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Confirmed Vivax Resistance to Chloroquine and Effectiveness of Artemether-Lumefantrine for the Treatment of Vivax Malaria in Ethiopia

Ambachew M. Yohannes,* Awash Teklehaimanot, Yngve Bergqvist, and Pascal Ringwald

World Health Organization Country Office, Addis Ababa, Ethiopia; Mailman School of Public Health and the Earth Institute, Columbia University, New York, New York; Dalarna University College, Borlänge, Sweden; Global Malaria Program, World Health Organization, Geneva, Switzerland

Abstract. Chloroquine (CQ) is still the drug of choice for the treatment of vivax malaria in Ethiopia, whereas artemether-lumefantrine (AL) is for falciparum malaria. In this setting, clinical malaria cases are treated with AL. This necessitated the need to assess the effectiveness of AL for the treatment of *Plasmodium vivax* with CQ as a comparator. A total of 57 (80.3%) and 75 (85.2%) cases treated with CQ or AL, respectively, completed the study in an outpatient setting. At the end of the follow-up period of 28 days, a cumulative incidence of treatment failure of 7.5% (95% confidence interval [CI] = 2.9–18.9%) for CQ and 19% (95% CI = 11–31.6%) for AL was detected. CQ resistance was confirmed in three of five CQ treatment failures cases. The effectiveness of AL seems lower than CQ; however, the findings were not conclusive, because the AL evening doses were not supervised.

INTRODUCTION

Malaria transmission in most parts of Ethiopia is seasonal and unstable, characterized by frequent focal and large-scale cyclic epidemics every 5–8 years.[1] In some parts of the western lowland areas and river basins, the transmission period may last for more than 6 months in a year.[2] *Plasmodium falciparum* accounts for 60–70% of the total malaria cases, whereas *P. vivax* accounts for 30–40%.[3] The first-line antimalarial drug for the treatment of uncomplicated *P. falciparum* malaria in Ethiopia was changed to artemether-lumefantrine (AL) in July 2004 after confirmation of the emergence and spread of resistance to chloroquine (CQ) and sulfadoxine/pyrimethamine (SP).[4,5] However, CQ remained as the drug of choice for the treatment of *P. vivax* malaria.[3] The effectiveness of AL for the treatment of *P. vivax* infections has not previously been evaluated in the Ethiopian setting. This study was, therefore, conducted to assess the therapeutic efficacy of CQ and effectiveness of AL for the treatment of vivax malaria in an outpatient setting where all the morning treatment doses of CQ and AL were supervised, whereas the evening doses of AL were handed to the patients to take at home.

The study was conducted from October 2004 to May 2005 in Debrezeit and Nazareth towns, located 60 km apart, in the Rift Valley area of Oromia Region, Central Ethiopia, at altitude ranges of 1,661–1,900 m above sea level. The malaria transmission pattern in the area is seasonal, with the major transmission months being from September to December after the main rainy season from June to mid-September. *Anopheles arabiensis* is the main malaria vector in the area, and *An. pharoensis* and *An. funestus* have also been incriminated as secondary vectors. Although there are no published evidences, the pattern of *P. vivax* recurrences in these sites is not expected to show differences.

METHODS

The study was conducted following the World Health Organization (WHO) protocol for the *in vivo* assessment of the therapeutic efficacy of antimalarial drugs, with a follow-up period of up to 28 days.[6] Patients who presented at the malaria laboratory centers were included in the study on fulfilling the following inclusion criteria: age of 1 year and above, positive for *P. vivax* mono-infection with asexual parasite density of > 250/μL of blood with fever or history of fever 48 hours before time of recruitment, absence of clinical conditions requiring hospitalization, no evidence of severe malnutrition, absence of pregnancy, absence of significant concomitant febrile or other illness that could interfere with the follow-up, absence of known allergy and/or intolerance to the drug being administered, and ability and willingness to participate in the study. Informed consent was obtained from all participants or their guardians in case of children.

Thick and thin blood smears stained with 3% Giemsa's stain for 30 minutes were used for microscopic examination. The thick blood smears were used for parasite count and parasite density estimation. Asexual parasites were counted against 200 leukocytes, and parasite density was estimated by considering 8,000 white blood cells (WBCs)/μL of blood as a multiplier. Duplicate blood films were examined simultaneously by two independent examiners, and discordant results were agreed on after demonstration of proof by each examiner.

CQ (batch no. 404055-2, expiration date of April 2007) and AL (batch no. 031146, expiration date of November 2005) were administered based on measured body weight for 3 days as recommended in the national malaria diagnosis and treatment guideline.[3] The evening dose of AL was not supervised, because this was handed to patients or guardians to take at home. Clinical and parasitological assessment was done on days 0, 1, 2, 3, 7, 14, 21, and 28. To minimize the loss to follow-up and withdrawals, patients and guardians were advised to complete the scheduled visits, and efforts were done to trace those who missed a follow-up appointment to ensure their continued participation and/or completion of the treatment. Treatment outcomes of the patients who completed the 28 days follow-up were classified based on the parasitological and clinical findings in accordance with the WHO protocol.[6] Subjects with confirmed treatment failure were given rescue treatment with quinine tablets.

Blood levels of CQ and desethylchloroquine for PCR confirmed cases[7] were determined with the high-performance liquid chromatography (HPLC) method using 100-μL dried blood samples collected on filter paper (Whatman 31ET Chr, Dalarna University College, Borlange, Sweden).[8] Treatment

* Address correspondence to Ambachew M. Yohannes, World Health Organization, 20 Avenue Appia, CH-1211 Geneva, Switzerland. E-mail: yohannesam@who.int

failure cases with CQ and desethylchloroquine blood level of above 100 ng/mL at the day of treatment failure were classified as CQ-resistant cases.[9]

RESULTS

A total of 71 patients in the CQ and 88 patients in the AL treatment group was enrolled in the study, and 57 (80.3%) and 75 (85.2%) completed the 28 days follow-up, respectively. During the follow-up period, 14 (19.7%) patients in the CQ group and 13 (14.8%) patients in the AL group defaulted from the study because of loss to follow-up and withdrawal. Of the 14 (19.7%) withdrawal and loss to follow-up cases in the CQ treatment group, all were in the 5 and above years of age category, with nine (64%) male and five (36%) female subjects. Of the 13 (14.8%) withdrawal and loss to follow-up cases in the AL treatment group, 12 (92%) were in the 5 and above years of age category, with eight (62%) male and five (38%) female subjects. Further information on the demographic characteristics of the study population is presented in Table 1.

After the first day of treatment, only 9.9% ($N = 7$) of subjects in the CQ and 10.2% ($N = 9$) of subjects in the AL treatment group had measured fever, and there were no cases with reported or measured fever on days 2 and 3 in both treatment groups. At the end of the follow-up period, 5 treatment failure cases in the CQ and 19 treatment failure cases in the AL treatment groups were detected. Kaplan–Meier survival analysis was done to calculate the cumulative incidence of treatment failure. The cumulative incidence of treatment failure for CQ was 7.5% (95% confidence interval [CI] = 2.9–18.9%) and for AL was 19% (95% CI = 11–31.6%), confirming significant difference ($P = 0.0145$).

Although all of the CQ treatment failure cases were detected on day 28 of the study, the treatment failure cases in the AL group were detected on or before day 21 (42%) and on day 28 (58%). Three of the CQ treatment failure cases had blood levels of CQ and desethylchloroquine of 256, 320, and 608 ng/mL, whereas the two treatment failures had blood levels of 32 and 64 ng/mL (Table 2). Prior arrangement to collect blood sample on day 7 to determine concentration of lumefantrine was not done, because significantly high treatment failures were not anticipated.

DISCUSSION

Findings from this study have confirmed a significantly higher cumulative incidence of treatment failure in *P. vivax* cases treated with AL than with CQ. The AL effectiveness study for the treatment of *P. vivax* was done for the first time in the Ethiopian setting, and there was no previous baseline data on which to compare. The CQ treatment failure detected in this study, however, is higher than the levels reported in earlier studies conducted in the same area, which reported treatment failure rates of 2% in 1996[10] and 2.3% in 2003.[11] None of the earlier studies reported if the treatment failure cases were because of drug resistance. In this study, CQ resistance has been confirmed in three of five CQ treatment failure cases, because the CQ and desethylchloroquine level at the day of treatment failure was higher than the minimum effective concentration (MEC) of 100 ng/mL.[9] The blood levels of the drug in the other two CQ treatment failure cases were below the MEC, and it is not known if this was related to drug malabsorption or other reasons. Although *P. vivax* resistance to CQ has been reported from several parts of the world, mainly Asia and South America,[12,13] a report on confirmed *P. vivax* resistance from Ethiopia is quite recent, and the findings from this study further confirm CQ resistance reported in two other recent studies.[14,15]

It is not known if the recurrent parasitemia cases detected in this study were caused by relapse, recrudescence, or reinfection. Based on the Indonesian experience, it has been suggested that failures after CQ therapy until day 16 are almost always caused by recrudescence, whereas failure between day 17 and 28 may be caused by recrudescence, reinfection, or relapse.[16] Distinguishing the origin of the recurrent parasitemia could be important to explain in the context of the treatment outcomes. Genetic analysis approaches have been used to compare the genotype of the parasites before and after treatment. However, it has been reported that more than 50% of the parasites causing relapse on day 28 do not match with the genotype at baseline,[17,18] making the comparison indeterminate.

The high level of cumulative incidence of treatment failure in the AL treatment group could possibly be attributed to underdosage cause by non-compliance to the evening doses, because the tablets were given to the patients or their guardians to take at home. As a follow-up to confirm compliance to the evening doses, patients were asked on each follow-up day to confirm that the correct doses were taken at the right time and that vomiting did not occur after drug intake. Although the issue of non-compliance is a real concern, a similar study conducted in Ethiopia in 2004 to assess the efficacy of AL for the treatment of uncomplicated falciparum malaria, where the evening doses of AL were not supervised, reported a 99% rate of treatment success[19] Another study conducted in Uganda

Table 1
Patients' characteristics

Parameters	Test drug and study site					
	Chloroquine (CQ)			Artemether-lumefantrine (AL)		
	Debrezeit	Nazareth	Total	Debrezeit	Nazareth	Total
Total enrolled	27	44	71	36	52	88
Gender						
Male	15 (55.6%)	19 (43.2%)	34 (47.9%)	18 (50.0%)	21 (40.4%)	39 (44.3%)
Female	12 (44.4%)	25 (56.8%)	37 (52.1%)	18 (50.0%)	31 (59.6%)	49 (55.7%)
Age group						
Under 5 years	0	5 (11.4%)	5 (7.0%)	2 (5.6%)	7 (13.5%)	9 (10.2%)
5 years and above	27 (100%)	39 (88.6%)	66 (93%)	34 (94.4%)	45 (86.5%)	79 (89.8%)
Median age in years (interquartile range)	21 (9.5–30)	17.5 (13–25)	19 (10–26)	17 (10–25)	17 (7.6–23.3)	17 (9.5–24)
Mean weight ± SD (range; kg)	42.8 ± 17.2	45.0 ± 16.9	44.1 ± 16.7	42.5 ± 17.1	41.7 ± 19.1	42.0 ± 18.7

TABLE 2
Patients enrolled and treatment outcomes

Parameters	Test drug and study site					
	Chloroquine (CQ)			Artemether-lumefantrine (AL)		
	Debrezeit	Nazareth	Total	Debrezeit	Nazareth	Total
Total patients enrolled	27	44	71	36	52	88
Follow-up completed	21 (77.8%)	36 (81.8%)	57 (80.3%)	30 (83.3%)	45 (86.5%)	75 (85.2%)
Loss and withdrawal	6 (22.2%)	8 (18.2%)	14 (19.7%)	6 (16.7%)	7 (13.5%)	13 (14.8%)
Treatment success	18 (85.7%)	34 (94.4%)	52 (91.2%)	23 (76.7%)	33 (73.3%)	56 (74.7%)
Treatment failure	3 (14.3%)	2 (5.6%)	5 (8.8%)	7 (23.3%)	12 (26.7%)	19 (25.3%)
Day of treatment failure						
Before day 21	0	0	0	0	1 (8.3%)	1 (5.3%)
Day 21	0	0	0	3 (42.8%)	4 (33.3%)	7 (36.8%)
Day 28	3 (100%)	2 (100%)	5 (100%)	4 (57.2%)	7 (58.3%)	11 (57.9%)
Blood level of CQ in treatment failure cases						
< 100 ng/mL (32–64 ng/mL)	1 (33.3%)	2 (100%)	3 (60%)	ND	ND	
> 100 ng/mL (256–320 ng/mL)	2 (66.6%)	0	2 (40%)	ND	ND	

ND = not done.

also reported comparable treatment success rates in both supervised and unsupervised treatment schedules.[20]

Contrary to the high level of efficacy of AL for the treatment of *P. falciparum,* its efficacy for the treatment of *P. vivax* does not seem as high. In a study conducted in Thailand[21] comparing the efficacy of CQ and AL combined with a 14-day course of primaquine, full treatment success was achieved in the CQ treatment group, whereas the treatment success with AL was slightly lower at 97.4%. Another study also reported greater post-treatment prophylaxis in averting *P. vivax* recurrences with dihydroartemisinin-piperaquine than with AL,[22] and dihydroartemisin-piperaquine has been indicated as highly efficacious as AL for the treatment of *P. vivax*.[23]

CONCLUSION

The coexistence of *P. falciparum* and *P. vivax* in Ethiopia and the different levels of effectiveness of the antimalarial drugs against the malaria parasite species demand administration of the right drug. In the Ethiopian setting, therefore, the current ongoing efforts to increase access to diagnostic services, including the use of appropriate rapid diagnostic tests (RDTs), are expected to have a significant contribution. Although another fully supervised study is required to assess the effectiveness of AL for the treatment of *P. vivax* in the Ethiopian setting, the coadministration of primaquine with CQ to avert subsequent attacks caused by relapse or recrudescence and its potential in delaying development of *P. vivax* resistance to CQ should be noted.[24]

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Authors' addresses: Ambachew M. Yohannes, World Health Organization, Geneva, Switzerland, E-mail: yohannesam@who.int. Awash Teklehaimanot, Mailman School of Public Health and The Earth Institute, Columbia University, New York, NY, E-mail: thawash@ei.columbia.edu. Yngve Bergqvist, Dalarna University College, Borlänge, Sweden, E-mail: ybq@du.se. Pascal Ringwald, Global Malaria Programme, World Health Organization, Geneva, Switzerland, E-mail: ringwaldp@who.int.

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