EVALUATION OF CHLOROQUINE (CQ) AND SULPHADOXINE / PYRIMETHAMINE (SP) THERAPY IN UNCOMPLICATED FALCIPARUM MALARIA IN NORTH LAKHIMPUR DISTRICT OF ASSAM, INDIA

By

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CERTIFICATION

This is to certify that this dissertation, entitled 'Evaluation of chloroquine (CQ) and sulphadoxine / pyrimethamine (SP) therapy in uncomplicated falciparum malaria in North Lakhimpur district of Assam, India', submitted by Dr. Pradyumna Kishore Mohapatra, in partial fulfillment of the requirements for the degree of Master of Applied Epidemiology, is the original work done by him and has not been submitted earlier, in part or whole, for any other (Publication or degree) purpose.

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for DIRECTOR

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Date: **Pradyumna Kishore Mohapatra**

DISSERTATION

Evaluation of chloroquine (CQ) and sulphadoxine / pyrimethamine (SP) therapy in uncomplicated *falciparum* malaria in North Lakhimpur district of Assam

Abstract

Background: During 2006, there was a large outbreak of malaria in North Lakhimpur district in Assam with high mortality. Chloroquine (CQ) and sulphadoxine / pyrimethamine (SP) are the first and second line of antimalarial drugs in the district. CQ failure was suspected to be factor for higher mortality. We compared a therapeutic efficacy of CQ, and SP -the first and second line of antimalarials to help National Vector Borne Disease Control Programme (NVBDCP) in modifying antimalarial drug policy

Methods: Patients with Pf mono-infection were allotted to either CQ (25mg per Kg in three divided doses in three days) or SP (25mg sulphadoxine with 1.25 mg pyrimethamine per kg as single dose) groups alternately after satisfying the inclusion and exclusion criteria. We recruited 50cases in each treatment assuming a treatment failure rate of 15% with an alpha error of 5% and a precision of 10%. We measured axillary temperature, asexual parasitaemia on Day 0, 2, 3, 7, 14, 21 & 28 or whenever patient reported any complaint. Adequate clinical and parasitological response was measured as per WHO criteria. All drug failure cases were treated with alternative therapy either with quinine or with artemisinin-SP combination therapy.

Results: 47 (94.0%) each of the 50 patients in the CQ and SP group completed the study and had 28% and 88% adequate clinical and

parasitological response after 28 days. Proportion of patients showing fever clearance and asexual parasite clearance was slower in CQ group as compared to SP group. No significant difference was observed in cure rate between intention to treat analysis and per-protocol analysis.

Conclusion: CQ has lost its relevance as first line of antimalarial treatment in North Lakhimpur. Using SP alone, as an alternative therapy will lead to development of quick resistance and limit its usefulness. Introduction of artemisinin based combination therapy can increase the therapeutic life span of SP and contain transmission. National Vector borne Diseases Control Programme changed the drug policy for the treatment of Pf cases to SP-artesunate combination as first line treatment in North Lakhimpur district.

Keywords: Assam, Artesunate, antimalarial, Chloroquine, combination therapy, drug failure, *falciparum*, malaria, North-east India

Early diagnosis and prompt treatment (EDPT) is one of the principal technical components of the global strategy to control malaria[1]. The effectiveness of this strategy is dependant on antimalarials which not only are safe but also available, affordable and acceptable to the population at risk[2]. The emergence and rapid spread of *Plasmodium falciparum (Pf)* strains resistant to commonly used antimalarial drugs poses a serious challenge to the effectiveness of this strategy[3, 4]. The first reports of chloroquine-resistant falciparum malaria was reported from Southeast Asia and South America almost half a century ago and since then resistance to antimalarials is spreading throughout the world, impending the efforts to control malaria[5]. Drug-resistant Pf is particularly serious problem in Southeast Asia, where strains are commonly resistant to chloroquine, antifolates, quinine, and mefloquine[6]. Among South and Southeast Asian countries, India reported 2.5—3 million cases annually during the last decade[7], accounting for more than 80% of malaria cases in the region. Half of the cases detected in India are due to Pf [8].

In Northeast region (NE) of India, malaria is a serious public health problem with an annual case load of 0.2–0.3 million, including 60–80% Pf cases[9]. The problem is further aggravated by the widespread resistance to chloroquine (CQ) to Pf. Chloroquine resistance to Pf was first detected in 1973 in Karbi-Anglong district of Assam state[10]. Resistance to sulphadoxine / pyrimethamine (SP) was first documented in 1992 in Changlang district of Arunachal Pradesh, NE India [11] though declining efficacy to this drug was reported earlier[12]. Since then CQ resistance has spread throughout the region and reports of SP resistance are on the increase[13]. Recently, treatment failure to CQ, SP and quinine, administered sequentially, was noted in 6% of Pf cases, indicating the presence of multi-drug resistant Pf strains in Changlang district bordering Myanmar[14]. Further, studies in different sites near Indo-

Myanmar border districts of Arunachal Pradesh in 2005 revealed that CQ has almost lost its therapeutic utility for the treatment of falciparum malaria in Myanmar-bordering districts of Changlang and Lohit in Arunachal Pradesh. SP is also losing its effectiveness and likely to fail in the near future, especially in areas closer to the Myanmar border. However, in areas further away the magnitude and spread of Pf resistance to CQ and SP is relatively slower, which may prolong the therapeutic life span of these drugs[15]. As per the Indian ational rug policy 1995[12] , CQ is the drug of choice in NE India, except in some selected areas where SP is used as the first line of treatment[11].

Epidemics of Pf malaria are common in foot hill areas of Assam. High mortality and morbidity during some of these epidemics might be due to increasing therapeutic failure to commonly used antimalarial drugs[16]. There was a large epidemic of malaria during May-June 2006 in Assam affecting eight districts. North Lakhimpur district was worst affected with 7906 malaria cases (Attack rate: 9%). 58% of the cases were due to Pf. The number of deaths reported from the district were higher (n=63, case fatality ratio=1.4%) than other districts in the state. Increase in vector population due to scanty rain fall and failure of timely implementation of vector control measures were most likely reasons for increased malaria transmission[17]. CQ and SP were used as drugs for first and second line of treatment during the epidemic. However, the local physicians who treated malaria cases during the epidemic expressed their suspicion of declining therapeutic efficacy of CQ and SP. Hence, we conducted a study with the objectives to (i) estimate the therapeutic efficacy of CQ and (ii) estimate the therapeutic efficacy of SP in uncomplicated Pf cases in the North Lakhimpur district of Assam. As a secondary objective we compared the therapeutic efficacy of the two treatments.

Methods

Study area and population

We conducted the study in the Nowbachia community health centre (CHC) area of North Lakhimpur district (Figure 1) in Assam having a population of 275,000. This CHC is located 380 Km, east of Guwahati - the state Capital and bordered Arunachal Pradesh on its northern side. The study area comprised of hills, foothills and plains with tropical rain forest cover and was inhabited by Hazong, Muttack and Nepali communities and tea tribe. Agriculture and forest-related activities are the primary occupations of the residents,

Patient enrollment

We collected thin and thick peripheral blood smears from fever patients attending the CHC, Nowbachia. We stained the blood smear slides with 3% Giemsa stain and examined them for presence of malarial parasites. We enrolled all the patients with Pf mono infection into study and conducted their complete physical examination. Patients who were not enrolled were treated according to the malaria species diagnosed and as per national antimalarial drug policy of National Vector Borne Disease Control Programme (NVBDCP), India.

Inclusion and exclusion criteria

Patients older than 1 year of age with acute fever (axillary temperature $\geq 37.5^{\circ}$ C) or history of fever in the preceding 24 hrs and a Pf mono-infection with an asexual parasite count between 1000 and 100 000/µl of blood were

eligible for inclusion in the study. We excluded those patients who presented with early signs of febrile illness of non- malarial origin; with general danger signs or signs of complicated or severe malaria [18]; those who had persistent vomiting or who vomited the anti-malarial drug after administration of a repeat dose after 1 hr; those with severe malnutrition; pregnant women; and patients with a history of sulfonamide sensitivity.

Sample size

We used WHO protocol "Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated Malaria" [18] for estimation of the sample size. Assuming a treatment failure rate of 15% with an alpha error of 5% and a precision of 10%, we required 49 subjects in each of the treatment groups.

Treatment schedule

We alternately allotted the Pf cases meeting the eligibility criteria to CQ or SP group. Both drugs were supplied by the Directorate of NVBDCP, Government of India. CQ was administered at 25 mg/kg body weight in three divided doses whereas SP was administered as a single dose at 25 mg sulphadoxine and 1.25 mg pyrimethamine/kg body weight. We administered both the drugs orally under direct supervision of the investigators. We followed-up the patients for a period of 28 days. Patients who did not get better were treated with parenteral quinine in 5% dextrose until they were able to take the drug orally. All patients were given 0.75 mg primaquine / kg body weight as a single dose, for radical cure, at the end of the study.

Clinical and parasitological evaluation

We collected thick and thin blood smears in duplicate on day 0, 2, 3, 7, 14, 21 and 28 from all the patients included in the study. We stained the blood smears with Giemsa stain and examined the stained smears for the density of malarial parasite by counting their number against 200 leucocytes. We expressed the asexual parasite density per µl of blood assuming an average leukocyte count of 8000/µl. Each blood smear was independently examined by two laboratory technicians. Inter-observer agreement between the two technicians was 98.5% (Kappa coefficient=0.97, p=0.0000).

We conducted a detailed clinical examination on all the follow-up days. We classified the clinical response as (i) Early treatment failure (ETF) if the patient developed danger signs (inability to drink or breastfeed, repeated vomiting, convulsions, lethargy or unconsciousness, inability to sit or stand up) or severe malaria on or before day-3 in the presence of asexual parasitaemia or when parasitaemia on day-2 was higher than day-0 count irrespective of axillary temperature or when parasitaemia on day-3 was present with axillary temperature ≥37.5^{o} C or when parasitaemia on day-3 was ≥25% of the count on day-0 (ii) Late clinical failure (LCF) if patient developed danger signs or severe malaria after day-3 or presence of parasitaemia and axillary temperature ≥ 37.5^{o}C between day-4 to day-28 (iii) Late parasitological failure (LPF): if there was presence of parasitaemia between day- 4 to day-28 irrespective of axillary temperature ≤37.5^{o}C (iv) Adequate clinical and parasitological response (ACPR): if there was absence of parasitaemia on day-28, irrespective of axillary temperature, without previously meeting any criteria of ETF, LCF or LPF[18].

Statistical analysis

We conducted primary analysis by intention to treat by including the case patients lost or withdrawn during the follow up by using excel data analysis sheet (www.rbm.who.int) for Kaplan Meier survival analysis[18]. We also conducted a per-protocol analysis of the primary outcome by excluding the case patients either lost or withdrawn during follow up. We also conducted the sub-group analysis according to age, sex and pretreatment asexual parasitaemia on Day0 to know the treatment outcome.

We analyzed the data with EPINFO (version 3.3.2) and SPSS (version 10.1) software. We used chi-square test to analyze difference in proportions in the two groups. We used independent t-test to analyze the difference in axillary temperature, age and weight of patients in both CQ and SP groups.

Human subject protection

The study was approved by the Ethical Committee of Regional Medical Research Centre, Dibrugarh, Assam. We obtained a written informed consent from the case-patients or from the parents/legal guardians in case of minors. We included only consenting patients in the study. The patients not responding to assigned treatment and not able take the drug orally were treated intravenously with 10mg of quinine per kg of body weight with 5% dextrose, 8 hourly till the patient is able to swallow the drug or with artemisinin combination therapy (artesunate plus SP combination drug) to those who can take the drug orally. Case patients who were found malaria positive but not included in the study were treated as per NVBDCP drug policy.

Results

We screened 3064 patients with acute fever or with history of fever within the preceding 24 hrs attending Nowbachia CHC area of North Lakhimpur district of Assam during April to July 2007. Of these, 203 (Figure-2) were positive for malarial parasite with a slide positive rate of 6.7%. Fifty nine (28.1%) patients had infection with *P. vivax*, whereas 131 (62.4%) had infection with *P. falciparum* and 13 (6.2%) had mixed infection.

Baseline characteristics

We enrolled 100 patients satisfying the inclusion criteria in the study, 50 in the CQ group and 50 in the SP group. The baseline characteristics of case patients with respect to age, sex, weight and axillary temperature were not different in two treatment groups (Table-1). Both CQ and SP were well tolerated and no adverse events observed. Three patients each from CQ and SP group were lost to follow-up as they moved out of the area.

Clinical response

Clinical response in CQ group

Per-protocol analysis: Of the 47 patients in the CQ group, 21 (44.7%) had ETF, 9 (19.2%) LCF, 4 (8.5%) LPF. The adequate parasitological clearance was 27.7% (13/47). Fifty percent (21/42) of the subjects became negative for asexual parasitaemia by day three, 15.4% (4/26) by day-7 and 18.8% (3/16) on day-28 (Figure 3). Age, sex, fever status or density of asexual parasitaemia on Day 0 did not show any effect on treatment outcome (Table – 2).

Intention to treat analysis: The cumulative success after 28 days follow up was 28% (95% CI 15 – 41). Survival analysis did not show significant difference from that of the per-protocol analysis (Figure 4).

Clinical response in SP group

Per-protocol analysis: In the SP group (n=47); 3 (6.4%) had ETF, 3 (6.4%) LCF and 0 (0.0%) LPF were respectively. The adequate parasitological clearance was 87.2% (41/47). Thirty eight percent (19/50) of the subjects became negative for asexual parasitaemia by day three, 0% (0/47) by day-7 and 4.5% (3/16) on day-28 (Figure 3). Therapeutic failure of SP was more marked in females (Table -2).

Intention to treat analysis: The cumulative success after 28 days follow up was 88% (95% CI 78 – 97). Intention to treat analysis did not show significant difference from that of the per-protocol analysis (Figure 4).

Comparison of CQ and SP drug

The adequate parasitological clearance varied significantly ($p<0.0001$) between SP and CQ group. The rate of asexual parasite clearance and fever clearance in patients on different days of follow up with chloroquine was found to be slower as compared to SP group (Figure 4).

Discussion

According to NVBDCP drug policy, chloroquine is the first line of treatment for Pf malaria in North Lakhimpur. However, the findings of our study indicated that the adequate clinical and parasitological response with CQ was only 28%. SP, which is the second line of treatment in the district, resulted in 88% cure rate.

As per the WHO[18] and NVBDCP guidelines[11], the drug policy needs to be changed, if the therapeutic failure rate of antimalarials is more than 25%. This study indicates chloroquine as first line drug in the treatment *P. falciparum* cases in North-Lakhimpur has lost its relevance. High mortality observed during the recent malaria outbreak in the district[17] was probably because of use of CQ as first line drug.

We observed low failure rate of SP drug during the study. This finding is tempting to substitute it for chloroquine which shows a failure rate which is not acceptable from a programmatic view. However, the long half-life coupled with increase in gametocytogenesis of SP provides a potent selective pressure for parasite resistance in areas of high transmission[19]. Widespread use of SP for the treatment of malaria might lead to development of high levels of SP resistance through continued accumulation of di-hydro folate reductase (dhfr) and di-hydro-ptorate synthatase (dhps) mutations and it may compromise the useful life span of newer antifolate combination drugs[20].

It is clear from this study that an effective, acceptable and sustainable antimalarial drug policy/strategy is urgently needed to contain the spread of drug resistant strains and to prolong the therapeutic lifespan of SP.

Combination of long acting antimalarial drugs, such as mefloquine, SP, or amodiaquine, with a short acting drug like artemisinin derivative can rapidly reduce parasite densities to very low levels. Such a combination reduces both the likelihood of parasites surviving initial treatment and exposure of the parasite to suboptimal levels of the longer acting drug[20]. Second, the use of artemisinin has been shown to reduce gametocytogenesis by 8-to 18-fold[21]. This reduces the likelihood that gametocytes carrying resistance genes are passed onwards and also reduce malaria transmission. In view of this, we recommend the use of SP-artesunate combination as a first line of treatment for Pf malaria in North-Lakhimpur district. Further, prevention of drug resistance can be achieved by focusing to reduce overall drug pressure through more selective use of drugs; improving the drug use through improvement in prescribing, follow-up practices, and patient compliance; or using drugs or drug combinations which are inherently less likely to develop resistance or have properties that do not facilitate development or spread of resistant parasites.

North-Lakhimpur district of Assam situated in an ecological niche where both the vectors i.e. *An. minimus* and *An. dirus* are transmitting the disease and appearance of high degree of chloroquine resistance and presence of SP resistance in this area should be a cause of concern. The spread of drug resistant strains of *P. falciparum* in Southeast Asia as well as in India remained limited to and corresponded with the geographical distribution of *Anopheles dirus* areas; thereafter, it moved out of the geographical limits of *An. dirus*. This was attributed to intense and selective transmission of resistant mutants of *P. falciparum* by *An. dirus*[16]. In areas where both vectors are prevalent, there is a possibility of spilling over of

resistant mutants from *An. dirus* to other vector species *An. minmus*[22]. This way such areas might act as a gateway for spread of drug resistant strains to other parts of the state and beyond. Adequate vector control measures can limit the spread of antimalarial drug resistance by curtailing transmission[23].

Our study had one major limitation. We did not randomize the allocation of the interventions. The baseline characteristics were similar in both the groups except density of asexual parasitaemia. The geometric mean density of asexual parasitaemia which indicates the severity of the disease was higher in SP as compared to CQ group. However, the adequate clinical and parasitological response in CQ group was significantly lower than SP. Hence, we believe that our conclusions would not have been influenced by the biased allocation procedure.

Based on our recommendations, NVBDCP, Govt. of India in its National antimalarial drug policy (2007)[24] has changed the anti-malaria drug policy in North-Lakhimpur district of Assam from CQ to Artemisinin based combination therapy (ACT).

References

1 World Health Organization. 1993. *A Global Strategy for malaria control, Geneva*. World Health Organization, Geneva.

2 Bloland PB et al. 1993. Beyond chloroquine: implications of drug resistance for evaluating malaria therapy, efficacy and treatment policy in Africa. *J Inf Dis*; 167 (4): 932 – 937.

3 Bloland PB & Ettling M. 1999. Making malaria treatment policy in the face of drug resistance. *Annals Trop Med Parasitol*; 93 (1): 5-23

4 White N. 1999. Antimalarial drug resistance and combination therapy. *Philosophical Transactions of Royal Society of London*; B(354) 739 – 749.

5 Breman, J. G. 2001. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am. J. Trop. Med. Hyg*. 64:1–11.

6 Wongsrichanalai, C., A. L. Pickard, W. H. Wernsdorfer, and S. R. Meshnick. 2002. Epidemiology of drug-resistant malaria. *Lancet Infect*. Dis. 2:209–218.

7 National Vector Borne Disease Control Programme (NVBDCP, formerly known as the National Anti-Malaria Programme)

8 Sharma, V.P., 2000. Status of drug resistance in malaria in India. In: Mahajan, R.C. (Ed.), Multi Drug Resistance in Emerging and Re-emerging Diseases. INSA Narosa Publication, New Delhi, pp. 191—203.

9 Mohapatra PK, Prakash A, Bhattacharyya DR & Mahanta J. 1998. Malaria situation in north-eastern region of India. *ICMR Bulletin*. 28, 21–30.

10 Sehgal PN, Sharma MID, Sharma SL & Gogai S. 1975. Resistance to chloroquine in falciparum malaria in Assam state, India. *J of Commun Dis* 5, 175–180.

11 NMEP, 1997. *Drug resistance and chemotherapy of malaria in India, an update*. In: National Malaria Eradication Programme (Enhanced Malaria Control Project) (eds GPS Dhillon, GS Sonal, U Arora & J Nandi) Ministry of Health & Family Welfare, Government of India, New Delhi, pp. 14–18.

12 Barkakaty, B.N., Chakravorty, N.K. and Narsimham, M.V.V.L. 1990. Efficacy of sulfalene and pyrimethamine combination drugs alone and with quinine in treatment of P. falciparum cases in chloroquine resistant areas of Northeast India. J Commun Dis; 72:178-84

13 Mohapatra PK, Prakash A, Bhattacharyya DR, Goswami BK & Mahanta J. 1999. Concurrent multi-drug resistant Plasmodium falciparum from northeast India, a case report. *Journal of the Association of Physicians in India* 47, 823–824.

14 Mohapatra PK, Namchoom NS, Prakash A, Bhattacharyya DR, Goswami BK & Mahanta J. 2003. Therapeutic efficacy of antimalarials in Plasmodium falciparum malaria in an Indo- Myanmar border area of Arunachal Pradesh. *Indian Journal of Medical Research;* 118, 71–76.

15 Mohapatra PK, Prakash A, Taison K, Negmu K, Gohain AC, Namchoom NC, Wange D, Bhattacharyya DR, Goswami BK, Borgohain BK and Mahanta J. 2005. Evaluation of chloroquine (CQ) and sulphadoxine/pyrimethamine (SP) therapy in uncomplicated falciparum malaria in Indo-Myanmar border areas. *Tropical Medicine and International Health*; 5 : 478–483

16 Nájera JA, Kouznetsov RL, Delacollette C. 1998. Malaria epidemics detection and control forecasting and prevention. World Health Organization, Geneva. WHO/MAL/98.1084

17 Mohapatra PK, Prakash A, Bhattacharyya DR, Murhekar M, Gupte MD & Mahanta M. 2007. Increased breeding of *Anopheles minimus* in slow running streams during an outbreak of malaria in North Lakhimpur, Assam, India. *Fourth TEPHINET Southeast Asia and Western Pacific Bi-Regional Scientific Conference*, Taipei, Taiwan. (Abstract no. OP36)

18 WHO, 2003. Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated Malaria. World Health Organization, Geneva, WHO/HTM/RBM/2003.50

19 Ogutu RB *et al*. The efficacy of pyrimethamine–sulphadoxine resistance of *Plasmodium falciparum*malaria in Kenyan children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, 94:83–84.

20 Bloland PB (2001) Drug Resistance in Malaria. World Health Organization, Geneva (WHO/CDS/CSR/DRS/2001.4).

21 Price RN et al. Effects of artemisinin derivatives on malaria transmissiblity. *Lancet* 1996;347:1654–1658.

22 World Health Organization. 2005. Susceptibility of Plasmodium falciparum to antimalarial drugs: Report on global monitoring 1996-2004. WHO/HTM/MAL/2005.1103.

23 Talisuna AO, Okello PE, Erhart A, Coosemans M, D'Alessandro U. Intensity of malaria transmission and the spread of Plasmodium falciparum-resistant malaria : A review of epidemiologic field evidence. Am J Trop Med Hyg 2007 ; 77 (6-Suppl): 170-180

24 Malaria Drug Policy (2007) Directorate of National Vector Borne Disease Control Programme, (Directorate General of Health Services), Ministry of Health and Family Welfare, 22-Shamnath Marg, Delhi-110054.

Fig 1: Location of North Lakhimpur district of Assam. India

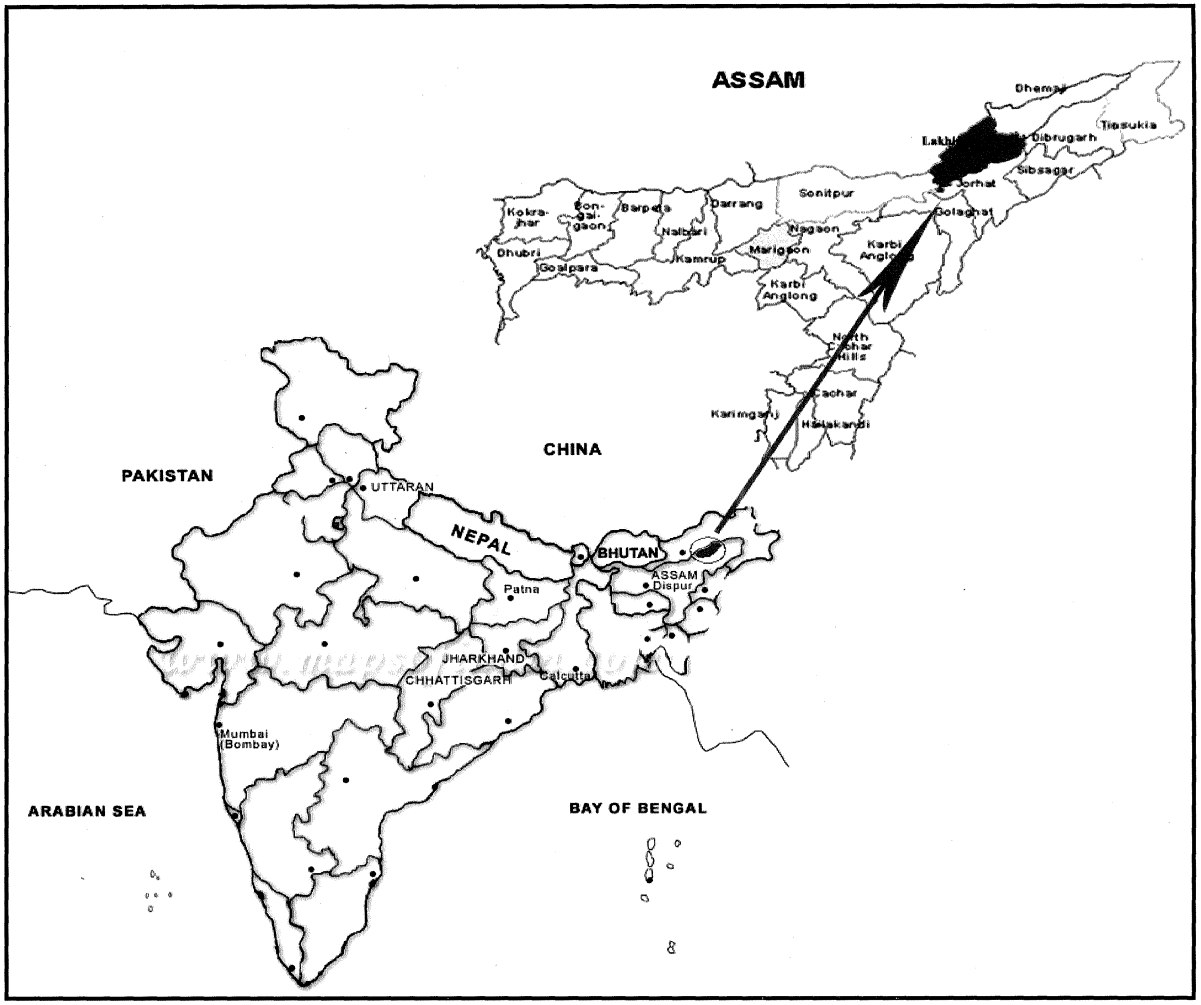


Table 1: Base line characteristics of study subjects

Characteristics		CQ Group	SP Group	P-value
Age	Mean (SD)	20.5 ± 13.4	22.8 ± 14.0	0.40
	Range	2-50	3-64	
	Under 5	6	3	1.0
	5-15 years	12	11	0.15
	Adult	32	36	0.92
Sex	Male	27	31	
	Female	23	19	
Weight	Mean (SD)	37.1 ± 16.0	39.8 ± 14.3	0.38
	Range	10 – 65	11 – 64	
Axillary temperature	Mean (SD)	38.6 ± 1.02	38.4± 0.5	0.37
	Range	36.6 – 41.4	37.2-39.6	
Asexual Parasiteamia	Geomean	9432 (95% CI 6779 –12777)	17107 (95% CI 13712–21343)	

CQ = Chloroquine, SP = Sulphadoxine / Pyrimethamine

Figure 2: Details of Therapeutic efficacy study of chloroquine and SP drugs carried out in Nowbachia PHC area of North Lakhimpur, Assam 2007

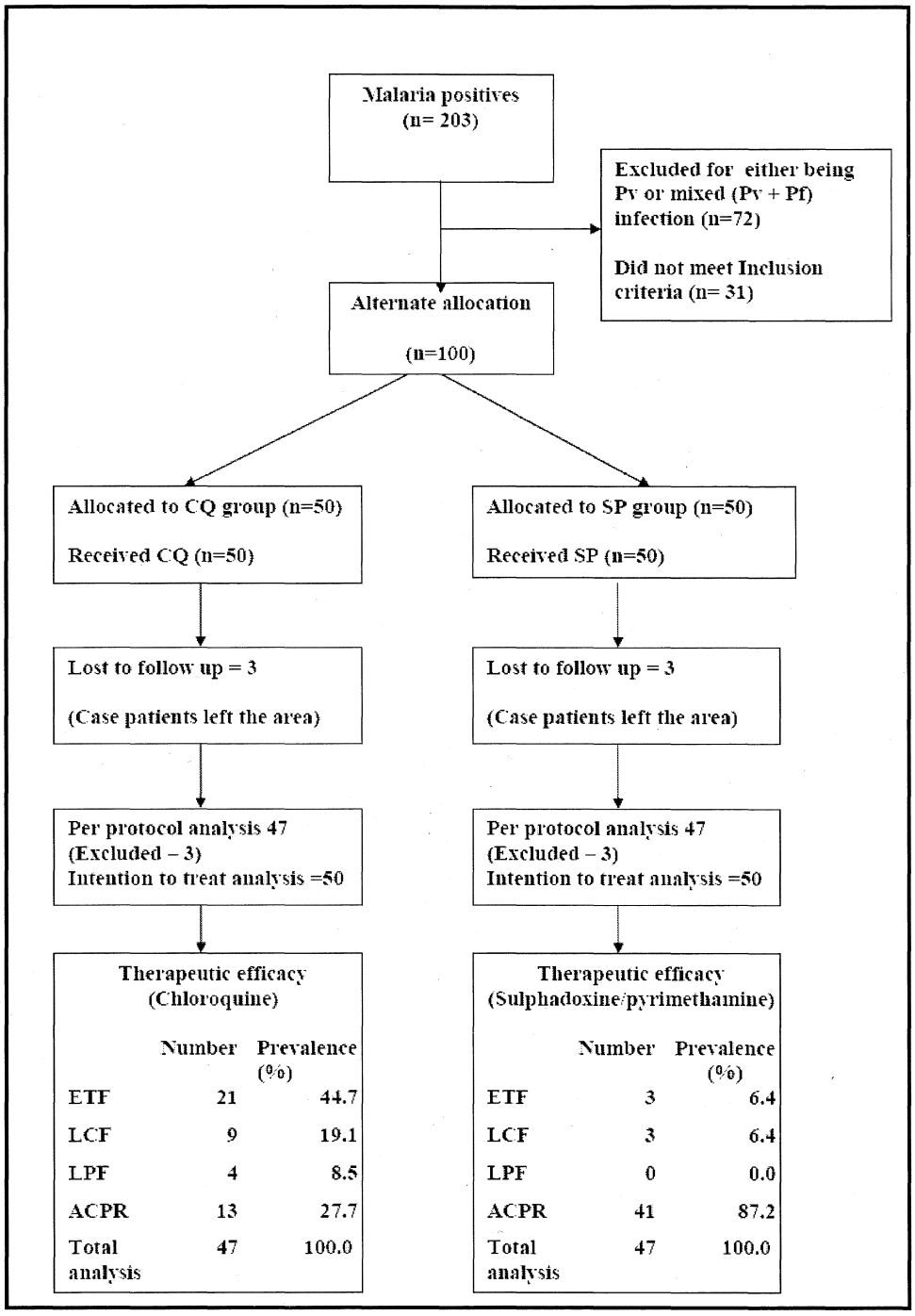
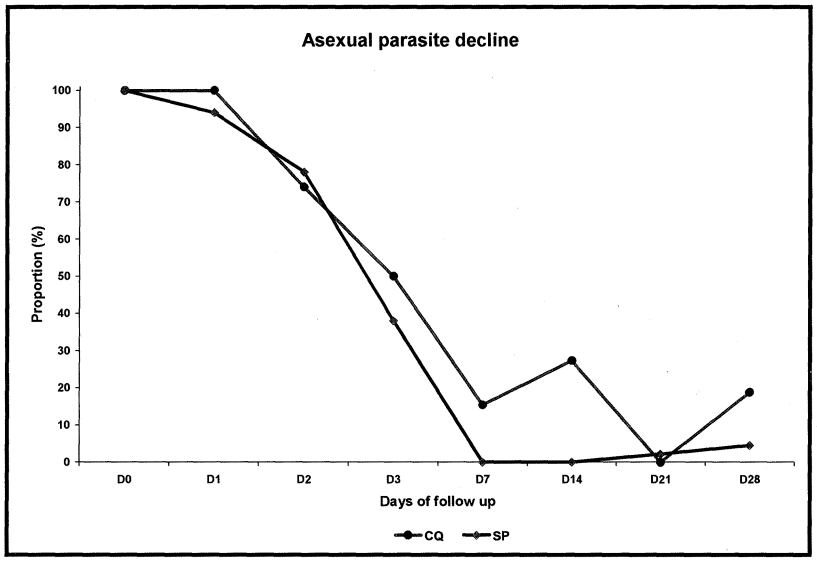
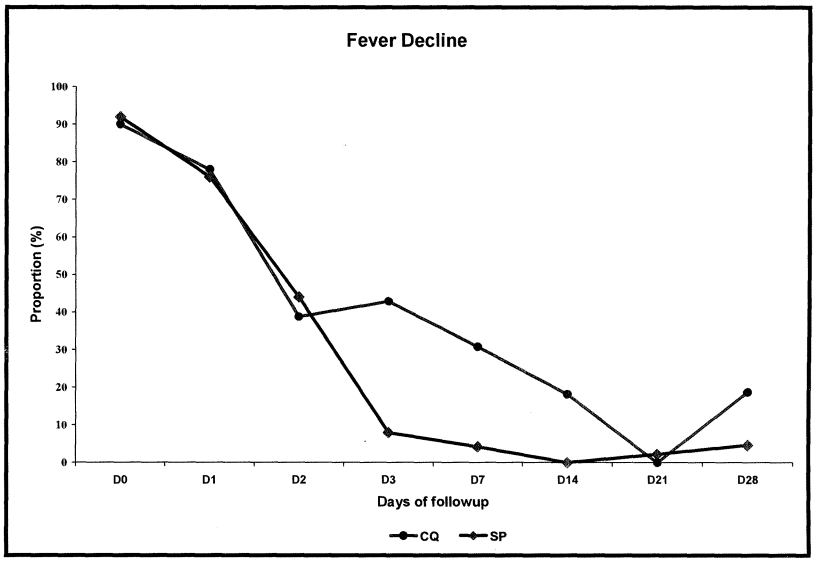


Figure 3: Proportion of patients with asexual parasitaemia (a) and fever (b) on different days of follow up.





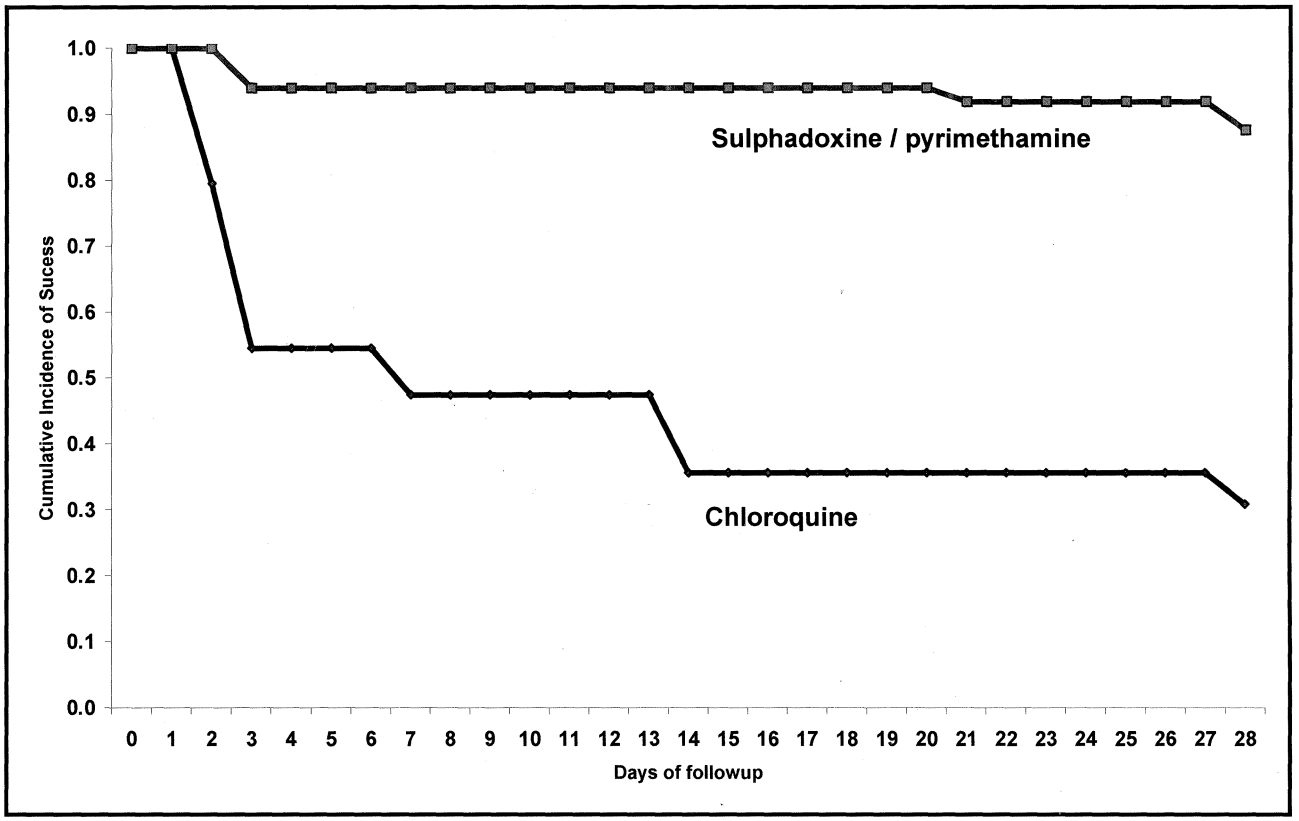
CQ = Chloroquine, SP = Sulphadoxine / Pyrimethamine

Table 2: Sub group analysis of therapeutic efficacy among chloroquine and SP drugs in North Lakhimpur district of Assam. India, 2007

Drug	Variable	Subgroup	Therapeutic failure (%)	ACPR (%)	RR	95% CI	
CQ	Sex	Female	16 (76.2)	5 (23.8)	1.1	0.8	1.56
		Male	18 (69.2)	8 (30.8)			
	Age group	≤15 Yrs	13 (72.2)	5 (27.8)	1.0	0.3	3.69
		>15 Yrs	21 (72.4)	8 (27.6)			
	Asexual parasite density on Day 0	≤10000 per µl	17 (70.8)	7 (29.2)	1.0	0.7	1.36
		> 10000 per µl	17 (73.9)	6 (26.1)			
SP	Sex	Female	5 (31.3)	11 (68.8)	9.7	1.2	76.1
		Male	1 (3.2)	30 (96.8)			
	Age group	≤15 Yrs	3 (18.8)	13 (81.3)	1.9	0.4	8.5
		>15 Yrs	3 (9.7)	28 (90.3)			
	Asexual parasite density on Day0	≤10000 per µl	2 (15.4)	11 (84.6)	0.96	0.67	1.36
		> 10000 per µl	4 (11.8)	30 (88.2)			

CQ = Chloroquine, SP = Sulphadoxine / Pyrimethamine

Figure 4: Comparison of cumulative therapeutic cure of chloroquine and SP drug in uncomplicated P. falciparum cases in North Lakhimpur, Assam 2007



Intention to treat analysis by Kaplan Mayer method

APPENDIX

Patient ID No **Drug ID No**

THERAPEUTIC EFFICACY EVALUATION REPORT FORM

Chloroquine

OR

Sulphadoxine / pyrimethamine

or

***P. falciparum* malaria**

(*uncomplicated*)

Dr. P. K. Mohapatra

MAE (FETP) Scholar

VI Cohort

Patient ID No

THERAPEUTIC EFFICACY REPORT
Drug – Chloroquine or Sulphadoxine/pyrimethamine

(Uncomplicated falciparum Malaria)

Summary

PHC/CHC/ HOSPITAL

INVESTIGATOR

AGE (Yrs)

SEX

WEIGHT (kg.)

DIAGNOSIS

DATE OF ADMISSION

DATE OF DISCHARGE

TREATMENT GIVEN **DRUG LEVEL CODE**

RESULT

TOLERABILITY
EFFICACY
SIDE EFFECTS

SIGNATURE OF THE INVESTIGATOR

Patient ID No

CONSENT

I.....

exercising my free power of choice, hereby give my consent to be included as a subject in the therapeutic assessment of the antimalarial drug, namely chloroquine (CQ) or sulfadoxine + pyrimethamine (SP) for the treatment of *P. falciparum* malaria. I understand that I may be treated with these drugs for the disease, I am suffering form. I have been informed to my satisfaction by the attending physician the purpose of the therapeutic efficacy assessment and the nature of drug treatment and follow up including the laboratory investigation to monitor and safeguard my body functions. I am also aware of my right to opt out of the trial at any time during the course of the trial without having to give the reasons for doing so. The consent form has been readout to me in my own language and I clearly understand the contents.

Signature of the attending physician Signature of the patient/ guardian

Date: Date:

Patient ID No

Case sheet no

Age **Sex**

Complaints with duration

History of present illness and details of therapy if any.

Patient ID ↓ / Time & Date → Category	Day 0			Day 1			Day 2			
	First	Second	Third	First	Second	Third	First	Second	Third	Fourth
Temp in °C										
Asexual Parasite (200 WBC)										
Pulse rate										
SBP										
DBP										

Signature of the Investigator / Treating Physician

CLINICAL DATA SHEET **Patient ID No**

Sl No	Parameter	Days							
		0	1	2	3	7	14	21	28
1	General Condition								
Vitals									
2	Pulse rate (per minute)								
3	Blood Pressure (systolic)								
4	Blood Pressure (diastolic)								
5	Respiration (per minute)								
6	Temperature (^{o} C)								
8	Pallor (Present / Absent)								
9	Jaundice (Present / Absent)								
10	Oedema / General Anasarca (Present / Absent)								
11	Dehydration (Present / Absent)								
ABDOMEN									
12	Spleen (palpable / Not palpable)								
	If palpable size in cms								

Contd **CLINICAL DATA SHEET**

Sl No	Parameter	Days							
		0	1	2	3	7	14	21	28
13	Chest								
14	Lungs								
15	Heart								
CNS									
16	Level of consciousness (normal/ abnormal)								
	If abnormal score as per Glasgow coma scale								
Pupils									
17	Size (Normal/Dilated/Constricted								
18	Reaction to light (Normal /Slugish / absent)								
19	Motor System								
20	Sensory System								
21	Plantar reflex (Normal / Absent / Extensor)								
22	Any other significant findings								

Date **Signature of the investigator**

Patient ID No

LABORATORY DATA SHEET

Sl No	Symptom	Days							
		0	1	2	3	7	14	21	28
	PARSITOLOGICAL		*						
1	Asexual parasite per 200 WBC		*						
2	Asexual parasite per μL of blood		*						
3	Sexual parasite per 200 WBC		*						
4	Sexual parasite per μL of blood		*						

* Not to be done

Date

Signature of the Investigator

Therapeutic assessment of antimalarial drugs (Counting Chart)

PLACE _____ PERIOD _____ Drug _____

Sl.No	CASE NO	Age	Sex	Asexual Parasite Count / 200 WBC with Gametocyte Count							
				D0	D1	D2	D3	D7	D14	D21	D28

Therapeutic efficacy trial of chloroquine / sulphadoxine /pyrimrtahamine

FOLLOW UP DATE RECORD CHART

Sl.No	Case No.	Age	Sex	Day0	Day1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28	Remarks

REVIEW OF LITERATURE

1. REVIEW OF LITERATURE

1.1 Malaria: The Disease Burden Scenario

Malaria is a leading cause of significant morbidity and mortality in developing countries and remains a major public health problem. Despite the impressive initial results of the WHO initiated malaria control and eradication programs in 1950's, technical, operational and socio-economic difficulties have led to setbacks and resulted in resurgence of malaria during 1970s in many parts of the world including India. Malaria ranks third (Incidence rate (IR) 2.3%) among major infectious disease after acute pneumococcal respiratory infections (IR 3.5%) and tuberculosis (IR 2.8%)[1]. Recently, Hay *et al.*, (2004)[2] have estimated that 48% of the global population remain exposed to the risk of malaria, a situation that has deteriorated since the early 1990s (~ 46%). However, this figure is substantially higher than the 40% global population widely cited earlier[3, 4, 5]. It is estimated that about 300-500 million people suffer from clinical malaria annually and about 1.5 – 3 million of these die every year, which includes one million children under the age of five years[6]. Every 40 seconds a child dies of malaria, resulting in a daily loss of more than 2000 young lives worldwide[7]. In addition, asymptomatic parasitaemia occur at any given time in a significant proportion of the population in different parts of the world where malaria is holo and/or hyper endemic[8]. Malaria in sub Saharan region and other countries in tropical Africa accounts for about 90% of the total malaria cases and a great majority of malaria deaths. Two third of the remainder are concentrated in six countries i.e. India, Brazil, Sri-Lanka, Afghanistan, Vietnam and Colombia, in decreasing order of prevalence[9].

In India malaria is endemic in all parts except at elevations above 1800 meters and in some coastal areas[10]. The incidence of malaria in the country has been fluctuating between 2-3 millions per year during the past two decades[11]. It has been estimated that economic loss due to malaria in the country during 1990-1993 was $506.82 million to $ 603.82 million[10]. India had spent up to 25% of its health budget on malaria control during 1977- 1991. In the five year plan started in 1997, budget for malaria control was increased up to $ 40 million, a 60% increase from the previous year[12]. In 1998, 20,000 people lost their life and about 577,000 DALYs (disability- adjusted life years) loss in the country, which indirectly turned in to big economic loss.

In India, the malaria control and eradication programmes started in 1953 (National Malaria Control Programme, NMCP) and 1958 (National Malaria Eradication Programme, NMEP) respectively, leading to enormous success and brought down incidence of malaria to less than 50,000 cases in few years and mortality due to malaria was completely eliminated in 1960s. Success in malaria control by the early 1960s was so overwhelming that malaria eradication seemed imminent. But due to reasons mentioned earlier the malaria eradication received setback and there was a resurgence of malaria in late 1960's and in early 1970's and malaria cases continued to rise unabated in the rural as well as urban areas. In 1976 a peak of 6.47 million malaria cases were reported in the country[13]. To arrest the rising trend of malaria, NMEP implemented the Modified Plan of Operation (MPO) in 1977. Simultaneously the MPO was strengthened by the *P. falciparum* Containment Programme (PfCP) funded by the Swedish International Development Agency (SIDA). There was an improvement in the malaria situation following the implementation of the MPO, which brought down the number of malaria cases

to 2 million during 1980. However, epidemiological analysis showed that improvement in malaria situation was due to selective reduction in *P. vivax* but not in *P. falciparum.* Since late 1970s, the proportion of *P. falciparum* cases has shown an increasing trend. The proportion of *P. falciparum* infections out of the total reported malaria cases was 9.34% in 1972[14], which rose to 43.4% in 1991[15]. The respective figures, during 1992, 1994 and 1996, were 41.2%, 38% and 38.89%[16]. In 1996, the incidence of *P. falciparum* in different states (as percentage of total cases of *P. falciparum* in the country) was: Northeastern states 12.3%; Orissa 33.6%; Madhya Pradesh 18.7%; Maharashtra 7.1% and Bihar 5.5%. Although contribution by some states to the total *P. falciparum* cases in the country may be small, such cases in many states were high, e.g. in 1996 *P. falciparum* percentage in Andhra Pradesh was 47.3; Assam 61; Bihar 61.9; Gujarat 22.3; Haryana 21.7; Madhya Pradesh 44.2; Maharashtra 26.4; Manipur 43.1; Mizoram 57.6; Nagaland 21.5; Orissa 86.6; Rajasthan 24.1; Tripura 72.3; Uttar Pradesh 12.4 and West Bengal 16.8[11]. National Anti Malaria Programme[17] have reported 48.53% *P. falciparum* cases in the country in year 2003 and documented the highest percentage of *P. falciparum* cases in Orissa (83.39), followed by Chattisgarh (73.91), Meghalaya (70.28), Tripura (67.61), Assam (57.69), Bihar (48.7), Jharkhand (44.6), West Bengal (34.98), Maharashtra (24.86) and Madhya Pradesh (24.04).

1.2 Drug Resistant *P. falciparum*: A Challenge for Malaria Control

Human malaria is a protozoan infection caused by five *Plasmodium* species including *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*), *Plasmodium malariae* (*P. malariae*), and recently discovered *Plasmodium knowlesi*[18]. However, *P. falciparum* is the one associated with the highest degree of morbidity and mortality. *P. falciparum* infection is characterized by comparatively higher degree of parasitaemia and potentially fatal complications such as cerebral malaria, renal failure, hypoglycemia, severe haemolysis, anemia and pulmonary edema. The problem of falciparum malaria has been further complicated by the appearance and spread of the strains, which are resistant to chloroquine (CQ) and other antimalarial drugs. The problem of CQ resistant malaria is mainly with *P. falciparum*, as there are very few reports of CQ unresponsiveness in *P. vivax* infection world wide[19, 20, 21, 22, 23, 24]. Widespread resistance to antimalarials throughout the world is impeding efforts to control malaria[25]. The first well-documented reports of *Plasmodium falciparum* resistant to chloroquine were made between 1957 and 1960 in South-East Asia and South America [26,27]. Resistance of *Plasmodium falciparum* to chloroquine appeared almost simultaneously in Colombia and on the frontier between Thailand and Cambodia. In Asia, chloroquine resistance was initially confined to the Indo-Chinese peninsula, until the 1970s, when it spread westwards and towards the neighboring islands in the south and east[28]. The advent of chloroquine resistance in Africa occurred much later, and it took a decade to cross the continent. Today, only countries in Central America north

of the Panama Canal and on the island of Hispaniola have not documented chloroquine-resistant *P. falciparum* malaria[28].

Drug-resistant *Plasmodium falciparum* is a particularly serious problem in Southeast Asia, where strains are commonly resistant to chloroquine, antifolates, quinine, and mefloquine[29]. The sulfadoxine–pyrimethamine combination was used as a second line of treatment for chloroquine in most countries. At the beginning of the 1980s, however, this drug combination found resistant to *P. falciparum* in Thailand and neighboring countries, and spread rapidly in South America. Resistance to mefloquine is found in Cambodia, Myanmar, Thailand and Viet Nam. Sporadic cases of prophylactic failure of mefloquine in travelers and therapeutic failure have been reported in Africa, other Asian countries and South America. Several studies have shown a diminution in sensitivity in vitro, and studies in vitro in West Africa showed the existence of strains with decreased sensitivity to mefloquine even before its introduction into the region for therapeutic use. Resistance to quinine is often overestimated, as the dosage of 30 mg base per kg for 7 days is rarely respected, and the threshold for resistance in vitro has not been clearly defined [30].

In India, the first focus of resistance to chloroquine was first detected in 1973 from Karbi- Anglong district in Assam[31], which gradually spread to all over the state. Subsequently, several such foci were detected from neighboring states of Meghalaya[32], Mizoram[33] and Arunachal Pradesh[34]. Thus, in Northeastern region of India comprising of seven states (Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland & Tripura), malaria is highly endemic, majority of cases (70 - 80%) are that of *P. falciparum*[35,36] which are resistant to chloroquine. Decline efficacy to

sulphadoxine/pyrimethamine (SP) was reported as early as 1990, however, confirmed cases of SP resistance in India reported during 1992 from Changlang district of Arunachal Pradesh[37, 38, and 39]. Since then CQ resistance has spread throughout the region and reports of SP resistance are on the increase[40]. Further, quinine was reportedly showing declined efficacy in northeast region of India (Karbi-Anglong, Assam) since 1990[41, 42]. Recently, treatment failure to CQ, SP and quinine, administered sequentially, was noted in 6% of Pf cases, indicating the presence of multi-drug resistant Pf strains[43]. Recent studies showed that areas farthest from Indo-Myanmar border in Arunachal Pradesh recorded lowest failure rates to chloroquine and SP drugs as compared to areas nearer to international border indicating an inverse relationship between magnitude of drug resistance and distance from the international border[44]. Chloroquine resistance, in northeastern states, was detected at different periods of time since 1973 (Table-1)

1.2.1 **Arunachal Pradesh:** Arunachal Pradesh is a thinly populated (population density< 12 per Sq Km) hilly tract on the eastern most part of India, surrounded on three sides by the international borders with Bhutan on the west, China to the north and Myanmar to the East. Towards the south is Assam and Nagaland. The state is predominantly tribal incharacter and among the main tribes are the Monpas, Apatanis, Miris, Mijis, Akas, Sherdukpens, Adi, Gallongs, Mishmis and Khamptis.

Malaria is highly endemic with preponderance of P. falciparum (Secondary data analysis) and particularly so in foothill areas bordering Assam

Chloroquine resistance to Pf was detected in 1982 from erstwhile Tirap (Jairampur, Changlang district) district during 1982. RII & RIII type of resistance has increased from 30% to 90% during 1986 to 2005. SP resistance was found during 1992 In-vivo resistance SP drugs has increased from 5.3% (1992) to 65% (2003) (Ref).

1.2.2 **Assam** is situated in the northeast corner of India and is surrounded by Bhutan and Arunachal Pradesh on the North; Nagaland and Manipur on the east; Meghalaya and Mizoram on the south and Bangladesh; Tripura and West Bengal on the west. Only a narrow strip of sub-mountain region of the Himalayas connects Assam with the Indian mainland. The state may be divided into two regions- the Barak Valley and Brahmaputra Valley. The climate is generally hot and humid. Assam has an area of 78438 Sq Km with a population of 27 million. The state is divided into 26 districts. The antimalarial drug resistance studies were carried out in 14 of Assam (Table Y1).

1.2.2.1 **Karbi-anglong**: The first report of chloroquine resistance in India was reported from this district in 1973. The presence of chloroquine resistant strains of Pf was consistently detected in the district since then in both in-vivo and in-vitro method. Declining efficacy of SP was first reported in 1979. Further, during 1991, SP resistance (RII & RIII together) to the tune of 12% was detected.

1.2.2.2 **Kamrup**: Chloroquine resistance was detected in 1982 (RI & RII) only. However, subsequent studies showed high level of resistance to chloroquine.

1.2.2.3 **Goalpara**: Chloroquine resistance was detected in tea gardens of the district and is widespread

1.2.2.4 **Sibsagar**: Chloroquine resistance is focal and mostly concentrated in tea gardens bordering Nagaland.

1.2.2.5 **Nowgaon**: RII and RIII level of chloroquine resistance has been consistently detected in the district since 1985. No significant difference in resistance was detected between chloroquine and amodiaquine in the district.

1.2.2.6 **Kokrajhar**: A hilly and forested district of Assam bordering Bhutan in its North is highly endemic for Pf malaria. The chloroquine resistance in the district is gradually increasing since 1985.

1.2.2.7 **Nalbari**: A hilly and forested district bordering Bhutan is highly epidemic prone with variable endemicity. RI type of resistance was first detected in 1986. However, subsequent studies in 1990, 1992 showed not only high resistance (RII & RIII) type to chloroquine. There is no difference in resistance pattern of chloroquine and amodiaquine in the district. Sporadic resistance to SP is also found in the district.

1.2.2.8 **Darrang**: The district shares international boundary with Bhutan and terrain is hilly and forested. RIII type of resistance to chloroquine was detected in the district in 1993 and persisting in subsequent studies.

1.2.2.9 **Dhubri**: This is highly endemic for malaria. Chloroquine resistance at RIII level was detected in 1990 and subsequently in 1993. RII and RIII together found to be 38.4%. There is no difference in sensitive status between amodiaquine and chloroquine in the district. Recent studies showed the sporadic appearance of SP resistance in Chhapar PHC area of this district.

1.2.2.10 **Cachar**: The topography of the district is mainly hilly and forested. The chloroquine resistance is RII and RIII type. However, limited studies were carried out in the district.

1.2.2.11 **Sonitpur**: The district is hilly and forested and borders Arunachal Pradesh. Foothill areas of the district bordering Arunachal Pradesh are having unstable malaria endemicity. Frequent epidemics reported from these areas. RIII level of chloroquine resistance was found in this district.

1.2.2.12 **Morigaon**: This is newly formed district carved out from erstwhile Nowgaon district. Chloroquine resistance of 15% of RIII level was found.

1.2.3 **Meghalaya** was made full fledged state on 21st January'1972. It is a mountainous and forested region with heavy rainfall and comprises of three hill ranges viz. Garo, Khasi and Jaintia hills. The Khasi, Jaintia, Bhoi, War collectively known as the Hynniewtrep people live in east Meghalaya, while Garos live in the western part. Garos belong to the Bodo family and said to have migrated from Tibet. The population of

the state 2318822 (2001 census) and covers an area of 22429 Sq Km. Malaria incidence of the state is very high. All the districts of the state are malaria endemic.

1.2.3.1 **Garohills**: It is a hilly forested district of Meghalaya. The entire district is highly endemic for malaria with P. falciparum preponderance Resistance to chloroquine is very high and focal resistance to SP is also have a focal presence in the district.

1.2.3.2 **Khasihills**: Studies conducted since 1980, chloroquine resistance was recorded at all levels in the district and it is quite widespread.

1.2.3.3 **Jaintiahills**: The chloroquine resistance was found to be low and focal

1.2.4 **Mizoram**: Mizoram means the land of highlanders. It became 23rd state of the Indian Union in 1987. Mizoram in the northeast corner of India is bounded on the north by Manipur and Assam, on the east and south by Myanmar, on the west by Bangladesh and Tripura. It has an area of 21031 Sq Km with 888573 populations and is the smallest state of northeastern region

1.2.4.1 **Aizwal (West)**: . Chloroquine resistance was first detected in the state from Sairong PHC area, Aizwal district in 1981.

1.2.4.2 **Lunglei**: A hilly forested district having chloroquine resistance of all levels since 1989 (33% RIII resistance)

1.2.5 **Nagaland:** Nagaland State became the sixteenth State of India on 1963. It is bounded by Assam in the West, Myanmar (Burma) on the East, Arunachal Pradesh and parts of Assam on the North and Manipur in the South. The State is inhabited by 16 major tribes along with other sub-tribes. The state covers an area of 16579 sq Km, with a population of 1990036 (2001 Census). Chloroquine resistance was first detected from Kohima district (RII and RIII) types in 1984. Subsequently, it was detected in Mon district (RIII, 1989) and Mokokchung district.

1.2.6 **Manipur:** The state with a population of 2166788 (as per 2001 census) covers an area of 22327 Sq Km. The state is bounded by Nagaland in the north, Mizoram in the South, Myanmar in the east and Assam in the west. Chloroquine resistance to Pf was first detected from Jiribam PHC area, Central district in 1994. Subsequently high resistance (RII and RIII) were found from Chandel and Senapati district in 1994 and 1999 respectively.

1.2.7 **Tripura:** The state with an area of 10486 Sq Km have a population of 3199203 (as per 2001 Census).Tripura is bounded on the north, west, south and southeast by Bangladesh and on the east by Assam and Mizoram states. The state is inhabited largely by 19 tribes, Bengalis and Manipuri communities. South Tripura district of the state bordering Bangladesh is highly endemic. Chloroquine resistance was first detected from Shantibazar area of South Tripura in 1981. Sulphadoxine / pyrimethamine resistance (RI level) was also detected from Nutunbazar area of the same district in 1993.

Early diagnosis and prompt treatment are fundamental components of the WHO global strategy for malaria control[45]. Correct use of an effective antimalarial drug will not only shorten the duration of malaria illness but also reduce the incidence of complications and the risk of death. Keeping this in view, NVBDCP has changed the drug policy in 2007. In 125 primary health centers in 22 districts of 7 northeastern states, alternative therapy (SP with artesunate i.e. ACT) has been introduced (Table 2).

The control of malaria by indoor residual insecticide spray has also suffered a setback as mosquito vectors have developed resistance to the popular and cheap insecticide DDT and hexachlorobenzene (HCH). Resistance in *An. culicifacies* (responsible for 60% rural malaria transmission in India) to DDT first appeared in 1959 in Gujarat[46] and has spread to almost all parts of the country[11]. The resistance to newer insecticides like HCH and malathion has also been reported from Gujarat, Maharashtra and parts of Rajasthan. *An. stephensi*, the vector of urban malaria has also developed resistance to residual insecticides[11]. Moreover the newer insecticides like malathion and pyrethroids are several times more expensive. Thus, the control of malaria has become a costly affair due to the high cost of alternative antimalarials and insecticides. As most malarious countries are developing nations, they can hardly afford the expenses of alternative drugs and insecticides. So the greater reliance on the prompt and successful treatment of falciparum malaria is the main tool to prevent morbidity and mortality from this infection.

1.3 Assessment of *P. falciparum* Antimalarial Susceptibility / Resistance

Chloroquine had become the treatment of choice for malaria since its discovery in the 1930s, there was a clear need to be able to assess the extent of resistance to this drug in areas where malaria was being transmitted. Chloroquine is a cheap and well-tolerated 4-aminoquinoline drug. It is still widely used to treat uncomplicated malaria even in areas where Plasmodium falciparum malaria parasites show significant levels of resistance to it[47].

A standardized in vivo test system for assessing the response of P. falciparum to chloroquine was first developed in 1965[48] and subsequently revised. A final set of revised system was available for field application during 1973[49, 50]. *In-vivo* response to drugs was originally defined by WHO in terms of parasite clearance [sensitive (S) and three degrees of resistance (RI, RII, RIII) (Figure 1)[48].

The methodology followed to assess this was fairly demanding and required daily blood examinations during the first week post-treatment followed by a prolonged period (28 days) of monitoring with weekly blood examinations. Because the outcome of primary interest was reappearance of parasites within the observation period, indicating treatment failure, patients were kept in a mosquito free environment to prevent reinfection. Later modifications allowed for a choice between a shortened observation period of 7 days (the "WHO standard test") and the longer 28-day observation period (the "extended test"), depending on whether the possibility of reinfection could be excluded. The short observation period allowed the test to be conducted under typical field conditions and constraints.

Subsequently, many a non-official modifications of the protocol began to show the usefulness of tracking clinical response to treatment, including calculating fever clearance times, initial symptom resolution, time to reappearance of parasites, and haematological response[51, 52,53,54]. The growing emphasis on clinical response, started taking into account issues, such as implications of acquired immunity and the appropriateness of inclusion of asymptomatic subjects. As a result, only symptomatic patients were included in the test and, in areas of intense transmission, focusing on the age group at greatest risk of severe morbidity and mortality (and least likely to have a well developed immune response to malaria), i.e. children less than 5 years of age. There was also a move to evolve an observation period of sufficient length (14 days or more) instead of short 7-day observation periods to allow observation of changes in clinical and haematological status. These efforts culminated in a new standardized protocol jointly developed by the Centers for Disease Control and Prevention, Atlanta, USA, and WHO at an inter-country workshop – Malaria Treatment and Resistance in Kenya, Zambia and Malawi – in Mangochi, Malawi, in 1996[55]. Simultaneously, a second protocol was developed specifically for areas of low to moderate transmission, beginning with a meeting in Manila (Philippines) in 1996 and continuing at an expert meeting in Manaus (Brazil) in 1998[56]. During 2001, a single, globally standardized protocol was developed and methodological problems that became apparent in the 1996 protocol were corrected[56].

The fundamental design of this protocol is intended to evaluate the therapeutic efficacy of a range of antimalarial drugs used for treating uncomplicated falciparum malaria, providing the minimum information

essential for programmatic decision-making as well as to develop a surveillance system capable of monitoring drug efficacy changes over time.

This protocol is intended to evaluate both the current first-line treatment as well as one or more potential replacement treatments. However, the protocol is not designed for the evaluation of new or experimental drugs; such studies, as clinical trials of new antimalarials or to assess drug regimens administered over periods longer than 3 days, such as quinine (given for 7 days), combinations of quinine and tetracycline or doxycycline (given over 7 days), or artemisinin monotherapy (given for 5–7 days) [57].

Antimalarial drug susceptibility can also be assessed by *in-vitro* assays measuring the intrinsic sensitivity of *P. falciparum* from the inhibition of growth or schizonts maturation[58, 6]. The *in-vitro* 'macro test' to measure the response of *P. falciparum* to CQ, quinine and cycloguanil was first developed by Rickman et al in 1968 (59) and subsequently, Reickman *et al.*, in 1978 (60) introduced the micro-test method for drug sensitivity testing which was adopted more or less in its basic form by WHO[61, 30]. The isotopic microtest method has also been developed for *in vitro* sensitivity testing[62]. Molecular techniques to determine the genetic alterations or polymorphisms associated with drug resistance in parasites like allele specific PCR (AS-PCR) and PCR-RFLP have also being employed. Modulation of expressions of genes associated with drug resistance is also being used to define the true resistance in field/ patients by using the most advanced techniques like real time polymerase chain reaction, reverse transcriptase polymerase chain reaction, and DNA microarrays61, [63, 64, 65, 66].

Each assessment method has its advantages and disadvantages, and the results might not be directly comparable with each other. In particular, *invitro* results do not necessarily correlate with *in vivo* outcomes, largely owing to the role of host immunity in the later. In addition, pharmacokinetic information may be required for differentiation between true resistance and failure to achieve adequate drug concentration profile (White, 1999).

Table 1: First time detection of chloroquine resistance in different states of N.E. Region of India

States of Northeast India	Year of Detection	District	PHC
Assam	1973	Karbi-Anglong	Manja PHC
Arunachal Pradesh	1982	Changlang	Jairampur (Nampong PHC)
Manipur	1990	Central	Jiribam PHC
Meghalaya	1990	West Garohill	Zikzak
Mizoram	1981	Aizwal	Sairong PHC
Nagaland	1984	Kohima	Dimapur
Tripura	1981	South Tripura	Santi Bazar

Table 2: PHCS and Districts of NE Region showing chloroquine resistance where drug policy has been changed

S.No.	State/UT	District	Number of PHCs	Name of PHCs
1	Assam	Karbi Anglong	11	Total district
		Nagaon	11	Total district
		Darrang	1	Orang
		Kamrup	1	Sonapur
		Nalbari	1	Tamalpur
		Sonitpur	1	Behali
2	Arunachal Pradesh	Lohit	7	Total district
		Tirap	1	Jairampur
3	Meghalaya	All districts except Jaintia Hills	39	Garohills, Khasi hills
4	Mizoram	Aizwal	1	Sairang
		Lunglei	1	Hnanthial
5	Nagaland	All districts	49	Whole state
6	Tripura	South Tripura	1	Santibazar
		22 districts	125	

Figure 1: Original Classification Antimalarial Drug Resistance (WHO 1965)

S (sensitive)	Reduction of <25% of initial parasitaemia on day 2 with smears negative for malaria from day 7 to the end of follow-up (28 days or longer for drugs with a long half life such as mefloquine)
RI response	Initial clearance of parasitaemia, a negative smear on day 7, followed by recrudescence 8 or more days after treatment.
RII response	Initial clearance or substantial reduction of parasitaemia (<25% of theinitial count on day 2) but with persistence or recrudescence of parasitaemia during days 4 – 7.
RIII response	No significant reduction in asexual parasitaemia following treatment.

Figure 2: Therapeutic Efficacy Classification Antimalarial Drugs

INTENSE TRANSMISSION AREA	LOW TO MODERATE TRANSMISSION AREA
***Early Treatment Failure* (ETF)**	
ETF • Development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitaemia • Parasitaemia on Day 2 higher than Day 0 count irrespective of axillary temperature • Parasitemia on Day 3 with axillary temperature ≥ 37.5 °C • Parasitemia on Day 3 ≥ 25 % of count on Day0	**ETF** • Development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitaemia • Parasitaemia on Day 2 higher than Day 0 count irrespective of axillary temperature • Parasitemia on Day 3 with axillary temperature ≥37.5 °C • Parasitemia on Day 3 ≥ 25 % of count on Day0
***Late Treatment Failure* (LTF)**	
***Late Clinical Failure* (LCF)** • Development of danger signs or severe malaria after Day 3 in the presence of parasitemia, without previously meeting any of the criteria of *Early Treatment Failure* • Presence of parasitemia and axillary temperature ≥ 37.5°C on any day from Day 4 to Day 14, without previously meeting any of the criteria of *Early Treatment Failure*	***Late Clinical Failure* (LCF)** • Development of danger signs or severe malaria after Day 3 in the presence of parasitemia, without previously meeting any of the criteria of Early Treatment Failure • Presence of parasitemia and axillary temperature ≥ 37.5 °C (or history of fever) on any day from Day 4 to Day 28, without previously meeting any of the criteria of Early Treatment Failure

Late Parasitological Failure **(LPF)**

- Presence of parasitemia on Day 14 and axillary temperature < 37.5°C, without previously meeting any of the criteria of *Early Treatment Failure* or *Late Clinical Failure*

Late Parasitological Failure **(LPF)**

- Presence of parasitemia on any day from Day 7 to Day 28 and axillary temperature < 37.5 °C, without previously meeting any
- of the criteria of *Early Treatment Failure* or *Late Clinical Failure*

Adequate Clinical and Parasitological Response **(ACPR)**

ACPR

- Absence of parasitemia on Day 14 irrespective of axillary temperature without previously meeting any of the criteria of
- *Early Treatment Failure*or *Late Clinical Failure*

o

r *Late Parasitological Failure.*

ACPR

- Absence of parasitemia on Day 28 irrespective of axillary temperature without previously meeting any of the criteria of
- *Early Treatment Failure*or *Late Clinical Failure* or *Late Parasitological Failure.*

References

1 World Health Organisation (1996a). Adhoc committee on health research relating to future intervention option. Inverting in health research and development (TDR/Gen/96.1), WHO.

2 Hay, S.I., Guerra, C.A., Tatem, A.J., Noor, A.M. and Snow, R.W. (2004). The global distribution and population at risk of malaria: past, present, and future. Lancet Infect Dis. 4 (6): 327-36.

3 Trigg, P.I. and Kondrachine, A.V. (1998). Commentary: malaria control in the 1990s. Bull World Health Organ. 76 (1): 11-6.

4 World Health Organization (1999). Rolling back malaria . The world health report 1999: making a difference. Geneva: World Health Organization: 49-63.

5 World Health Organization (2000). WHO expert committee on malaria: twentieth report. Geneva: World Health Organization.

6 World Health Organization (2001). *Invitro* micro-test (mark III) for the assessment of the response of *P. falciparum* to chloroquine, mefloquine, quinine, amodiaquine, sulfadoxine / pyrimethamine and artemisinin. CTD/MAL/97.20 Rev.2.

7 Sachs, J. and Malaney, P. (2002). The economic and social burden of malaria. Nature. 415 (6872): 680-5.

8 Ibhanesebhor, S.E., Otobo, E.S. and Ladipo, O.A. (1996). Prevalence of malaria parasitaemia in transfused donor blood in Benin city, Nigeria. Ann. Trop. Paed. 16: 93-95.

9 Lou, J., Lucas, R. and Grau, G.E. (2001). Pathogenesis of cerebral malaria: recent experimental data and possible applications for humans. Clin Microbiol Rev. 14 (4): 810-20.

10 Sharma, V.P. (1996). Re-emergence of malaria in India; Indian J. Med Res. 103: 26-45.

11 Sharma, V.P. (1997). Drug resistance: Mechanism and management. Radhey L, Singal and Sood, O.P. (eds.). Ranbaxy Science foundation, New Delhi, India 67-72.

12 Jayaraman, K.S. (1997). India plans $200 million attack on malaria. Nature. 386 (6625): 536.

13 National Malaria Eradication Programme (NMEP). (1986). Malaria and its control in India, Volume III. Delhi: Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India.

14 Sharma, G.K. (1983). Review of malaria and its control in India. In proceedings of the Indo-UK workshop on malaria (ed.) by Dr. V.P. Sharma Director MRC (ICMR): 13-40.

15 World Health Organization (1994). World malaria situation in 1992. Part II. Wkly Epidemiological Rec. 69: 317-21.

16 World Health Organisation, Geneva. (1996b). Weekly epidemiological record. 71: 25-32.

17 National Anti Malaria Programme (2003). Annual Report. Directorate of National Anti Malaria Programme Annexure-II. Downloaded from http://www.whoindia. org /EN/ Section3/section128.htm.

18 Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, Thomas A, Conway DJ. A large focus of naturally acquired *Plasmodium knowlesi* infection in human beings. *Lancet*. 2004; 364 (9441): 1217

19 Rieckmann, K.H., Davis, D.R. and Hutton, D.C. (1989). *Plasmodium vivax* resistance to chloroquine? Lancet. 2 (8673): 1183-4.

20 Schwartz, I.K., Lackritz, E.M. and Patchen, L.C. (1991). Chloroquine-resistant *Plasmodium vivax* from Indonesia. N Engl J Med. 324 (13): 927.

21 Schuurkamp, G.J., Spicer, P.E., Kereu, R.K., Bulungol, P.K. and Rieckmann, K.H. (1992). Chloroquine-resistant *Plasmodium vivax* in Papua New Guinea. Trans R Soc Trop Med Hyg. 86 (2): 121-2.

22 Garg, M., Gopinathan, N., Bodhe, P. and Kshirsagar, N.A. (1995). Vivax malaria resistant to chloroquine: case reports from Bombay. Trans R Soc Trop Med Hyg. 89 (6): 656-7.

23 Dua, V.K., Kar, P.K. and Sharma, V.P. (1996). Chloroquine resistant *Plasmodium vivax* malaria in India. Trop Med Int Health. 1(6): 816-9.

24 Singh, R.K. (2000). Emergence of chloroquine-resistant vivax malaria in south Bihar (India). Trans R Soc Trop Med Hyg. 94 (3): 327.

25 Breman, J. G. 2001. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am. J. Trop. Med. Hyg.* **64:**1–11.

26 Moore, D.V. and Lanier, J.E. (1961). Observations on two *P. falciparum*, infections with an abnormal, infections with an abnormal response to chloroquine. Am J Trop Med Hyg. 10: 5-9.

27 Harinasuta T, Suntharasamai P, Viravan C. Chloroquine-resistant falciparum malaria in Thailand. *Lancet*, 1965, 2:657–660.

28 World Health Organization. Susceptibility of Plasmodium falciparum to antimalarial drugs: Report on global monitoring 1996-2004. WHO/HTM/MAL/2005.1103.

29 Wongsrichanalai, C., A. L. Pickard, W. H. Wernsdorfer, and S. R. Meshnick. 2002. Epidemiology of drug-resistant malaria. *Lancet Infect.* Dis. **2:**209–218.

30 World Health Organization. 2001. The use of antimalarial drugs: An informal consultation. WHO, Geneva. WHO/CDS/RBM/2001.33

31 Sehgal PN, Sharma MID, Sharma SL & Gogai S (1975) Resistance to chloroquine in falciparum malaria in Assam state, India. Journal of Communicable Diseases 5, 175–180.

32 Pattanayak, S., Roy, R.G. and Sen, N. (1979). Response to chloroquine with and without pyrimathamine in *Plasmodium falciparum* in West Bengal, Tripura, Mizoram, Manipur and Arunachal Pradesh. Ind. J. Med. Res. 70 Suppl: 48-51.

33 Das, S., Roy, R.G. and Pattanayak, S.A. (1979). Note on chloroquine resistance tests on *P.falciparum* in Nagaland. Indian J. Med. Res. 70 (Suppl): 30-33.

34 Wongsrichanalai, C., Webster, H.K., Wimonwattrawatee, T., Sookto, P., Chuanak, N., Thimasarn, K. and Wernsdorfer, W.H. (1992). Emergence of multidrug-resistant *Plasmodium falciparum* in Thailand: in vitro tracking. Am J Trop Med Hyg. 47(1): 112-6.

35 Mohapatra, P.K., Prakash, A., Bhattacharyya, D.R. and Mahanta J. (1998). Epidemiological importance of younger age group during malaria epidemic in PHC Tamulpur, Assam. J Commun Dis. 30(4):229-32.

36 Mohapatra, P.K., Namchoom, N.S., Prakash, A., Bhattacharya, D.R., Goswami, B.K. and Mahanta, J. (2003). Therapeutic efficacy of anti-malarials in *Plasmodium falciparum* malaria in an Indo-Myanmar border area of Arunachal Pradesh. Indian J Med Res. 118: 71-6.

37 Barkakaty, B.N. and Narsimham, M.V.V.L. (1990). Efficacy of low dose oral quinine therapy in *Plasmodium falciparum* cases of Assam, Meghalaya and Arunachal Pradesh in India. J. Commun. Dis. 23: 248-53.

38 Garg, M.R., Gogtony, N.J. and Kshirsagar, N.A. (1996). Efficacy of sufadoxin-pyremethamine in chloroquine resistant falciparum malaria in Mumbai. J. Assoc. Physicians India. 44:683-5.

39 Shivlal, Dhillon GPS, Sonal GS, Arora U, Nandi J. Editors. *Drug Resistance and Chemotherapy of Malaria in India, an Update 1997.* National Malaria Eradication Programme (Enhanced Malaria Control Project), Ministry of Health and Family Welfare. Government of India; 1997.

40 Mohapatra PK, Prakash Anil, Bhattacharyya DR, Goswami BK & Mahanta J. Concurrent multi drug resistant *Plasmodium falciparum* from India: a case report. J Assoc Physicians India. 1999, 47(8): 823 – 824.

41 Barkakaty, B.N., Chakravorty, N.K. and Narsimham, M.V.V.L. (1990). Efficacy of sulfalene and pyrimethamine combination drugs alone and with quinine in treatment of *P. falciparum* cases in chloroquine resistant areas of Northeast India. J Commun Dis. 72:178-84.

42 Clyde, D.F. (1987). Variations in response of malaria parasites to drugs in Asia and Oceania. La Medicina Tropica 3 (1): 3-21.

43 Mohapatra PK, Namchoom NS, Prakash Anil, Bhattacharyya DR, Goswami BK & Mahanta J. Therapeutic efficacy of anti-malarials in Indo-Myanmar border areas of Arunachal Pradesh. Indian J Med Res. 2003, 118: 71 – 76.

44 Mohapatra PK, Prakash Anil, Taison K, Negmu K, Gohain AC, Namchoom NC, Wange D, Bhattacharyya DR, Goswami BK, Borgohain BK and Mahanta J. 2005. Evaluation of chloroquine (CQ) and sulphadoxine / pyrimethamine (SP) therapy in uncomplicated falciparum malaria in Indo-Myanmar border areas. *Tropical Medicine and International Health*. 10 (5): 478-483.

45 World Health Organization. 1993, *A global strategy for malaria control*, WHO Geneva.

46 Rahman, J., Roy, M.L. and Singh, K. (1959). Development of increased tolerance to DDT in *An. Culicifacies* Giles, in the Punch Mahals district of Bombay state (India). 13: 125-30.

47 McIntosh HM, Jones KL. 2005. Chloroquine or amodiaquine combined with sulfadoxine-pyrimethamine for treating uncomplicated malaria. *Cochrane Database of Systematic Reviews* 2005, Issue 4. Art. No.: CD000386. DOI: 10.1002/14651858.CD000386.

48 *Resistance of malaria parasites to drugs. Report of a WHO Scientific Group*. Geneva, World Health Organization, 1965 (WHO Technical Report Series, No. 296).

49 *Chemotherapy of malaria. Report of a WHO Scientific Group*. Geneva, World Health Organization, 1967 (WHO Technical Report Series, No. 375).

50 *Chemotherapy of malaria and resistance to antimalarials. Report of a WHO Scientific Group*. Geneva, World Health Organization, 1973 (WHO Technical Report Series, No. 529).

51 Brandling-Bennett AD et al. Chloroquine treatment of falciparum malaria in an area of Kenya of intermediate chloroquine resistance. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1988, 82:833–837.

52 White NJ, Krishna S. Treatment of malaria: some considerations and limitations of the current methods of assessment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1989, 83:767–777.

53 Bloland PB et al. Chloroquine in Africa: critical assessment and recommendations for monitoring and evaluating chloroquine therapy efficacy in sub-Saharan Africa. *Tropical Medicine and International Health*, 1998, 3:543–552.

54 *Antimalarial Drug Policies: data requirements, treatment of uncomplicated malaria and management of malaria in pregnancy. Report of an Informal Consultation.* Geneva, World Health Organization, 1994 (document WHO/MAL/94.1070).

55 *Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malariain areas with intense transmission*. Geneva, World Health Organization, 1996 (document WHO/MAL/96.1077).

56 World Health Organization . 2003. *Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria*. WHO, Geneva, WHO/HTM/RBM/2003.50

57 World Health Organization. 2002. *Monitoring antimalarial drug resistance. Report of a WHO consultation*. WHO, Geneva (document WHO/CDS/CSR/EPH/2002.17-WHO/CDS/RBM/2002.39).

58 Wernsdorfer, W.H. and Payne, D. (1991). The dynamics of drug resistance in *Plasmodium falciparum*. Pharmacol Ther. 50(1): 95-121.

59 Rieckmann, K.H., McNamara, J.V., Friescher, H., Stockert, T.A., Carson, P. and Powell , R.D. (1968). Effects of chloroquinine and cycloguanil upon the maturation of asexual erythrocytic forms of two strains of *P. falciparum invitro*. Am J Trop Med Hyg. 17: 661-71

60 Rieckmann, K.H., Sax, L.J., Campbell, G.H. and Mrema, J.E. (1978). Drug sensitivity of *P. falciparum*. An *invitro* micro technique. Lancet1: 22-23.

61 Bruce- Chwatt, L.J., Black, R.H., Canfield, C.J., Clyde, D.F., Peters, W. and Wernsdorfer, W.H. (1981). Chemotherapy of malaria, 2nd edn. Geneva: World Health Organization.

62 Desjardins, R.E., Canfield, C.J., Haynes, J.D., Chulay, J.D. (1979). Quantitative assessment of antimalarial activity *invitro* by a semiautomated microdilution technique. Antimicrob Agents Chemother. 16(6): 710-8.

63 Peel, S.A., Bright, P., Yount, B., Handy, J. and Baric, R.S. (1994). A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (*pfmdr*) of *Plasmodium falciparum* in *invitro*. Am J Trop Med Hyg. 51 (5): 648-58.

64 Adagu, I.S. and Warhurst, D.C. (1999). Association of *cg2* and *pfmdr1* genotype with chloroquine resistance in field samples of *Plasmodium falciparum* from Nigeria. Parasitology 119 (4): 343-8.

65 Djimde, A., Doumbo, O.K., Cortese, J.F., Kayentao, K., Doumbo, S., Diourte, Y., Dicko, A., Su, X.Z., Nomura, T., Fidock, D.A., Wellems, T.E., Plowe, C.V. and Coulibaly, D. (2001). A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med. 344 (4): 257-63.

66 Purfield, A., Nelson, A., Laoboonchai, A., Congpuong, K., Mcdaniel, P., Miller, R.S., Welch, K., Wongsrichanalai, C. and Meshnick, S.R. (2004). A new method for detection of *pfmdr1* mutations in *Plasmodium falciparum* DNA using real time PCR. Malar. J. 3:9

67 Wilson. P.E., Kazadi. W., Kamwendo, D.D., Mwapasa, V., Purfield, A. and Meshnick, S.R. (2005). Prevalence of pfcrt mutations in Congolese and Malawian *Plasmodium falciparum* isolates as determined by a new Taqman assay. Acta Trop. 93(1): 97-106.

68 White, N. (1999) Antimalarial drug resistance and combination chemotherapy. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 354: 739-749.