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Assessment of therapeutic efficacy of chloroquine and sulphadoxine-pyrimethamine in uncomplicated falciparum malaria

Sukla Biswas[a*], Neena Valecha[a], Prajesh K. Tyagi[b], Shobhan Phookan[c], Vas Dev[c], Surya K. Sharma[b] & Sarala K. Subbarao[a]

[a]*Malaria Research Centre (ICMR), 22 Sham Nath Marg, Delhi*; [b]*Malaria Research Centre (Field Station), Rourkela;* [c]*Malaria Research Centre (Field Station), Sonapur, India*

A standardised protocol has been developed by World Health Organization (CDS/RBM/2002) to assess the efficacy of common antimalarials in the treatment of clinically manifested infection with uncomplicated *P. falciparum* malaria for areas with low to moderate transmission. The therapeutic efficacy protocol is based on clinical and parasitological responses of the patients and it has the purpose of determining the practical efficacy of the drug regimen in study areas with the ultimate objective of ascertaining its continued usefulness or the necessity for replacing it in the routine treatment. Present study has been conducted at seven sites—Kathiatali and Simonabasti of District Nowgaon, Assam; Sonapur and Boko of District Kamrup, Assam; Keonjhar Town, Padampur and Basudebpur of District Keonjhar, Orissa. In order to reduce the patient recruitment time, health centre close to well-defined community was identified to conduct the activities at peak malaria season by selecting local pockets and organising mobile clinics. Microscopically confirmed cases of *P. falciparum* were enrolled according to the criteria for inclusion and exclusion. Treatment with recommended drug was given under supervision and a follow-up schedule at various intervals for 28 days was maintained. In chloroquine (CQ) study areas, wherever patients showed treatment failure, they were treated with second line drug—sulphadoxine-pyrimethamine (SP) combination and then followed-up as per study protocol. It was observed that 30% cases showed treatment failure to CQ in District Nowgaon, where revised drug policy has already been introduced. In Kamrup district, treatment failure with CQ was found to be less than 25%, which denotes the said regimen is still effective. Almost all the patients from Padampur and Basudebpur of District Keonjhar responded to CQ, treatment failure was noticed only in two patients (3%). The antifolate combination found to be fully effective as second line and also as first line wherever revised drug policy has been introduced.

Key words Drug resistance – *in vivo* monitoring – malaria – *P. falciparum*

Assessment of the efficacy of a course of treatment on malaria patients carries significant importance in the field of disease management. Several procedures are available for use in the field and clinic. The selection of appropriate method depends on the level of immunity of the subjects, clinical condition, the period of time within which they can be followed-up and the chances of getting re-infection during the observation period. The standardised test protocol for assessment of *in*

*Corresponding author

vivo response in *Plasmodium falciparum* has come to use after the first suspect of increase in CQ resistant *P. falciparum* malaria in Thailand in 1957 and found in patients in Colombia in 1960, thereafter the test systems were subsequently revised to make them suitable in various transmission zones with different degrees of endemicity[1–7]. Present test system for assessing the therapeutic efficacy has been simplified, where the number of parasitological tests is reduced, but supplemented with clinical observation.

There are large areas in southeast Asia where malaria transmission is of low to moderate intensity. In these areas the level of immunity is generally low, adults attain immunity after repeated exposures. As these areas are also having drug resistant *P. falciparum* and clinical consequences of such resistance are more marked than those in areas with stable malaria, the protocol has been reviewed and further revised to make it suitable for use in areas of low to moderate transmission. In the present form the test is simple and feasible, provides essential information and requires only moderate resources[7]. At all times, proper patient management and good clinical practice are the priorities over continuation of the test[8].

Among southeast Asian countries, India alone contributes more than 80% malaria cases and *P. falciparum* accounts for 35–40% cases[9]. In the northeastern states of India proportion of *P. falciparum* cases is much higher. Chloroquine resistant *P. falciparum* was first reported in 1973 from Karbi Anglong, Assam[10]. Presently, RI level CQ resistant *P. falciparum* strains are common in India barring a few states in northern India, RII level is not so common and a few focal RIII level of resistance has also been reported[9]. In CQ resistant areas SP combination drug is introduced as a second line of treatment[11]. With the introduction of SP combination and its mass use treatment failure has been noticed. The long half-life of SP combination favours the selection of resistant parasites. There is a general concern that in areas where the intensity of malaria transmission is considerably high, the effective life of SP may even be shorter[12,13]. India has extensive borders on the north with China, Nepal and Bhutan and towards east side with Myanmar and Bangladesh. Around 8–9 million population are from border areas. These border areas generally harbour migrant population. Movement of population between the neighbouring countries and neighbouring states of variable malaria endemicity also results in the spread of malaria particularly resistant *P. falciparum* malaria.

Nationwide routine surveillance to determine the antimalarial drug efficacy and tolerance is seldom feasible. Therefore, a site-specific surveillance is required. Present study based on standard test protocol at seven different sites provides information about the incidence of *P. falciparum* infection, number of positive patients and clinical outcome of the treatment with CQ and SP drugs. Data thus generated may provide an opportunity for assessing the emergence and dispersal of CQ and SP resistant parasites and also to help in determining the drug policy for treatment in areas of low/moderate/high malaria transmission.

Material & Methods

Study sites : Study was undertaken from July to November 2002. Study sites were identified in two northeastern and eastern zones of the country based on the available epidemiological data. Mainly the criteria followed were: (i) area with *P. falciparum* transmission (seasonal or perennial); (ii) area from where therapeutic failure to CQ has been reported; (iii) area with malaria mortality due to *P. falciparum*; and (iv) area where revised drug policy with SP has been introduced.

Four sites, Sonapur and Boko of Kamrup district, Kathiatali and Simonabasti of Nowgaon district were chosen in northeastern state, Assam (Fig. 1). These areas are highly endemic for malaria. Majority of the malaria infections is due to *P. falciparum* (>70%) and is responsible for enhanced morbidity and mortality. *An. minimus* is the major vector species, which is highly anthropophilic thus responsible for enhanced transmission[14]. Malaria cases are detected throughout

the year having a peak transmission during May to October. In Kamrup district, CQ is the drug of choice whereas in Nowgaon SP is the first line drug for the treatment of malaria.

Three sites, Keonjhar Town, Padampur and Basudebpur of District Keonjhar, Orissa were chosen for the study (Fig. 1). In Keonjhar Town, CQ resistant *P. falciparum* cases at the level of RII and RIII were detected. Therefore, the revised drug policy with SP has been introduced[11]. In Padampur and Basudebpur, CQ is the drug of choice for the treatment of malaria. Keonjhar is situated in the Garhjat hills of eastern plateau at an altitude range of 100–800 m above the sea level. All the areas are characterised by tropical climate and receive high rainfall during south-east monsoon and retreating northeast monsoon. About 34% of the areas are covered with forest. *P. falciparum* constitutes more than 90% of the total malaria cases and death cases are also high. The whole district is under the influence of two malaria vectors, *An. culicifacies* and *An. fluviatilis*. The peak prevalence period of *An. culicifacies* is between April and September, and for *An. fluviatilis*— October and January, which coincides with peak transmission season.

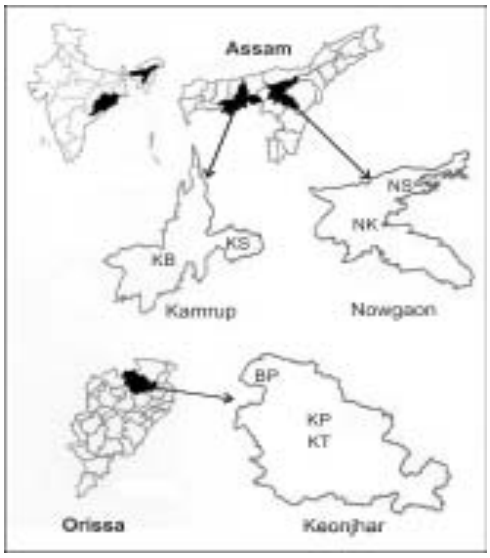

Fig. 1: Study sites

In order to reduce the patient recruitment time, health centre/dispensary close to well-defined community were identified to conduct the activities at the peak malaria transmission season by selecting local pockets or organising mobile clinics, which received at least 15–20 febrile patients per day. The study was conducted at seven sites from July–October and efficacy of CQ and SP were assessed in patients as per treatment policy (Table 1).

Test protocol : The basic test system consists of recording essential patient information, clinical assessment, axillary temperature, parasitaemia, body weight and supervised treatment with the stipulated antimalarial, thereon a follow-up for a period of 28 days (Table 2). Each enrolled patient was examined for above mentioned test parameters.

Study subjects and clinical examination : Peripheral blood smears were prepared from all age groups dur-

Table 1. Study sites

Site	Code	District	State	Efficacy study of the drugs — CQ/SP	
Keonjhar Town	KT	Keonjhar	Orissa	–	SP
Padampur	KP			CQ	–
Basudebpur	BP			CQ	–
Kathiatali	NK	Nowgaon	Assam	CQ	SP
Simonabasti	NS			–	SP
Sonapur	KS	Kamrup	–do–	CQ	SP
Boko	KB			CQ	SP

Table 2. Study design

		Days								Other day
		0	1	2	3	7	14	21	28	
Treatment (mg/kg of body weight)	CQ	10	10	5						
	SP	25/1.25								
Clinical examination		Y	Y	Y	Y	Y	Y	Y	Y	Y
Axillary temperature		Y	Y	Y	Y	Y	Y	Y	Y	Y
Parasitaemia		Y		Y	Y	Y	Y	Y	Y	Y
Body weight		Y								

Y—Yes; CQ—Chloroquine; SP—Sulphadoxine-pyrimethamine.

ing transmission season. All necessary information was recorded in prescribed proforma. Preparation and staining of the blood slides were done following the procedures of WHO outlined in basic malaria microscopy[15]. Thick and thin blood smears were checked after staining with Giemsa stain. Parasite density was estimated by counting the number of parasites per 200 leucocytes. Parasite counts were converted to number of parasites/µl blood taking 8000 leucocytes/µl as standard. Microscopically confirmed cases of *P. falciparum* were undergone pre-treatment examination. Patients meeting all the criteria for inclusion were enrolled as per the guidelines (Box 1, 2 and 3). Treatment with recommended drug was given under supervision and patients were followed for 28 days as per test protocol.

Box 1. Inclusion criteria

- All patients above six months of age
- The patients should have mono-infection with *P. falciparum*, at least 1000 to 1,00,000 asexual parasites/µl
- History of fever during the present illness, axillary temperature >37.5°C
- Absence of general danger signs of severe and complicated falciparum malaria
- Absence of severe malnutrition
- Ability to come for the stipulated follow-up visits
- Informed consent by the patient or by parents/guardians for children

Box 2. Exclusion criteria

- Patient who need change of treatment during follow-up or develop sign of complications
- Patient who have consumed other antimalarials during the period of study
- Pregnancy
- Individuals with febrile diseases other than malaria
- Patient reporting with sign of complications, history of allergic reaction to sulfonamides

Box 3. General serious symptoms

- Not able to drink or feed
- Repeated vomiting
- Convulsions during the present illness
- Lethargic or unconscious
- Unable to sit or stand up

Treatment : Patients were treated with recommended drug regimen as per the National policy. The enrolled patients were administered orally, either CQ, a total dose of 25 mg base/kg body weight over 3 days (10 mg/kg on Day 1 and Day 2 followed by 5 mg/kg on Day 3) or a single dose of SP combination having sulfadoxine/sulfalene 25 mg/kg with pyrimethamine 1.25 mg/kg body weight.

The test system : The tests on patients were carried out as per recommendation[6,7]. The essential information of patients as axillary temperature, clinical assess-

ment, body weight on Day 0, parasitaemia, medication on various days were recorded. The data on clinical examination, temperature and parasitaemia were obtained by following up the patient on Days 1, 2, 3, 7, 14, 21 and 28 (Table 2). Microscopic results of the enrolled patients and of those who showed treatment failure were assessed by two different microscopists to determine the reproducibility in parasite counts and test outcome. Clinical measurement and other parameters for assessment remained same for CQ and SP. Axillary temperature was measured using digital electronic thermometer and patient with temperature less than 37°C were re-examined. Treatment with recommended drug was given under supervision and patient was observed for at least 30 minutes post administration to be sure the retention of drug. Drugs used for the study were produced under good manufacturing practice (GMP) and same batch of the drugs were used throughout the study. The basic test schedule is presented in Box 4.

Box 4. Basic test schedule

Day 0	— Clinical assessment
	— Axillary temperature measurement
	— Determination of parasitaemia
	— Informed consent and enrollment
	— Body weight and treatment, first dose
Day 1	— Clinical assessment–referral in case of severe malaria/serious signs
	— Axillary temperature measurement
	— Parasitaemia monitoring
	— Treatment, second dose or alternative in case of early treatment failure (ETF)
Day 2	— Same as Day 1, except treatment, third dose or alternative in case of ETF
Day 3,	— Clinical assessment
7, 14,	— Axillary temperature
21, 28	— Parasitaemia
& any other day	— Alternative treatment in case of late treatment failure (LTF)

Sample size : Determination of sample size was done initially on the basis of lot quality assurance sampling (LQAS) method. For the prospective study, sample size was based on a balance including a large enough population of all age groups. Patients included in the study were chosen randomly and as many patients were found suitable to fulfill the criteria were recruited, based on the point prevalence of malaria at each study site.

Classification of therapeutic response

Patients' response to the antimalarial drug has been classified as per WHO guidelines. For low to moderate transmission area, classification and characteristics are as follows:

Early treatment failure (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; Parasitaemia on Day 3 with axillary temperature >37.5°C; and Parasitaemia on Day 3 >25% of count on Day 0

Late treatment failure (*LTF*) : This type of response was classified into two sub groups as:

Late clinical failure (LCF) : Development of danger signs or severe malaria after Day 3 in the presence of parasitaemia, without previously meeting any of the criteria of ETF. Presence of parasitaemia 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 ETF.

Late parasitological failure (LPF) : Presence of parasitaemia on any day from Day 7 to Day 28 and axillary temperature < 37.5°C, without previously meeting any of the criteria of ETF or LCF.

Adequate clinical and parasitological response (ACPR) : Patients fully responded to the drug fell under

this category—absence of parasitaemia on Day 28 irrespective of axillary temperature without previously meeting any of the criteria of ETF or LCF or LPF.

Results & Discussion

Study was conducted at seven selected sites from July to November 2002. All the patients reported with fever were checked for malaria parasite by microscopy. A total of 3872 fever cases were screened, 417 found *P. falciparum* positive, 78 patients had *P. vivax* and two had both *Pf* and *Pv* infection (Table 3).

In study areas an overall positive cases with *P. falciparum* were 10.77%. Of the 417 *Pf* cases, 365 met the criteria for inclusion and they were enrolled for the study with informed written consent, 52 patients who could not be included, were given full course of treatment with the respective drugs as per recommendation. For assessment of CQ treatment efficacy, 224 patients were included among them 10 were dropped during the period of study. In SP treatment efficacy group, 166 patients were enrolled, almost all of them could be monitored for a period of 28 days, one patient was dropped, because of tetracycline intake due to cold and cough, one patient could not come for complete follow-up. The details of the test results are summarised in Tables 4–6.

Patients failed to respond to CQ were given second line of drug—SP and they were then followed-up as per the protocol. In NK group, along with 41 fresh enrolled patients, 12 CQ treatment failure cases were also included. In KS and KB sites only CQ treatment

Table 3. Microscopic examination of blood smears

Study site	Site code	BSE	*Pf*	*Pv*	Mixed	%*Pf*	Cases selected
Keonjhar Town	KT	439	67	3	0	15.26	63
Padampur	KP	560	58	9	1	10.36	54
Basudebpur	BP	467	29	4	0	6.21	22
Kathiatali	NK	701	102	9	1	14.55	93
Simonabasti	NS	235	31	0	0	13.19	25
Sonapur	KS	843	72	48	0	8.54	50
Boko	KB	627	58	5	0	9.25	58

Table 4. CQ study

Site code	Cases enrolled	Parasites/µl (range)	Axillary temp (^{o}C) (range)	ETF	LTF	ACPR	L	W
KP	54	1040–25000	37.6–40.2	1	1	47	5	N
BP	22	1200–78000	37.4–39.6	N	N	17	5	N
NK	40	1200–37600	37.5–39.5	2	10	28	N	N
KS	50	1280–72000	37.3–40.2	9	8	33	N	N
KB	58	1200–96000	37.7–40.2	3	5	50	N	N

ETF—Early treatment failure; LTF—Late treatment failure; ACPR—Adequate clinical and parasitological response; L— Lost to follow-up; W—Withdrawn; N—None.

Table 5. CQ treatment failure

Site code	No. of cases	Treatment failure on Days							
		Day 2	Day 3	Day 6	Day 7	Day 9	Day 14	Day 21	Day 28
KS	17	–	9	–	1	–	3	3	1
KB	8	2	1	–	2	–	2	1	–
NK	12	2	–	3	5	1	1	–	–
KP	2	–	1	–	1	–	–	–	–

Table 6. SP study

Site code	Sample size	Parasites/µl (range)	Axillary temp (°C) (range)	ETF	LTF	ACPR	L	W
KT	63	1040–75000	37.5–40.4	N	N	61	1	1
NK	53	1040–90000	37.3–40.6	N	N	53	N	N
NS	25	1000–50000	37.2–40.5	N	N	25	N	N
KS	17	1000–21920	37.2–38.5	N	1	16	N	N
KB	8	1200–16000	37.8–39.6	N	N	8	N	N

ETF— Early treatment failure; LTF— Late treatment failure; ACPR—Adequate clinical and parasitological response; L—Lost to follow-up; W—Withdrawn; N— None.

failure cases were given SP, they were monitored for 28 days. In KS group, one patient was found feverish with parasitaemia on Day 14. This patient was referred to Central Health Facility and was treated with alternative drug.

Data obtained from the present study showed 30% treatment failure with CQ in District Nowgaon. There are reports of CQ resistance of RII and RIII grades in this district[16]. In Kamrup district CQ is the first line of treatment and 23.15% patients found non-responsive to this drug. In Keonjhar district, though malaria cases are reported in high number, but only 3.03% patients showed treatment failure in Padampur and Basudebpur areas. In the study sites, SP combination found to be fully effective as a second line drug and also as a first line drug wherever revised drug policy has been introduced.

Host related variables, such as the vector and the treatment affect the selection, survival and propagation of drug resistant parasites. Selection of drug resistance may occur if large numbers of infected people are treated, if treatment continues for a long time, and if many parasites are exposed to the drug in each patient. Propagation of a resistant strain depends on wide-spread use of the drug to which resistance has been developed, which may act as an important factor in restraining competition from drug susceptible strains of the parasites[1]. The focus of roll back malaria is based on greater intensity of efforts and its main emphasis on the operational issues of implementing malaria control by adding value to existing efforts. Therapeutic strategy with common or newly introduced drugs should be designed in such a way so as to minimise the threat of resistant parasites. Specific strategies should be applicable to the patient, the communi-

ty and the region. The selection of drugs and treatment protocols must be based on efficacy of clinical and epidemiological assessments. Findings of the present study would help in determining the practical efficacy of the drug regimens with ultimate objective of ascertaining their continued usefulness or the necessity for replacing them in routine treatment[6]. Data thus generated on drug resistance using standard protocol would help in determining drug policy for the treatment in areas of low/moderate/high malaria transmission. This would be a step towards promoting evidence-based actions against malaria at country level to fulfill one of the main objectives of roll back malaria initiative.

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References

1. Black RH, Canfield CJ, Clyde DF, Peters W, Wernsdorfer WH. Drug resistance in malaria. In : Bruce-Chwatt LJ, editor. *Chemotherapy of Malaria*, 2nd ed. Geneva : WHO 1981; p. 102–18.
2. Resistance of malaria parasites to drugs. *WHO Tech Rep Ser* No. 296. Geneva : WHO 1965.
3. Chemotherapy of malaria. *WHO Tech. Rep Ser* No. 375. Geneva : WHO 1967.
4. Chemotherapy of malaria and resistance to antimalarials. *WHO Tech Rep Ser* No. 529. Geneva : WHO 1973.
5. Antimalarial drug policies : data requirements, treatment of uncomplicated malaria and the management of malaria in pregnancy. WHO/MAL/94. Geneva : WHO 1994; p. 1070.
6. Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated malaria. WHO document, draft 28.2.97. Geneva : WHO 1997; p. 1–30.
7. Monitoring antimalarial drug resistance. Report of a WHO consultation. CDS/RBM/2002.39. Geneva : WHO 2002; p. 1–29.
8. Guidelines for good clinical practice for trials on pharmaceutical products. *WHO Tech Rep Ser* No. 850. Geneva : WHO 1995.
9. Sharma VP. Status of drug resistance in malaria in India. In : Mahajan RC, editor. *Multi-drug resistance in emerging and re-emerging diseases*. New Delhi : Indian National Science Academy 2000; p. 191–202.
10. Sehgal PN, Sharma MID, Gogai S. Resistance to chloroquine in falciparum malaria in Assam state. *J Com Dis* 1973; *5* : 175–80.
11. Dhillon GPS, Sonal GS. Status of drug resistance. In : Gupta S, Sood OP, editors. *The changing face of malaria* : Proceedings of the IV round table conference. New Delhi : Ranbaxy Science Foundation 1998; p. 95–101.
12. Plowe CV, Cortese JF, Djimde A. Mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine-sulfadoxine use and resistance. *J Infect Dis* 1997; *176* : 1590–6.
13. Watkins WM, Mberu EK, Winstanley PA, Plowe CV. The efficacy of antifolate antimalarial combinations in Africa : a predictive model based on pharmacodynamic and pharmacokinetic analyses. *Parasitol Today* 1997; *91* : 456–60.
14. Dev V, Phookan S. Epidemiology and control of malaria in the Brahmaputra valley of Assam. *Adv Med Entomol Human Welfare* 1998; *1*(Suppl) : 59–65.
15. Basic malaria microscopy, Pt I & II. Geneva : WHO 1991.
16. Misra SP. *In vivo* resistance to chloroquine and sulfadoxine-pyrimethamine combination in *Plasmodium falciparum* in India. *Proc Natl Acad Sci India* 1996; *66* : 123–38.