Transactions of the Royal Society of Tropical Medicine and Hygiene (2006) 100, 108–118

ELSEVIER

www.elsevierhealth.com/journals/trst

A randomized trial comparing the efficacy of four treatment regimens for uncomplicated falciparum malaria in Assam state, India

P. Campbell[a,*], S. Baruah[a,1], K. Narain[b], C.C. Rogers[a,2]

[a] *Medecins sans Frontieres-Holland, India Section, R.G. Baruah Road, Guwahati, Assam 781024, India*
[b] *Regional Medical Research Centre, N.E. Region (ICMR), Post Box #105, Dibrugarh, Assam 786001, India*

Received 28 November 2003; received in revised form 28 June 2005; accepted 28 June 2005
Available online 14 November 2005

KEYWORDS
Malaria treatment;
Chloroquine;
Sulfadoxine–pyrimethamine;
Mefloquine;
Artesunate;
India

Summary A four-arm drug sensitivity study compared chloroquine, sulfadoxine–pyrimethamine (SP), mefloquine and mefloquine–artesunate in Sonitpur and Karbi Anglong districts in Assam state, India. Two criteria were used to ascertain outcome: success of clinical treatment and parasitologic cure. In Sonitpur, at 14 days, there were 36/56 early and late treatment failures plus late parasitologic failures to chloroquine and 16/56 for SP. In Karbi Anglong, combined treatment failure at 14 days was 16/56 to chloroquine and 8/60 to SP. Mefloquine and mefloquine–artesunate demonstrated 93.9% and 93.6% sustained responses respectively at 42 days. High failure rates to both chloroquine and SP preclude the use of these drugs as first-line treatment for uncomplicated falciparum malaria in this region. A mefloquine–artesunate combination presents an effective alternative utilizing the currently recommended higher dose of mefloquine.

1. Introduction

Current estimates by WHO indicate that there may be as many as 15 million cases of malaria and 6000 deaths per year in India (Sharma, 1999). However, the true number of cases is likely to be even higher since many persons do not seek care in government facilities due to inadequate performance, lack of easy access and shortage of medicines. Self-medication is widespread. Malaria is one of the most prevalent health problems in the seven northeastern states of India where it is endemic and widely distributed. Although it contains only 3.7% of India's population, this region accounts for 10% of confirmed malaria cases and 13–41% of all malaria-related deaths. The mortality rates reflect

* Corresponding author. Present address: 6A Chateaux Circle, Scarsdale, NY 10583, USA. Fax: +1 914 948 7795.
E-mail address: patricia10334@yahoo.com (P. Campbell).
[1] Present address: Tezpur University, Napaam, Tezpur 784 928, Sonitpur, Assam, India.
[2] Present address: International Rescue Committee, Central and South East Afghanistan, Gardez, Paktia Province, Afghanistan.

0035-9203/$ – see front matter © 2005 Published by Elsevier Ltd on behalf of Royal Society of Tropical Medicine and Hygiene.
doi:10.1016/j.trstmh.2005.06.016

the rising predominance of falciparum malaria, which now constitutes 60–80% of malaria in this area. Assam is the largest and most developed of the seven states, its 26 million population comprising 71% of the population of the entire region. Although malaria transmission is perennial, the pattern of transmission is primarily seasonal with the largest peak in May–August followed by a less intense post-monsoon period during October to late November (Dev, 2001; Mohapatra et al., 1998a). It is considered a region of low to moderate transmission intensity. The principal vectors, *Anopheles dirus* and *A. minimus*, are well adapted to the ecology of the deep forest and forest-fringe environment which covers extensive areas of northeast India (Prakash et al., 1998). It has been estimated that these ecological sites contribute as much as 53% of the malaria cases in the region. In 2000, Assam recorded 85 000 slide-confirmed cases of malaria, 61% due to *Plasmodium falciparum*, the remainder due to *P. vivax*. In the past 20 years, northeast India has witnessed an increase in both geographical extent and number of cases of falciparum malaria as populations have moved into the malarious reservoirs to pursue cultivation, exploitation of natural resources and infrastructure development. As a result, Assam has experienced recurrent focal outbreaks of falciparum malaria during the past decade (Das et al., 1997; Mohapatra et al., 1995, 2001a; Prakash et al., 2000). Transmission across international borders shared with Bangladesh and Bhutan are included in this paradigm.

An important factor in the increase of falciparum malaria has been the emerging resistance to chloroquine. In 1973, Assam was the first state in India to report a case of chloroquine-resistant malaria which was found in Karbi Anglong district (Sehgal et al., 1975). Since that time, increasing chloroquine resistance has been documented throughout the Indian subcontinent, including Assam (Barkakaty and Narasimilam, 1992; Dev and Phookan, 1998). Most of these studies have involved a small number of subjects. The most recent national compilation of drug sensitivity studies in 1997 reviewed 12 863 cases of falciparum malaria. There were 3065 cases with R1, R2 or R3 levels of in vivo chloroquine resistance which translates into a cumulative resistance of 24% for the entire country. The National Antimalaria Program (NAMP) concluded that the increase in malaria morbidity and mortality during the 1990s was, in part, due to increasing resistance to chloroquine (Shiv et al., 1997).

The NAMP recommends chloroquine as a first-line treatment for clinically or parasitologically diagnosed uncomplicated falciparum malaria. Sulfadoxine-pyrimethamine (SP) is employed for clinical failures or as the first-line treatment in districts where >25% R2/R3 level chloroquine resistance has been confirmed parasitologically. A 7-day course of quinine is used for third-line treatment. Primaquine is advised for transmission reduction for both vivax and falciparum species. DDT is used as a vector control agent (Government of India, 1995). Both mefloquine and artesunate are widely available in the marketplace, but neither is generally prescribed due to cost and, in the case of mefloquine, a reputation for adverse drug reactions. Artemether is approved for treatment of severe malaria in hospitalized patients but is also prescribed by private practitioners for those who can afford the medication. The retail cost of 250 mg mefloquine is US$1, and for 40 mg artesunate approximately US$0.56. Neither mefloquine nor artesunate are approved for routine use by the national guidelines and are thus not available in government health centers. A compelling factor in treatment-seeking behavior is the overwhelming preference for intravenous injections. Quinine is the most common alternative even for those whose monthly family income may be as low as 1000 Rs. The cost of three adult doses (the usual affordable course) is 350–450 Rs (US$7.50–9.50). The WHO has recommended the introduction of artesunate-containing treatment into endemic areas where the R2/R3 level of resistance exceeds 25% (Bloland et al., 2000). Previous reports have documented the utility of mefloquine–artesunate regimens in multidrug-resistant areas in Southeast Asia (Nosten et al., 1994, 2000). The present study was undertaken to assess the efficacy of chloroquine and SP in comparison with mefloquine and with mefloquine–artesunate in order to identify alternative choices for the management of drug-resistant malaria.

2. Materials and methods

2.1. Study design

The study was designed as a prospective randomized non-blinded trial utilizing four arms for the treatment of uncomplicated falciparum malaria in an outpatient setting. The follow-up period was 6 weeks (42 days) for all treatment arms. The primary endpoint was parasitologic cure and the secondary outcome variable was clinical response utilizing the WHO definitions for treatment response and failure (WHO, 2003). The occurrence of adverse reactions was monitored and characterized for each of the regimens.

2.2. Study sites

One site was selected in each of two widely separated districts, Karbi Anglong and Sonitpur. The investigation was conducted from 30 June to 11 September 2001 and spanned the peak transmission season, which was uninterrupted in 2001 due to the absence of the usual summer monsoon.

Karbi Anglong district has historically been one of the more highly endemic regions in Assam. The estimated district population of 800 000–900 000 is widely dispersed in the hilly and forested terrain, which is dotted with coffee and tea plantations. The study catchment area was centered around the Bokajan Community Health Center (latitude 26°1.5′N, longitude 93° 47′E) which serves a population of 60 000. This location exhibits a particularly high malaria prevalence with an annual slide positivity rate of 11–12%. More than 80% of cases are due to *P. falciparum*. Bokajan was the site of a longitudinal chloroquine resistance study conducted from 1979–1989, thus providing a baseline for comparison with data from the current sensitivity study (Barkakaty and Narasimilam, 1992). Sulfadoxine–pyrimethamine was substituted for chloroquine as the first-line treatment in 1983. Subsequently, in vivo resistance to SP of 12% within the first week of treatment was detected in 1991. Unlike chloroquine, which is provided to community workers and health posts for empiric treatment of fever cases, SP should be prescribed by a physician. Nevertheless, both SP and chloroquine are available for purchase in the local drug shops and are the most inexpensive antimalarials available.

Sonitpur district is located in western (lower) Assam. The area is predominantly hilly and forested with 300 000–400 000 of the 1.7 million population living in malarious areas, close to the foothills of Arunachal Pradesh. The highest cumulative incidence of malaria in the district occurs in North Jamuguri (latitude 26°43′N, longitude 92°56′E), where the annual slide positivity rate is 9–10% (number of positive slides per total number of slides examined). More than 50% of confirmed cases are due to *P. falciparum*. The study catchment area was centered around the North Jamuguri Community Health Center serving a largely rural population of 150 000. This location had been one of three areas in the district designated as 'high risk' with the onset of the malaria season in April 2001. The national malaria control program recommends that chloroquine be provided to village health workers and health posts for presumptive treatment of fever cases at the onset of the high transmission season. In addition, mobile clinics are initiated in high risk areas that are selected on the basis of preliminary district mortality/morbidity reports for the current year. Sonitpur was designated as one of these sites and a number of mobile fever clinics had taken place during the month prior to the commencement of the current drug sensitivity study. Although resistance to chloroquine had previously been reported to be high and widespread in directly adjacent districts (Shiv et al., 1997), Sonitpur had not been included in those studies. Therefore, chloroquine continued as the first-line treatment.

2.3. Study patients

Patients were recruited from the local, largely rural population in the vicinity of the North Jamuguri and Bokajan community health centers. Many were directly referred to MSF fever clinics upon presentation to the community health center outpatient clinic with fever or history of fever within the previous 48 h. Subjects with a positive slide for *P. falciparum* asexual forms and with a parasite density of 150–100 000/μl were entered into the study. Each patient (or guardian) gave written consent to receive treatment allocated on a random basis (day 0) and agreed to return for follow-up examination on days 1, 2, 7, 14, 21, 28, 35 and 42. Exclusion criteria included the following: pregnancy, children under 1 year of age, treatment with antimalarials within the past 48 h, signs of severe malaria, concurrent febrile illness, history of psychiatric illness, known hypersensitivity to any of the medications used in the study, severe malnutrition and inability/refusal to attend clinic for follow-up visits. Patients with mixed falciparum/vivax infections were included. Since hemoglobin determinations were not performed, the level of anemia was not used in the selection criteria. Those patients excluded from recruitment were referred to the health center doctors for evaluation and treatment.

2.4. Sample size

Sample size calculations were based on the difference in the proportion of parasitologic failure between chloroquine and SP. An expected success rate of 50% for chloroquine and 80% for SP was selected. To achieve a two-sided alpha of 0.05 and a power of 0.80, 45 patients were needed in each treatment arm for a total of 200 patients in each study site. Assuming a default/withdrawal rate of at least 20%, an enrolment of 60 patients in each treatment arm in each study site was adopted, bringing the total to 486 subjects.

2.5. Treatment randomization

Patients were stratified into three age groups: 1–4 years, ≥5–14 years and ≥15 years. Within each group, subjects were randomly assigned to one of the four treatment groups: (1) chloroquine 10 mg/kg on day 0 and day 1 and 5 mg/kg on day 2; (2) SP 25 mg/kg sulfadoxine and 1.25 mg/kg pyrimethamine on day 0; (3) mefloquine 15 mg/kg on day 0; and (4) mefloquine 15 mg/kg day 0 and artesunate 4 mg/kg day 0, on day 1 and day 2. All medications were given under direct observation and the patient observed for 1 h. A full dose of medicine was given again if the patient vomited within 30 min and a half dose of medication was given if vomiting occurred between 30 min and 1 h after administration of the medication. Persistent vomiting was a reason for being excluded from the study. Patients who exhibited clinical or parasitologic failure on the study drug were given alternative treatment as follows: those failing chloroquine or SP were given mefloquine + artesunate. Those failing the mefloquine or mefloquine–artesunate arms were treated with a 7 day course of artesunate (2 mg/kg/day) plus doxycycline (4 mg/kg/day) except in children aged <8 years who received a 7 day course of artesunate alone.

2.6. Data collection and laboratory methods

Each subject was weighed, the medical history obtained, and a physical examination including axillary temperature was performed. Information regarding previous type and duration of treatment with antimalarials was recorded. At each clinic visit, patients were examined and temperature recorded along with any complaints. Patients who failed to return after 48 h were classified as defaulters and were dropped from the study. Blood slides were prepared for quantitative counts of asexual forms on days 0, 1, 2, 7, 14, 21, 28, 35 and 42, and on any other day that the patient returned with fever or other symptoms. Thick and thin blood films, were stained with 12% Giemsa and the density of asexual parasites was determined based on an assumed WBC of 8000/μl (WHO, 1991). Mixed infections were not speciated individually on the quantitative counts. Following completion of the study, slides of parasitologic failures were recounted by MSF laboratory staff who were not informed of the original results. External quality control on 966 study slides (365 positive and 601 negative) was performed at the Regional Laboratory for the Northeast in Shillong, Meghalaya State, India. Discordant results were re-examined by both laboratories and retabulated.

2.7. Definitions used in outcome variables

The following definitions were used in ascertaining the outcome of treatment.

2.7.1. Parasitologic response

Sensitive (S): absence of parasites by day 7 and no reappearance of parasites by day 42.
R1: reappearance of parasites between 7 and 42 days of completing treatment, following initial complete resolution of symptoms and clearing of parasitemia.
R2: reduction of parasitemia by >75% at 48 h but failure to clear parasites within 7 days.
R3: parasitemia does not decrease by more than 75% within 48 h.

2.7.2. Therapeutic failure

Clinical deterioration at any moment or day: presentation with general danger signs, i.e. inability to drink or eat, repeated vomiting, convulsions during the present illness, lethargy or unconscious, inability to sit or stand up (these patients were referred to hospital for immediate admission).

The above definitions of parasitologic failure assume that reinfection has not occurred, a possibility which could not be excluded in the absence of PCR determinations in this study. To address the lack of PCR studies in distinguishing between reinfection and recrudescence, data was also analyzed according to WHO definitions of therapeutic failures as follows. Early treatment failure (ETF): (a) parasitemia and general danger signs on day 3 or earlier; (b) parasite density on day 2 > than that on day 0; (c) fever of >99.5 and parasitemia day 3; and (d) parasite density on day 3 >25% that on day 0. Late treatment failure (LTF) using a cut-off of 14 days: danger signs appearing after day 3 in the presence of parasitemia or parasitemia and fever on any day 4–14, excluding patients meeting the definition for ETF. Late parasitologic failure (LPF): parasitemia on any day 4–14 without fever. Since the subjects were ambulatory, fever clearance time was not obtained. The proportion of patients demonstrating parasite clearance was determined at 48 h.

2.8. Data entry and analysis of parasitologic failure

Data were coded as numerical variables and entered in an EpiInfo 6.0 database (CDC, Atlanta, GA, USA). Categorical data were analyzed for differences in proportions using the χ^2 statistic or

Fisher's exact test. The Monte Carlo method was used to obtain an unbiased estimate of the exact significance level. Normality assumption of continuous variables was tested using Kolmogorov–Smirinov one sample test. The Mann–Whitney *U* test and Kruskal–Wallis analysis of variance were used for comparing continuous data not following normal distribution. Survival times were interpreted as the time between the start of treatment and the day of failure or the date of last follow-up using the Kaplan–Meier method (SPSS for Windows, SPSS Inc., Chicago, IL, USA). Cumulative proportion of patients without treatment failure were evaluated by the product-limit method of Kaplan and Meier (Kalbfleisch, 1980) and compared by the log-rank test for ascertaining significant difference in survival times in the four treatment groups. Multivariate analysis using Cox proportional hazards regression was used to find the influence on treatment outcome of the following variables: treatment regimen, age, sex, density of asexual parasites on day 0, presence or absence of gametocytes on day 0 and axillary temperature on day 0. The final multivariate model was chosen using a backward elimination procedure removing non-significant determinants ($P > 0.10$). The best subset of variables included age, presence of gametocytes on day 0 and the treatment regimen employed. All other variables considered in the initial model but not found to be significant in the final model by this analysis were excluded. There were enough significant differences between the two study sites to warrant separate analysis for each location.

2.9. Ethical considerations and consent

The study was approved by the Health Secretary for the Assam State Government, the Ministry of Home Affairs New Delhi and the National Antimalaria Program. Informed consent, written in Assamese, was obtained from each patient or guardian. If necessary, consent was verbally translated into the local language. Decisions regarding management of patients were based on their clinical condition and were independent of the study. Drugs used in the study were produced under the principles of Good Manufacturing Practice (GMP) and were supplied by International Dispensary Association (IDA), Amsterdam, The Netherlands.

3. Results

3.1. Bokajan: Karbi Anglong district

Of a total of 774 persons screened for malaria in the MSF fever clinic, 46.6% had positive slides and 87% of these were classified as *P. falciparum*. A total of 244 were entered in the study. Table 1 summarizes the characteristics of this group. A total of 46 (18.6%) patients were lost to follow-up primarily due to the advent of the planting season when both men and women work in the rice paddies. However, they are included in the event-free survival curves up to the date of loss. An additional 10 were disqualified due to misclassification and entry errors and were not included in the 234 cases analyzed for event-free survival. Of the 186

Table 1 Patient characteristics

	Bokajan, Karbi Anglong ($n = 244$)	North Jamuguri, Sonitpur ($n = 242$)
Sex ratio (M/F)	0.92 (111/123)	1.66 (143/89)[a]
Median (range) age (years)	12.0 (1–70)	17 (1–75)
Mean (SD) age (years)	15.7 (12.3)	19.5 (13.3)
<5 = 36		
5–15 = 105		
>15 = 93		
Mean (range) axillary temperature (°F)	98.1 (95.6–103.8)	98.8 (96–102.8)
Geometric mean (range) parasitemia at day 0 (/μl)	3561 (479–32 075)	5157 (661–37 833)
Gametocytes at day 0	34/244 (13.9%)	37/205 (15.3%)
Percentage febrile	16 (47%)	17 (46%)
Percentage previously treated	14 (41.2%)	13 (35%)
Mixed *P. vivax*/*P. falciparum* infection	5 (2.0%)	5 (2.1%)
Previous medication intake	74/244 (30.3%)	100/242 (41%)

[a] The skewed sex ratio represents a selection bias since men were more likely to keep the bus transportation fee provided for the study and walk or cycle to the fever clinic.

Table 2 Parasitologic treatment response

	Failure	95% CI	Adjusted HR[a]	*P* value
Bokajan, Karbi Anglong				
Chloroquine	29/44 (65.9%)	48.8–78.1	11.67 (5.4–30.4)	<0.001
SP	20/51 (39%)	25.3–53.98	4.88 (1.84–12.96)	0.0015
Mefloquine	2/45 (4.4%)	0.54–15.15	0.38 (0.07–1.94)	0.2434
Mefloquine–artesunate	5/46 (10.9%)	3.62–23.56	1 (reference)	
Total	56/186			
North Jamuguri, Sonitpur				
Chloroquine	46/48 (95.8%)	83.5–98.7	167.98 (22.7–1245)	<0.001
SP	28/49 (57.1%)	42.2–71.2	61.6 (82.5–460.3)	<0.001
Mefloquine	4/51 (7.8%)	2.2–18.9	4.35 (0.49–38.95)	<0.001
Mefloquine–artesunate	1/53 (1.8%)	0.5–9.9	1 (reference)	
Total	79/201			

[a] Adjusted hazard ratio: multivariate analysis after backward elimination.

completing the 6-week follow-up period, there were 56 treatment failures (30.1%). Total loss due to misclassification (10) or default (46) was 56/244 (23%). Treatment response (Table 2), resistance levels to the drug regimens (Table 3) and time to treatment failure (Table 4) demonstrated significant differences among the four arms. Data were also analyzed utilizing WHO criteria and 56 patients on the chloroquine arm were evaluable at day 14. There were five ETF, three LTF and eight LPF for a total of 26.8% therapeutic failures. Chloroquine resistance in Bokajan had been monitored over a 10-year period from 1979 to 1988 utilizing parasitologic response during a 28 day study period in previously untreated subjects (Barkakaty and Narasimilam, 1992). Despite the substitution

Table 4 Time to treatment failure

	Mean day of failure	(95% CI)
Bokajan, Karbi Anglong		
Chloroquine	25	(22–29)
SP	33	(30–37)
Mefloquine	42	(40–43)
Mefloquine–artesunate	40	(38–42)
North Jamuguri, Sonitpur		
Chloroquine	15.5	(11–18)
SP	28	(24–31)
Mefloquine	40.6	(39–42)
Mefloquine–artesunate	41.8	(41–42)

Table 3 Parasitologic resistance level

	R3	R2	R1	S	Clinical failure	Lost	Total
Bokajan, Karbi Anglong							
Chloroquine	4 (8.9%)	1 (2.2%)	24 (53%)	15 (33%)	1	12	57
SP	3 (5.8%)	2 (3.8%)	15 (29%)	31 (60%)	1	9	61
Mefloquine	–	–	2 (4.4%)	43 (96%)		13	58
Mefloquine–artesunate	1 (2%)[a]	–	4 (8.7%)	41 (89%)		12	58
Total	8	3	45	130	2	46	234
North Jamuguri, Sonitpur							
Chloroquine	14 (29%)	14 (29%)	18 (37.5%)	2 (4.2%)		10	58
SP	3 (6.1%)	7 (14.3%)	18 (36.7%)	21 (43%)		8	57
Mefloquine	–	1 (2%)	3 (5.9%)[b]	47 (92%)		8	59
Mefloquine–artesunate	–	–	1 (1.9%)[b]	52 (98%)		5	58
Total	17	22	40	122		31	232

[a] The single R3 failure in the mefloquine–artesunate arm was a clinical failure on day 1 in a 3-year-old child who was unable to stand or walk at the time of the clinic visit. However, quantitative parasite count on day 2 was less than 25% of entry count and she recovered fully without additional treatment.

[b] The four R1 level failures in the mefloquine-containing arms occurred at or after day 28.

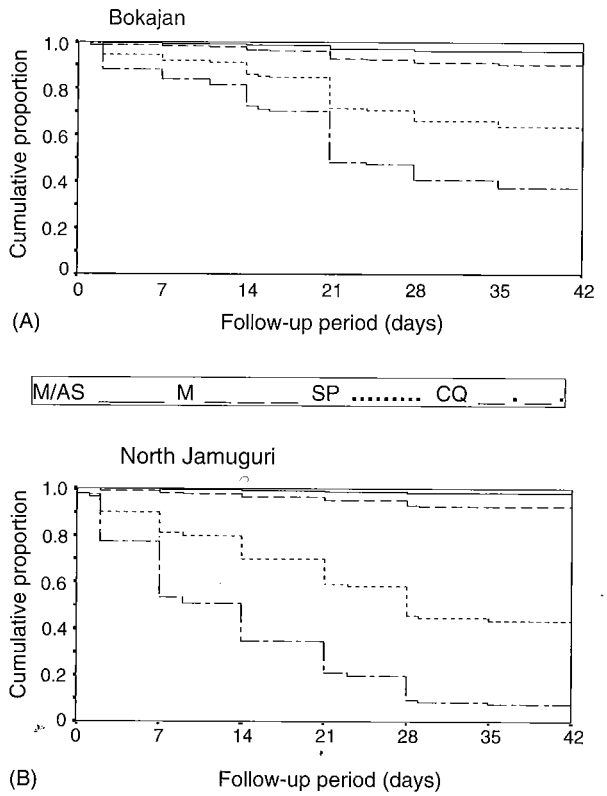

Figure 1 Patients without reappearance of parasitemia up to date of last follow-up visit in (A) Bokajan, Karbi Anglong and (B) North Jamuguri, Sonitpur.

of SP as the first-line therapy in 1983, R2/R3 chloroquine resistance remained at 14–16%, possibly because chloroquine continued to be available on the market. Sulfadoxine–pyrimethamine R2/R3 parasitologic failure was found in 9.6% cases. At the end of 14 days, there were four ETF, two LTF and two LPF among 60 patients (total 13.3%). The relapse-free survival during the 6-week follow-up period (Figure 1A) includes each subject up to the time of reappearance of parasitemia/fever or default from the study and demonstrates the superiority of both mefloquine-containing arms. Younger age was correlated with increased risk of treatment failure: ($P \leq 0.001$ ages 0–5 years; $P = 0.0367$ ages 5–15, multivariate analysis). The presence of gametocytes on day 0 was not correlated with treatment response ($P = 0.08$). Other characteristics were compared in the chloroquine and SP groups between treatment failures and successes. They include initial temperature, initial parasite count, sex and history of antimalarial medication in the month prior to study entry. None of the differences were statistically significant.

3.2. North Jamuguri: Sonitpur district

A total of 815 slides were screened for malaria in the MSF fever clinic at the North Jamuguri Community Health Center from 30 June to 27 July inclusive and 348 were positive for malaria (42.7%) and 75% of these were due to *P. falciparum*. The characteristics of the study group are summarized in Table 1. A total of 31 patients were lost to follow-up (12.8%). They are included in the event-free survival curves up to the date of loss. An additional 10 patients were disqualified due to entry error or misclassification and are not considered in the event-free survival data. Of the 201 persons who completed the 6-week follow-up period, there were 79 treatment failures (39.3%). Total loss due to default (31) and misclassification (10) is thus 41/242 or 16.9%. Treatment response (Table 2), resistance levels to the drug regimens (Table 3) and time to treatment failure (Table 4) demonstrated significant differences among the four arms. The level of chloroquine resistance in North Jamuguri (58% R2/R3) was twice that of Bokajan. The overall mean time to treatment failure of 15 days is also consistent with a high level of chloroquine-resistant parasites circulating in the community. Utilizing WHO criteria for treatment failure at day 14, similar evidence emerges. Of 56 evaluable patients, 14 were ETF, 2 LTF and 20 LPF giving a combined total of 64% therapeutic failures. In the month prior to the current study, North Jamuguri had been the site of at least five mobile clinics for the distribution of chloroquine to any patients presenting with fever. Reported non-compliance to previous treatment in the sample was nearly 40%. Both of these factors are recognized as major determinants in selecting drug-resistant parasites (Mohapatra et al., 2001b; White, 1998; Wongsrichanalai et al., 2002). Sulfadoxine–pyrimethamine was also ineffective: 20% of subjects treated with this drug failed to clear parasitemia by the end of the first week. There were 56 evaluable patients at day 14: five were ETF, three LTF and eight LPF for a combined total of 28.6% therapeutic failures. The relapse-free survival during the 6-week follow-up period (Figure 1B) includes each subject up to the time of reappearance of parasitemia/fever or default from the study and demonstrates the great superiority of the mefloquine-containing arms. Young age and the presence of gametocytes on day 0 were the only pre-treatment characteristics showing statistical significance. Treatment failure was highest in children <5 years old ($P = 0.001$ by multivariate analysis; Cox proportional hazards regression). The presence of gametocytes on day 0 was associated with a higher risk of failure ($P = 0.05$

Table 5 Clearance of parasitaemia at 48 h/evaluable subjects

	Bokajan, Karbi Anglong	North Jamuguri, Sonitpur
Chloroquine	32/60 (53%)	18/59 (31%)
SP	44/60 (73%)	30/53 (57%)
Mefloquine	47/55 (85%)	49/58 (84%)
Mefloquine–artesunate	48/54 (89%)	51/58 (88%)

by multivariate analysis), an observation that was not noted in the Bokajan data set. Initial parasite count, initial temperature, sex, and history of antimalarial treatment during the month prior to study entry did not reveal significant differences between responders and non-responders in the chloroquine and SP arms.

3.3. Parasitologic clearance

There was a rough inverse correlation between clearance of parasites at 48 h and treatment failure in the chloroquine and SP arms at both sites (Table 5).

3.4. Gametocyte carriage

Gametocytemia was found at entry in 15.3% of subjects at both sites, indicating that malaria had been present for 7–10 days. Clearance of gametocytes was more rapid in the mefloquine–artesunate arm at both study sites and slowest in the SP arm. Since the latter drug fosters the development of gametocytes, this is not unexpected. Treatment with mefloquine alone, a slower acting drug, was also associated with persistence of gametocytes as long as 28 days after the initiation of treatment and was similar to chloroquine in the rate of gametocyte clearance (Figure 2). In North Jamaguri, age distribution, (45% <15 years), history of previous treatment and mean parasitemia (10 000/μl) were similar between those subjects with and without gametocytes at study entry. In Bokajan, those with gametocytes at study entry were more likely to have been previously treated (41.2% compared to 28.6%), were more often younger than 15 years old (62% compared to 49%) and had lower parasite density (5800/μl) compared to those without gametocytemia (10 033/μl). The 5–14 years age group exhibited the highest gametocyte carriage (17%) compared to 12% in those younger than 5 years or 10.4% in those older than 15 years. Of the risk factors for gametocyte carriage identified in other studies, gametocytemia was associated with younger age, resistant infection, history of illness longer than 2 days, previous drug treatment or

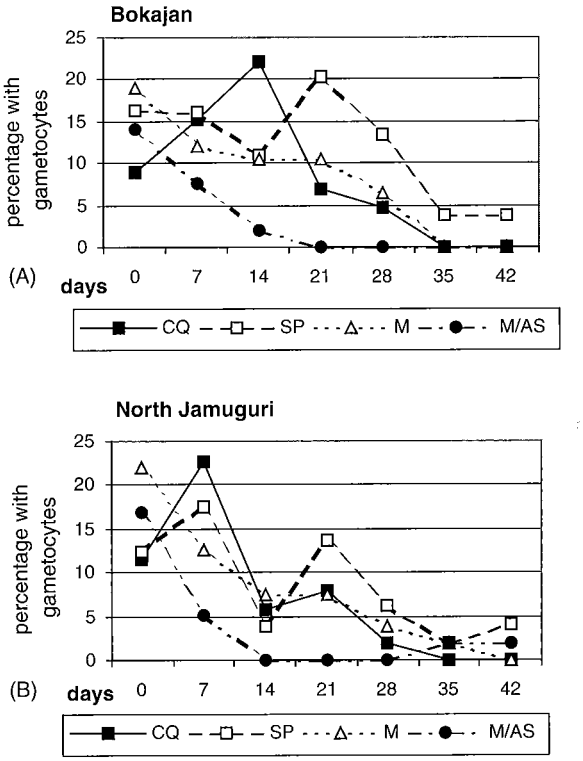

Figure 2 Gametocyte carriage in four treatment groups in (A) Bokajan, Karbi Anglong and (B) North Jamuguri, Sonitpur.

mixed vivax-falciparum infection (Bousema et al., 2003; Mohapatra et al., 1998b; Price et al., 1999). The presence of gametocytes at study entry was correlated with a higher risk of treatment failure only in North Jamuguri (multivariate analysis, $P = 0.05$). This observation would need confirmation in a larger number of subjects.

3.5. Quality control

A total of 936 slides were submitted for quality control of which 30 could not be evaluated due to slide deterioration: 261/283 of the positive slides and 565/653 of the negative slides submitted were in agreement with the MSF technicians. Review of discordant interpretations in consultation with the MSF laboratory supervisor and the reference laboratory established low parasite counts as the principal reason for lack of agreement. Revised positive slide agreement was 277/283 = 98% sensitivity and 633/653 = 97% specificity. Data was processed after all errors were corrected.

3.6. Clinical failures

Three patients developed signs of severe malaria within 1 day of beginning treatment: one had received mefloquine–artesunate, one had received

chloroquine and one had received SP. Two were children less than 5 years old and one was 9 years old. All of them survived. None of the patients in North Jamuguri returned to the clinic specifically for symptoms related to malaria outside of the follow-up schedule. Only 12 (15%) of the 79 treatment failures were febrile on the date of parasitologic failure. Of the 56 treatment failures in Bokajan, only 9 (16%) were febrile at the time of parasitologic failure. Three of these patients returned because of clinical symptoms of malaria as the initial indication of treatment failure.

3.7. Adverse effects

In general, all of the medications were well tolerated. The most serious adverse effect was in one male patient who exhibited aggressive behavior on the second day after receiving mefloquine alone. He was lost to follow-up. Nausea and dizziness lasting several days up to 1 week was a specific complaint in adults on the mefloquine-containing arms, particularly in women. In Karbi Anglong, 16/111 women and 3/123 men complained of these symptoms and 8/89 women in Sonitpur. Four children aged <5 years and one child aged >5 years vomited the first dose after receiving mefloquine and two children aged <5 years vomited the dose of SP. After treatment with paracetamol and observation for an interval of 2 h, all medications were successfully re-administered. Other symptoms such as weakness, fatigue, anorexia and headache were present in all four groups and were attributed to malaria infection. There were no deaths or complications directly related to malaria in any of the patients that were followed to study completion. Since hemoglobin/hematocrit determinations were not routinely obtained in this study, the advent of anemia as a complication could not be evaluated.

4. Discussion

The current investigation is the largest drug resistance study from this geographical area and the only one to include mefloquine and a mefloquine–artesunate combination in the study design. It supports the presence of drug resistance as a primary factor leading to treatment failure. PCR genotyping of alleles of MSP1, MSP2 and glutamine-rich protein (GLURP) can distinguish between reinfection and recrudescence in areas of low transmission intensity (hence limited genetic diversity) such as occur in Thailand and the Indian subcontinent (Brockman et al., 1999; Paul et al., 1998). The current study spanned the period of maximum seasonal transmission. It was not possible to exclude reinfection in parasitologic/clinical failures occurring after day 14 since PCR analysis was not carried out. However, the sustained parasitologic response in the mefloquine-containing arms suggests that novel infection was an infrequent event, even assuming that all of the late failures in these arms were due to reinfection. Determination of clinical and parasitologic failures at day 14 underestimates the true resistance. Since Assam is considered to be an area of low–moderate transmission intensity, a significant number of failures after day 14 are likely to be due to recrudescence rather than novel infection. Moreover, recrudescent malaria is often associated with less severe illness, an observation which is supported by the lack of clinical symptoms in those patients in whom recurrent parasitemia was documented during the 6-week follow-up period. Asymptomatic infection and the associated prolongation of gametocyte carriage also promotes increased transmission of drug resistant parasites (Bousema et al., 2003; Mohapatra et al., 1998b; Price et al., 1999). The true resistance levels are also likely to have been underestimated due to the relatively low number of young children in this study. Studies from the Indo-Bhutan border have documented attack rates as high as 110/1000 in the 2–9 years age group compared to 40/1000 in those aged 10–20 and 65/1000 in those aged 21–30 years. The parasite index in children aged 1–5 years from forest fringe villages in eastern Assam during peak transmission may reach 610/1000 compared to those aged >15 years (200/1000) (Mahanta, personal communication).

The role of transmission intensity in the development and spread of drug resistance is complex. Areas of low transmission intensity appear to favor increased rates of drug resistance such as has occurred in Thailand and South America, as well as Assam which has a similar transmission pattern (Hastings, 2001; Wongsrichanalai et al., 2002). However, high intensity transmission levels have also been linked with the rapid emergence of drug resistance such as has occurred in some East African countries. Recent studies and models predicting the evolution of drug resistance related to transmission intensity have led to conflicting results. Nevertheless, they appear to support the conclusion that the successful emergence of a drug-resistant clone is favored in a low transmission site (low genetic diversity), where a large number of infections are symptomatic and thus associated with the widespread use of antimalarial drugs (Hastings, 2003; Talisuna et al., 2002).

Chloroquine resistance in both study sites has reached a level at which it should no longer be

used to treat falciparum malaria. Moreover, SP efficacy is already too limited to warrant its continued use as a single agent (WHO, 2003). Rapid increase in SP resistance due to selection of mutant alleles has been documented in Southeast Asia since the 1970s (Hurwitz et al., 1981). More recent studies from Africa have established waning efficacy in East Africa as well as some West African countries (Checci et al., 2002; Dorsey et al., 2002; Ronn et al., 1996; Staedke et al., 2001; Terlouw et al., 2003). Analysis at 14 days significantly underestimates SP resistance since most failures occur after this period (Dorsey et al., 2002). One study found 18% clinical and 32% parasitologic failures with SP alone and 10% failure with an SP–artesunate regimen in African children aged <5 years (Dorsey et al., 2003). However, extrapolation of results from African studies to the Indian subcontinent, where patterns of transmission, levels of immunity and risk of infection are quite different must be undertaken with caution. Although WHO recommends combining a longer-acting antimalarial agent with the rapid-acting artesunate, the combination may not salvage this situation if background resistance to the longer acting drug is already too high (>25%) to be useful (Von Seidlein et al., 2000; WHO, 2002).

Mefloquine alone was highly efficacious at both sites, with 93.9% sustained response at 42 days. Studies on mefloquine resistance in Thailand have shown that cure rates fell to less than 70% within 4 years of monotherapy at 25 mg/kg (Nosten et al., 2000). Although monotherapy is no longer recommended, the present study demonstrated that even the lower dose of mefloquine (15 mg/kg) is almost 94% effective when given as a single agent. Since mefloquine has been on the Indian market for the past decade, it is unlikely that the use of mefloquine had been widespread in the current study sites. The mefloquine–artesunate combination was equally effective with 93.6% disease free at 6 weeks. This combination has been used successfully in Southeast Asia and represents an appropriate choice for the management of falciparum malaria in Assam. A major obstacle to the introduction of artesunate combination treatment into the public sector is the largely unregulated nature of drug distribution and utilization in India and therefore the difficulty in controlling the availability of monotherapies in the market place. Private practitioners, pharmacies and drug vendors provide medication according to ability to pay, promoting under-dosage thus increasing the risk of treatment failure. The adoption of a mefloquine–artesunate preparation in a single tablet is likely to be effective since monotherapy with mefloquine is uncommon in India. Moreover, there has been no resistance to artemesinin derivatives, which is related to the short half-life and the reduction of gametocyte carriage in this class of drugs. Finally, fewer than 50% of febrile patients were found to have malaria in this study. Insufficient treatment from unqualified providers and widespread lack of compliance emphasize the need for intensive community education and better access to health services.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgements

The authors wish to thank Dr Borgohain, Regional Director of Health Services for providing laboratory assistance for quality control on study samples and Dr Frank Smithuis, MSF Myanmar, for assistance with the study design. The cooperation provided by Dr Hira, the former Joint Director of Health Services for Malaria, Assam, the district medical directors for Sonitpur and Karbi Anglong and the medical personnel at Bokajan Community Health Center is gratefully acknowledged.

References

Barkakaty, B.N., Narasimilam, M.V.V.I., 1992. A longitudinal study to monitor chloroquine resistant malaria in Bokajan and Manja PHC areas of Karbi Anglong District, Assam. Ind. J. Malariol. 29, 173–183.

Bloland, P., Ettling, M., Meek, S., 2000. Combination therapy for malaria in Africa: hype or hope? Bull. World Health Organ. 78, 1378–1388.

Bousema, J.T., Gouagna, L.C., Meutstege, A.M., Okech, B.E., Akim, N.I.J., Githure, J.I., Beier, J.C., Sauerwein, R.W., 2003. Treatment failure of pyrimethamine–sulphadoxine and induction of *Plasmodium falciparum* gametocytaemia in children in western Kenya. Trop. Med. Int. Health 8, 427–430.

Brockman, A., Paul, R.E., Anderson, T.J., Hackford, I., Phaiphin, L., Looareesuwan, S., Nosten, F., Kay, K.P., 1999. Application of genetic markers to the identification of recrudescent *Plasmosium falciparum* on the northwestern border of Thailand. Am. J. Trop. Med. Hyg. 60, 14–21.

Checci, F., Durand, R., Balkan, S., Vonhm, B.T., Kollie, J.Z., Biberson, P., Baron, E., Le Bras, J., 2002. High *Plasmodium falciparum* resistance to chloroquine and sulfadoxine–pyrimethamine in Harper, Liberia: results in vivo and analysis of point mutations. Trans. R. Soc. Trop. Med. Hyg. 96, 664–669.

Das, N.G., Baruah, I., Kamal, S., Sarkar, P.K., Das, S.C., Santhanam, K., 1997. An epidemiological and entomological investigation on malaria outbreak at Tamulpur PHC, Assam. Ind. J. Malariol. 34, 164–170.

Dev, V., 2001. Malaria-attributable morbidity in Assam, northeastern India. Ann. Trop. Med. Parasitol. 95, 789–796.

Dev, V., Phookan, S., 1998. Epidemiology and control of malaria in the Brahmaputra valley of Assam. Adv. Med. Entomol. Hum. Welf., 59–66.

Dorsey, G., Njama, D., Kamya, M.R., Cattamanchi, A., Kyabayinze, D., Staedke, S.G., Gasasira, A., Rosenthal, P.J., 2002. Sulfadoxine/pyrimethamine alone or with amodiaquine or artesunate for treatment of uncomplicated malaria: a longitudinal randomized trial. Lancet 360, 2031–2038.

Dorsey, G., Vlahos, J., Kamya, M.R., Staedke, S.G., Rosenthal, P.J., 2003. Prevention of increasing rates of treatment failure by combining sulfadoxine–pyrimethamine with artesunste or amodiaquine for the sequential treatment of malaria. J. Infect. Dis. 188, 1231–1241.

Government of India, 1995. Operational Manual for Malaria Action Programme (MAP) 1995. Directorate of National Malaria Eradication Programme, New Delhi, 1–30.

Hastings, I.M., 2001. Modelling parasite drug resistance: lessons for management and control strategies. Trop. Med. Int. Health 6, 883–890.

Hastings, I.M., 2003. Malaria control and the evolution of drug resistance: an intriguing link. Trends Parasitol. 19, 70–73.

Hurwitz, E.S., Johnson, D., Campbell, C.C., 1981. Resistance of *Plasmodium falciparum* malaria to sulphadoxine–pyrimethamine (Fansidar) in a refugee camp in Thailand. Lancet 1, 1068–1070.

Kalbfleisch, J.D., 1980. The Statistical Analysis of Failure Time Data. Wiley Co., New York, pp.10–19 and 87–89.

Mohapatra, P.K., Prakash, A., Mahanta, J., Das, J., Srivastava, V.K., 1995. Malaria outbreak in Lower Assam: an epidemiological appraisal. J. Commun. Dis. 19, 175–178.

Mohapatra, P.K., Prakash, A., Bhattacharyya, D.R., Mahanta, J., 1998a. Malaria situation in northeastern region of India. ICMR Bulletin 28, 22–30.

Mohapatra, P.K., Prakash, A., Bhattacharyya, D.R., Mahanta, J., 1998b. Epidemiological importance of younger age group during malaria epidemic in PHC Tamulpur, Assam. J. Commun. Dis. 30, 229–232.

Mohapatra, P.K., Narain, K., Prakash, A., Bhattacharyya, D.R., Mahanta, J., 2001a. Risk factors of malaria in the fringes of an evergreen monsoon forest of Arunachal Pradesh. Natl. Med. J. India 14, 139–142.

Mohapatra, P.K., Prakash, A., Bhattacharyya, D.R., Hazarika, N.C., Mahanta, J., 2001b. Treatment seeking behaviour in rural areas of Assam, India and its impact on malaria during the epidemic. J. Hum. Ecol. 12, 195–199.

Nosten, F., Luxemburger, C., ter Kuile, F.O., Woodrow, C., Pa Eh, J., Chongsuphajaisiddhi, T., White, N.J., 1994. Treatment of multidrug resistant *P. falciparum* malaria with 3 day artesunate–mefloquine combination. J. Infect. Dis. 170, 971–977.

Nosten, F., van Vugt, M., Price, R., Luxemburger, C., Thway, K.L., McGready, R., ter Kuile, F., Looareesuwan, S., White, N.J., 2000. Effects of artesunate–mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. Lancet 356, 297–302.

Paul, R.E.I., Hackford, I., Brockman, A., Muller-Graf, C., Price, R., Luxemburger, C., White, N.J., Nosten, F., Day, K.P., 1998. Transmission intensity and *Plasmodium falciparum* diversity on the northwestern border of Thailand. Am. J. Trop. Med. Hyg. 58, 195–200.

Prakash, A., Bhattacharya, D.A., Mohapatra, P.K., Mahanta, J., 1998. Anopheline fauna in the northeastern states of India with notes on vectors of malaria. Proc. Natl. Acad. Sci. India 68, 217–228.

Prakash, A., Mohapatra, P.K., Bhattacharyya, D.R., Sharma, C.K., Goswami, B.K., Hazarika, N.C., Mahanta, J., 2000. Epidemiology of malaria outbreak (April/May 1999) in Titabor primary health center, district Jorhat (Assam). Indian J. Med. Res. 111, 121–126.

Price, R., Nosten, F., Simpson, J., Luxemburger, C., Phaipun, L., Kuile, F.T., Van Vugt, M., Chongsuphajaisiddhi, T., White, N.J., 1999. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. Am. J. Trop. Med. Hyg. 60, 1019–1023.

Ronn, A.M., Msangeni, H.A., Mhina, J., Wernsdorfer, W.H., Bygbjerg, I.C., 1996. High level of resistance of *Plasmodium falciparum* to sulfadoxine–pyrimethamine in children in Tanzania. Trans. R. Soc. Trop. Med. Hyg. 90, 179–181.

Sehgal, P.N., Sharma, M.I.D., Sharma, S.I., Gogoi, S., 1975. Resistance to chloroquine in falciparum malaria in Assam state. J. Commun. Dis. 5, 175–180.

Sharma, V.P., 1999. Current scenario of malaria in India. Parassitologia 41, 349–353.

Shiv, L., Dhillon, G.P.S., Sonal, G.S., Arora, U., Nandi, J., 1997. Drug Resistance and Chemotherapy of Malaria in India: an Update. Government of India, New Delhi, pp. 2–18.

Staedke, S.G., Kamya, M.R., Dorsey, G., Gasasira, A., Ndeezi, G., Charlebois, E.D., Rosenthal, P.J., 2001. Amodiaquine, sulfadoxine–pyrimethamine and combination therapy for treatment of uncomplicated falciparum malaria in Kampala Uganda: a randomized trial. Lancet 258, 1218–1223.

Talisuna, A.O., Langi, P., Bakyaita, N., Egwang, T., Mutabingwa, T.K., Watkins, W., Van Marck, E., D'Alessandro, U., 2002. Intensity of malaria transmission, antimalarial-drug use and resistance in Uganda: what is the relationship between these three factors? Trans. R. Soc. Trop. Med. Hyg. 96, 310–317.

Terlouw, D.J., Nahlen, B.L., Courval, J.M., Kariuki, S.K., Rosenberg, O.S., Oloo, A.J., Kolzak, J.S., Hawley, W.A., Lal, A.A., ter Kuile, F.O., 2003. Sulfadoxine in treatment of malaria in western Kenya: increasing resistance and underdosing. Antimicrob. Agents Chemother. 47, 2929–2932.

Von Seidlein, L., Milligan, P., Pinder, M., Bojang, K., Anyalebechi, C., Gosling, R., Coleman, R., Ude, J.I., Sadiq, A., Duraisingh, M., Warhurst, D., Alloueche, A., Targett, G., McAdam, K., Greenwood, B., Walraven, G., Olliaro, P., Doherty, T., 2000. Efficacy of artesunate plus pyrimethamine–sulfadoxine for uncomplicated malaria in Gambian children: a double-blind randomized controlled trial. Lancet 355, 352–357.

White, N.J., 1998. Why is it that antimalarial drug treatments do not always work? Ann. Trop. Med. Parasitol. 4, 449–458.

Wongsrichanalai, C., Pickard, A.L., Wernsdorfer, W.H., Meshnick, S.R., 2002. Epidemiology of drug-resistant malaria. Lancet Infect. Dis. 2, 209–219.

WHO, 1991. Basic Malaria Microscopy. World Health Organization, Geneva.

WHO, 2002. Press Release, 25 April. World Health Organization, Geneva, WHO/31.

WHO, 2003. Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria. World Health Organization, Geneva, WHO/HTM/RBM/2003.50.