4

Clinical Research

The treatment of malaria has been a challenge in south-east Asia since 1950s when the first case of chloroquine resistance in *P. falciparum* was detected in Thailand. The rapid change of treatment policy in some countries to new drugs led to multi-drug resistance, and few other countries including India continued to use chloroquine due to various technical and operational reasons. However, in the present scenario of the increased donor support and strong recommendations of the World Health Organization (WHO) for use of Artemisinin-based combination therapy (ACT), movement to ban production, marketing of artemisinin monotherapy and raising the desired cure rate level from 75 to 90%, it has become a necessity for all countries to change their treatment policies.

In India, ACT (Artesunate + Sulphadoxine- Pyrimethamine) has been recommended in more than 160 districts in 2008. Therefore, the efficacy of the new regimen was evaluated using WHO protocol, at different sites in India. In addition, the efficacy of fixed dose combination registered in India (Artemether + Lumefantrine) was also evaluated.

"ACT is highly effective in the treatment of uncomplicated falciparum malaria (cure rate 95–100%)"

It is well-known that compliance is better with fixed dose combinations than blister packs. Therefore, to facilitate the introduction of new fixed dose ACTs, Phase II and III clinical trials were also conducted in association with hospitals in endemic states. New linkages have been developed, for example with Tata Main Hospital in Jamshedpur, Jharkhand.

4.1 Assessment of Therapeutic Efficacy of Antimalarial Drugs against Uncomplicated *Plasmodium falciparum* Malaria

Antimalarial drug resistance is a challenge in the treatment of falciparum malaria. It is, therefore, necessary to evaluate the efficacy of first and second line antimalarial drugs at several sites, so as to help adopt alternative strategies for treatment as per need. The present studies were conducted to evaluate efficacy of ACT used/marketed in India with following objectives: (i) to assess the therapeutic efficacy of combination therapy in uncomplicated *P. falciparum* malaria in endemic districts in Assam, Jharkhand and Orissa; and (ii) to validate the *in vivo* drug resistance data using molecular markers.

The study was conduced according to WHO protocol for therapeutic efficacy. This was one arm prospective evaluation of parasitological and clinical response to directly observed treatment for uncomplicated malaria. All patients reporting to local clinics at study sites with complaint of fever were examined for prevalence of parasites in blood smear. Peripheral smear was examined, and those positive cases for *P. falciparum* were enrolled and given treatment. They were observed for 30 min and if they vomited during this period, full dose was repeated.

Re-assessment was done on Day 0 and on Days 1, 2, 3 and 7 after enrolment, and then weekly up to 28 days. The dosing response was classified as adequate clinical and parasitological cure (ACPR), early treatment failure (ETF) and late treatment failure (LTF). Rescue medication was given to treatment failures. Genotyping of blood spots on Day 0 and on the day of recrudescence by MSP-1, MSP-2 and GLURP. Nested PCR with family-specific assay was done to differentiate new infection from recrudescence. The study was conducted at Garden Hospital, Amchong Tea Estate, PHC Sonapur, District Kamrup, Assam; Angara PHC at Ranchi and Jaldega PHC at District Simdega, Jharkhand; and Keonjhar Town and PHC Banspal of District Keonjhar, Orissa (Fig. 4.1.1).

Therapeutic efficacy of Artemether- Lumefantrine (AL) was tested at two sites, namely Garden Hospital, Amchong Tea Estate, PHC Sonapur, District Kamrup, Assam and Keonjhar Town, District Keonjhar, Orissa. Therapeutic efficacy of Artesunate+ Sulphadoxine-pyrimethamine (AS+SP) was tested at three sites, namely Angara PHC, District Ranchi, Jaldega PHC, District Simdega, Jharkhand and PHC, Banspal of District Keonjhar, Orissa.

4.1.1 Assam

A total of 53 patients were enrolled for the study with Artemether and lumefantrine (AL) combination therapy from Sonapur PHC, District Kamrup. The cumulative success rate (ACPR) by survival analysis was 100% (Table 4.1.1). The drug combination was well-toler-

Table 4.1.1. Therapeutic efficacy of AL in District Kamrup, Assam

Response	Number	Prevalence
ETF	0	0
LCF	0	0
LPF	0	0
ACPR	53	1
Total analysis	53	
Withdrawn	0	
Lost to follow-up	0	0
Total	53	

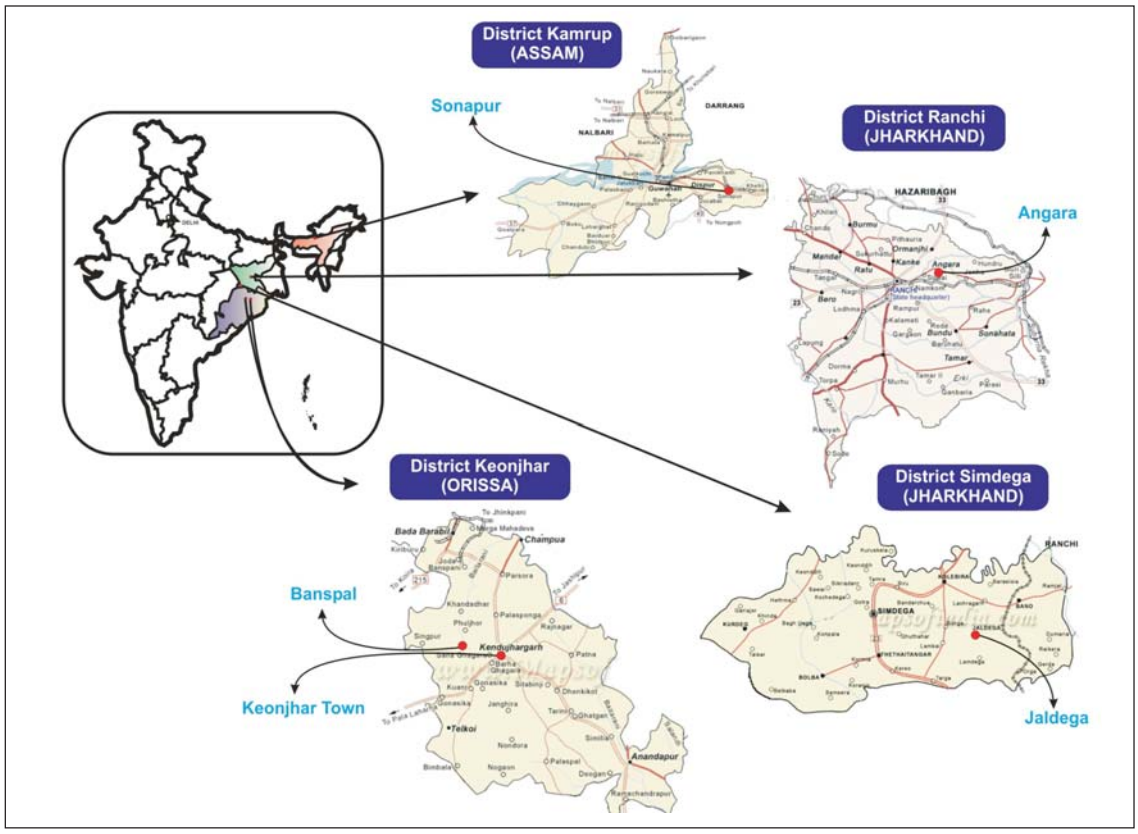

Fig. 4.1.1: Map showing study sites of therapeutic efficacy studies

ated. No adverse effects were reported with AL. Parasite clearance was also rapid.

4.1.2 Jharkhand

In PHC Angara, District Ranchi, two patients were late treatment failures, but were classified as re-infection after PCR leading to corrected cure rate of 100% (Table 4.1.2). At PHC Jaldega, District Simdega, there was no early or late treatment failure indicating that AS+SP is an effective ACT (Table 4.1.3).

Table 4.1.2. Therapeutic efficacy of AS + SP at District Ranchi, Jharkhand

Response	Number	Prevalence
PCR Uncorrected Data		
ETF	0	0
LCF	0	0
LPF	2	0.039
ACPR	49	0.961
Total analysis	51	
Withdrawn	2	
Lost to follow-up	0	0.038
Total	53	
PCR Corrected Data		
ETF	0	0
LCF	0	0
LPF	0	0
ACPR	49	1
Total analysis	49	
Withdrawn	4	
Lost to follow-up	0	0.075
Total	53	

Table 4.1.3. Therapeutic efficacy of AS + SP at District Simdega, Jharkhand

Response	Number	Prevalence
ETF	0	0
LCF	0	0
LPF	0	0
ACPR	45	1
Total analysis	45	
Withdrawn	0	
Lost to follow-up	0	0
Total	45	

4.1.3 Orissa

A total of 71 patients were enrolled at each site in PHC Banspal and Keonjhar Town in Orissa. The cumulative success rate by survival analysis was 100% with AS+SP in PHC Banspal. At Keonjhar there were two failures with AL, one on Day 7 and the other on Day 28. The latter turned out to be re-infection after PCR analysis. Therefore, the corrected cure rate was 98.6%. It can be concluded that AS+SP and Artemether- Lumefantrine combinations are highly effective in the treatment of falciparum malaria in India.

"AS+SP and Artemether-Lumefantrine combinations are highly effective in the treatment of falciparum malaria in India"

4.2 Clinical Trials

4.2.1 Multi-centre, Open-label, Randomized Clinical Study of Efficacy and Tolerability of the Fixed-dose Artesunate/Amodiaquine (AS/AQ) Combination Therapy and Amodiaquine (AQ) Monotherapy for the Treatment of Uncomplicated *P. falciparum* Malaria in India

In view of development of resistance to current antimalarials, WHO recommends use of Artemisinin-based combination therapy for the treatment of falciparum malaria. At present, only one ACT (Artemether-Lumefantrine–Coartem®) is available in India as a fixed dose drug in which both compounds are co-administered. Drug for Neglected Diseases *initiative* (DND*i*) in association with UNICEF-UNDP-World Bank-WHO (TDR) is developing a new fixed-dose combination of artesunate and amodiaquine that will allow a simple treatment of three days, with a single daily administration of two tablets.

An open-label, randomized clinical study was conducted at two sites in India to evaluate the efficacy and safety of this combination in India. A total of 300 subjects were randomized

(2:1) to Group A (fixed-dose combination of AS/AQ tablets), and Group B (Amodiaquine). The subjects recruited were those presenting with uncomplicated falciparum malaria defined by *P. falciparum* mono-infection with fever >37.5ºC and parasitaemia of 1000–100,000 asexual parasites/µl. The dose administration in both groups was according to age criteria and two formulations of AS/AQ containing 25 mg/67.5 mg and 100 mg/270 mg were used for children and adults respectively. The eligible subjects were recruited for the study after they signed the Institutional Ethical Committee (IEC) approved informed consent document. The treatment period was for three days with follow-up of 25 days. The safety reporting was done through the entire conductance of the study. The clinical study was conducted according to the local regulations including Schedule Y, ICMR guidelines, Indian Good Clinical Practice (GCP) and the standards conforming to the ICH and GCP.

The two participating centres screened 327 patients, out of them 27 were screen failures and 300 were randomized into the study. The intention-to-treat (ITT) population at both the sites was 298 and the per-protocol (PP) population was 292.

The cure rate based on ACPR (before PCR correction) was 92.4% in Group A (AS/AQ) and 83% in Group B (AQ). The difference seen between the two groups was statistically significant (p = 0.0144). The cure rate based on PCR genotyping (after PCR correction) was 97.5% in Group A (AS/AQ) and 88.3% in Group B (AQ).

"Cure rate of AS/AQ is according to WHO recommendation for ACT and AQ has desired efficacy of partner drug"

The study demonstrates that cure rate of AS/AQ is according to WHO recommendation for ACT and AQ has desired efficacy of partner drug. The study provides evidence for a fixed dose combination of AS+AQ which is easy to administer, cost-effective, well-tolerated and a short duration regimen. The study provides evidence that AS/AQ combination can be an option for implementation of this combination as an effective and safe drug.

4.2.2 A Phase III, Randomized, Non-inferiority Trial to Assess the Efficacy and Safety of Dihydro-artemisinin + Piperaquine (Artekin) in Comparison with Artesunate + Mefloquine in Patients Affected by Acute, Uncomplicated *Plasmodium falciparum* Malaria

Artekin™ (Dihydroartemisinin + Piperaquine) is a second generation of Artemisinin-based combination therapy (ACT) with similar efficacy to that of Artemether + Lumefantrine or Artesunate + Mefloquine, but with a simpler dosing scheme that will aid better compliance. A new phase III trial using Artekin produced according to good manufacture practice (GMP) and European regulatory standards was conducted in Thailand, India and Laos.

The study was conducted at three sites in India, namely Goa, Mangalore and Guwahati. A total of 1150 patients were recruited at all the sites in India and other countries. In order to ensure concealment of treatment allocation and avoid other biases, the randomization was under blind conditions and the treatment allocation was concealed until the final recruitment of the patients. The primary end point was the PCR-corrected adequate clinical and parasitological response (PCR corrected ACPR) on Day 63.

Males and females aged ≥18 years having microscopically confirmed monoinfection of *P. falciparum* (asexual forms parasitaemia ≥1000/µl – ≤100,000/µl or mixed infection), history of fever or presence of fever (temperature ≥37.5°C) were included. Dihydroartemisinin+ Piperaquine tablets containing

40 mg of Dihydroartemisinin and 320 mg of Piperaquine for adult patients were administered for three days and on Day 63 cure rate of 98% was observed, and parasite clearance time ranged between 1 and 3 days. The drug was well-tolerated. It was concluded that Artekin is non-inferior to standard treatment of Artesunate + Mefloquine.

4.2.3 A Phase II, Randomized, Open-label, Multi-centre Study to Assess the Antimalarial Efficacy and Safety of Arterolane (RBx 11160) Maleate and Piperaquine Phosphate Co-administration and Coartem® in Patients with Acute Uncomplicated *Plasmodium falciparum* Malaria

RBx 11160, (Arterolane) a new peroxide, is a synthetic trioxolane that is easy to synthesize, inexpensive, achiral and orally rapid acting with high antimalarial activity. It produces antimalarial action by reductive activation of haem, released as a result of haemoglobin digestion and irreversible redox reaction produces carbon-centered free radicals, leading to alkylation of haem and proteins (enzymes). Preclinical and phase I human studies have confirmed safety of the drug. The Phase II studies with Arterolane alone and in combination with Piperaquine have shown excellent efficacy against *P. falciparum*. A Phase II clinical trial was then carried out to assess the antimalarial efficacy of Arterolane (RBx 11160) maleate and Piperaquine phosphate co-administration and Coartem in patients with acute uncomplicated *P. falciparum* malaria at Rourkela, Ranchi and Jamshedpur. The collaborating hospitals were Ispat General Hospital, Rourkela, Mahadevi Birla Hospital, Ranchi and Tata Main Hospital, Jamshedpur. Primary objective of the study was to estimate the Day 28 PCR corrected adequate clinical and parasitological response (ACPR) of three dose regimen of arterolane (RBx 11160) maleate 150 mg and piperaquine phosphate 750 mg co-administration and six dose regimen of Coartem in patients with acute uncomplicated *P. falciparum* malaria. In all, 200 patients were enrolled in the study including from India and results are encouraging and comparable to standard treatment with ACT.

"Pyramax was found as effective as chloroquine in the treatment of vivax malaria"

4.2.4 A Phase III Comparative, Open-label, Randomized, Multi-centre, Clinical Study to Assess the Safety and Efficacy of Fixed dose Formulation Oral Pyronaridine Artesunate (180 : 60 mg Tablet) versus Mefloquine (250 mg Tablet) plus Artesunate (100 mg Tablet) in Children and Adult Patients with Acute Uncomplicated *Plasmodium vivax* Malaria

Pyramax is a combination of Artesunate and Pyronaridine which has shown to be effective for the treatment of malaria in phase II trials. The present phase III study was designed as a multi-centre, randomized, comparative, parallel group study of the efficacy and safety of a three day regimen of the fixed combination of pyronaridine artesunate (180 : 60 mg tablets) versus chloroquine in vivax malaria. Patients between 3 and 60 years with minimum parasitaemia of 250 parasites/µl were enrolled and followed for 28 days after the first study drug administration. The primary efficacy end point was at 28 days. A total of 456 patients (228 in each group) were enrolled at all sites including Thailand, Cambodia, Indonesia and India. The cure rates were as good as standard treatment with chloroquine. The parasite clearance was faster than that of chloroquine. It can be concluded that Pyramax is as effective as chloroquine in the treatment of vivax malaria.

4.3 Operational Research on Drug use Practice and Pre-packaged Blister Pack Drugs

Therapeutic efficacy studies were conducted in Jharkhand state and high CQ resistance in

Workshop on operational research on drug use practice

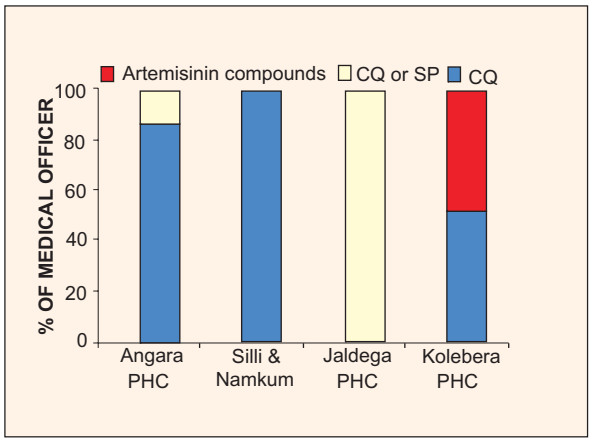

Fig. 4.3.1: Drugs prescribed by medical officers for the treatment of uncomplicated falciparum malaria

P. falciparum in study areas was observed. On this basis, the NVBDCP recommended change of drug policy in the affected PHCs of three districts to ACT (Artesunate and Sulphadoxine + Pyrimethamine). Blister packs for the radical treatment of adult patients (15 yrs and above) have been introduced in the national programme to improve acceptance of antimalarial drugs and compliance of the full course. A study was undertaken in Jharkhand state to document information on drug use practice and compliance of blister packs. The selected districts were Simdega and Ranchi. In Ranchi district, Angara PHC (Changed drug policy) and Silli and Namkum PHCs (No change in drug policy) were included. In Simdega district, Jaldega PHC (Changed drug policy) and Kolebera PHC (No change in drug policy) were included.

The first objective of the study was to evaluate drug-use practice with emphasis on districts with change in drug policy in Jharkhand state. Secondly, to study the knowledge and skills of paramedical personnel in the use of blister packs (including ACT), its acceptance by paramedical personnel and in the community; and to study the compliance for blister pack and the serious adverse events, if any, with the usage of blister packs. Pre-tested questionnaire for observations on diagnosis and treatment of malaria was developed. At all sites, in addition to verification of the prescribing patterns, the records and investigations were retrospectively analysed.

In the studied PHCs, all Medical Officers (100%) used blood slide examination to diagnose malaria and were aware of rapid diagnostic kits (RDKs). The reporting of malaria cases to higher authorities was found to be 100%. However, awareness about the training course on malaria is low. Treatment of *P. vivax* malaria in all the studied PHCs is with chloroquine (100%). Despite drug policy change, the drugs prescribed for *P. falciparum* malaria were irrational (Fig. 4.3.1). The awareness about drug dose and duration is inadequate at sub-centre level. Availability of RDK at sub-centre is scanty.

In Jharkhand, recently all the DDCs are converted to FTDs. Majority of the staff at FTDs are not well qualified and are unaware of the drug policy. The supply of RDKs to sub-centres located at remote places is inadequate. The overall knowledge about new drug policy and treatment guidelines was insufficient. Medical Officers should be made aware of treatment guidelines through various means.

4.4 *In vitro* Sensitivity of Indian *Plasmodium falciparum* Strains to Antimalarial Agents

This is a cross-sectional sample survey to estimate the *in vitro* drug resistance of *P. falci-*

Table 4.4.1: Success rate of culture according to origin

Origin	No. attempted	No. successful	Percent
Orissa	43	28	65.1
Jharkhand	38	24	63.2
Other states	22	9	40.9
Parasite Bank	19	7	36.8
Total	122	68	55.7

parum to antimalarials. The study is being conducted at various field units of NIMR at Rourkela, Guwahati, Ranchi, Bengaluru, Goa and Raipur.

Subjects having *P. falciparum* monoinfection with a parasitaemia of 1000 to 80000 per µl blood and not having a history of antimalarial consumption were recruited for the study. Sensitivity was carried out for dihydroartemisinin, chloroquine, amodiaquine and mefloquine by using the WHO microtest mark III. The mean inhibitory concentrations (IC_{50}) of individual samples for each drug were determined by non-linear regression analysis.

Table 4.4.2: *In vitro* susceptibility of *P. falciparum* to four drugs

Drug	IC_{50} mean (nM/l)	IC_{99} mean (nM/l)	Resistance threshold (nM/l)
Chloroquine	20.8	154.48	>160[a]
Monodesethyl amodiaquine	4.04	27.60	>80 [a]
Dihydroartemisinin	2.59	5.26	>10.5[b]
Mefloquine	24.90	56.15	>640 [a]

a— WHO, Threshold refers to IC_{99}; b—Restrepo-Pineda *et al*, Threshold refers to IC_{50}.

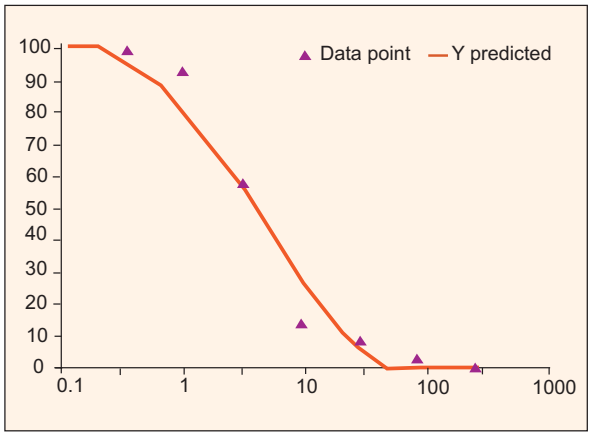

Fig. 4.4.1: Response of a *P. falciparum* strain to chloroquine

For this, HNnonLin version V1.1 software (downloaded from www.malaria.farch.net) model was used.

In all, 122 samples have been studied from four different sites, namely Jharkhand, Orissa, Karnataka and Goa (Table 4.4.1). The male : female ratio was 1 : 2.6. The average parasite count in the patients subjected to *in vitro* testing was 17540/µl blood. In addition, 19 isolates have also been studied from the malaria parasite bank of NIMR. The proportions of successful assays were 59.2 and 36.8% in fresh and cultured isolates respectively.

The geometric mean IC_{50} were 20.8 nmol/l, 4.04 nmol/l, 2.59 nmol/l and 24.9 nmol/l, for chloroquine, monodesethyl amodiaquine, dihydroartemisinin and mefloquine respectively (Table 4.4.2). Figure 4.4.1 shows a representative graph illusrating the response of a *P. falciparum* strain to chloroquine. The level of resistance in chloroquine was 50% (30 isolates), while that of in amodiaquine was 25% (15). Of these 15 isolates, seven were also re-

Table 4.4.3: Number of drug resistant isolates/total isolates in different places according to origin

	Chloroquine (%)	Amodiaquine (%)	Artemisinin (%)	Mefloquine (%)
Orissa	20/28 (71.4)	9/28 (32.1)	1/28 (3.6)	0/28 (0)
Jharkhand	6/18 (33.3)	4/17 (23.5)	0/17 (0)	0/17 (0)
Others	4/14 (28.6)	2/15 (13.3)	0/15 (0)	0/15 (0)
Total	30/60 (50)	15/60 (25)	1/60 (1.66)	0/60 (0)

sistant to chloroquine. One isolate was resistant to dihydroartemisinin. The isolate also showed resistance to chloroquine as well as amodiaquine. No isolate showed resistance to mefloquine.

"Very high resistance to chloroquine (71%) was found in Orissa followed by Jharkhand (33.3%) among the study states"

Table 4.4.3 shows the drug sensitivity pattern according to the origin. Very high resistance to chloroquine was seen in Orissa, followed by Jharkhand and other states. It is important to note that there is a change in the drug policy in both Jharkhand and Orissa for the treatment of malaria; while there is no change in the policy in the other studied states. The sole case of artemisinin resistance was detected in Orissa. ❑