3. Epidemiology

MALARIA CLINICS

At 22-Sham Nath Marg, Delhi

A total of 67 patients attended the Malaria Clinic at 22-Sham Nath Marg or were referred from hospitals for blood examination and treatment of malaria during the period of January to December 2003. Of these, five patients were positive for malaria, one was diagnosed as *Plasmodium vivax* and four as *P. falciparum* infection.

At 2-Nanak Enclave, Delhi

A total of 2575 patients attended the Malaria Clinic at 2, Nanak Enclave during January to December 2003, of which 56 patients were found positive for malaria. Among these 43 were positive for *P. vivax* and 13 were positive for *P. falciparum*. Slide positivity rate (SPR) and slide falciparum rate (SfR) are given in Table 3.1. Clinical examination was done and specific and symptomatic treatment was given wherever necessary. Blood samples were collected for host parasite interaction studies from 37 patients (34 *Pv* and 3 *Pf*) and for genetic diversity studies from 46 patients (34 *Pv* and 12 *Pf*). The month-wise distribution of malaria cases are given in Table 3.1.

Diagnosis and treatment services were provided to more than 2600 patients at Malaria Clinics

Table 3.1 Data from Malaria Clinic, Nanak Enclave (2003)

Month	BSE	Total	*Pv*	*Pf*	Mix	SPR	SFR
Jan	45	0	0	0	0	0	0
Feb	123	0	0	0	0	0	0
Mar	177	1	0	1	0	0.56	0.56
Apr	225	2	1	1	0	0.89	0.44
May	191	1	0	1	0	0.52	0.52
Jun	88	5	5	0	0	5.68	0
Jul	170	3	3	0	0	1.76	0
Aug	266	9	8	1	0	3.38	0.37
Sep	410	22	17	5	0	5.36	1.21
Oct	603	11	8	3	0	1.82	0.50
Nov	196	2	1	1	0	1.02	0.51
Dec	81	0	0	0	0	0	0
Total	2575	56	43	13	0	2.17	0.50

CLINICAL TRIALS

Operational Activity for the Assessment of Therapeutic Efficacy of Chloroquine and Sulfadoxine-pyrimethamine in Uncomplicated falciparum Malaria

Antimalarial drug resistance is a major public health problem, which hinders control of malaria. In India, after the detection of first case of *P. falciparum* resistant to chloroquine in 1973, resistance has been reported to this drug from several parts of the country. Twelve *P. falciparum* monitoring teams from National Anti Malaria Programme (NAMP) now NVBDCP monitor resistance to chloroquine and in case RII/RIII resistance exceeds 25%, second line drug sulfadoxine-pyrimethamine is introduced in that particular PHC/district. However, the data is still limited and needs updating. Recently new protocols have been developed by WHO to have uniform data globally on the problem of drug resistance. Thus the present studies envisage generating data systematically in different parts of the country.

Therapeutic efficacy studies revealed only 50% cure rates with chloroquine in CHCs of Sundargarh district Orissa and still lower rates in Goa. However, chloroquine is still effective in Udaipur district (Rajasthan)

The present study was conducted at urban and rural sites– Districts Sundargarh (CHC Bisra and Kuarmunda) in Orissa; District North Goa (Panaji) in Goa and District Udaipur (PHC Rishabdev) in Rajasthan, which are located in different ecoepidemiological zones of the country.

Since study was also aimed at developing skills at local level in addition to generating data, therefore, Malaria Research Centre conducted orientation training for technical staff and medical officers of PHC, at the respective sites at the time of initiation of the study. The objectives were : to familiarise the team with the protocol; to orient the medical staff about the study procedures and their role in case of emergencies and setting up the study site and field laboratory.

In Orissa, preinitiation meeting was conducted on 20 August 2003 at MRC, Rourkela, to discuss study protocol and logistics. Co-investigators and staff of Malaria Research Centre, Doctors of Primary Health Centres, Consultants of IGH Hospital, District Malaria Officers of Sundargarh, Kuarmunda and Bisra

Table 3.2 Baseline characteristics of patients in Sundargarh district (Orissa)

Classification	Dose 25 mg/kg over 3 days	
	Bisra CHC	Kuarmunda CHC
No. of cases	63	70
Male/Female	37/26	28/42
Age in years (Range)	3.5–65	9 months–58 yrs
Parasitaemia/?l(Range)	1040–88800	1120–88800

Table 3.3 Classification of therapeutic response

Classification	Bisra CHC		Kuarmunda CHC	
	No. of patients	Prevalence	No. of patients	Prevalence
ETF	1	0.018	2	0.034
LCF	10	0.179	17	0.288
LPF	14	0.250	8	0.136
ACPR	31	0.554	32	0.542
Loss	6	0.1	3	0.2
Withdrawl	1	–	8	–
Total	63		70	

CHCs attended the meeting. In Goa, meeting was held on 21 August 2003 at the Office of the Director, Health Services, Goa. The meeting was attended by the Director of Health Services Goa, Deputy Director (Malaria & Filaria), Deputy Director (Public Health), Health Officer (Malaria) and State Entomologist. In Rajasthan, an orientation workshop was conducted at District Udaipur on 1 September, 2003. The Additional Chief Medical Officer, Dy. CMHO, Senior Medical Officers, Medical Officers of PHCs and technical staff attended the meeting.

High percentage of treatment failure to first line drug sulfadoxine-pyrimethamine was found in areas along the West Bengal-Nepal border

The therapeutic efficacy of first line drug chloroquine was determined using WHO 28-day protocol. Subjects were enrolled according to the inclusion and exclusion criteria and weekly peripheral smears and clinical examination conducted. The results of the study indicate that cure rates with chloroquine were about 50% in study CHCs of Orissa (Tables 3.2 and 3.3) and still lower in Goa. However, chloroquine was found to be still effective in Udaipur district (Rajasthan).

Assessment of Therapeutic Efficacy of Antimalarial Drugs against Uncomplicated *P. falciparum* Malaria in West Bengal as Part of Indo-Nepal Cross Border Activity

Among the areas with high transmission of falciparum malaria, areas along international borders pose serious problem. India and Nepal share their border along Bihar and West Bengal and there is free population movement across the border. On both sides of the border the presence of drug resistance has been documented. In West Bengal the first line drug is still chloroquine except in PHCs Ajodhya hills and Uttarlatabari of Purulia and Jalpaiguri districts respectively. In Nepal, the total number of laboratory confirmed cases are 10,000 annually, out of which 6–20% infections are due to *P. falciparum*. Drug policy was modified in 1998 following reports of resistance to chloroquine. First line drug is now sulfadoxine-pyrimethamine (SP). Since it is well-known that

distribution and prevalence of drug resistance parasites can grow quickly, there is urgent need to generate data on drug resistance in these areas.

The sites selected for the study were Sukna and Naxalbari PHCs of District Darjeeling, based on the baseline epidemiological data and the logistics. The medical officers and technical staff of the block primary health centre (BPHC) were identified as Co-investigators for the study.

The standard procedures were followed for the study. A total of 91 patients were enrolled at two study sites (Table 3.4). The analysis of the data revealed high percentage of treatment failure in these PHCs of border district (Fig. 3.1). Thus from the results of the studies it can be concluded that efficacy of first line drug is compromised in some parts of the country and there is an urgent need to review drug policy in these areas.

Table 3.4 Baseline characteristics of patients

Drug: Chloroquine (Dose 25 mg / kg over 3 days)	Sukna	Naxalbari
No. of cases	50	41
Male / Female	29 / 21	21 / 20
Mean age in yrs [M±SD]	21.6±13.1	22.1±12.7
(Range)	(0.5–50)	(2.5–56)
Mean parasitaemia / ?l on D0	3894.4±2731.9	7453.7±7035
[M±SD] (Range)	(1000–9640)	(1000–32,280)

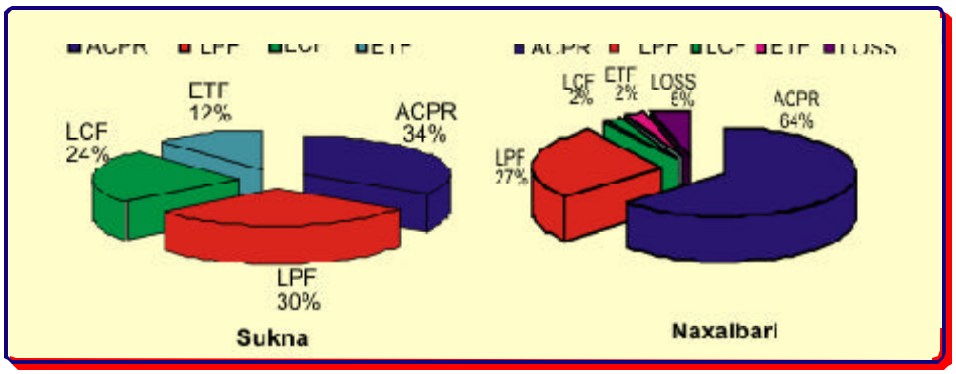

Fig. 3.1: Classification of response (% of total) in Sukna and Naxalbari PHCs, District Darjeeling

DIAGNOSTICS

Paracheck[â], a rapid diagnostic kit showed 100% sensitivity and > 90% specificity in the detection of P. falciparum

Quality check of rapid diagnostic test "Paracheck[?] " was conducted on request from NVBDCP/RITES. Approximately 56–58 test kits, each from seven batches of the dipstick were checked for sensitivity and specificity. The rapid test has 100% sensitivity for detection of *P. falciparum* for parasitaemia range of 100–19000/?l, which is within acceptable standards. The specificity is also more than 90% and is acceptable for HRP II based kits since the antigenaemia persists even after treatment. Thus the tests from above batches have been found to be of high quality in terms of sensitivity and specificity. The report has been submitted and kits were procured by NVBDCP.

Association of Leptospirosis in Patients of Severe falciparum Malaria

Severe and complicated malaria present with many complications like cerebral malaria, jaundice, renal failure, etc. Among these, acute renal failure occurs in less than 1% cases but the mortality from these cases is reported up to 45%. Data from Ispat General Hospital show nearly two fold increase in the number of patients with severe complications like acute renal failure and jaundice over a period of five years. Leptospirosis is an acute anthropozoonotic infection prevalent worldwide and is emerging as an important public health problem in India. The clinical picture of leptospirosis mimicks severe and complicated malaria especially that of acute renal failure and jaundice. The recent increase of acute renal failure and jaundice among malaria patients at Rourkela may be due to leptospirosis alone or in combination with malaria. Hence, a collaborative study was undertaken to rule out the presence and/or association of leptospirosis among these patients.

Detailed haematological and biochemical examinations were done in these patients. There were 23 severe malaria patients (microscopy/ICT +ve 12; microscopy/ICT –ve 11) and 16 uncomplicated malaria (microscopy/ICT +ve). Serum samples were tested for leptospirosis by leptospira IgM specific agglutination test and Lepto Tek Dri Dot test at NICD, Delhi. None of these were positive by serology for leptospirosis. The study will be continued with larger sample size.

REMOTE SENSING AND GIS

RS and GIS for Decision Support in Malaria Control

Blind field surveys in GIS predicted areas were conducted for *An. minimus* in Kamrup and Karbi Anglong districts of Assam. In GIS predicted favourable areas in Kamrup district *An. minimus* was found whereas in non-favourable areas in Karbi Anglong, the species could not be found. A team of experts visited Banbasa, Champawat district of Uttaranchal during May 2003. The team validated the site for collection of adults and larvae of *An. minimus*. Adults and larvae were collected and identified in Haldwani Field Unit laboratory by the expert team. Out of 117 larvae collected, 103 (88%) belonged to *An. minimus* group and in 45 adult specimens of *An. minimus* group collected, 2 specimens were identified as *An. minimus*. As per the recommendation of the group the specimens of *An. minimus* have been given for confirmation by molecular technique– PCR.

Electronic album on GIS predicted distribution of 58 anophelines was prepared

Electronic album on GIS based distribution of Indian anophelines was tested and final product on CD was prepared. This album consists of 58 maps each showing the GIS predicted distribution of individual species in India along with the blow-up map of GIS predicted districtwise favourable areas and the validation through reported surveys.

Delineation of Breeding Habitats and Landscape Features Suitable for *An. culicifacies* Abundance using Satellite Data

In continuation of ongoing work, satellite data of January 2002 and May 2001 was analysed in respect of 27 villages of high, moderate and non-malarious areas (or lowest) of three PHCs for generating statistics of landscape features. The difference in extent of land use features in villages of high and least malaria categories indicated that in villages of high malarious area, presence of water in water bodies (0.36–35.78%), permanent vegetation cover ranging from 24.9–85.72%, less barren area and scrub (0–12.9%), less barren rocks (0.13–9.68%) as compared to 1.44–3.2% water bodies, 15.07–34.19% vegetation cover; 8.57–30.21% barren area and scrub, and 0.35–20.33% barren rocks in least malarious area (Byalya). It was found that the presence of water in water bodies in the month of May is the most important landscape feature followed by less barren area and acacia plantation associated with high malarious area. Based on landscape features regrouping of villages from high risk malarious area to low risk malarious and vice versa is desirable.

Comparision of NDVI images of years 2000 and 2001 was also done to find out the significance of vegetation index in predicting malaria. It was observed that apparently in low malarious areas, vegetation index is low as compared to high malarious area, however, mapping of vegetation index at village level is desired for application of NDVI parameter in predicting malaria.

Remote sensing may be used for stratification of malaria at village level

necessitating the need for operationalisation of this technique in different problematic paradigms of malaria.

Impact of Climate Change on Malaria in India

In continuation of work, field visit was undertaken in Banaskantha district to find out the terrain features and distribution of breeding habitats and vector species supporting malaria endemicity. Meteorological data for 1985 to 2000 was procured from IITM, Pune. Correlation coefficient between malaria incidence and meteorological parameters was calculated. It was found that in monthly correlation between rainfall and *P. vivax* cases, positive correlation was found in general, while with *P. falciparum* it was negative.

Outbreaks of malaria occurred due to fluctuations in rainfall and not due to temperature fluctuations as evidenced in meteorological data of Tumkur and Bikaner districts

Retrospective analysis of temperature increase in 15–20 years was studied vis-a-vis malaria incidence in respect of Banaskantha, Tumkur and Bikaner districts. It was found that there was only 0.9°C increase in annual temperature over the years. Preliminary analysis indicated that outbreaks of malaria occurred due to fluctuations in rainfall (indirectly increasing RH) and not because of increase/decrease in temperature. Study is in progress for identifying transmission windows of malaria and correlation of meteorological parameters and malaria in all problematic districts of Rajasthan and Karnataka.

Independent Assessment of the Status of the Use of Larvivorous Fish as an Integrated Vector Control Measure under the Enhanced Malaria Control Project in Kota district

The project was undertaken to: (i) assess and describe the process of the establishment and distribution of larvivorous fishes in malaria endemic areas; (ii) to assess the impact of fishes in the control of mosquito breeding, adult densities and malaria-incidence; and (iii) to describe the sustainability and reproducibility of the fish introduction programme within the integrated vector control framework of NVBDCP.

IEC activities, community participation and GR at subcentre level are desired for use of larvivorous fish for malaria control in Kota district

Two Block PHCs namely Kaithun which reported highest API (7.03) and Pipalda where least GR work was done and fish hatcheries were selected for detailed survey under rural area. In Kota city also a few sites were selected for evaluation. The entomological surveys were conducted as per WHO procedures. Pre-designed questionnaires to elicit information on the establishment of fish hatcheries, method of distribution of fish, fish density, infrastructure available, involvement of other sectors, monitoring and evaluation of epidemiological and entomological parameters at district and PHC level were filled up.

The establishment of fish hatcheries in Kota city and endemic rural areas of Kota district is in process since August 2003. Hatcheries comprising mainly Guppy fish (*Poecilia reticulata*) and *Gambusia affinis* have been established in available step wells/irrigation wells/bavries mainly up to PHC level. Geographical reconnaissance (GR) of potential breeding habitats of mosquitoes has been done

to some extent in all the Block PHCs. Ponds, road-side ditches, seepage from canals, riverine stagnant pools (Chambal river which passes through Kota city) are the prevalent breeding habitats of anophelines. Therefore, release of fish up to village level and in all the habitats– Roadside ditches, ponds, pools, etc. still remains to be done.

It is premature to study the impact of fish on adult anopheline density / malaria incidence keeping in view that the fish are not released in all the prevalent breeding habitats of mosquitoes. As regards sustainability and reproducibility of fish introduction programme within the integrated vector control framework of NVBDCP, the programme of fish hatcheries established / release done so far in the available aquatic habitats was found successful. However, detailed GR of breeding habitats is required at sub-centre level and fish hatcheries should be established in ponds, specially constructed tanks at all sub-centres. From sub-centres the fish may be released in potential breeding habitats. IEC activities and involvement of other sectors like fishery department, irrigation and village Panchayats are needed for sustainability of fish introduction programme.

Serological assessment was found well-correlated with slide positivity rate and API in determining the malaria endemicity in Goa

Sero-epidemiological Assessment of Malaria in Migratory Population in Goa

Sero-epidemiological survey was conducted in a large number of migratory labour populations engaged in construction work in Goa. Results of the antimalarial seroreactivity against *P. falciparum* crude antigen and synthetic peptide AR1 along with the slide positivity rate (SPR) and annual parasite index (API) have been compared. A total of 841 samples from nine areas of Goa like Panaji, Porvarim, Caranzalem, Mapusa, Parnem, Vasco-da-Gamma, Margaon, Canacona and Ponda were collected during January 1998. API from 1999–2003 was also collected. API value for 1997–98 was found to be 42.7, ELISA O.D. against AR1 and *Pf* were 0.67 $\pm$ 0.23 and 0.65 $\pm$ 0.22 respectively. SPR value before and after sample collection was compared with ELISA O.D. Comparison of SPR (parasite load) and malarial antibody response (Table 3.5) in migratory population have been analysed as follows:

Table 3.5 Classification of malaria transmission in different study sites based on serological assessment

Area	SPR (1994-97)	AR1 ELISA O.D.	SPR (1999-2003)	Malaria transmission
Panaji	+++	+++	+++	High
Caranzalem	+++	+++	+++	High
Mapusa	+++	+++	+	Low
Parnem	+	+++	+	Low
Vasco-da-Gamma	++	+++	+	Moderate
Margaon	+++	++	+++	High
Canacona	++	++	+	Moderate
Ponda	+	+	+	Low
Porvorim	+++	+++	–	High

+++	High ELISA O.D.	>0.7	SPR >2
++	Moderate ELISA O.D.	0.4–0.7	SPR=2
+	Low ELISA O.D.	<0.4	SPR<2

The results confirm Panaji, Caranzalem and Mapusa had high malaria transmission, while in Parnem and Vasco-da-Gama antibody levels were higher compared to parasite load indicating labourers had high antibody titre though they had low infection rate. In Margaon and Canacona ELISA O.D. were moderate but parasite load was high. The possible explanation is that these groups of labourers are susceptible to malaria infection. In Ponda, transmission was very low both serologically and parasitologically, confirm the same. The results indicate that serologically it is possible to study malaria transmission dynamics.

Development of a Field Site for Malaria Vaccine Trial (A Collaborative Project with International Centre for Genetic Engineering and Biotechnology, New Delhi –Funded by Department of Biotechnology, Govt. of India)

Phase II studies were initiated for preparation of site for malaria vaccine trial in Sundargarh district, Orissa. Longitudinal studies indicated API—280.9 and 21.8, IPR—69.2 and 12.5, CPR—49.7 and 89 in forest and plain area villages respectively.

This is a collaborative project with International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi and is being funded by the Department of Biotechnology (DBT), Govt. of India under Jai Vigyan Mission. Longitudinal epidemiological studies were continued in two sets of villages in forest and plain areas characterised by hyper- and meso-endemic malaria situations respectively. During the year, new study villages for phase II studies were identified on the basis of baseline surveys and 22 new villages (forest–15 and plain–7) with a total population of 11,304 were included in the study area. Now there are 35 villages (forest–23 and plain–12) with a total study population of 15,525. The demographic information of the new villages was collected through census surveys and the demographic information of phase-I study villages was updated.

Entomology and Parasitology

Longitudinal parasitological surveys were conducted in all the 13 villages of phase-I study area. The malaria incidence was measured through weekly surveillance with the help of village volunteers. The month-wise malaria incidence in forest and plain areas was ranging from 7.7 to 43.1 and 0 to 3.8 respectively. The annual parasite index (API) in forest and plain areas was 280.9 and 21.8 respectively. The age-wise distribution of malaria incidence in forest and plain areas showed highest malaria incidence in 1–5 years age group and the annual parasite index (API) was 1053.3 and 36.5 respectively. In forest area the malaria incidence was inversely proportional to the increase in age but in plain area, malaria incidence was more or less the same in all other age groups and the difference was not very significant. Infant parasite rate (IPR) and child parasite rate (CPR) in the forest area were high throughout the year with a yearly average of 69.2 and 49.7 respectively. The average annual IPR and CPR in plain area

were 12.5 and 8.9 respectively. The highest attack rate due to *P. falciparum* – number of episodes per person per year in forest area was recorded in 1–5 years age group (0.82 episodes per child per annum). The average attack rate in the total population was found to be 0.24 and 0.02 in forest and plain area respectively. During the year peak transmission was observed during post monsoon months– October–November with another peak during March–April which was due to spring transmission. The proportion of different *Plasmodium* species in forest area was 85, 14 and 1 for *P. falciparum, P. vivax and P. malariae* respectively whereas it was 75, 25 and 0 respectively in plain area.

Malaria prevalence in the study population during different transmission seasons was measured through cross-sectional point prevalence surveys in all the study villages during March, June and November characterised by moderate, low and high malaria transmission seasons respectively. About 40% of the houses were selected randomly and all occupants of these houses were examined for malaria parasite irrespective of clinical symptoms. The clinical case history of each individual screened during point prevalence surveys was recorded on patient data sheet. The parasite rate in the forest area during these surveys was found to be 10.6, 11.5 and 14.5 respectively, whereas it was 1.4, 1.1 and 1.2 respectively in plain area. In forest area, about 40 percent of falciparum cases were asymptomatic out of which 5.2 percent were asymptomatic carriers. The proportion of children having >10,000 parasites / ?l was more as compared to adults. The spleen rate in children and adults in the forest area was 72.9 and 14.5 respectively, whereas in plain area it was 14.6 and 1.0 respectively. The average enlarged spleen (AES) in

The spleen rate in children was 72.9 and 14.6 in forest and plain areas respectively

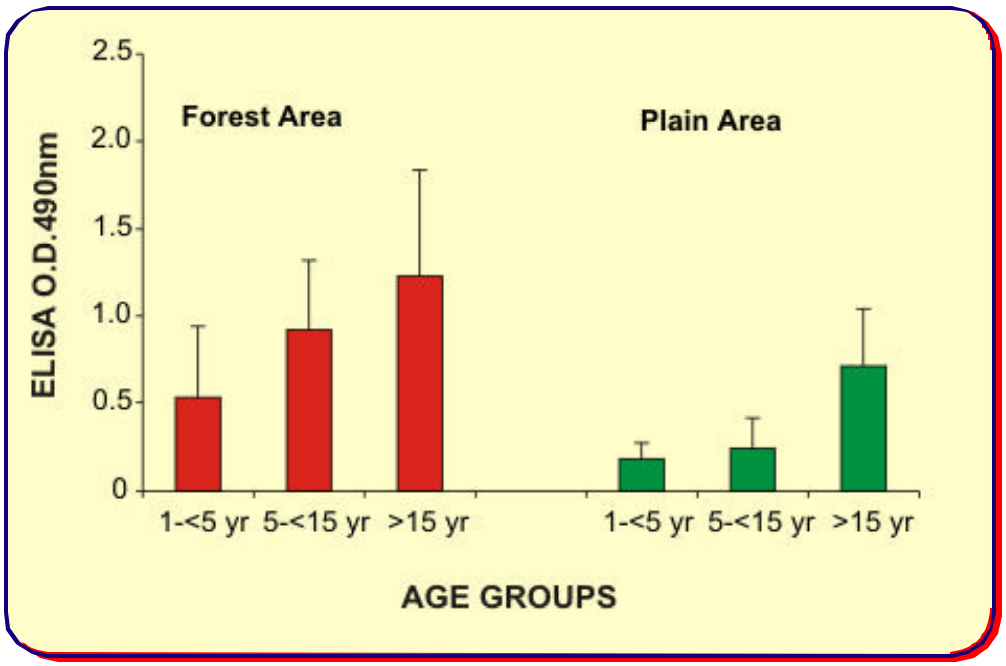

Fig. 3.2: Anti MSP-1_{19} antibody profile in different age groups of forest and plain areas during low transmission season

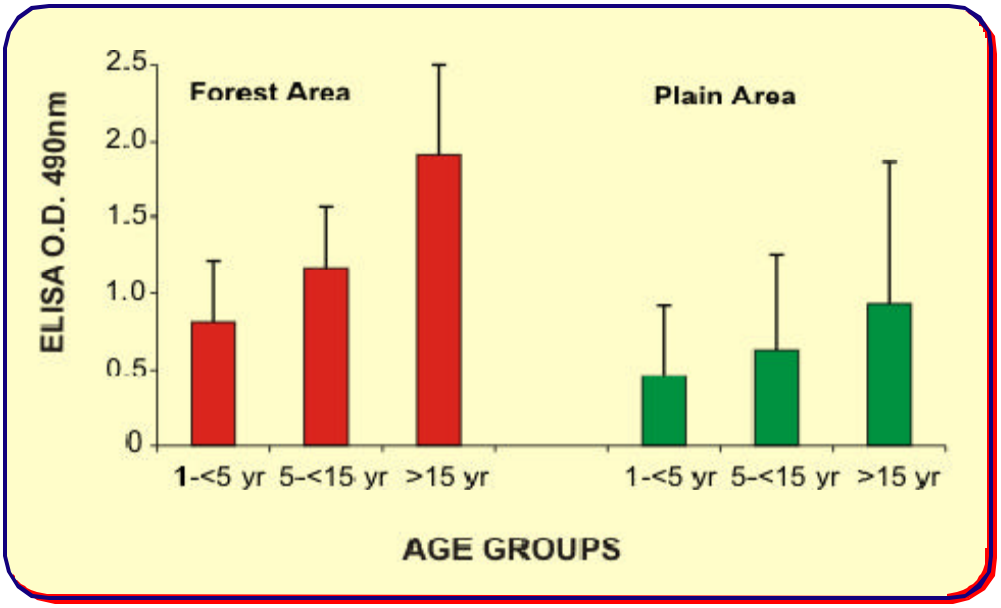

Fig. 3.3: Anti MSP-1_{19} antibody profile in different age groups of forest and plain areas during high transmission season

children in forest and plain areas was 1.8 and 1 respectively. During malaria prevalence surveys, blood samples were also collected for parasite diversity and immune response during different seasons and these studies were carried out at MRC, Delhi.

Age-dependent increase of specific antibody level was observed in individuals in both the areas during high as well as low malaria transmission seasons as revealed from host immune response studies.

Entomological surveys were carried out in two indicator villages each from forest and plain area villages of phase-I study area and one village each selected randomly from forest and plain areas of phase-II study villages. A total of 14 anopheline species from forest area and 10 species from the plain area were recorded. *An. culicifacies* was widely prevalent throughout the year in both forest and plain areas with prevalence rate of 38.8 and 24.9 per cent respectively. *An. fluviatilis* was restricted to the forest area and its prevalence rate was 6.3 percent. The density of *An. culicifacies* in the forest and plain areas was ranging from 0.5–36.2 and 0.7–35.7 respectively. The density of *An. fluviatilis* in the forest area ranged between 0–7.7. The mosquito-landing rate of *An. culicifacies* on human baits in both the areas was 0.50 bites per person per night, whereas that of *An. fluviatilis* in the forest area was 6.4 bites per person per night. The sporozoite rate and human landing rate of vector species were used to calculate entomological inoculation rate (EIR) in the study area during different transmission seasons. The EIR in plain area was nil whereas, in forest area it was 0, 0.085 and 0.35 infective bites per person per day during low, intermediate and high transmission seasons respectively.

Host Immune Responses

In continuation of earlier work, repeated cross-sectional surveys were conducted at two sites of forest area and two sites of plain area for the collection of finger-prick blood samples from different age groups during low and high

transmission seasons. In forest area, *P. falciparum* infection was detected in 11.25% (9/80) persons during low transmission phase, whereas in plain area among 57 none was found positive. During high transmission, 26.14% (23/88) were *P. falciparum* positive in forest area and 9.09% (5/55) in plain area. Indirect ELISA was conducted to measure the antibody level in 280 blood samples collected in both the surveys. It was observed that overall anti-MSP-1_{19} IgG profile was higher in study subjects of forest area than that of in plain area in both low and high transmission seasons. The age-dependent increase of specific antibody level was noticed in individuals of two areas in both seasons (Figs. 3.2 and 3.3). However, acquisition of anti-MSP-1_{19} antibodies during the time of high transmission phase was more compared to low transmission phase. The results demonstrated that during high transmission more than 95% of the sera contained antibodies to MSP-1_{19} in forest area, whereas about 89% of the sera contained anti-MSP-1_{19} antibody in plain area, though at low level. Thus the results suggest that there was enhanced antibody production against this molecule by natural infections among these individuals. The level of antibodies in study groups appeared to be related to their exposures to the parasite during high transmission phase.

MOI of 1.8 for MSP-1 and 2.0 for MSP-2 was reported among *P. falciparum* isolates.

Multiplicity of Infection

To understand the epidemiology of malaria it is important to understand the diversity existing among the malaria parasite species and its transmission intensity. Results obtained during 9th plan period has shown highly polymorphic nature of *P. falciparum* isolates in respect of MSP-1 and 2 gene with about more than 60% of the isolates harbouring more than one parasite genotype suggesting multiplicity of infection is mostly greater than 1. To confirm if phenomenon is universal in the area, analysis of multiplicity of infection among *P. falciparum* was continued and results confirmed our earlier observations. Analysis revealed MOI of 1.8 for MSP-1 and 2 for MSP-2 among the *P. falciparum* isolates.

Fifteen novel sequence variations of vaccine candidate antigens— MSP-$1_{19,}$ EBA-175 and TRAP were reported for the first time

Sequence Diversity in Vaccine Candidate Antigen Genes

For the success of development and testing of malaria vaccine it is important to know diversity existing among the parasite population of the area. Our earlier results revealed sequence variations in all the three vaccine candidate antigens MSP-$1_{19,}$ EBA175 and TRAP. Maximum variations were observed in EBA. Among 16 field isolates, 20 sequence variations were observed and of what 15 were novel, not reported earlier from any area. To answer the question if these variations effect the binding function of the parasite to human RBC. Isolates with mutations were expressed in expression vectors and results revealed binding pattern of sequence mutations is same as observed for control (camp-EBA-F2) sequence.

Also sera raised against control (camp-EBA-F2) sequence binding (rosette formation) was equal to the binding of the control sera. Studies provide support for the development of a sexual blood stage vaccine based on recombinant PfF2 expressed and purified from *E. coli* which may prevent free *P. falciparum* merozoites from entering into the red blood cells and thus prevent pathogenesis of malaria.