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THERAPEUTIC EFFICACY OF QUININE PLUS SULFADOXINE-PYREMETHAMINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN BANGLADESH

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Abstract. In terms of drug resistance Bangladesh acts as an important gateway to the Indian Subcontinent. However, little is known about the current status of drug resistance in this country. The aim of this study was therefore to determine the therapeutic efficacy as well as *in vitro* drug sensitivity of quinine for 3 days plus a single dose of sulfadoxine/pyrimethamine (Q3F), an affordable alternative to the previously used chloroquine, for the treatment of uncomplicated falciparum malaria. Sixty-three patients were enrolled in the study; the overall cure rate in a 42-day follow-up after PCR adjustment was 87.3% (95% CI: 77.6–94.1). One patient was classified as early treatment failure (1.7%, 95% CI: 0.0–8.9%); 6 patients (10%; 95% CI: 3.8–20.5%) had late treatment failures within a median time of 27 days. HRP2 *in vitro* drug sensitivity tests were performed on all samples. Significantly higher ($P = 0.008$) *in vitro* IC_{50}s for pyrimethamine in treatment failures reflect the somewhat compromised drug sensitivity to this drug. These data suggest that the combination of 3 days of quinine with a single dose of sulfadoxine/pyrimethamine is an interesting and affordable alternative as long as or whenever ACT is not available.

INTRODUCTION

Resistance of *Plasmodium falciparum* to antimalarial drugs is spreading throughout many countries of Southern and Southeast Asia and is impeding efforts to control malaria. However, drug sensitivity reports from countries like Bangladesh are sparse. So far very limited data are available on the efficacy of currently used malaria treatments and the current situation of antimalarial drug resistance in Bangladesh, a country that in addition to financial and logistical constraints in malaria control must cope with increasing levels of drug resistance. The World Health Organization (WHO) reports that the malaria situation in Bangladesh is worsening, particularly in the hilly and forested areas in the Hill Tract Districts and also along the border areas in 13 high endemic districts with reported chloroquine and sulfadoxine/pyrimethamine (S/P) resistance.[1] In fact about two thirds of the laboratory confirmed malaria cases in Bangladesh occur in the Chittagong Hill Tracts (CHT), a relatively small area bordering Myanmar and India in the Southeast of the country. Up to 57,000 laboratory-confirmed and 400,000 clinical cases of malaria with more than 500 deaths per year have been reported from Bangladesh.[1] These numbers might underestimate the diseases burden due to shortcomings in surveillance and the information systems.[2,3] In its country profile the WHO report concludes that many problems and constraints exist for the malaria control program including: (i) lack of trained staff particularly as a result of retirement of experienced eradication staff; (ii) weak surveillance, supervision, and monitoring at various levels of program implementation; and (iii) increasing drug resistance in high endemic areas, especially the CHT districts.[1]

Only an integrated approach based on *in vivo* and *in vitro* drug susceptibility tests can provide clinical treatment response parameters as well as intrinsic drug sensitivity data to elucidate the background of clinical treatment failures. As a public health tool *in vivo* tests can be used to monitor drug resistance and to compare drug susceptibility status at different points in time or in different endemic areas. Polymerase chain reaction allows for the distinction of reinfection and recrudescence in outpatient trials. *In vitro* tests on the other hand are an excellent tool to demonstrate biologic resistance to antimalarials independent of host immune reaction and patient compliance.

The aim of this study was therefore to determine the therapeutic efficacy of quinine plus sulfadoxine-pyrimethamine for the management of uncomplicated falciparum malaria in Southeastern Bangladesh and to elucidate the background of treatment failures by correlating clinical findings with *in vitro* drug sensitivity data.

MATERIALS AND METHODS

The study was conducted at the field site of the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B) in Chakaria, in Cox's Bazar district, Chittagong, a malaria endemic area in southeastern Bangladesh close to the Chittagong Hill Tracts, between June and September 2004. The study was performed by the ICDDR,B in collaboration with the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok. Written informed consent was obtained from all study participants and the study protocols were approved by the Ethical Review Committee of the ICDDR,B and the Human Use Review Committee of the US Army (HURC). Patients 18 years or older with laboratory confirmed falciparum malaria were invited to participate in the study. All participants had monoinfections with *Plasmodium falciparum*, confirmed by rapid diagnostic devices (Now® Malaria, Binax, USA) and microscopy. Pregnant women and patients with prior anti-malaria treatment within the preceding 8 weeks were excluded. All patients agreed to undergo a 42-day follow-up.

In total, 72 subjects were screened and 63 were enrolled

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into the study. The minimum sample size was calculated to be 61 following WHO guidelines for the assessment and monitoring of antimalarial drug efficacy assuming an anticipated population proportion of clinical failures of 20%, a confidence level of 95%, and a precision of 10%.[4] No patients were withdrawn. One patient was lost to follow-up during the 42-day follow-up. Thirty-one (49.2%) patients were female; 32 (50.8%) were male. The median age was 20 years (range 18–63).

All patients received treatment with quinine 3 times a day (10 mg/kg per dose) for 3 days, followed by a single dose of sulfadoxine (25 mg/kg) co-formulated with 1.25 mg/kg of pyrimethamine on the fourth day. All treatment was directly observed. The first dose on day 0 was administered by the study team; the subsequent doses were supervised by local health care workers and village volunteers.

The *in vivo* study design roughly followed the WHO guidelines for the assessment and monitoring of antimalarial drug efficacy with an extension of follow-up until day 42.[4] Patients were asked to return on days 3, 7, and then weekly until day 42. If the patients did not return for the scheduled visits or missed an appointment they were visited at their homes by the study staff. Blood smears were performed and the temperature was recorded at each visit. Thick smears were used to quantify parasite densities per 200 white blood cells. Slides were declared negative based on reading 200 high-power fields. Patients were advised to return to the center in case of any symptoms consistent with malaria.

Primary outcome measure was cure. Absence of parasitemia until day 42 irrespective of axillary temperature was categorized as adequate clinical and parasitological response. Patients who showed a presence of parasitemia and axillary temperature ≥ 37.5°C on any day from day 4 to day 42 were classified as late clinical treatment failure; presence of parasitemia on any day from day 7 to day 42 and axillary temperature < 37.5°C were classified as late parasitological failure. Patients who were parasitemic on day 3 with an axillary temperature ≥ 37.5°C or $\geq 25\%$ of the parasite count on day 0 were categorized as early treatment failure.[4]

Venous blood was drawn for the assessment of antimalarial drug susceptibility on enrollment and whenever a patient developed parasitemia during follow-up. All samples were tested in the HRP2 *in vitro* drug susceptibility assay. The culture was performed as previously described.[5] In brief, the fresh *P. falciparum* parasite isolates were cultured in the presence of serial dilutions of the antimalarial drugs dihydroartemisinin (DHA), mefloquine (MEF), quinine (QNN), chloroquine (CHL), pyrimethamine (PYR), and sulfadoxine (SDX) at 1.5% hematocrit in complete RPMI 1640 with 0.5% Albumax (Albumax® I, Gibco, Bangkok, Thailand) and 25 mg/L of gentamycin without freezing, washing, dilution, addition of serum, or preculturing. For sulfadoxine and pyrimethamine a specially prepared RPMI1640 medium was used with low content of folate and PABA (1/100 as compared with standard RPMI1640). After 72 hours of culture the plates were frozen and stored at –20°C. The plates were then thawed and parasite growth inhibition was quantified using an HRP2 ELISA based on 2 commercially available monoclonal antibodies (Immunology Consultants Laboratory Inc., Newberg, OR) directed against *P. falciparum*-specific HRP2: MPFM-55A, an IgM antibody used as the capture antibody and MPFG-55P, a horseradish peroxidase conjugated IgG antibody, which was used as the indicator antibody. The ELISA was performed as previously described.[6] Optical density was measured at 450 nm using a field-deployable ELISA plate reader.

Gene loci of pre-treatment and post-treatment sample pairs (MSP2) were compared in polymerase chain reaction (PCR) to determine whether the genotype before and after reappearance of parasites in the peripheral blood was identical, indicating a recrudescence.

Kaplan-Meier analysis was performed to calculate the proportion of aparasitemic patients for each point in time (later referred to as cure rates). Patients who were lost to follow-up, who had reinfections diagnosed by PCR, or who developed *P. vivax* parasitemia during the follow-up were censored. Inhibitory concentrations were calculated using nonlinear regression analysis based on a polynomial regression model.[7]

RESULTS

From a total of 72 screened subjects 63 (87.5%; 95% CI: 77.6–94.1) were enrolled in the study. Fifty-nine subjects were evaluable for primary end point on day 42; only 1 patient (1.6%; 95% CI: 0.0–8.5) was lost to follow-up after day 35. One patient was censored after a reappearing *P. falciparum* parasitemia could not be confirmed by PCR. Two patients were censored after being treated with chloroquine and primaquine for *P. vivax* parasitemia on days 14 and 31, respectively (Figure 1).

The overall cure rate prior to PCR adjustment calculated by Kaplan-Meier analysis after 42 days was 82.1%. The cure rates for days 7, 14, 21, 28, and 35 were 98.4, 96.8, 93.6, 85.5, and 83.9%, respectively. After PCR adjustment the overall

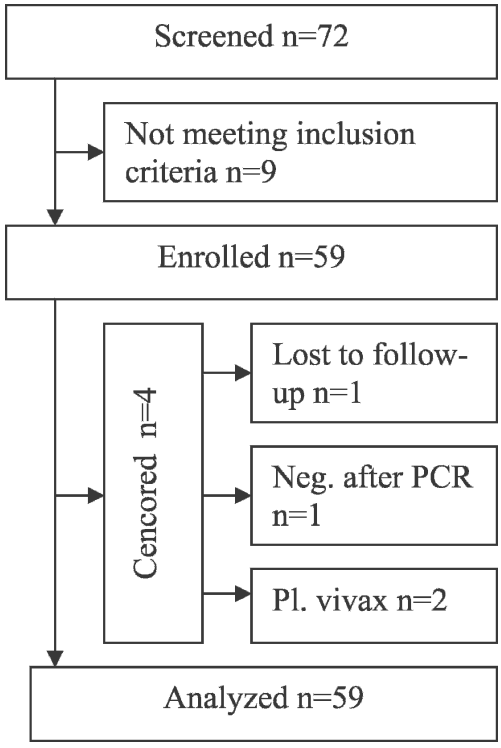

FIGURE 1. Flow diagram of the progress through the phases of the clinical study.

cure rate was 87.3% the corresponding cure rates were 98.4, 98.4, 95.1, 89.8, and 87.7%, respectively (Figure 2).

Eleven of the 59 patients who completed the 42-day follow-up (18.6%; 95% CI: 9.7–30.9%) developed a reappearance of parasites during follow-up. One (1.7%; 95% CI: 0.0–9.1) of these patients was characterized as early treatment failure (ETF) with a parasite density on day 3 of over 25% of the parasite count of day 0. The remaining 10 (16.9%; 95% CI: 8.4–29.0) patients showed reappearance within a median time of 27 days (range: 13–42 days). Four (6.8%; 95% CI: 1.9–16.5%) patients were classified as reinfection and 6 (10.2%; 96% CI: 3.8–20.8%) as late treatment failures. Of these 6 patients, 2 (33.3%; 95% CI: 4.3–77.7%) subjects showed late parasitological failure (LPF), whereas late clinical treatment failures (LCF) were observed in 4 patients (66.7%; 95% CI: 22.3–95.7%). In addition, 2 patients who fulfilled the criteria of ETF (i.e., parasitemia on day 3 $\geq$ 25% of that on day 0) but who did not show any clinical signs and symptoms and who cleared parasites without requiring additional therapy were classified as cured with delayed parasite clearance. Parasite densities were not significantly higher ($P > 0.05$) in failures than in patients who were cured. By day 3 all patients had cleared fever and the majority of all patients showed a heavily reduced parasite load. All patients who presented with treatment failures were treated following the national treatment guidelines (third-line regimen: 7 days quinine TID).

The *in vitro* data suggest high levels of chloroquine resistance among the samples tested in the course of this study. The geometric mean IC_{50} and IC_{90} were 93.06 nM (95% CI: 80.38–107.76) and 214.76 nM (95% CI: 175.64–262.62), respectively. In contrast the isolates were relatively sensitive to quinine and mefloquine. The geometric mean IC_{50} for quinine was 73.24 nM (95% CI: 65.26–82.21) and the IC_{90} 157.75 nM (95% CI: 134.16–185.5). The IC_{50} for mefloquine was 11.26 nM (95% CI: 9.75–13.0) and the IC_{90} 19.55 nM (95% CI: 15.73–24.29). The dihydroartemisinin ICs were equally low suggesting high sensitivity of the parasites to that drug (IC_{50}: 1.33 nM; 95% CI: 1.08–1.63, IC_{90}: 2.65 nM; 95% CI: 2.13–3.29). For pyrimethamine and sulfadoxine the geometric mean IC_{50s} were 1.7 μM (95% CI: 1.25–2.3) and 40.46 μM (95% CI: 31.15–51.97), respectively; the IC_{90s} were 4.83 μM (95% CI: 3.17–7.37) and 173.48 μM (95% CI: 120.78–249.17).

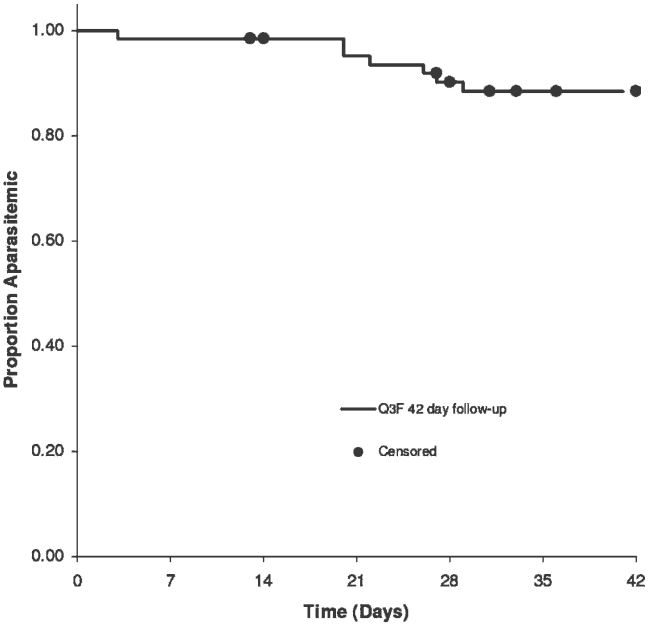

FIGURE 2. Kaplan-Meier curve for the PCR-adjusted cure rate for the combination of quinine with S/P in 63 uncomplicated falciparum malaria patients in Southeastern Bangladesh.

Significant correlations suggesting cross sensitivity were found between DHA and mefloquine at IC_{50} and IC_{90} level ($R = 0.65$; $P < 0.001$; $N = 49$ and $R = 0.57$; $P < 0.001$; $N = 49$) as well as between pyrimethamine and sulfadoxine at IC_{90} level ($R = 0.88$; $P = 0.009$; $N = 7$).

Close relations were also found between *in vitro* drug sensitivity and clinical treatment response parameters, suggesting a significant impact of intrinsic drug sensitivity on the treatment outcome. In Mann-Whitney *U* tests isolates taken on admission from patients who later developed treatment failures showed significantly higher IC_{50} levels for PYR ($P = 0.008$) than those patients who were cured (Geometric means: 1199.25 nM 95% CI: 731.66–1965.66 and 365.08 nM 95% CI: 253.29–536.22, respectively). As the definite parasite clearance is not related to quinine sensitivity, the quinine IC_{50s} were not higher in patients who later developed treatment failures ($P > 0.05$).

DISCUSSION

In spite of known high levels of chloroquine resistance, particularly in the southeastern parts of the country along the border with Myanmar, until very recently chloroquine was used as the first-line therapy for uncomplicated falciparum malaria in Bangladesh.[8,9] The Ministry of Health therefore revised the guidelines for malaria treatment with the introduction of artemisinin combination therapy (ACT) for areas with reported drug resistance. However, so far financial constrains and shortcomings in production of adequate quantities of drugs limited the availability of ACT in these regions.

Our data suggest that the combination of 3 days of quinine with a single dose of S/P, formerly the official second-line therapy in Bangladesh, is an interesting and affordable alternative as long as or whenever ACT is not available. A relatively faster acting antimalarial with a short half-life combined with a slower acting, long half-life drug has proven to be a good strategy for eliminating falciparum parasites from the bloodstream. The role of quinine in this combination is to reduce the initial parasite biomass, whereas the role of S/P is to eliminate the remaining parasites and keep up drug levels above the minimum inhibitory concentration long enough to prevent recrudescence.

S/P shares mechanisms of action with Cotrimoxazole and they are therefore likely to develop cross-sensitivity patterns. One study reports that treatment with S/P resulted in increased colonization with cotrimoxazole-nonsusceptible *S. pneumoniae*. However, the clinical impact of cotrimoxazole resistance in these pathogens is not fully understood.[10] Moreover in recent studies Cotrimoxazole did not appear to select for SP-resistant parasites.[11]

The majority of failures in this study were categorized as late treatment failures. The significantly higher pyrimethamine IC_{50}s found in parasite samples from patients who later developed recrudescences clearly show that these were largely caused by reduced sensitivity to S/P. In spite of emerg-

ing resistance to S/P in this area, in combination with quinine the activity of S/P is still sufficient to clear more than 87% of the infections. However, for the future it will be important to strictly limit the use of S/P to combination therapies. Its use as monotherapy could quickly lead to high levels of resistance. Efficacy of S/P in combination with chloroquine was found to be only 63.4% and is therefore probably not a viable option.[12]

Only 1 patient was characterized as early treatment failure. Due to its short half-life the activity of quinine has relatively little impact on the overall cure rate. In this combination quinine resistance would be expected to lead to early treatment failures. Although another 2 patients had similarly slow parasite clearance, they were classified as cured with delayed parasite clearance as they cleared parasites quickly after day 3 and did not require salvage therapy. These findings are consistent with the generally low quinine IC_{50}s found in this study.

The high chloroquine IC_{50}s found in this study are similar to earlier reports of *in vitro* and clinical chloroquine resistance in this area.[8,9,13] Chloroquine resistance in Bangladesh has been known since the 1970s and is likely to have increased since then.[14] Our *in vitro* data once again confirm the fact that chloroquine should not be used for the management of *P. falciparum* malaria in this region. They are directly comparable to chloroquine drug sensitivity data from Thailand from the same year (unpublished data), a country with some of the highest levels of antimalarial drug resistance in the world.

The relatively low ICs for DHA and mefloquine suggest that both drugs still show a high activity against *P. falciparum* parasites from that area. So far artemisinin derivatives have never been used on a large scale in Bangladesh. Our data suggest that they will be an interesting option for combination regimens (e.g., with lumefantrine) in the future. Although previous data indicate that mefloquine sensitivity may be compromised due to the import of mefloquine-resistant parasites across the near border with Myanmar these new data show that mefloquine sensitivity in the area is still comparatively high.[13] However, due to its extremely long half-life mefloquine may not be a good choice for an area with relatively high malaria endemicity like the Chittagong Hill Tracts.

The recrudescence rate in the 42-day follow-up is similar to failure rates found in a previous study with a 28-day follow-up from a nearby area.[15]

Although failures from S/P may occur also 4–6 weeks after the course of treatment we found no recrudescence after day 30.[16] In our study the median time until recrudescence was 27 days and most of the failures actually happened on or around day 28. These data suggest that the minimum follow-up for S/P efficacy studies should therefore be 35 days, thereby confirming previous observations with the same drug combination.[16] With longer follow-ups in outpatient studies, however, the role of PCR for the distinction of recrudescence/reinfection becomes even more important.

The overall compliance was very good; only 1 patient was lost to follow-up. No severe adverse events (SAE) were observed. Probably due to the short administration of quinine relatively few patients complained about cinchonism (e.g., tinnitus) during their course of treatment.

There are only limited data about the use and the resistance patterns of Q3F in Africa, but due to the known safety in the management of children and pregnant women it might also be an affordable option for sub-Saharan Africa.

In conclusion, our data suggest that Q3F is an interesting alternative as long as or whenever ACT is not available. Both drugs used in this combination are available from local producers and are relatively inexpensive. However, it is important to reiterate that S/P sensitivity is compromised and that S/P should only be used in combination with faster-acting antimalarials that have a different mechanism of action to prevent a rapid progression of drug resistance. Continuous surveillance of antimalarial drug resistance in Bangladesh, both *in vivo* and *in vitro*, will therefore be essential.

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