A total of 2771 persons were examined in Puri district for disease status, out of which 31.5% in male and 32.4% in female had varied manifestations of filariasis. In Balasore district, 26.9% males and 26.8% females showed disease manifestation out of 346 persons examined. The prevalence of disease was found to be age dependent irrespective of sex. Balasore district, with predominant *B.malayi* infection, showed disease prevalence (26.9%) equivalent to that of infection (27.1%). Lower than infection. But a reverse trend was observed in Puri district where both *B.malayi* and *W.bancrofti* exist and the disease prevalence (31.5%) was found to be greater than the prevalence of infection (12.3%). Endemicity rate was nearly equal for both male and female i.e., 43.9% and 40.6% in Puri district, 36.8% and 29.3% in Balasore district respectively.

6.2.2. Transmission parameters

i. **Host Efficiency Index:** The mean host efficiency index in *Ma.annulifera* and *Ma.uniformis* was observed to be 0.213 and 0.154 respectively.

ii. **Risk of Infection Index:** The risk of infection index of *Ma.annulifera* and *Ma.uniformis* was calculated to be 0.054 and 0.013. Among the two vectors, it was observed that risk of infection in case of *Ma.annulifera* was 4 times more than that of *Ma.uniformis*.

iii. **Annual Transmission Potential:** Annual transmission potential was calculated as 583.02 in *Ma.annulifera* and 196.76 in *Ma.uniformis*.

iv. **Vectorial Capacity:** Vectorial capacity of *Ma.annulifera* and *Ma.uniformis* was 6.84 and 3.40 respectively.

6.2.3. Development of filarial parasites in *Aedes aegypti* (Liverpool strain)

Scientists : Dr A.P.Dash
Dr N.Mohapatra
Mr S.K.Parida

Starting date : October, 1991

Infected *Mastomys* were fed to black eyed Liverpool strain in successive batches throughout the year. During January 94 to March 95, 38 batches of *Aedes aegypti* (5389) mosquitoes were fed. The mosquitoes were dissected on the 14th day and 3147 number of L_3 were procured which were further used for reinfection (Table - 2).

Table - 2 Number of mosquitoes dissected and larvae obtained

Filaria parasite	Mf density/ 20 µl(range)	Total no. of mosquitoes fed	Total no. of mosquitoes dissected	Total no. of L_3	L_3 load/ mosquito
Brugia malayi	15-220	5389	1838	3147	1.7

6.3 STUDIES ON MOSQUITOES OF ORISSA IN RELATION TO FILARIASIS AND MALARIA : MOSQUITO REGISTRY

Scientists	:	Dr A.P.Dash Dr N.Mohapatra Mr R.K.Hazra Mr S.K.Parida
Technical staff	:	Mr H.K.Tripathy
Starting date	:	June, 1992

6.3.1. Mosquitoes were collected from different areas of Puri district (endemic for filariasis), Keonjhar and Gajapati district (endemic for malaria). The species composition reveals the presence of 22 species belonging to six genera. The detailed results are presented in Table-3.

6.3.2. Feeding habits

Blood meals of 1538 mosquitoes belonging to twelve species were analyzed by gel diffusion technique. The anthropophilic indices were 66.6%. 7.24%, 17.6%, 7.6%, 70.0%, 72.7%, 5.0%, 88.3%, 11.5%, 3.15%, 65.5% and 56.8% respectively for *Ae.aegypti, An.nigerimus, An.subpictus, An.vagus, Ar.theobaldi, Cx.epidesmus, Cx.gelidus, Cx.quinquefasciatus, Cx.tritaeniorhynchus, Cx.vishnui, Ma.annulifera* and *Ma.uniformis*. Apart from human and cow blood meal, 1.28% of *Cx.tritaeniorhynchus* and 1.05% of *Cx.vishnui* were found to have fed on pigs while 0.2% of *Cx.quinquefasciatus* and 1.2% of *Ma.uniformis* had avian blood.

Table - 3 Mosquito species collected and their PMHD in different areas

Sl.No.	Species	Areas				
		Pipili	Khurda	Puri (Dt.)	Gajapati (Dt.) (Kashinagar)	Keonjhar (Dt.) (Jhumpura)
1.	*Ae.aegypti*	1.3	1.4	-	-	-
2.	*Ae.albopictus*	0.1	0.3	-	-	-
3.	*Ae.edwards*	-	-	-	-	-
4.	*Ae.vittatus*	-	0.1	-	-	-
5.	*An. aconitus*	0.2	1.3	1.0	-	-
6.	*An annularis*	2.2	1.9	2.5	0.2	4.0
7.	*An.culicifacies*	0.3	0.1	0.2	0.5	2.0
8.	*An.fluviatilis*	-	-	-	0.2	-
9.	*An.hyrcanus*	7.5	6.2	5.4	-	-
10.	*An.maculatus*	-	-	-	0.1	-
11.	*An.subpictus*	5.1	4.3	5.2	1.16	29.0
12.	*An.vagus*	4.2	3.9	3.0	1.1	5.5
13.	*Ar.theobaldi*	0.8	0.3	1.0	-	-
14.	*Cx.(lutzia) fuscanus*	0.7	1.8	-	-	-
15.	*Cx.quinquefasciatus*	47.0	17.9	34.8	0.1	6.0
16.	*Cx.tritaeniorhynchus*	0.9	10.5	-	0.4	3.5
17.	*Cx.vishnui*	2.2	2.3	5.2	0.4	-
18.	*Cx.whitmorei*	0.3	0.7	-	-	-
19.	*Ma.annulifera*	7.6	0.7	1.5	-	-
20.	*Ma.longipalpis*	-	0.1	-	-	-
21.	*Ma.uniformis*	7.3	0.1	3.8	-	-
22.	*Coq.crassipes*	0.3	-	-	-	-

6.4. FIELD EVALUATION OF *B.SPHAERICUS* AGAINST *CULEX QUINQUEFASCIATUS* IN ORISSA AND FILARIASIS TRANSMISSION

Scientists : Dr A.P.Dash
Dr N.Mohapatra
Mr.R.K.Hazra
Ms.Saswati Rup

Technical staff : Mr. H. K. Tripathy

Starting date : March, 1992

Completion date: October, 1996

Bacillus thuringiensis var *israelensis* and *B.sphaericus* are two important pathogenic bacteria which are being developed commercially for the control of mosquito borne diseases. Important attributes of *B.sphaericus* seem to be its persistence in the environment following application and activity in heavily polluted areas, which have promoted its use as a biocontrol agent.

B.sphaericus is a gram positive, rod shaped and aerobic bacteria. The end of vegetative growth is associated with the formation of an endospore. The bacterial spore is ingested at the water surface by feeding larvae. Once in the midgut, the spore releases toxic proteins and generates pathological changes in the larvae, ultimately causing death of the larvae. The cadaver decomposes releasing bacterial spores for further intoxication. The biocide is known to be active against culicines especially *Cx.quinquefasciatus*, the vector of filariasis. The WHO/TDR supported five global projects including one at the RMRC, Bhubaneswar.

Field evaluation of *B.sphaericus* against filariasis transmission started since April 1992 with WHO/TDR support. After collection of base line data for one year, intervention with biocide started from April 93. Successive application of biocide was done during August 94 and February 95. Entomological data and various transmission parameters like

larval and adult densities, adult landing rate, parous rate, infection, infectivity rate and L_3 load have been collected in pre spray and during the spray.

Immediately after the application of the biocide in late March, 93 and August, 93 around 90% reduction in larval and adult densities was achieved. During the 3rd application more than 70% larval and adult densities reduction was seen. However, after the 4th and 5th application only 50% reduction was seen in both larval and adult densities. The man biting rate was drastically reduced immediately after the application of the biocide and maintained at that level for more than a year. However, the fourth and fifth application did not give the same benefit though significant reduction was marked.

Though L_1 and L_2 stages of filarial parasite were found in the mosquitoes throughout the year, no infective larvae was seen in Khurdha area. The results in a nutshell are presented in Table-4.

Annual transmission index came down to 433 from 25,544 and risk of infection index was reduced to 0.04 from 1.29 during the second year of post intervention in Khurda. In Pipili (control area) these indices did not decline. The details are given in Table-5.

Exploratory analysis revealed some interesting patterns in the data. Mosquito density could be predicted with high accuracy based on environmental information. The same was true of biting rate. This is encouraging in that a potential for modelling appears to be a possibility (Courtsey: University of Pune). The project will be completed by October, 1996.

6.4.2. Toxicity of *B.sphaericus* against non-target species

Toxicity of *B.sphaericus* against non-target species like water bugs, frog tadpoles and Swiss mice were studied, where 250 ppm and 300 ppm of *B.sphaericus* were found to be lethal towards water bugs and frog tadpoles respectively, while it is nontoxic to Swiss mice even at a dose of 10,000 ppm daily for 10 days.

Table - 4 Entomological and transmission data in Khurdha and Pipili area

Month	Khurda						Pipili					
	Larval density	Adult density	Infection rate	Infect-vity rate	L_3 load	Man biting rate	Larval density	Adult density	Infec-tion rate	Infec-tivity rate	L_3 load	Man biting rate
Jan 94	8.2	13.1	1.4	-	-	52	42.0	49.2	8.4	4.4	3.5	253
Feb.94	8.5	12.9	0.8	-	-	43	33.6	41.8	7.2	2.9	2.6	234
Mar.94	5.3	11.8	1.9	-	-	23	27.8	35.2	6.0	2.4	3.0	223
Apr.94	4.1	7.6	1.0	-	-	18	19.9	31.1	5.0	1.8	3.5	181
May.94	2.8	6.3	1.1	-	-	28	32.4	28.7	3.5	0.8	2.0	148
June.94	14.2	20.1	1.4	-	-	72	38.0	50.1	1.4	0.6	2.0	199
July.94	17.6	21.2	1.3	-	-	84	41.0	59.8	9.2	2.4	2.5	323
Aug.94	18.5	20.5	1.4	-	-	79	40.0	52.3	10.5	2.6	3.0	380
Sept.94	14.6	20.2	1.8	-	-	87	44.0	53.0	9.4	3.2	3.0	412
Oct.94	15.5	22.8	2.0	-	-	93	48.6	59.0	10.6	4.9	3.0	368
Nov.94	18.2	23.2	2.1	-	-	91	44.8	57.5	9.4	4.7	3.0	285
Dec.94	18.8	24.0	2.6	-	-	98	42.0	52.1	6.3	3.4	2.5	310
Jan.95	18.5	23.8	2.1	-	-	93	43.0	50.2	8.6	4.6	3.0	247
Feb.95	18.8	22.5	1.4	-	-	91	34.0	42.8	7.4	3.1	2.5	232
Mar.95	10.1	14.5	0.8	-	-	65	29.8	34.9	6.1	2.5	3.0	218
Mean	12.93	17.63	1.54	–	–	67.80	37.3	46.51	7.26	2.95	2.84	267.53
± SD	5.66	6.03	0.52	–	–	28.00	7.81	10.16	2.60	1.31	0.48	76.87

Table - 5 Filariasis transmission indices in Khurda and Pipili

	Annual transmission index			Risk of infection index		
	Pre trial year1	Post trial year 2	Post trial year 3	Pre trial year 1	Post trial year 2	Post trial year 3
Khurda	25,544	538	433	1.29	0.08	0.04
Pipili	17,428	18,961	20,158	1.06	1.08	1.2

6.5. MOSQUITOCIDALS FROM PLANTS : THEIR BIOACTIVITY AND BIOEFFICACY

Scientist : Dr.A.P.Dash

Technical staff : Ms.U.L.Mohanty

Starting date : January, 1992

The efficacy and bioactivity of the effective plant extracts of *Anacardium occidentale* and *Pongamia pinnata* were tested against the three laboratory reared mosquito species *Cx.quinquefasciatus*, *An.stephensi* and *Ae.aegypti* at sublethal doses and the following observations were recorded.

6.5.1. Ovicidal activity

The ovicidal activity of LD_{50} doses of the two extracts, Anacardium methanol extract (AME) and Pongamia methanol extract (PME) for three mosquito species are as follows:

Mosquito species	Hatching inhibition (%) AME	PME
Ae.aegypti	100 ± 0.76	83 ± 0.01
An.stephensi	72 ± 0.32	67 ± 0.57
Cx.quinquefasciatus	63 ± 0.81	58 ± 0.48

Being exposed to LD_{90} dose of both AME and PME 100% inhibition in hatching of the eggs have been observed.

6.5.2. Abnormalities

It was observed that when the larvae hatched from treated eggs were allowed to develop as adults, the longevity was affected. The following abnormalities were observed during the development.

1. Larval mortality with black posterior part
2. Some larvae were twisted after exposure of 24 hours (effect of AME)
3. Larval-pupal intermediates
4. Pupal death
5. Adult mortalities due to unsuccessful emergence as:
   a) Abdomen inside exuviae
   b) Legs and wings inside exuviae and
   c) Some fully emerged dead adults have been found floating on water surface
6. Delayed egg laying and fed females dying without egg laying.

6.5.3. The early third instar larvae were treated with the sub lethal dose of AME and PME to observe their effect on the biochemical parameters like protein, lipid and carbohydrates (glucose and glycogen) following Lowny and Van Handle (1985) procedure. Some parts of this work is under progress.

6.5.4. Ovipositional active index (OAI) for the three mosquito species due to 5% AME solution is as follows

Mosquito species tested	OAI	Remarks
Ae.aegypti	- 0.55	The result shows that 5% AME solution is significantly ovipositional deterrent.
An.stephensi	- 0.42	Significantly ovipositional deterrent.
Cx.quinquefasciatus	- 0.45	Significantly ovipositional deterrent.

6.5.5. Toxicity to non-target species

The effective plant extracts were tested against the non-target species to observe their range of toxicity.

Plant extracts	Non-target species	LD_{50} (ppm) ± S.E.	LD_{90} (ppm) ± S.E.
AME	*Bufo melanostictus*	15.68±3.27	45.31±1.32
	Cirrhinus mrigala	11.23±1.35	33.11±0.57
PME	*Bufo melanostictus*	35.37±2.52	78.29±1.67
	Cirrhinus mrigala	42.31±1.92	87.15±0.13

The toxicity test against lower mammals are being studied.

6.5.6. Cyto-toxic effect of plant extracts against Mf of *Setaria digitata*

The lethal effect of the two plant extracts AME and PME were tested against Mf of *Setaria digitata* following a cytotoxicity method.

Test Extracts	LD_{50} (ppm) ± S.E.	LD_{90} (ppm) ± S.E.
AME	16.94 ± 0.04	56.06 ± 0.11
	18.28 ± 0.16	77.14 ± 0.05

6.5.7. Mosquitocidal activity of marine samples

Out of the five samples supplied by CDRI, Lucknow; CDR 134/F008/A/KO II was highly active against *An.stephensi* larvae causing 100% mortality at 30 ppm. CDR-234-F006/F008/A was active against *Cx.quinquefasciatus* with LD_{50}/LD_{90} doses being 40.09 ± 0.16/111.64 ± 0.38 ppm.

6.6. COMPARATIVE EFFICACY OF APHID EXTRACTS AND SOME JUVENOIDS AGAINST THE DEVELOPMENT OF MOSQUITOES

Scientist : Dr.A.P.Dash

Technical staff : Ms.Rajashree Mohapatra

Starting date : October, 1992

6.6.1. Effects of OMS 3031 on the development of *Brugia malayi* in *Aedes aegypti*

Aedes aegypti (black eyed Liverpool strain) were fed on *B.malayi* infected *Mastomys* blood in two batches, one normal and the other treated with sublethal doses of OMS 3031 at larval stages. The development of Mf was studied and the results revealed that infection rate, infectivity rate and L_3 load were significantly reduced ($P<0.001$) in case of treated mosquitoes.

Mosquito population	No. of mosq. fed on *Mastomys*	Mf density in *Mastomys*	No. dissected	Infection rate (%)	Infectivity rate (%)	L_3 load/ mosquito
Normal	95	28/10 µl of blood	31	90.3	51.6	2.9
Treated	70	28/10 µl of blood	30	73.3	33.3	1.4

6.6.2. Toxicity of OMS 3031 to non-target animals

The toxicity effect of OMS 3031 on non-target species viz., Tadpoles (*Bufo melanosticuts*), and Fish fry (*Cirrhinus mrigala*) was studied. Both the non-target species were subjected to different concentrations of OMS 3031 and the LD_{50}/LD_{90} values were calculated by probit regression equation. The results reveal that OMS 3031 has very low toxicity to both the non-target species when compared to mosquitoes.

Species tested	LD_{50} (Mean ± S.E.)	LD_{90} (Mean ± S.E.)
Fish fry (*Cirrhinus mrigala*)	0.137mg/lit. ± 0.076	0.228mg/lit. ± 0.198
Tadpole (*Bufo melanostictus*)	0.097mg/lit ±0.036	0.145mg/lit. ± 0.073

6.6.3. Biochemical characterisation of *Culex quinquefasciatus* after application of OMS 3031

The effect of OMS 3031 on protein, lipid, glucose and glycogen metabolism of larvae, pupae and adults of *Cx.quinquefasciatus* have been studied. Treatment of this compound at third instar larvai stage of the mosquito species decreases the protein contents in pupae and adults, decreases the lipid contents in larvae and pupae,and increases significantly the lipid contents in adults. It has no effect on the glycogen content in all the developmental stages while it reduces the glucose content in all stages. The results are presented in Table - 6.

Table - 6 Influence of EI_{30} dose of OMS 3031 on different biochemical parameters (mg/gm of tissue) of *Cx.quinquefasciatus*

Develop-mental Stages	Protein		Lipid		Glucose		Glycogen	
	CONTROL Mean ±SD	TREATED Mean±SD	CONTROL mean±SD	TREATED Mean±SD	CONTROL Mean±SD	TREATED Mean±SD	CONTROL Mean±SD	TREATED Mean±SD
Larvae	155.82 7.8	149.08 3.09	58.52 2.58	7.012 0.35	3.162 0.27	1.654 0.39	2.35 1.54	0.476 0.27
Pupae	147.88 3.4	137.26 1.45	77.61 2.80	56.1 0.81	3.14 0.36	0.906 0.19	3.94 1.23	1.006 0.116
Adult Male	191.08 3.38	141.92 1.69	11.44 1.39	101.17 5.49	10.38 2.36	4.016 0.02	3.762 0.87	2.378 0.71
Female	173.0 3.67	99.43 3.98	33.16 1.21	80.22 1.29	2.968 0.24	1.93 0.23	3.404 1.49	2.004 0.81

6.7. FIELD STUDY ON THE PERSISTENCE MALARIA TRANSMISSION IN FOUR PHYSIOGRAPHICAL REGIONS OF ORISSA IN COMPARISON WITH BORDERING AREA IN ANDHRA PRADESH

Investigators : Dr. A.P. Dash
Dr.N.Mohapatra
Sri S.K.Parida
Sri.R.K. Hazra

Starting date : March, 1995

6.7.1. The project started in March, 1995. Entomological and parasitological studies were initiated in 3 index villages viz., Siali, Gariaguda and Kitting in Kashinagar PHC. Out of 81 blood slides examined, 18 (22.2%) slides and 4 (4.9%) slides were positive for *P.falciparum* and *P.vivax* respectively. The detailed observations are presented in Table 7 and 8.

Entomological survey revealed (Table - 7) the presence of 6 Anophelines and 4 Culicines including *An.fluviatilis* and *An.culicifacies* the main vector species. Results of fever survey are tabulated in Table-8.

Table - 7 Mosquito fauna of three villages of Kashinagar (PHC)

Villages	Species	No. collected			PMHD		
		HD	CS	Mean	HD	CS	Average
SIALI	*An.subpictus*	0	3	1.5	0	1.1	0.55
GARIAGUDA	*An.annularis*	0	1	0.5	0	0.7	0.33
	An.fluviatilis	0	1	0.5	0	0.7	0.33
	An.subpictus	0	2	1.0	0	1.3	0.66
KITTING	*An.annularis*	1	0	0.5	0.17	0	0.1
	An.culicifacies	3	2	2.5	0.5	0.5	0.5
	An.fluviatilis	1	0	0.5	0.17	0	0.1
	An.maculatus	1	0	0.5	0.17	0	0.1
	An.subpictus	20	4	12.5	3.3	1.0	2.4
	An.vagus	6	5	5.5	1.0	1.25	1.1
	Cx.quinquefasciatus	1	0	0.5	0.17	0	0.1
	Cx.tritaeniorhynchus	2	2	2.0	0.33	0.5	0.4
	Cx.vishnui	4	0	2.0	0.67	0	0.3
	Ma.uniformis	0	1	0.5	0	0.25	0.1

Table - 8 Result of fever survey of Kashinagar (PHC)

Name of the village	BSE	Total positive	Pf	Pv	Pm	P.O.	SPR	SFR
SIALI	24	5	5	0	0	0		
GARIAGUDA	20	3	2	1	0	0		
KITTING	37	14	11	3	0	0		
TOTAL	81	22	18	4	0	0	27.16	22.22

6.8. NEW PROJECTS FOR INITIATION

6.8.1. INVESTIGATIONS ON THE OUTBREAK AND TRANSMISSION OF J.E. IN SUNDARGARH DISTRICT OF ORISSA AND PRAKASHAM DISTRICT OF A.P.

Investigators : Dr.A.P. Dash
Dr.S.S.S. Mohapatra
Dr.(Mrs.) N. Mohapatra
Sri. R.K. Hazra

Starting date : This project was recommended by the Scientific Advisory Committee in Feb., 95. The project will start from May'95 (Pre monsoon).

6.8.2. INTERRUPTION OF BANCROFTIAN FILARIASIS TRANSMISSION IN SELECTED AREAS OF ORISSA, INDIA BY PYRETHROID IMPREGNATED BED NETS AND CURTAINS

Investigators : Dr.A.P. Dash
Dr.N.Mohapatra
Sri.R.K. Hazra
Sri.H.K. Tripathy

Starting date : This project was recommended by the SAC in Feb.95. The project will be started after obtaining the extramural funding.

The area of Khurdha district viz., Durgapur and Simor with 8000 population each (1991 Census) are selected for the study. A preliminary study revealed the PMHD of vector and Mf rate as 50 and 15% respectively. A survey in February/March, 95 revealed that 50% of people in the locality use mosquito-nets regularly and 15% of population are willing to procure mosquito-nets for their use. Another 25% belong to the poorer section and will not be able to buy mosquito-nets. The rest 10% will need sufficient motivation to procure and use mosquito-nets.

SEMINARS/SYMPOSIA ATTENDED	PAPER PRESENTED
Dr. A.P. Dash	
8th All India Congress of Zoology, Bhubaneswar, April, 1994.	Participated
Round Table conference on Impregnated mosquito-nets at British Council Division/ ODA, Bhubaneswar.	Participated in discussion.
TDR/ICMR Filariasis Research Review Meeting, December, 1994.	*B.sphaericus* studies in India
Third Annual Conference of Indian Association of Epidemiologists, New Delhi, February, 1995.	Epidemiological evaluation of *B.sphaericus* application on Bancroftian filariasis transmission in Orissa.
X Annual Congress on Man and Environment, Goa, March, 1995.	Host selection pattern of twelve mosquito species in Orissa, India.
Dr. N. Mohapatra	
8th All India Congress of Zoology, Bhubaneswar, April, 1994.	Human blood index and forage ratio of *Culex quinquefasciatus*.
IV Symposium on Vectors and Vector Borne Diseases at RMRC, Bhubaneswar, November, 1994.	Titre of different batches of *Bacillus sphaericus*.
Workshop on Cultivation of Parasites at CDRI, Lucknow, December, 1994.	Participated in discussion.
S.K. Parida	
VIII All India Congress of Zoology, Bhubaneswar, April, 1994.	Man biting rate of two species of *Anopheles* in Orissa.
Workshop on "Cytotaxonomic technique in mosquitoes" at MRC, Delhi, 1994.	Participated in discussion
IV Symposium on Vectors and Vector Borne Diseases, RMRC, Bhubaneswar, Nov.'94.	Anthropophilic indices of seven anopheline species of Orissa, India.

R.K. Hazra

8th All India Congress of Zoology, Bhubaneswar, April, 1994.	Development of *B. malayi* in *Aedes aegypti* L.P. strain.
IV Symposium on Vectors and Vector Bome Diseases, RMRC,Bhubaneswar, November, 1994.	Susceptibility of three species of mosquitoes to different strains of *Bacillus sphaericus.*
Third Annual Conference of Indian Association of Epidemiologists, New Delhi. February, 1995.	Participated.

Utkal Laxmi Mohanty

8th All India Congress of Zoology, April, 1994, Bhubaneswar.	Susceptibility of mosquitoes to Chromatographic fractions of *Anacardium* extracts.
IV Symposium on Vectors and Vector Bome Diseases, November, 1994, Bhubaneswar.	Larvicidal activity of marine organism (mangrove) against the mosquitoes *Culex* and *Anopheles* species.
XIII Annual Conference of Toxicological Society of India, OUAT, Bhubaneswar.	Participated.
X Annual Congress of National Environmental Science Academy, Goa, March, 1995.	Cyto-toxicity of two plant extracts against *Setaria digitata*.

Rajashree Mohapatra

8th All India Congress of Zoology, April, 1994, Bhubaneswar.	Toxicity of the insect growth regulator (OMS 3031) on mosquitoes and swiss mice.
IV Symposium on Vectors and Vector Bome Diseases, November, 1994, Bhubaneswar.	Toxicity effect of OMS 3031, an insect growth regulator on some non-target species.
X Annual Congress of National Environmental Science Academy at Goa, March, 1995.	Non-toxicity of OMS 3031 to non-target animals.

OTHER ACTIVITIES

Dr. A.P. Dash

Was invited by British Council as a consultant on prevention and control of Malaria in Andhra Pradesh during August/September, 1994.

Invited as the Chief Speaker on the occasion of the Annual day function held on 22.02.94 at M.P.C. College Baripada and delivered a lecture on "Role of Biocide in Vector Control".

Organising Secretary of the International Symposium on Vectors and Vector Borne Diseases held on 12th-15th November, 1994 at Regional Medical Research Centre, Bhubaneswar.

Acted as the resource person at the workshop on Malaria Control Strategy, held on 23.12.94 at Visakhapatnam.

Acted as Ph.D. examiner of various Universities.

Invited as a Chief Guest of the Annual Seminar, organised by the Department of Zoology, Vyasanagar College held on January, 1995.

Dr. N. Mohapatra

Acted as Joint Secretary of the International Symposium on Vector and Vector Borne Diseases held on 12th to 15th November, 1995 at Regional Medical Research Centre, Bhubaneswar.

R.K. Hazra

Acted as Treasurer of the International Symposium on Vector and Vector Borne Diseases held on 12th to 15th November, 1995 at Regional Medical Research Centre, Bhubaneswar.

Training Programme

Ms. Julia Haward from the University of Bristol, U.K. has joined in the Department of Medical Entomology on 09.03.95 for three months to work on Triflumoron.

Papers published

Dash, A.P., Mohapatra, N., Hazra, R.K. and Rup, S. (1994) Tolerance of *Culex quinquefasciatus* Say to *Bacillus sphaericus* in Orissa, India., *Asian J. Zool. Science,* 2: 63-65.

Mohapatra, N., Dash, A.P. and Hazra, R.K. (1994) Feeding patterns of eight mosquito species in Orissa. *Asian J. Zool. Science*, 2: 17-24.

Ranjit, M.R., Dash, A.P. and Chhotray, G.P. (1994) Morphological and histopathological effects of Aphid extracts and Juvenoids in developing mosquitoes. *Asian J. Zool. Science*, 2: 25-25.

Mohanty, U.L., Dash, A.P., Mohapatra, N. and Ranjit, M.R. (1994) Bio-efficacy of *Anacardium occidentale* L. against mosquito species. *Biological Control of Insect Pest,* pp: 139-142.

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VII. HUMAN GENETICS DIVISION

According to the decision taken in the 6th SAC meeting and subsequently the endorsement of the same in the 8th SAC meeting, attempts have been made to bring the laboratory and the staff from the Field Station, Burla to RMRC (ICMR), Bhubaneswar. The staff have been transferred to RMRC, Bhubaneswar and re-allocated for different activities of the Centre.

7.1. STUDIES ON HEALTH STATUS OF KONDH TRIBE OF MOHANA BLOCK IN GAJAPATI DISTRICT OF ORISSA

Scientists : Dr.R.S.Balgir
Dr.G.P.Chhotray
Mr.A.Mohapatra
Dr.M.R.Ranjit
Mr.N.Marai

The International Labour Organisation (ILO) had prepared a note on the death of men folk in a Kondh village and this note was referred to ICMR, New Delhi for investigation by the WHO representative of India. It was alleged that a village 'Tinigharia' had lost most of its men from its 10 households and similar situation may be seen in other villages. Endemicity of malaria, G6PD deficiency, undernutrition and other causes were suggested to have precipitated such a peculiar social situation (leaving widows as heads of households). The note raised doubts regarding the possible occurrence of such isolated observations in the surrounding villages as well (quoting the lone male who survived).

The village 'Tinigharia' was traced as the hamlet of a main village, 'Kandhakalameri'. This hamlet is situated about 7 km. away (on eastern side) from Mohana block head-quarters. The main village is situated about half km. away from the hamlet. Another tribal hamlet, Chapatipanka, situated 3 km. away from Mohana block head-quarters (on western side) was selected for comparison.

Demographic particulars collected, revealed striking differences between the two hamlets under study. There were 17 females and 20 males in Chaptipanka, while there were 18 females and 7 males only in Tinigharia (Table - 1). Only difference is, one is towards west (Chaptipanka) and the other is towards east (Tinigharia) of Mohana block head-quarters. The other village, Kandhakalameri had 40 females and 33 males (Kondhs). The male:female ratios varied widely in the three settlements. The number of females per 1000 males or the ratio of females per one male varied by three fold for Kondhs in these three settlements (Table-1). Comparison hamlet, Chaptipanka had normal sex ratio (1:0.85), whereas the Tinigharia hamlet had abnormal sex ratio (1:2.57).

Table - 1 Demographic profile of Kondhs in 3 tribal settlements studied

Name of settlement	Male	Female	M:F ratio
Kandhakalameri	33	40	1:1.2
Chapatipanka	20	17	1:0.85
Tinigharia	7	18	1:2.57
Total	60	75	1:1.25
Major Groupings			
Mohana block tribal population			1:1.05
Luhagudi Gram Panchayat			1:1.12
Mohana block total population			1:1.02

Mohana block had a total population of 92,931 and M:F ratio was observed to be 1.0:1.02. This block had a total of 53,196 scheduled tribe (ST) population, with a M:F ratio of 1.0:1.05. The Gram Panchayat (GP) Luhagudi, where our study villages are located had a total population of 1670, with a M:F ratio of 1.0:1.04. Six villages of this GP had Kondhs, with a total population of 827 Kondhs and their M:F ratio was 1.0:1.12. The ILO report was describing the "Tinigharia" situation accurately for sex ratio. However, comparison hamlet (Chapatipanka) and main village (Kandhakalameri) were having near normal sex ratios. Kondhs in six villages of GP had normal sex ratios for tribals.

Age disparity between partners of a couple (10-15 or more years) and greater proportion of female child births (18 girls and 10 boys), greater loss of boys (4 boys and 2 girls), resulted in girls (16) out numbering boys (6) by about 2.7 times in Tinigharia (Appendix-I) . Absence of unmarried men (bachelors) between 20-30 years and presence of 5 unmarried women in the same age range, coupled with the fact that 4 households out of 5 are headed by widows, produced a peculiar social structure and was recorded by the ILO note. However, such peculiar aberrations were not seen in other two settlements studied by us. Hence, the demographic observations made on Tinigharia need not to be generalised.

Clinical examination and haematological investigations

A total of 21 persons from Tinigharia hamlet and Chapatipanka hamlet were examined to document obvious clinical signs. Ten individuals (6 males and 4 females) belonged to Tinigharia and 11 belonged to Chapatipanka (9 males and 2 females). The total sample consisted of 15 males and 6 females for clinical examination and haematological investigations.

More than one-third (38.0%) of the subjects examined had either splenomegaly or hepatomegaly or both. Three persons (14.3%) had both hepatomegaly and splenomegaly and one of them is a female. Three boys had lymphadenopathy (14.3%) and one of these had splenomegaly as well. Vitamin B-complex deficiency signs were seen in 19.0% of subjects, with a male preponderance. No frank cases of protein energy malnutrition (PEM) were seen in the hamlets. Body mass index (BMI) values of adults examined were in the normal range. There were no major differences between the two hamlets for their health and nutritional status. Malarial parasite was not seen in blood smears of all 21 persons screened during the survey time, which was a slack period for malaria. However, higher splenomegaly prevalence indicates endemicity of malaria in the hamlets studied.

None of the persons investigated revealed any abnormal hemoglobin in Tinigharia, whereas, two males from comparison hamlet, Chapatipanka had sickle cell trait. However, two persons (mother and son) of one family in Tinigharia hamlet were glucose-6-phosphate dehydrogenase enzyme deficient. The percentage prevalence for these deficiencies are in line with other tribal groups of Orissa.

It would appear from our studies that the five families of Tinigharia were not having any particular health problem in excess, apart from social and sex ratio peculiarity seen in two of the five families.

APPENDIX - I

Three brothers Pua Mallick, Tua Mallick and Kumbya Mallick have migrated from the main village Kandhakalameri and started a new settlement named as Tinigharia (meaning three houses). Pua Mallick's two sons built 2 more houses for themselves and established their own families. Among the five men, Hai Mallick is the youngest (now about 53 years) and is the lone survivor. Deaths among these men occurred at about 50 to 80 years of age, after some prolonged illness or otherwise, any where before 1989. Pua Mallick's daughter was married twice and lost both of her husbands.

Tua Mallick died 5 years back, leaving his widow (uncle's daughter), who is about 46 years of age. They had 5 daughters and 4 sons. Three sons and one daughter expired at very young age - probably at ages less than 8 years. Infection and under-nutrition might have some role in these cases. The oldest daughter is married and two daughters (22 and 26 years) are yet to be married. Thus there are higher number of male child deaths and two girls are awaiting marriage in this family.

Kumbya Mallick expired 8 years back leaving his widow (Kharinga) aged about 38 years. Kumbya is father's sister's son of Kharinga and it is a consanguineous marriage. This couple had four daughters and three sons and lost one daughter. Eldest daughter (17 yrs) is married into the main village (uncle's son).

Pua Mallick, brother of original three migrants and his wife died long back. They had three children, Bari Mallick (expired 10 years back), Hai Mallick (53 years now) and one female child Chipunga (46 years now). Bari Mallick had two daughters and three sons and lost one son. Chipunga got married twice and lost both of her husbands (10 to 15 years back). These two deaths have taken place in a distant village (8 km. away) named Kamliguda. She had no children and stays single. She joins the ranks of widows comprising of her two uncles wives and elder brother's wife. Thus there are four elderly ladies in four households of the village, widowed mostly between 1989 and 1980.

Hai Mallick is the only elderly male (53 years) available in the village. This couple has produced 7 girls and 1 boy and there is no child loss. Three of his daughters (22, 26 and 30 yrs) are awaiting marriage and there are no eligible bachelors in the village. Suitors had to come from neighbouring villages and pay Rs.1000 to Rs.3000/- bride price and arrange for a feast, etc. for taking his wife. Often this amount had to be earned by the young man himself. Higher bride price custom, lack of suitors in the immediate vicinity and greater proportion of female child births seem to have complicated matters. In Hai Mallick's own version, this village is for unattached women (Randas or widows).

Apart from the old couple, Pua Mallick and his wife, there were 9 deaths in the hamlet Tinigharia. None of these deaths occurred in the recent 5 years (1989-1994). The latest adult death was that of one of the original brothers i.e. Tua Mallick, during 1989. He had fever and pain in the mouth. Hai Mallick's brother i.e. Bari Mallick died during 1984. No specific reasons could be assigned to their deaths. The youngest of original three brothers, Kumbya Mallick died during 1986. He suffered (for 10 years) from swelling of abdomen and leg and progressive weakness.

It was very difficult to obtain specific reasons for these deaths. Mortality pattern does not indicate any specific disease or consequence of treatment of illness in the index village.

7.2. INVESTIGATIONS FOR TWO DECLINING TRIBES OF ORISSA

Scientists : Dr.R.S.Balgir
Mr.A.Mohapatra

To an enquiry from the ICMR Headquarters for investigations among two primitive tribes of Orissa, i.e., Didayi and Mankirdia, which seem to show a population declining trend as per the 1981 Census report, this Centre had thoroughly examined their case.

DIDAYI TRIBE

The name Didayi, means 'wild people', has been applied to them by the outsiders. The origin of Didayi, according to a legend, reveals that the Bondo were their elder brothers

and other tribes like the Gadaba and the Paraja were their other brethren. The Didayis have their own dialect which has much similarity with those of the Gadaba and the Bondo. Till to-day, the Didayis live in the midst of these tribal communities and have much cultural similarities with those of the Gadaba and the Bondo in particular. The Didayi is a typical tribe, described as "wild tribe" in the stratum of primitive semi-nomadic shifting cultivators and mostly found in the Konda Kamberu hill ranges of Eastern ghat in Malkangiri district of Orissa.

The area inhabited by the Didayi is intersected by the well known Bondo hills, forming a portion of Konda Kamberu hill ranges of Eastern ghats. The river Machhkund which flows along the ghats forms three well-marked sub-regions. The first sub-region lying on the left bank of the river Machhkund is comparatively plain where Didayi villages like Orapadar, Bhojaguda, Sanyasiguda, Totaguda, Jantra, Dakepada and Dabugam are located. The second sub-region lying between the river Machhkund and the Bondo hills is characterised by rugged and mountainous terrain where the elevation of land increases from the north-west, and slopes towards south-east. Here hill Didayi villages like Bayapada, Suripada, Ganjapada, Badkiaguda, Khajunguda, Amblibeda, Gisingijhola and Damarbeda are located. The third sub-region lying between the Bondo hills and the Jeypore-Chitrakunda road is partly plain and partly hilly. Muduliguda, Oringi, Purunaguma and Chillipada are important villages located in this sub-region.

In 1941, their population in Orissa was 1661 whichrose to 1978 in 1961, registering an average annual growth rate of 0.95%. They have a localized habitat in the erstwhile district of Koraput and now in the district of Malkangiri. From 1961 to 1971, the annual growth rate of 0.94% had been recorded according to census reports. In 1981 Census, their number has shown decline from 2164 in the year 1971 to 1977 in the year 1981 with annual reduction rate being 0.86%. This might had happened due to under-reporting. Since Bondo Paraja, Didayi and Gadaba who are related and live together in the same ecological environment and show socio-cultural similarities might have the chance of intermixing with these populations (Table - 2). When the other two tribes, i.e. Bondo Paraja and Gadaba are growing very well and showing population expansion, there is no reason why the Didayis under the similar bio-ecological environment should not grow.

Table - 2 District-wise distribution of Didayi, Bondo Paraja and Gadaba Tribes of Orissa in different Censuses

Districts of Orissa	Population											
	Census, 1961				Census, 1971				Census, 1981			
	Didayi	Bondo	Gadaba	Total	Didayi	Bondo	Gadaba	Total	Didayi	Bondo	Gadaba	Total
Sambalpur	-	-	24	24	-	-	-	-	3	5	6	14
Sundargarh	-	-	89	89	-	-	1	1	3	-	9	12
Bolangir	-	-	-	-	-	-	-	-	-	3	8	11
Keonjhar	-	-	-	-	-	-	-	-	-	4	-	4
Mayurbhanj	-	-	17	17	-	-	-	-	-	-	-	-
Balasore	-	-	-	-	-	-	-	-	-	8	2	10
Cuttack	-	-	-	-	-	-	-	-	-	2	104	106
Dhenkanal	-	-	-	-	-	-	-	-	-	-	7	7
Phulbani	-	-	-	-	-	4	-	4	-	1	1	2
Kalahandi	-	-	1188	1188	-	-	152	152	-	18	349	367
Koraput	1978	4677	42560	49215	2164	5334	46237	53735	1971	5854	56413	64238
Ganjam	-	-	45	45	-	-	204	204	-	-	10	10
Puri	-	-	-	-	-	-	-	-	-	-	4	4
Total	1978	4677	43939	50578	2164	5338	46594	54096	1977	5895	56913	64785

Further, as per the survey of Tribal and Harijan Research-Cum-Training Institute, Bhubaneswar under the Tribal Welfare Department, Government of Orissa which was conducted in 1982 for the Action Plan for Didayi Development Agency, Bayapada (Koraput district), their number in 42 villages in the same area has been recorded to be 3994 (Table-3). This further supports our suspicion of under-reporting of Didayi population in 1981 Census. Recently, in a similar survey in 38 villages in the month of August 1994 by the same Institute has shown their population to be 4460 persons. From 1982 to August 1994, the annual growth rate of Didayi comes out to be 1.17%. This again confirms positive growth rate and eliminates doubts regarding declining trend of Didayi population in Malkangiri district of Orissa.

Table - 3 Didayi Population in Koraput district of Orissa

Census	Didayi Population
1941	1661
1961	1978
1971	2164
1981	1977
1982*	3994
1991	Not Published
1994(August)**	4460

* A Report on "Action Plan for Didayi Development Agency, Bayapada (Koraput District)" by the Tribal and Harijan Research-Cum-Training Institute, Bhubaneswar, Orissa. 1982-83.

** A Report on "Action Plan for Didayi Development Agency, Bayapada (Malkangiri district)" by the Tribal and Harijan Research-Cum-Training Institute, Bhubaneswar, Orissa. 1994-95.

Although 1991 Census figures for Didayi population are not yet published, but the available information does not suggest any decline of Didayi population. However, the fertility, morbidity and mortality studies with health status would be able to appraise of the exact position of the tribe.

MANKIRDIA

In fact, both the Mankirdia and Mankidi are the one and the same and both types are none but the Birhors. The Birhors fall in to the category of hunting and gathering group having trade relations and exchange transactions with the local peasants. Instead of being settled at particular place permanently, the Birhors wander from place to place within a circumscribed area and known as nomadic tribe. It may be noted that the local people used to call the Birhors in different ways. In the district of Kalahandi and Sundargarh, they are named as Mankidi, whereas, in Mayurbhanj and Sambalpur districts, they go by the name Mankirdia.

The reason for calling the Birhors as Mankidi or Mankirdia is that they are experts and skilled in catching monkeys. When monkeys create havoc in the rural area and destroy crops, fruits and vegetables, the local people employ these Birhors to catch and kill them. Hence this wandering community was named as Mankidi or Mankirdia. They use nets made of 'siali' creepers for catching monkeys and eat the flesh of monkeys and sell the skin to local scheduled caste people for cash.

There are two types of Birhors, the Uthals (nomadic) and the Jagi (settled) Birhors. These nomadic or mobile Birhors move from one place to another in different groups in a specified area within the state. Often, they go out of the state and come back after 2 to 3 years. Each group comprises on an average 10 to 15 households which are related consanguineally and affinally. Their movements from place to place centre around three seasons, i.e. rainy, winter and summer, but more frequent in summer than in other seasons. Their frequent change of settlements is primarily in search of livelihood.

Birhors are found in Bihar where their population was 3464 in 1971 Census. In Orissa, their number was noted 98 in 1971 which rose to 142 in 1981 Census. As Birhor, Mankidi and Mankirdia are different terms used for the one and the same group of people, their number has been recorded under these heads as per the knowledge of the enumerator. When Birhor, Mankidi and Mankirdia population is combined in different censuses, they show progressive population growth during the decade from 1961 to 1981 in the state of Orissa (Table - 3). There is no population decline of the combined group in Orissa. The total population moved up from 1069 in 1961 to 1307 in 1971 and 1349 in 1981.

Further, because of the nomadic nature of the tribe and their migration from place to place within the state and outside the state also, there is likelihood of reporting them in one census at one place and at another place in the next census and so on (Table - 4). For example, in Sambalpur district in 1961, there was no Mankirdia tribe, but in 1971 Census, it has been reported to be 241 persons and in 1981 Census, their number was reduced to 69 in the same district. Similarly, in Mayurbhanj district in 1961, their (Mankirdia) number was 2, which rose to 483 in 1971 and again reduced to 256 in 1981 Census. In Bolangir district, in 1961 and 1971 Census, there was no Mankirdia, but in 1981 Census, they were reported to be 142 persons. Since inter-district and inter-state migrations do take place, it is quite

possible that they may be recorded in one state in one census and in another state in the next census and so on. Under these circumstances, it is very difficult to ascertain their exact number in one state which may obviously be misleading. This may create discrepancy for assuming the population decline of the tribe.

As already noted, when three closely related tribes were taken together (Birhor, Mankidi and Mankirdia), there was no population decline in this group of population in the state of Orissa. Recent surveys by the Tribal and Harijan Research-Cum-Training Institute,

Table - 4 Distribution of Mankirdia tribe in different districts of Orissa

District of Orissa	Population											
	Census, 1961				Census, 1971				Census, 1981			
	Birhor	Man kidi	Man kirdia	Total	Birhor	Man kidi	Man kirdia	Total	Birhor	Man kidi	Man kirdia	Total
Sambalpur	140	137	-	277	-	-	241	241	70	30	69	169
Sundargarh	-	248	-	248	90	128	3	221	33	38	151	222
Bolangir	-	23	-	23	-	23	-	23	1	1	142	144
Keonjhar	81	-	96	177	-	-	104	104	1	-	224	225
Mayurbhanj	38	131	2	171	3	-	483	486	-	123	256	379
Balasore	-	-	71	71	-	-	41	41	11	-	65	76
Cuttack	1	-	-	1	-	-	4	4	11	-	1	12
Dhenkanal	-	88	-	88	-	-	-	-	-	4	66	70
Phulbani	-	-	-	-	-	-	-	-	-	-	-	-
Kalahandi	-	-	-	-	5	149	-	154	-	-	25	25
Koraput	13	-	-	13	-	22	-	22	3	6	3	12
Ganjam	-	-	-	-	-	11	-	11	11	-	-	11
Puri	-	-	-	-	-	-	-	-	1	-	3	4
Total	273	627	169	1069	98	333	876	1307	142	202	1005	1349

Bhubaneswar indicate that there was no decline of the population in the other tribe (Didayi) under consideration. In view of the normal situation and normal growth rates for both the groups when related tribes were also examined carefully, the need for special investigations was not felt necessary.

7.3. DISTRIBUTION OF HEMOGLOBINOPATHY, G6PD DEFICIENCY AND BLOOD GROUPS IN SOME SELECTED TRIBAL POPULATIONS OF ORISSA

Scientists : Dr.R.S.Balgir
Dr.B.P.Dash
Mr.B.Murmu

Duration : Two years

Starting date : April, 1995

The sickle cell disorder is a molecular abnormality of the beta globin chain with substitution of glutamic acid by valine at the 6th codon position. This mutation grossly affects the solubility and crystallization of sickle cell hemoglobin under the conditions of hypoxia. The first report of sickle cell was made by a physician named J.B. Herrick based at Chicago in 1910 and discovered these peculiar elongated sickle shaped cells in the blood of an anemic person hailing from the West Indies.

The sickle cell disorder is an autosomal hereditary anomaly of erythrocytes. In heterozygous state (HbAS), the carriers are perfectly fit, healthy, and do not suffer from anemia, but compared to normal individuals (HbAA), these persons are more prone to infections, vaso-occlusive episodes under stress and hypoxia. The red cells of sickle cell trait persons survive usually from 100 to 120 days, blood looks normal but when deoxygenated by sealing a drop of blood under a coverslip after addition of a reducing substance like sodium metabisulphite (2%), the red cells show a peculiar bizarre sickle shape called sickling. The homozygous sickle cell disease (HbSS) results in hemolytic anemia, i.e. shortening of

life span of red cells, leading to severe and often fatal anemia. In some cases, the patient survives upto 2nd or 3rd decade depending upon the severity of the disease. The disease is further characterised by enlarged spleen, painful crisis, organ damage, impaired mental functions, increased susceptibility to infection and early death under stressful conditions.

The prevalence of sickle cell gene in India was first simultaneously demonstrated among the tribals of Nilgiri hills in South India and the tea garden tribals of Assam in 1952. Initially, a few sporadic studies on sickling were carried out without doing electrophoresis by the Anthropological Survey of India. Subsequently, the studies among the tribals of Bastar and Koraput in Central India showed fairly high frequency of sickling among various tribal and non-tribal communities. Later studies have found that it is common among the general castes, scheduled castes and scheduled tribes of India. The sickle cell gene is now widely prevalent throughout India barring a few states in Northern India (Jammu & Kashmir, Himachal Pradesh, Punjab and Haryana) and North Eastern India.

As per the estimates based on 1981 Census data, the sickle cell gene is prevalent in 75 districts in various states of India. Estimates revealed a figure of 1,21,375 homozygote (HbSS) sufferers and 2,434,170 heterozygotes/carriers (HbAS) among the tribal populations of India. As per these estimates there were 94,410 heterozygotes and 1726 homozygotes in 1981 in the state of Orissa. However, these estimates are far from the actual figures in Orissa.

The Sickle Cell Research Centre (ICMR) at Burla has studied the natural history, clinical and hematological aspects of the sickle cell disease in Orissa. As per the Sickle Cell Clinic records at Burla, the cases of homozygous Sickle cell disease, Sickle cell trait, hemoglobin D, E traits, Sickle cell-ß-Thalassaemia, Beta Thalassaemia major, Beta Thalassaemia trait and double heterozygosity like SD disease, SE disease were encountered (RMRC, Annual Report 1992-93). The sickle cell hemoglobinopathy is more common in Western Orissa than the Coastal region of Orissa. Further, it has been observed based on the Sickle Cell Clinic data at Burla that the sickle cell disease is equally common in general castes like Agharia, Kulita, Chasa, Gaud, Teli, etc. and scheduled castes like Ganda, Dhoba, Ghasi/Ghasia, Dumal, Pan, etc. and scheduled tribes namely, Gond, Munda, Bhuyan, Kharia, etc. Similar picture has emerged during a camp organised recently at Kania block, Kania.

To the best of our knowledge, apart from some of these hospital based reports, no systematic survey work has been done among different population groups of Orissa, especially the tribals. Available data reviewed by Dr.B.C.Kar (1987) revealed that conclusions on the prevalence of sickle cell gene for three districts was based on a sample size of more than 500. For another group of three districts in Orissa this conclusion was drawn from 200 - 500 subjects. Majority of other districts were covered with meagre number and the conclusion can be very different and far from reality. For example, the sickle cell gene prevalence in Koraput district was estimated to be very high (16.6%) based on 18 samples studied. It is now realised that at least 20-30 clusters with 60-80 persons per cluster had to be covered for giving an ultimate figure for a region in a state. A few surveys have been carried out among the tribals. There was no sickle cell disorder detected in the tribals like Juang, Bhuyan, Munda of Banspali Block of Keonjhar district of Orissa. It is important that randomly selected subjects from adequate number of clusters had to be covered for giving genuine figures.

In some states like Andhra Pradesh, Madhya Pradesh, Maharashtra, Gujarat, Rajasthan, etc. it was observed that the sickle cell hemoglobinopathy is more common among the tribals. The scheduled tribes constitute 22.4% of the total population of Orissa as per 1991 Census. The present study has been designed to know the prevalence and the actual magnitude of this disorder among major tribes of Orissa. This year, we have selected the Sundargarh district of Orissa for our study.

OBJECTIVES

1. To study the distribution of abnormal hemoglobins, G6PD deficiency and some blood groups in the tribal groups, based on adequate number of clusters per region or per group.
2. To look for some rare and abnormal genetic characters of clinical importance.
3. To suggest the best preventive and intervention measures.

MATERIALS AND METHODS

Though it is necessary to select villages or group of villages (like sub-centre) for PPS method (cluster sampling), as a preliminary step Ashram schools were chosen for our studies. We have collected data on Ashram schools and the student strength in each Ashram school in the state of Orissa. It has been observed that there are 10 Ashram schools in the district of Sundargarh with highest student strength of 1060 students as compared to Mayurbhanj district which has 7 Ashram schools with a total school strength of only 540. Since these Ashram schools are falling in the tribal area with 90% scheduled tribes and 10% scheduled caste students, these will be representing the local communities.

This year, therefore, we have selected Sundargarh district for our project on hemoglobinopathy and related disorders to carry out the studies among three tribes, namely, Munda, Oraon and Bhuyan. Tribal children attending Ashram school can be taken as representatives of the predominant tribes of the area. Adequate children in the age group of 6-18 years of both sexes will be covered for our studies from each tribe. In case, the school sample is found inadequate, some of the surrounding villages of the same tribe will be covered.

Blood samples will be collected by finger prick technique, when abnormal hemoglobin detected, intravenous blood sample will be taken for detailed study. In the field, total hemoglobin level, sickling test, solubility test and blood grouping will be done. Rest of the blood will be transported to the main laboratory for further analysis. All the standard techniques and procedures standardised in the laboratory will be strictly followed for analysis.

7.4. PRELIMINARY SURVEY CONDUCTED

Preliminary survey work was carried out in one of the Ashram school of Sundargarh district of Orissa. The findings of the survey have been reported here.

There is a preponderance of B, followed by A, O and AB blood groups among the tribes, namely, Oraons, Bhuyans and Mundas of Sundargarh district in Orissa (Table - 1). These findings are compatible with the general pattern of the Indian populations. One case of Rhesus negative was recorded among the Oraons. The prevalence of Rhesus negative is low among the tribals of Orissa.

Table - 1 Distribution of ABO and Rhesus blood groups among the Aleikera Ashram school tribal children of Sundargarh district, Orissa

System			Tribal groups		
			Oraon	Bhuyan	Other tribes
ABO blood groups :					
	O	No.	17	8	23
		%	23.3	22.9	53.5
	A	No.	20	9	11
		%	27.4	25.7	25.6
	B	No.	26	14	8
		%	35.6	40.0	18.6
	AB	No.	10	4	1
		%	13.7	11.4	2.3
	Total	No.	73	35	43
		%	100.0	100.0	100.0
Rhesus blood groups :					
	Rh+	No.	72	35	23
		%	98.6	100.0	100.0
	Rh-	No.	1	0	0
		%	1.4	0.0	0.0
	Total	No.	73	35	23
		%	100.0	100.0	100.0

The incidence of sickle cell hemoglobin has been found to be low (0.8%) among the tribals of Sundargarh district in Orissa (Table - 2). Only one case of sickle cell trait was detected in Kharia tribe. However, the occurrence of glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency is high among the Oraons (14.3%) of Orissa. The over-all prevalence of 12.7% for G6PD enzyme deficiency has been found in the present study.

Table - 2 **Distribution of hemoglobinopathy and G6PD enzyme deficiency among the Aleikera Ashram school tribal children of Sundergarh district, Orissa**

Parameters		Tribal groups		
		Oraon	Bhuyan	Other tribes
Sickle cell hemoglobinopathy:				
HbAA	No	73	35	22
	%	100.0	100.0	95.7
HbAS	No	0	0	1
	%	0.0	0.0	4.3
HbSS	No	0	0	0
	%	0.0	0.0	0.0
Total	No	73	35	23
	%	100.0	100.0	100.0
G6PD enzyme :				
Gd+	No.	36	3	16
	%	85.7	100.0	88.9
Gd-	No	6	0	2
	%	14.3	0.0	11.1
Total	No.	42	3	18
	%	100.0	100.0	100.0

About one-fourth of school children had normal Hb values (≥ 12 g/dl, Table-3). More than one-third of day-scholars were having normal Hb values, while only one-eighth of hostelers had normal Hb status. Two thirds (65.1%) of school children were observed to suffer from mild anemia (10-12 g/dl). The proportion of children with less than 10 g Hb/dl was found to be very low (3.0% only) among day-scholars, whereas it was 15.9% in hostelers. Overall prevalence of anemia (74.4%) was similar to rural Hyderabad prevalences, though its magnitude of moderate and severe anemia was found to be low in this group of school children. This picture of iron nutritional status is slightly better than rural Hyderabad school children, especially for the proportion with less than 10 g Hb./dl.

Table - 3 **Incidence of anemia among the Aleikera Ashram school (tribal) children aged 6-14 years of Sundargarh district, Orissa**

Grades of Anemia	Ashram school children					
	Day scholars		Boarders		Combined group	
	No.	%	No.	%	No.	%
Severe	0	0.0	1	1.6	1	0.8
Moderate	2	3.0	9	14.3	11	8.5
Mild	39	59.1	45	71.4	84	65.1
Normal	25	37.9	8	12.7	33	25.6
Total	66	100.0	63	100.0	129	100.0

Severe anemia = < 7.0 g/dl

Moderate anemia = 7.0 - 10.0 g/dl

Mild anemia = 10.0 - 12.0 g/dl

Normal = > 12.0 g/dl

AWARDS/HONOURS/OTHER RECOGNITIONS

1. Dr.R.S.Balgir, Assistant Director received Dr.B.C.Mehta Diamond Jubilee Endowment Fund Award of the K.E.M. Hospital and Seth G.S.Medical College Society, Bombay 1993.

2. Dr.R.S.Balgir, Assistant Director was selected for publication of his Biography in the directory entitled "Indo-American Who's Who Men and Women of Achievements 1994", which has been published.

3. Dr.R.S.Balgir, Assistant Director was invited to become an elected Active Member of the New York Academy of Sciences, New York, U.S.A. for the year 1994-95.

4. Dr.R.S.Balgir, Assistant Director received the Gold Medal Award 1995 in recognition and appreciation of contribution rendered towards community welfare by the Royal Society of Medicos and All India Medicos Society at the 8th National Medicos Congress held during 18-19th March 1995 at Jaipur, Rajasthan.

5. Dr.R.S.Balgir, Assistant Director was included as Member of the Scientific Advisory Committee of the 8th National Medicos Congress held during 18-19th March 1995, Jaipur.

AWARD OF Ph.D. DEGREE

Mr.B.P.Dash, Research Assistant has qualified for the Ph.D. degree of the Sambalpur University, Jyoti Vihar under the guidance of Prof.B.C.Kar and Dr.A.Mitra. The title of his Ph.D. thesis was "Study of glucose metabolism in sickle haemoglobin containing human red blood cells".

CONFERENCES/WORKSHOPS, ETC. ATTENDED AND PAPERS PRESENTED

1. Dr.R.S.Balgir, Assistant Director was invited to present a paper entitled "Population, Environment and Sickle cell Hemoglobinopathy in Orissa" in the Seminar on Population, Health and Environment held on 11th July 1994, Bhubaneswar.

2. Dr.R.S.Balgir, Assistant Director presented a paper entitled "Gene frequencies and spatial distribution of three predominant abnormal hemoglobins in India" in the Third International Conference on DNA Fingerprinting held during 13-16 December 1994, Hyderabad.

3. Dr.R.S.Balgir, Assistant Director participated in the 8th National Medicos Congress held during 18-19 March 1995, Jaipur.

4. Dr.B.P.Dash, Research Assistant attended the 5th Network Group meeting on Hemoglobinopathy on 20th March 1995, Bombay.

5. Dr.R.S.Balgir, Assistant Director and Dr.B.P.Dash, Research Assistant attended the IV International Symposium on Vectors and Vector Borne Diseases organised during 12-15 November 1994, Bhubaneswar.

PUBLICATIONS

1. Dash BP, Das RK, Pattanaik G and Kar BC (1993) Foetal haemoglobin in normal, sickle cell trait and sickle cell disease patients in Western Orissa, India. Ind. J. Hematol. Blood Transf. 11:232-236.

2. Kar BC and Dash BP (1993) Sickle cell disease with Diabetes mellitus. Ind. Practr. 46:913-15.

3. Mohapatra BN, Dash BP and Kar BC (1993) Serum immunoglobulins in sickle cell disease. JAPI 41:418-19.

4. Kar BC, Devi S, Pattanaik G, Dash BP and Das RK (1994) HbF levels do not determine the clinical course of SS disease. Ind. J. Hematol. Blood Transf. 12:225-29.

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VIII. ANTHROPOMETRY AND NUTRITION SECTION

8.1. HEALTH AND NUTRITIONAL PROFILE OF SCHOOL CHILDREN

Scientists : Mr.A.Mohapatra
Mr.N.Marai
Mr.B.Murmu
Ms.G.Mallick

Starting date : March 1994

Nutritional status of school children is one of the parameters to assess the nutritional status of a given area. School children constitute a major and convenient segment whose health and nutritional status will indicate the trends of changing profile in different regions. Nineteen schools were covered so as to document the nutritional status of school children by this Centre. Standard equipments were used for the measurement of height, weight, mid-arm circumference and skinfold at triceps. Methodologies for these measurements were standardized. Clinical nutritional status parameters were standardized as per the protocol used for NNMB by NIN, Hyderabad. Haemoglobin (Hb) estimation was carried out by cyanmethaemoglobin method, using filter paper to transport 20 µl of finger prick blood samples as per NIN, Hyderabad field based method.

A total of 4004 school children aged between 4-14 years were examined for clinical nutritional status and nutritional anthropometry. Girls constituted 47.2% of the total (Table - 1). Vitamin A deficiency was recorded in 10.6% children and more than half of these children had Bitot's spots (6% prevalence). Vitamin A deficiency prevalence was higher (15.3%) in the semi-urban schools. B-complex deficiency was seen in 13.5% of children, dominated by angular stomatitis. Prevalence of Vitamin A deficiency was found to

Table - 1 Total number of students covered

Schools	Total no. of students	No. of boys	No. of girls
Rural (12 schools)	1538	773	765
Semi-urban (6 schools)	1582	830	752
Urban (1 school)	884	513	371
Total (19 schools)	4004	2116	1888
No.as % of total	100.0 %	52.8%	47.2%

be more than the general pattern seen in such surveys in other parts of the country (Table - 2). Dental caries was observed only in urban school children (10.1%). A total of 3394 children were covered for Hb estimation. About one-fifth of children had normal haemoglobin (Hb) status i.e., $\geq$ 12gm Hb/dl of blood (22.2% of boys and 20.8% of girls).

Table - 2 Percentage of clinical signs among boys and girls of 5 to 14 years school going children

Type of school	Vit.A	Vit.B	Fluorosis	Scabies	Caries
Rural (n=1538)	10.6	19.7	4.0	1.2	NIL
Semi-urban(n=1582)	15.3	11.7	1.2	0.1	NIL
Urban (n=884)	2.1	6.1	NIL	0.2	10.1
Total (n=4004)	10.6	13.5	1.9	0.5	2.2
Male	11.8	13.9	2.2	0.5	2.4
Female	9.3	13.1	1.7	0.4	2.0

Anemia of varying degrees was seen in about 78.5% of boys and girls. Mild anemia (10.0 - 11.9.0 g Hb/dl) was seen in 45.6% boys and 42.0% girls. Significant anemia (less than 10 g Hb/dl) both moderate and severe anemia was seen in 32.2% of boys and 37.2% of girls under study. In this context it may be mentioned that tribal school children from Ashram schools had a lower prevalence (<10%) of significant anemia, compared to about one-third (34.6%) of children studied in and around Bhubaneswar. Correction of anemia may need therapeutic phase also, along with fortified salt in these schools, where as fortified salt alone may be adequate for anemia control in tribal Ashram schools. The prevalence of anemia was nearly similar (70.1% in tribal Ashram school and 78.5% in Bhubaneswar schools), though the quantum of moderate and severe anemia was three times greater in schools around Bhubaneswar.

Growth and development studies are in progress in these schools. Anthropometric data is under processing to assess the degree of growth retardation in different schools. In

Table - 3 Percentage distribution of anemia by different grades in school going boys and girls

Hb g/dl (Status) Sex	≥ 12.0 (Normal)	10.0-11.9(Mild anemia)	8.0-9.9 (Moderate anemia)	<8.0 (Severe anemia)	Total (All grades)
Boys					
Number	397	815	432	145	1789
Number as % of total	22.2%	45.6%	24.1%	8.1%	100.0%
Girls					
Number	334	674	449	148	1605
Number as % of total	20.8%	42.0%	28.0%	9.2%	100.0%
Both sexes					
Number	731	1489	681	293	3394
Number as % of total	21.5 %	43.9%	26.0%	8.6%	100.0%

the clinical pathology section the investigation on the prevalence of intestinal parasite infestations has been reported from these schools.

This study is in progress and the second round of measurements on heights and weights have been taken up.

8.2. ASSESSMENT OF NUTRITIONAL STATUS OF PRE-SCHOOL CHILDREN AND MOTHERS IN GANIA P.S. OF NAYAGARH DISTRICT

Scientists : Mr. A. Mohapatra
Dr. S.S.S. Mohapatra
Mr. N. Marai
Mr. B. Murmu
Mrs.G.Mallick

Starting date : April, 1994

This Centre was gearing itself in early 1994 to carry out studies on community nutritional status, when the request came from an NGO to study the population served by its programmes. Local logistics support was provided by this NGO named Gania Unayan Committee (GUC), through its village volunteers.

Gania Unayan Committee (GUC), an NGO has been working in these villages for more than a decade. Overall development was emphasized by this NGO with health volunteers placed in most of the villages. Supervisors of this NGO have been trained in identifying health and nutritional problems and they encouraged parents to initiate early health action. The GUC president approached this Centre to help them by documenting the health and nutritional problems of Gania Panchayat Samithi (G.P.S.) area.

Our team examined all the available children between 1-5 years and women aged between 15-45 years from 12 villages of three Gram Panchayats (G.P.) of the Gania P.S. A total of 519 pre-school children and 334 women were examined in this survey. These subjects

were clinically examined for nutritional deficiency signs/symptoms. Anthropometric measurements were recorded to assess nutritional status using standardised techniques and standard equipments.

Body weights of pre-school children were expressed as percentage of NCHS American standards of equal age and sex. The weight- for - age percentage values of children were classified into four grades of nutritional status (Gomez grading). Those children with 90% or more of weight- for - age values were designated as having 'normal' nutritional status. The group with 75-90% values were referred as 'mildly undernourished'; those with 60-75% values were referred to as 'moderately undernourished' and those with less than 60% as "severely undernourished". The two lower grades were combined and those with severe and moderate under-nutrition were referred to as significant under-nutrition. In case of women, body mass index (BMI) values were computed and classified into chronic energy deficiency (CED) grades as explained below.

Table - 1 Malnutrition grades (Gomez grading) in children aged 1-5 years in Gania Panchayat Samithi (G.P.S.)

Gomez grade Groups	Grade. III < 60% (severe)	Grade. II 60-75% (moderate)	Grade. I 75-90% (mild)	Normal ≥ 90%	Total
Girls	8 (3.2%)	88 (34.6%)	81 (31.9%)	77 (30.3%)	254 (100%)
Boys	12 (4.5%)	103 (38.9%)	101 (38.1%)	49 (18.5%)	265 (100%)
Both Sexes	20 (3.9%)	191 (36.8%)	182 (35.0%)	126 (24.3%)	519 (100%)
NNMB	8.7%	43.8%	37.6%	9.9%	100%

Out of 519 children classified by weight-for-age as percentage of NCHS standards and by Gomez grading for malnutrition, 3.9% of the pre-schoolers were found to be severely malnourished. The mild and moderate (grade I and Grade II) under-nutrition together was observed is about three-fourth of children (71.8%), each grade sharing equal number of children respectively. Normal nutritional status was observed in about one-fifth (24.3%) of children studied. These figures are definitely better than the rural picture reported by NNMB for 1 to 5 years children (Normal 9.9%; mild and moderate 37.6 + 43.8 = 81.4% and severe under-nutrition in 8.7% children; (Nutrition trends in India, NIN, Hyderabad, 1993).

Moreover, the quantum of significant under-nutrition in the two lower grades together (moderate and severe) constituted more than half of the total (43.8 + 8.7 = 52.5%) in the NNMB report survey of 1988-90, while these two grades together contributed about two-fifth of the total (36.8 + 3.9 = 40.7%) in this survey area in Orissa. Our study children thus not only had nearly half of severe under-nutrition (3.9% versus 8.7%) but also had more than double the quantum of normal nutrition status (24.3% versus 9.9%), when compared to NNMB figures for several states.

It was observed that the boys in this area of Orissa had slightly higher prevalence of severe under-nutrition (4.5% versus 3.2%) that was seen in the girls, which is in contrast to the popular beliefs in India. Our observations were not peculiar, because NNMB reported that magnitude of malnutrition was similar among boys and girls and this was also noted to be contrary to the general belief (Nutrition Trends in India, NIN, Hyderabad, 1993). In our study it was also noted that the prevalence of normal nutrition status was also higher in the girls (30.3%), compared to that of boys (18.5%).

A total of 334 non-pregnant women aged 15-45 were measured for heights and weights and BMI values were computed and classified according to standard classification given in the table - 2. The results were also compared with NNMB data on adult women.

Table - 2 **Grades of chronic energy deficiency (CED) in adult females (15-45 yrs) based on BMI***

BMI value (CED grade)	Number of females	Percentage prevalence	
		Gania	NNMB
≤ 16.0 (Third)	20	6.0	12.7
16 - 17 (Second)	32	9.6	13.2
17 - 18.5 (First)	84	25.1	25.9
18.5-25 (Normal range)	195	58.4	44.8
≥ 25 (obesity)	3	0.9	3.4

$$*BMI = \frac{\text{Weight (Kg)}}{\text{Height}^2\text{ (m)}} \times 100$$

The assessment of chronic energy deficiency (CED) in adult women aged 15-45 years (under-nutrition grades like Gomez grades for children) was undertaken by using BMI values (Table-2). The categories were, the least grade with BMI less than 16.0 (third degree); 16.0 - 17.0 (second degree); 17.0 - 18.5 (first degree); 18.5 -25.0 (normal nutritional status) and the highest grade with BMI values 25.0 or more (obesity).

The women of Gania had about 10% lower prevalence of significant under-nutrition (15.6% versus 25.9% ; third + second degree together) compared to average figures for rural India reported by NNMB. Also significant is the fact that Gania P.S. women had 13% higher prevalence of normal nutrition(58.4% versus 44.8%) and much lower prevalence of obesity (0.9% versus 3.4%) as against NNMB data. These data are in line with the finding of better nutritional profile of children in this P.S. area of Orissa.

Clinical nutritional deficiency signs of PEM like emaciation (1.9%) and marasmus (0.8%) were seen at a lower prevalence in the children examined. Bitot's spots were seen in less than 1% children, though xerosis was recorded in 6% of children. Bitot's spots and night blindness was recorded in less than 1% of the mothers examined, though xerosis was recorded in about 3% of the mothers. The clinical nutritional profile of mothers and children in this area is in line with better anthropometric measurements outlined above.

The NNMB surveys recorded 8.7% severe under-nutrition for the combined 10 states in the country and about 12% severe under-nutrition for Orissa pre-school children. Striking change towards improvement was observed in the Gania P.S. children with about 4% severe under-nutrition. Similarly, women from Gania P.S. had lower prevalence of third grade and second grade CED, compared to NNMB figures. On the other extreme the proportion of children and mothers with normal nutritional status was also found to be higher in the Gania P.S. area. This survey raises new hopes of encouraging results by blending government resources for developmental infrastructure and NGOs towards health development in human societies. This may be taken as the indicator, heralding new development with the opening up of economy and interaction between Government agencies, NGOs and people themselves. It would appear that we have probably recorded the advent of a new beginning in the nutritional uplift in the country and that too from Orissa which has the highest IMR and the highest poverty profile.

SEMINARS/CONFERENCES ATTENDED

1. Mr. A. Mohapatra was invited 8 times from 17.1.94 to 29.12.94 to train the ICDS supervisors at State Institute of Rural Development, Bhubaneswar

2. Mr. A. Mohapatra attended a National Seminar on "Prevention of Drug Abuse & Alcoholism" during 12-15 Sept, 1995 at Bhubaneswar and presented a paper entitled "Opium addiction-Rajasthan experience" as a special invitee.

3. Mr. A. Mohapatra presented a paper entitled " Anthropological perspective of filariasis" in the International Symposium of Vector and Vector Borne Disease during 12-14 Nov. 94 at Bhubaneswar.

4. Mr. A. Mohapatra was invited to talk on AIDS during a workshop organized by GUC on 23.1.95 at Gania.

5. Mr. A. Mohapatra organized a Training Course on "Nutritional Anthropometry" as the Convenor during 23-24, February 1995 to the members of Nutrition Society of India, Bhubaneswar Chapter.

IX. EXTENSION AND EDUCATION SECTION

Scientist: Dr. D. Das

9.1. HEALTH EDUCATION

9.1.1. Health Fair Camp at Konark

Dr.D.Das attended a Health Fair Camp as resource person, organized by SOLAR at Government High School, Konark from 14-15th Jan. 1995. There were about 60 student participants in this fair from 17 different schools who have opened stalls on various aspects of health and environment. During the programme he demonstrated to the students about the causative agents of filariasis, malaria and described methods of prevention/control of the disease.

9.1.2. AIDS Awareness Camp at Gania

RMRC, Bhubaneswar extended all technical expertise to Gania Unayan Committee for organizing the AIDS awareness camp at Gania on 23.1.95. Around 200 young men and women attended the programme. During the workshop they were briefed through slide-shows, posters and video programmes about the HIV, it's mode of transmission and mode of prevention by Mr.A.Mohapatra, R.O., Dr.D.Das, R.A., Dr.B.B.Pal, R.A. and Mr.C.C.Rath, SRF of RMRC, Bhubaneswar. The programme was also attended by local health personnel.

9.1.3. Educational visit of Sainik School students

About 130 students (Class VIII), of Sainik school, Bhubaneswar visited RMRC, Bhubaneswar on 24.3.95. After a brief introduction by Dr. K. Satyanarayana, Director, RMRC, to the students they were divided into 4 groups. Through practical demonstrations and lectures, health education was imparted on the causation and prevention of various diseases

like filariasis, malaria, AIDS, etc. Animal experimentation, mosquito rearing and vector control procedures were also shown to them. Dr.D.Das, Ms.R.Mohapatra, Mr.C.C.Rath and Mr.P.K.Sahoo organized the programme.

9.2. TRAINING PROGRAMMES

9.2.1. One day training course on HIV/AIDS

One day training programme on HIV/AIDS was arranged for four core-workers of Gania Unayan Committee. Information was presented with the help of posters, slides and video-shows regarding the mode of transmission and clinical aspects of AIDS and its preventive measures. It was emphasized that universal sanitation, safer blood transfusion and safer sex practices are the need of the hour to prevent transmission. This was followed up with demonstration of testing procedure for laboratory diagnosis of HIV seropositivity (ELISA). The programme was co-ordinated by Dr.Das, Mr.A.Mohapatra, Dr.B.B.Pal and Mr.C.C.Rath.

9.2.2. A short training programme (23.2.95 and 24.2.95) on Malnutrition and anemia to University students

RMRC in collaboration with NSI, Bhubaneswar chapter organised a short training course on 23 & 24 Feb. 1995 at RMRC. About 50 students from BJB College (Utkal University) and Home Science College (OUAT) participated in the course. Lectures were arranged to transfer knowledge on the importance of "Nutritional assessment at community level". "Nutritive value of Indian foods and planning of balanced diet" (By Dr.K. Satyanarayana), "Anthropometric methods for nutritional assessment", "Assessment of dietary intakes" (By Mr. A. Mohapatra) and "Anemia and it's control" (By Dr. M.R. Ranjit). Mr. A. Mohapatra and Dr. D. Das co-ordinated the training programme.

9.3. PUBLICATIONS/COMMUNICATIONS

9.3.1. Dr. D. Das has prepared manuscripts (for folders)in common local language (Oriya) on filariasis, immunization, fluorosis and AIDS. The folder on filariasis has been circulated to several scientists for modifications and suggestions.

9.3.2. Dr. D. Das participated in a T.V. programme on filariasis which was telecast by the Doordarshan, Bhubaneswar.

9.4. TRAINING

Dr. D. Das attended training on Gas Chromatography and H.P.L.C. at Institute of Microbial Technology, Chandigarh from 6.3.95 to 10.3.95.

9.5. PAPERS PUBLISHED AND CONFERENCE/TRAINING PROGRAMME ATTENDED

9.5.1. D. Das and Manoj K. Das (1995) Isolation of microfilariae and eggs of *Setaria digitata*. J. Helminthol., 69: 89-90.

9.5.2. D. Das and N.M. Pattnaik (1995) Separation of *Wuchereria bancrofti* microfilariae from blood by sedimentation". J. Helminthol. (Communicated).

9.5.3. Conference

Dr.D.Das attended IV Symposium on Vectors and Vector Borne Diseases. November 1994, Bhubaneswar and presented a paper entitled "Separation of viable microfilariae of *Setaria digitata*".

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