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A Controlled Trial on the Efficacy of Mefloquine in an Area with Reported Prevalence of Multi-drug Including Mefloquine Resistant Falciparum Malaria in Bangladesh

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Summary:

An open controlled chemotherapeutic trial to describe the efficacy of mefloquine in an area with reported mefloquine resistance in a multi-drug resistant falciparum area of Bangladesh was done. Subjects were symptomatic patients, 12 years or older, with parasite density between 500-250,000/cmm and no history of taking antimalarials during the previous week. The regimens used were mefloquine 25 mg/Kg, divided into two doses six hours apart in 70 cases, and oral quinine sulphate tablets (10 mg/Kg eight hourly) for seven days in seven cases. Five healthy controls were recruited. Subjects were kept in a reasonably reinfection free environment in a makeshift hospital for 28 days. Drug administration was supervised and subjects were followed up clinically and with blood films in the hospital. Blood films were examined daily on days 1-8, then on days 14, 21 and 28, and on unscheduled days in case of relapse of symptoms. A total of 83 (71+ 7+ 5) cases were enrolled and one patient failed to complete the follow up. The drugs were well tolerated in all cases (except reversible cinchonism in two cases on quinine) and there was no clinical failure in the mefloquine group. One patient in the quinine group had late clinical failure. Five patients in the mefloquine group showed R III type of parasitological response (D2 parasitaemia more than 25% of D0), and in all of them radical cure was achieved. The study area can be considered to have preserved sensitivity to mefloquine in 25 mg/Kg dosage.

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Introduction:

Malaria constitutes a significant public health problem in Bangladesh and situation has slowly deteriorated since 1988[1]. Out of a total population of about 125 million, 10 million people living in south-eastern region are at the highest risk, with *P. falciparum,* the predominant species. In 1997, there were about 889,000 clinical malaria cases, about 69,000 laboratory confirmed cases (*P. falciparum* rate was about 62%) and 457 confirmed malaria deaths, reported through the primary healthcare set up (PHC) of the country[2]. This represents a gross underestimate of the total burden as many people do not attend the government health care facilities for various reasons, mostly due to lack of transportation facilities and shortage of medicines. A revised National Malaria Control Policy was adopted in 1995, which included the anti-malaria drug policy[3]. Three clinical case definitions and their respective treatment guidelines were adopted[4]. Objective information on drug sensitivity pattern of *P. falciparum* to various antimalarials are not available from most of the areas of the country, but resistance to chloroquine (CQ) and sulfadoxin/pyremethamine (SP) is known to be present for long[5,6,7].

In the recent years, multi-drug resistant falciparum malaria emerged as a major public health problem in

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Bangladesh, with both the first and second line drugs showing unacceptable proportions of treatment failures[8]. In one study, the respective rates of clinical failures to first line agent CQ, second line agent three days oral quinine followed by single-dose fansidar (Q3+SP), proposed second line agent mefloquine (Mef) and third line agent oral quinine for seven days (Q7) were 77%, 28%, 27% and 18% in 1996-1997 in the present study area[8]. The group recommended Q3+SP regimen for slide-positive falciparum malaria cases as first line agent in the area. Mefloquine was proposed as the second line drug for treatment failure cases in the same area.

Mefloquine was registered and introduced in the country market in late 1997. The National Malaria Control Programme is not using the drug and the drug is not widely available in the local medicine shops because of the cost, and the area could be considered an area without any selection pressure for development of resistance to the drug. A clinical failure rate of 11% and parasitological failure rate of 27% (95% CI, 17-49) observed with mefloquine in 20 mg/Kg single dose was alarming for the National Control Programme, which was already in problem with multi-drug resistance. However, there is no data on the resistance to mefloquine when used with an increased dose of 25 mg/Kg in two divided doses, which is well tolerated[8]. The study was therefore carried out to check whether there is resistance to high dose mefloquine within 28 days following treatment in the area where multi-drug resistance is present.

Materials and methods:

*Study design***:** The study was designed as an unblinded, controlled trial to describe the cure rate for the high dose of mefloquine. A few concurrent positive (blood slide confirmed falciparum malaria treated with seven days oral quinine) and negative controls (blood slide negative for *P. falciparum* and were not treated) were included primarily to see the re-infection rate. However, in the positive controls, clinical and parasitological cure as outcome were also recorded.

*Study site***:** The study was conducted in a primary care hospital [Ramu Upazila Health Complex (RUHC)] situated in a high-risk malarious area of the country, between January and August 1998. The RUHC is the only in-patient facility for about 200,000 population over an area of about 191 square kilometers. The area consisted mostly of forested, deforested and forest-fringe areas and some plain areas with valleys. The transmission is considered intense and perennial, with a largely stable population with malaria accounting for about half of the in- and out- patient load in the study hospital.

*Study population***:** The patients, screened at the outpatient department, presented with fever or history of fever over the previous 48 hours, suspected of having uncomplicated malaria, and were subjected to blood film examination. Positive cases were checked for exclusion criteria. A trained laboratory technician measured parasite density immediately. Male patients aged between 12 and 60 years with asexual *P. falciparum* parasite density between 500-200,000/cmm were initially selected. Those agreeing with written informed consent to take part in the investigation, to choose the treatment regimen by lottery and to remain in hospital for 28 days were enrolled, admitted and assigned to treatment regimen. Pregnant and lactating women, and patients with a history of taking antimalarials over the previous one week, presence of any of the severe manifestations, co-existence of other infections, co-infection with *P. vivax*, and known hypersensitivity to quinine or mefloquine, and patients with history of psychiatric illness were excluded.

*Study size and allocation of treatment***:** Patients fulfilling the eligibility checklist were enrolled, admitted and assigned to treatment regimens by lottery. The sample size chosen was 70 for mefloquine. In the same setting it was decided to recruit five negative (healthy) controls, who got no treatment but monitored with blood film throughout the study, to ensure that there was no transmission in the ward. In addition, there was a control group of seven patients who were given Q7 regimen just to monitor the efficacy of quinine. Codes for treatment were prepared, kept in a jar with 10 codes for mefloquine and one code for Q7. Each time a patient drew his own treatment regimen, and the jar was re-filled in the same manner after being exhausted.

*Hospital arrangements***:** Patients were accommodated in a dormitory in the immediate neighborhood of the hospital, used earlier for accommodating hospital nurses. The whole dormitory was mosquito protected by wire-netting with smallest hole nets for all doors, windows and exhaust holes. The doors were provided with automatic door closers. The dormitory and

adjacent areas were insecticide-sprayed twice daily. The enrolled patients were allowed to go out of room after 7 AM and had to enter the dormitory by 4 PM. In addition to compensation for the work loss, the enrolled patients were provided with entertainment arrangements like TV, VCP, newspapers, news magazines and other indoor games facilities (carom, ludu, chess and playing cards), popular in the area.

Drug regimens: The treatment groups were group I (mefloquine, 25 mg/Kg, in two divided doses, six hours apart) and group II [oral quinine sulfate tablets 10 mg/Kg/dose eight hourly for seven days (21 doses)]. Single dose primaquine 45 mg base, was used in all cases on the last day of treatment according to the national guidelines. The drugs used were Larium® from F. Hoffman-la-Roche, Basel, Switzerland and Quinine tablets from Jayson Pharmaceuticals Ltd., Bangladesh. Patients were weighed and doses were rounded to the nearest half tablet. The maximum dosage of drugs used were 1800 mg/day for quinine and 1500 mg for mefloquine. Each treatment dose was administered by a team nurse and observed for one hour thereafter for vomiting. The total dose was replaced in case of vomiting within one hour of ingestion and again observed in the same manner. The patients had daily clinical follow-up and blood slide examination during days 0 to 7 and thereafter on days 14, 21 and 28.

Recrudescent treatment: Patients who had fever (more than 100^0F) and parasitaemia (clinical treatment failure) from day 3 through day 28, were given alternative antimalarial treatment regimens, i.e., seven days oral quinine for the mefloquine and Q7 failures with seven days of oral quinine plus seven days of oral oxytetracycline. Voluntary withdrawal, development of severe manifestations and overt toxicity (hypersensitivity) to drugs were considered reasons for dropping patients from the study.

Definitions (WHO, 1995) used in outcome measurement:

Clinical response was categorized as –

1) Early treatment failure (ETF): Study subjects with parasitaemia and persistent fever from day 3 onwards, as well as those whose condition has worsened before day 3;
2) Late treatment failures (LTF): Study subjects with initial clearance of fever (body temperature less than 100^0F) by Day 3 but with persistent/recurrent parasitaemia and fever (body temperature more than 100^0F or history of fever) at a later time over the observation period; and
3) Adequate clinical response (ACR): The remainder (excepting those withdrawn because of a change of diagnosis or lost to follow-up).

Parasitological response was categorized as –

1) R III: Density of parasite on day 2 more than 25% of density on day 0, or alternative antimalarial therapy was required on or before day 2;
2) R II: Positive on day 2, with a density less than 25% of density on day 0, and either positive on day 7 or alternative antimalarial therapy was required on any of days 2 to 7;
3) R I: Negative or positive (less than 25% of day 0) on day 2, negative on day 7 and positive anytime thereafter (within 28 days); and
4) S: The remainder including those positive on day 2 (with density less than 25% of density on day 0) and negative thereafter.

Fever clearance time (FCT) was defined as the time from admission to the start of at least 48 hours when the temperature remained below 100^0F. Parasite clearance time (PCT) was defined as the time between admission and the first of the two consecutive negative blood slides. FCT and PCT were calculated from the initial responders (those who failed to clear fever and parasitaemia and received alternative treatment were not followed). Haemoglobin level was estimated by colour matching technique and done on days 0, 14 and 28 to observe the haematological response.

Statistical analysis: Data analysis was done using EPI INFO 6.04 statistical package. Statistical tests used were *chi-square* for frequency, ANOVA (*t-test*) for continuous variables and Kruskal Wallis as non-parametric test. Confidence intervals were determined by exact binomial distributions. Means ($\pm$ standard error of the mean), median (range) and percentages (with 95% CI) were used to describe the parameters.

Results:

Completed data from 77 cases and five healthy controls were available for analysis. One case was

lost to follow up in the mefloquine group; it was excluded and an additional case was recruited. The baseline percentiles of the study population characteristics and actual drug dosage per kilogram received after breaking down the tablets to the next half pill are shown in Table-I. The socio-demographic characteristics of the populations (positive and negative controls) in the control group were similar to the study population (data not shown).

Table-II describes the outcome parameters. No clinical failure was observed in the mefloquine group. One patient in the quinine group had clinical failure on day 27. None of the healthy controls developed any clinical illness. Mean PCT and FCT were significantly shorter with mefloquine compared to quinine. RIII type of parasitological response was observed in four cases with mefloquine and one case with quinine, however all of them showed radical cure up to 28 days of follow up without alternative antimalarial treatment.

Both the drug regimens were well tolerated. Two patients on Q7 had reversible cinchonism from day 5 onwards. Twenty two patients complained of nausea, nine had vomiting, four had loose motion and in none of them additional treatment was required to relieve the symptoms.

Table-I

Description of study population and actual drug-doses received in baseline percentiles (n=70)

Variables	Minimum	25%ile	50%ile	75%ile	Maximum
Age of subjects (years)	12.0	18.0	20.0	26.0	60.0
Body weight (Kg)	27.0	40.0	46.5	50.0	60.0
Duration of fever at inclusion (days)	1.0	3.0	4.0	7.0	45.0
Initial parasite count (n/cmm)	960	4260	12000	24624	150,400
Haemoglobin at inclusion (g/dl)	7.0	9.5	10.6	11.7	14.5
Actual total drug-dose received (mg/kg)- Mefloquine	23.1	24.0	25.0	25.0	26.5
Actual total drug-dose received (mg/kg/day)- Quinine	25.0	27.0	27.6	32.7	33.7

Table-II

Response to therapy in the four groups (n=77)

Type of response to therapy	Group I	Group II	P-value
	N (%) (95% CI)	N (%) (95% CI)	
Adequate clinical response (ACR)	70/70 (100) (95-100%)	6/7 (86) (42-100%)	
Early treatment failure (ETF)	0/70	0/7	
Late treatment failure (LTF)	0/70	1/7 (14) (.4-58%)	
S (Radical cure)	66/70 (94) (86-98%)	06/07 (86) (42-100%)	
R I	0/70	0/07	
R II	0/70	0/07	
R III	04/70 (06) (1-14%)	01/07 (14) (.4-58%)	
PCT* (days, mean+SD)	2.49 + .76	2.57+ .79 (n=7)	<.001
FCT** (hours, means+SD)	27.17+21.05	33.43+ 32.65	< .005

Discussion:

The study showed a 100% (95% CI, 95-100) in-vivo cure rate for mefloquine high-dose in uncomplicated falciparum malaria in malaria endemic study area in Bangladesh. In the previous study using mefloquine, the rate of clinical failure was 11% and that of parasitological failure was 27% (R III, R II and R I failures were 10%, 4% and 13% respectively)[8]. The reasons for clinical and parasitological failures found in the earlier study could be due to the lower dosage used of mefloquine and/or due to re-infections acquired in the community. However, with mefloquine observed R III failures (6%) were still observed, which could suggest onset of *P. falciparum* resistance, and should be kept under continued monitoring over time.

It is alarming to find even one clinical failure out of seven patients on Q7, in a situation free from re-infections. The recrudescence during the earlier study in Q7 group was considered re-infections, but they could be recrudescence as well. R III response was also observed in another case on Q7, which ultimately showed radical cure during follow up.

In the earlier study, the PCT was significantly ($p < .02$) lower with Q7 regimen compared to mefloquine and the FCT was not significantly different. But now, a significant prolongation of the PCT and FCT was observed in the Q7 group. Though, the number of cases in the Q7 group was very small, clinical failure, R III response and prolonged PCT and FCT, all could suggest beginning of resistance to Q7 in the study area.

Resistance to mefloquine appeared in regions at border between Thailand and Cambodia within a few years, perhaps owing to widespread use of quinine, to which it is structurally related[9]. Resistance in that border region remains high[10]. However, in nearby regions resistance to mefloquine remains relatively low[11]. Smithius et al reported more than 90% sensitivity to mefloquine (at a dose of 15 mg/Kg) from the western border region of Myanmar[12]. In Bangkok 86% of cases treated with mefloquine (at a dose of 25 mg/Kg) was sensitive[12]. The findings of failure to mefloquine 15 mg/Kg in an earlier study and also finding of failure to quinine could represent beginning of failure to both these drugs, and demands a continued monitoring of efficacy of these drugs over time. The present recommendations of WHO encourages not to use single antimalarials alone in order to preserve efficacy of the new drugs, instead combination of antimalarials preferably artemisinin based one is now recommended[13]. The high efficacy of large dose mefloquine in the present study suggests that mefloquine plus artesunate may be an alternative option for such a regimen for uncomplicated falciparum malaria instead of artemether lumefantrine. Both the regimens have been found to be highly effective in another high endemic area of malaria in Bangladesh[14].

It is apprehended that results of this study could be skewed, since the study population represent only those seeking treatment at a government facility, an estimated 20% of total malaria cases. A pre-condition for 28 day hospitalization could also impose a selection bias. However, since the majority of cases attend private drug vendors prior to reporting to health centres, the patients here may be selected for high levels of drug resistance.

The two consecutive studies with eight days hospitalization versus 28 days hospitalization, suggest that a higher failure rate could be detected by the 8-day trial. Thus, on a programme basis, and for a rapid assessment of drug efficacy situation, much simplified tests like 14-day in-vivo test on an outpatient basis[11] may be sufficient. But for new drugs (like mefloquine in the study area), a 28-day test with prevention of re-infection is required as an optimal test of efficacy of antimalarials.

The recommendation for use of mefloquine in the earlier study as the second line agent in the area appears justified. However, the drug should not be used alone in order to conform to the WHO policy. The efficacy trials should be continued in the area to specially document the possible declining sensitivity to Q7 regimen and the status of Q3+SP regimen should be re-evaluated in the area, including the sensitivity of the component parts.

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