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Therapeutic Efficacy of Chloroquine for the Treatment of Uncomplicated *Plasmodium falciparum* in Haiti after Many Decades of its Use

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Abstract. Chloroquine (CQ) has been used for malaria treatment in Haiti for several decades, but reports of CQ resistance are scarce. The efficacy of CQ in patients with uncomplicated *Plasmodium falciparum* undergoing treatment in Haiti was evaluated. Malaria patients were enrolled, treated with CQ, and monitored over a 42-day period. The treatment outcomes were evaluated on day 28 by microscopy. The *P. falciparum* slide-confirmed rate was 9.5% (121 of 1,277). Malaria infection was seasonal, with peak observations between October and January; 88% (107 of 121) of patients consented to participate. Sixty patients successfully completed the 42-day follow-up, whereas 47 patients withdrew consent or were lost to follow-up. The mean parasite density declined rapidly within the first few days after treatment. Seven patients did not clear their malaria infections and were clinically asymptomatic; therefore, they were considered late parasitological failures. About 90% (95% confidence interval = 84.20–97.90) of patients had no detectable parasitemia by day 28 and remained malaria-free to day 42. Testing for recrudescence, reinfection, and CQ serum levels was not done in the seven patients, and therefore, their CQ resistance status is unresolved. CQ resistance surveillance by patient follow-up, in vitro drug sensitivity studies, and molecular markers is urgently needed in Haiti.

INTRODUCTION

Malaria in Haiti is a significant public health concern, with an estimated 80% of the population at risk of infection.[1] *Plasmodium falciparum* is the predominant malaria parasite species in Haiti, and it accounts for more than 98% of all confirmed malaria infections.[2] The transmission of malaria in Haiti is tightly linked with rainfall patterns,[3] with the peak transmission season occurring between November and January.[4] The treatment of malaria in Haiti is based on chloroquine (CQ) given at a total dose of 25 mg base per kilogram body weight over a 3-day period. Recently, the treatment policy was revised to incorporate a single dose of primaquine[2] for malaria patients found to harbor gametocyte stages that are transmissible to mosquitoes.

Despite being rendered ineffective for treating malaria in many endemic areas because of resistance, CQ is still used in Haiti because of the apparent sensitivity of the *P. falciparum* strains in Haiti. The use of CQ might have been continued based on drug sensitivity studies conducted in the 1980s that found *P. falciparum* to be sensitive to CQ.[5,6] However, the recent findings of genetic markers of CQ resistance in *P. falciparum*[7,8] might indicate a change in sensitivity and emergence of CQ resistance in Haiti. However, there are no recent in vivo CQ efficacy data. To provide up to date information about the efficacy of CQ in the treatment of *P. falciparum* malaria infections, this study evaluated the treatment outcomes in patients with uncomplicated *P. falciparum* undergoing CQ therapy. The data obtained in this study might be useful in guiding future malaria treatment policy in Haiti.

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METHODS

Ethical approval. This study was approved by the Haiti Ethical Review Board, the University of Florida Institutional Review Board, and the Office of Research Protections, United States Army Medical Research and Material Command (USAMRMC).

Study site. This study was conducted between September of 2011 and December of 2012 at two clinics in Haiti: (1) Hospital Saint Croix in Leogane, a regional reference hospital managed by the Episcopal Church of Haiti and (2) the Family Medical Health Ministries Medical Center (Blanchard Clinic), which is located in Port-au-Prince in Blanchard (Terre Noire) 1 mile northwest of the Port-au-Prince International Airport (Figure 1).

Identification and enrollment of patients in the follow-up study. The participants in this study were identified from patients seeking treatment at the clinics. Febrile patients presenting with malaria symptoms were tested by rapid malaria diagnostic tests (CareStart, AccessBio, Somerset, NJ) and followed by standard microscopy for the confirmation and quantification of *P. falciparum* parasitemia in positive cases. To participate in the study, patients had to meet the following inclusion criteria: (1) between the ages of 2 and 100 years old, (2) not severely malnourished (defined as a child whose weight for height is below 3 standard deviations or less that 70% of the median of World Health Organization [WHO] reference values), (3) have microscope slide-confirmed infection with *P. falciparum* malaria with a parasite density of at least 1,000 asexual parasites/μL blood, (4) absence of general danger signs among children < 5 years old and other signs of severe complicated malaria (e.g., inability to drink or breastfeed, vomiting everything, recent history of convulsions, lethargy or unconsciousness, and inability to stand), (5) absence of hypersensitivity reactions to CQ, and (6) no history of previous antimalarial use within the previous 2 weeks. The exclusion criteria included (1) pregnant or lactating women, (2) existence of underlying chronic severe illness (e.g., human immunodeficiency virus [HIV] and hepatic

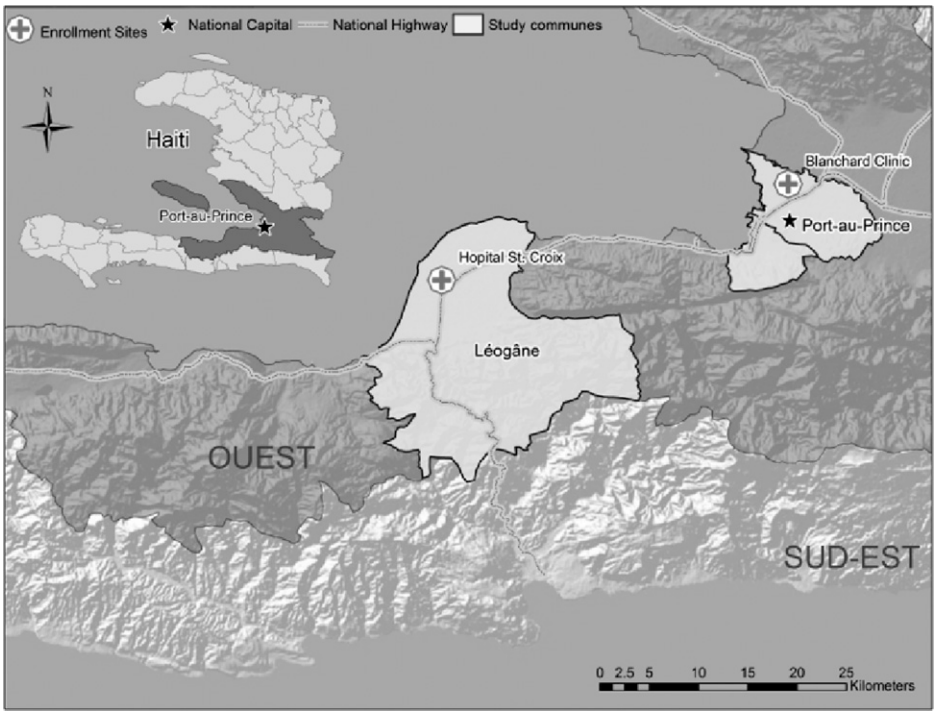

FIGURE 1. Map showing the communes and the clinics where the study was conducted.

diseases), and (3) refusal to give consent to participate in the study. The details of the study protocol were explained to the prospective participants or the guardians of children in a language that they could understand (Kreyol), and those giving consent were requested to sign an informed consent form to affirm their agreement to participate in the follow-up study. All malaria-positive subjects were treated with 25 mg CQ per kilogram body weight according to the standard protocol at the clinics under the direct supervision of the study site physician. The number of participants enrolled and followed-up is illustrated in Figure 2.

Clinical study. After enrolling in the study on day 0, the participants were followed up on days 1, 2, 3, 7, 14, 21, 28, 35, and 42. CQ (Ranbaxy Pharmaceuticals Inc, Jacksonville, FL) treatment was given at 10 mg per kg body weight on day 0, 10 mg per kg body weight on day 1, and 5 mg per kg body weight on day 2. Patients were requested to come back on the pre-appointed days for clinical monitoring and assessment of parasite clearance by microscopy. In addition, community health workers followed up with the participants at their homes. *P. falciparum* parasitemia was assessed by standard microscopy techniques by counting the number of asexual *P. falciparum* parasites per 2,000 leukocytes.[9] On each follow-up day, the temperature of the subjects was recorded, and the hemoglobin concentration was measured using a hemoglobin meter (Hemacue Hb 201; Angelholm, Sweden).

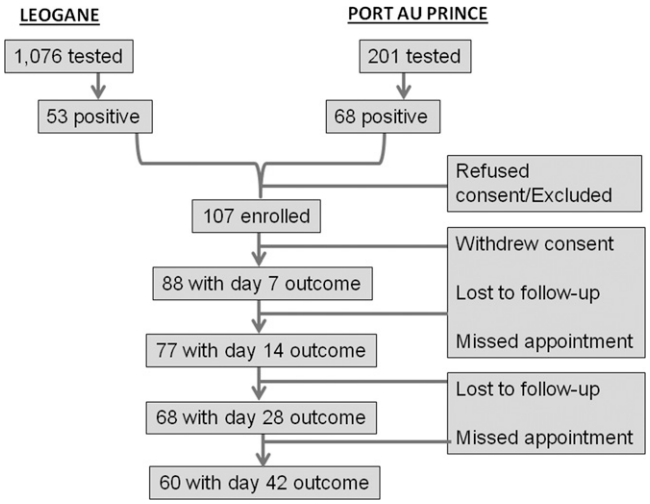

FIGURE 2. Patient identification, enrollment, and follow-up schematic. d = Day.

Treatment outcomes. The WHO standard classification of treatment outcomes in low-malaria transmission settings[10] was used as shown in Table 1. The primary treatment outcomes were assessed on day 28. The treatment outcomes were also assessed on day 7 following the simplified WHO standard 7-day in vivo field test as used by Duverseau and others[5] and day 14 as suggested by WHO.[10]

Statistical analysis. Mean values of age, hemoglobin content, and parasitemia of enrolled patients were computed on SPSS version 21 software. Only patients who completed the study with no major protocol violation were used in the analysis of the treatment outcomes. Those lost to follow-up or who withdrew consent were excluded from the analysis.

RESULTS

Low *P. falciparum* malaria infection rates in febrile patients. In total, 1,277 symptomatic patients presenting with malaria-like fevers were tested for *P. falciparum* infection (Table 2). The positivity rate for *P. falciparum* by slide-confirmed microscopy was 9.5% (121 of 1,277). There was seasonal variation in malaria-like symptoms and *P. falciparum*

TABLE 1

Treatment endpoints and patient condition as described in the WHO manual for the assessment and monitoring of antimalaria drug efficacy for the treatment of uncomplicated *P. falciparum*[10]

Treatment outcome	Patient condition
ETF	Development dangers signs or severe malaria on day 1, 2, or 3 after drug treatment in the presence of parasitemia
	Malaria parasite count on day 2 is higher than on day 0, irrespective of axillary temperature of the patient
	Axillary temperature ≥ 37.5°C on day 3 in the presence of parasitemia
	Parasitemia on day 3 is ≥ 25% of the count on day 0
LTF	Development of danger signs or severe malaria in the presence of parasitemia on any day from day 4 to 14 after treatment without previously meeting any of the criteria for ETF
	Presence of parasitemia on any day from day 4 to 28 and a measured axillary temperature > 37.5°C (or fever) without previously meeting any of the criteria of ETF
LPF	Presence of parasitemia on any day from day 7 to 28 and axillary temperature of < 37.5°C without previously meeting criteria for LTF
ACPR	Absence of parasitemia on day 28, irrespective of axillary temperature, without previously meeting any of the criteria of ETF, LTF, or ACPR

ACPR = adequate clinical and parasitological response; ETF = early treatment failure; LTF = late clinical failure; LPF = late parasitological failure.

malaria cases, with peak numbers of cases observed in October and January, respectively (Table 2).

Patient characteristics at enrollment and follow-up. Eighty-eight percent (107 of 121) of malaria-positive patients identified by microscopy gave consent and were enrolled to participate in the study. The mean age of the enrolled patients was 31.7 years (range = 11–85 years). The mean temperature at enrollment was 37.9°C, whereas the hemoglobin content was 13.1 g/dL. The mean parasite density in enrolled patients at day 0 was 1,967.29 ± 131.5. The mean temperature reduced to 37.2°C by day 42, whereas the hemoglobin content remained stable over all subsequent follow-up days; 60 patients successfully completed the 42-day follow-up period, whereas 47 patients withdrew consent or were lost to follow-up (Table 3). The number of patients who withdrew consent or were lost to follow-up was highest in the first 1 week and then stabilized with time (Table 3). The number of patients with assessed clinical endpoints who completed follow-up is presented in Table 4.

Parasitological outcomes of malaria patients treated with CQ. The mean parasite density declined rapidly after treatment as shown in Figure 3. Seven patients did not clear their malaria infections but were clinically asymptomatic, and therefore, they were considered late parasitological failures (LPFs). These patients were positive until day 42, but their parasitemia levels continued to steadily decrease. On day 28, 91.0% (95% confidence interval [95% CI] = 84.20–97.90) of patients had no detectable parasitemia by microscopy. These patients remained clear of malaria parasites until the end of the extended follow-up period (day 42). In the simplified WHO in vivo field test, 92.1% (95% CI = 86.4–97.7) of patients cleared their parasitemia by day 7, and following the WHO-suggested 14-day follow-up period,[10] 91% (95% CI = 84.5–97.3) of patients were aparasitemic.

TABLE 2

Monthly data of patients with malaria-like fevers screened for *P. falciparum* infection at the two clinics in Haiti

	Hospital Saint Croix		Blanchard Clinic		Total number of cases	
Year and month	Fever	Malaria	Fever	Malaria	Fever	Malaria
2011						
September	34	1	11	3	45	4
October	43	3	13	5	56	8
November	103	13	11	7	114	20
December	105	8	25	18	130	26
2012						
January	101	6	4	3	105	9
February	74	5	3	1	77	6
March	70	4	15	3	85	7
April	81	4	24	1	105	5
May	103	4	28	7	131	11
June	135	2	15	2	150	4
July	59	2	13	3	72	5
August	81	0	11	2	92	2
September	33	0	10	2	43	2
October	54	1	18	11	72	12
Total	1,076	53	201	68	1,277	121

Malaria infection was detected by rapid detection kit and confirmed by microscopy.

DISCUSSION

Our study observed high treatment success rates (adequate clinical and parasitological responses) in malaria patients after CQ therapy, although a few patients did not respond to treatment and were categorized as treatment failures. Molecular analysis of these seven cases did not find the *pfcrt* K76T mutation[11] that is widely associated with CQ resistance. It is not clear why the patients remained chronically parasitemic, because we did not test for reinfection or recrudescence. In addition, we were not able to test for the blood concentration levels of CQ in the patients, and therefore, we do not know if these patients rapidly metabolized and eliminated the antimalarial medicine. The *P. falciparum* densities in the patients who participated in the study were generally low (approximately 0.6% parasitemia). On CQ therapy, a rapid decline in the parasite densities was observed within the first 3 days, suggesting high sensitivity to CQ. Our findings support the notion that high CQ sensitivity of *P. falciparum* malaria exists in Haiti, but the status of resistance to CQ is inconclusive. There is a great need to continue monitoring efforts of

TABLE 3

The characteristics of patients diagnosed with *P. falciparum* on day 0 of enrollment grouped by the treatment outcome observed in the study

	Follow-up day			
	7	14	28	42
Completion of study procedures	88	77	68	60
Voluntary withdrawals/loss to follow-up (weekly withdrawals/loss to follow-up)	19 (19)	30 (11)	39 (9)	47 (8)
Total number of enrolled patients	107	107	107	107

TABLE 4
Therapeutic outcomes in malaria patients undergoing CQ treatment in Haiti

Study endpoint	Day 7	Day 14	Day 28	Day 42
Early treatment failure	0	0	0	0
Late clinical failure	0	0	0	0
Late Parasitological Failure	7	7	7	7
Adequate clinical and parasitological response	81	70	61	53
Total number of patients	88	77	68	60
Treatment failure rate (%)	7.9	9.1	10.3	11.6
Parasite cure rate/clearance rate (%)	92.1	90.9	89.7	88.4

The treatment outcome endpoints were assessed on days 7, 14, 28, and 42 as described in Methods.

the emergence of resistance to antimalarial drugs that may be in use in Haiti.

The drastic decline of parasitemia on treatment with CQ observed in many patients in our study is suggestive of widespread sensitive malaria strains in Haiti. In areas with CQ-sensitive malaria, similar rapid declines in parasitemia have been observed. In a CQ efficacy trial in Honduras, where CQ-sensitive *P. falciparum* is prevalent, the parasitemia rapidly reduced after CQ treatment, and by the third day, less than 10% of the malaria patients had malaria parasites in their blood.[12] In our study, the high proportion of patients who were successfully treated with CQ supports the assertion that malaria parasites in Haiti are sensitive. In the earlier in vivo CQ efficacy study conducted by Duverseau and others[5] in Haiti, 81% of the patients had no detectable parasitemia in their blood by the seventh day. Our study observed a high percentage (92%) of patients with no detectable parasitemia in their blood by the seventh day, mirroring the findings by Duverseau and others.[5] Moreover, the proportion of patients remaining positive after CQ treatment reduced very rapidly within the first week (57% on day 1, another 25% on day 3, and another 18% on day 7), which is very consistent with treatment efficacy data from areas reported with CQ-sensitive *P. falciparum*, like Honduras.

Despite observing a few cases that did not respond to treatment, the evidence for CQ resistance is not clear cut in our study. Molecular testing did not find the K76T mutation in the *Pfcrt* gene[11] in these seven patients. It is not exactly clear why these patients did not respond to treatment, although the patients were compliant with the treatment and follow-up protocol and completed their medications under direct supervision of the medical doctors. However, it is possible that host factors (such as differences in individual pharmacokinetics) might have led to suboptimal CQ concentrations in the blood, but we were unable to test for serum levels of the CQ in these patients. Although we did not test for reinfections or recrudescent parasites because of a lack of sufficient samples, these are unlikely to be the case, because the parasite densities in these seven patients decreased rapidly and did not increase at any point during the follow-up, as would be expected with reinfections or recrudescent parasites.

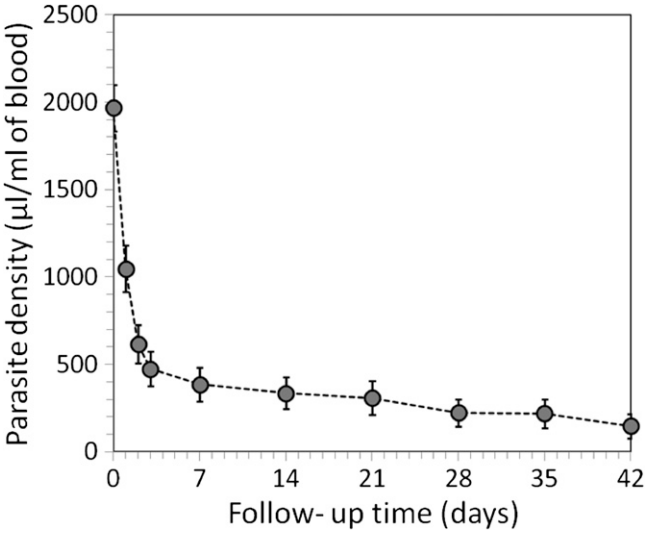

FIGURE 3. The parasitemia rates in *P. falciparum* malaria patients undergoing CQ therapy in Haiti.

The high loss to follow-up and withdrawal rates of the participants impacted this study. The extension of the follow-up period from 28 to 42 days caused an increase in the number of patient withdrawals and follow-up loss. Only patients with no major protocol violation were included in the analysis; those lost to follow-up or who withdrew consent were excluded from the analysis. Therefore, overall cure rates dropped from 92.1% on day 7 to 89.7% on day 28, which is largely attributed to patient loss to follow-up. The losses observed in our study were somewhat higher at 42 days than has been observed in other studies.[10] However, such high dropout rates are to be expected in study areas, such as Haiti, where clinics can be far away from patients.[13] Although a tremendous amount of effort was put into minimizing the dropout rates (for example, hospital staff would visit patients at their homes, patients were given reimbursements for food and travel costs, and overnight accommodations were also available for the enrolled patients), in the end, the patients' right to withdraw their consent at any time was paramount, and their decisions had to be respected in accordance with the consent documents that they signed.

In conclusion, our study presents data that suggest that CQ may still have an important role to play in the treatment of uncomplicated *P. falciparum* in Haiti but are inconclusive about CQ resistance because of the unresolved status of the seven cases that did not respond to treatment. Because we did not to detect the *pfcrt* K76T mutation, the CQ resistance status of these seven patients is unresolved; we did not determine the presence of other single-nucleotide polymorphisms (SNPs) in the *Pfcrt* gene that have also been associated with CQ resistance,[14] and therefore, the CQ resistance issue in Haiti remains an open question. In addition, there are still unanswered questions surrounding the bioavailability of CQ (CQ serum levels) in the patients with parasitological failure. Although the patients were complaint with the study protocols and procedures, the individual differences in drug metabolisms may have influenced the effectiveness of CQ for malaria treatment in these patients. It is also very likely that a low-malaria transmission setting, like Haiti, is likely to slow the spread of CQ resistance.[15] Finally, we urge that surveillance activities for antimalarial drug resistance be increased in Haiti and whenever possible, that malaria patients undergoing treatment be followed up or requested to return for follow-up visits. Additionally, in vitro sensitivity testing should be performed if resources allow, and sample collection through dried blood spots should be done to enable future investigations on molecular markers for antimalaria drug resistance.

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