Therapeutic efficacy of chloroquine for uncomplicated *Plasmodium vivax* malaria in southeastern and western border areas of Myanmar

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Abstract

Background—In the Greater Mekong Subregion of Southeast Asia, *Plasmodium vivax* malaria is endemic and causes significant morbidity. In this study, the efficacy of chloroquine for treating uncomplicated *P. vivax* malaria at the eastern and western borders of Myanmar was investigated.

Methods—A total of 197 participants with microscopically confirmed *P. vivax* infection were enrolled from three townships of the southeastern (Thanbyuzayat and Kawthoung) and western (Kyauktaw) borders of Myanmar. Patients were treated with chloroquine according to the national malaria treatment guidelines and followed for 28 days.

Results—Among the 197 enrollments, 172 completed the 28-day follow-up. Twelve recurrent *P. vivax* infections, all occurring in the third and fourth week, were detected, resulting in an overall cumulative rate of recurrence of 4.7% [95% confidence interval (CI): 1.5% – 7.8%]. The incidence rate of recurrence varied among the three sites. In Thanbyuzayat township, no patients

had recurrent parasitemia between days 7 and 28. In contrast, Kyauktaw township had a day 28 cumulative incidence rate of recurrence of 7.2% (95% CI 0.6%–13 .9%) compared to 6.9% (95% CI: 0.6%–13.2%) in Kawthoung township.

Conclusion—While this study confirmed the relatively high clinical efficacy of chloroquine for treating *P. vivax* in Myanmar with modest rates of recurrent infections within 28 days of the treatment, it also revealed considerable geographical heterogeneity of chloroquine efficacy, which warrants continuous surveillance efforts.

Keywords

Chloroquine; *Plasmodium vivax* ; clinical efficacy; recurrent parasitemia; Myanmar

Introduction

Among the five *Plasmodium* species infecting humans, *Plasmodium vivax* is the most geographically widespread species outside Africa [1]. Although it is generally considered less virulent than *Plasmodium falciparum*, *P. vivax* can cause multiple relapsing episodes of the disease, increasing its morbidity substantially [2]. In recent years, especially under malaria elimination settings, the resilience of *P. vivax* to conventional control measures has resulted in increased proportions of this parasite in many endemic areas [3], stressing the importance of effective management of vivax malaria. In the Greater Mekong Subregion (GMS), the ongoing malaria elimination campaign, aiming to eliminate *P. falciparum* by 2025 and all malaria by 2030, has also led to a dramatic change in the landscape of malaria in the region [4]. In the last decade, the dominant parasite has shifted to *P. vivax* in all the GMS countries [5]. Spatially, malaria is concentrated along the international borders [6–8]. With a disproportional distribution of malaria across these borders, cross-border spread and introduction of the parasites by migrating human or mosquito populations have become a paramount concern. Especially, the aptitude of *P. vivax* to form silent hypnozoites in the host liver enables the persistence of infections and facilitates the concealed migration of the parasites, presenting a major challenge to malaria elimination.

Chemotherapy plays a key role in the effective management of malaria. Chloroquine (CQ) combined with primaquine (PQ) has been used as the frontline drug for the radical cure of *P. vivax* malaria for several decades. CQ-resistant *P. vivax* parasites have been detected globally.[9] In Indonesia, the high prevalence of CQ-resistant parasites led to the replacement of CQ with an artemisinin combination therapy (ACT) as the first-line treatment for *P. vivax* [10]. The first CQ-resistant *P. vivax* cases in the GMS were reported in Myanmar almost thirty years ago [11], followed by additional reports [12, 13]. In recent years, although the efficacy of CQ/PQ for uncomplicated *P. vivax* remained high in Myanmar and its border areas [14, 15], there have been increasing numbers of studies showing clinical failures in various regions of Myanmar [16–19], indicative of RI or even RII type resistance according to the WHO defined criteria [20]. Despite the increasing trend of CQ resistance in *P. vivax*, the molecular mechanisms of resistance are poorly understood [21], making molecular surveillance of resistance difficult. Thus, management of drug resistance in *P. vivax* relies heavily on monitoring the clinical efficacy of the drugs. Yet, clinical efficacy studies also have limitations, including variable CQ pharmacokinetics

in different patients and the inability to distinguish recrudescence due to treatment failure, relapse due to activation of hypnozoites, and reinfection [22].

Efficacy studies conducted in different vivax-endemic areas of Myanmar showed that the clinical failure rates of CQ in treating *P. vivax* had been relatively low (below 10%), but there was substantial spatial heterogeneity with some areas (e.g., southern Myanmar) showing increased failure rates. Thus, the objective of this study was to continuously monitor the efficacy of CQ for treating uncomplicated cases of vivax malaria at sentinel sites located at the eastern and western borders of Myanmar. We showed that although CQ generally remained effective, the day-28 cumulative incidence rate of recurrence at two study sites reached ~7%.

Materials and methods

Ethical Consideration

The research protocol of this study was approved by the Institutional Review Board at the Department of Medical Research under the auspice of the Ministry of Health (10-a/Ethnics 2010). Written informed consent/assent was obtained from all participants and guardians in case of minors after a full explanation of the research activities.

Sample size consideration

Sample size, based on a conserved 28-day treatment failure rate of 4%, a precision of 5% and a 95% confidence level (CI), was estimated to be 59. If assuming a 5% lost-to-follow-up rate, 62 patients were planned to enroll at each site for the study.

Study sites and populations

This study was conducted near the western Myanmar-Bangladesh border (Kyauktaw Township, Rakhine State) and eastern Myanmar-Thailand border (Thanbyuzayat Township, Mon State and Kawthoung Township, Tanintharyi Region) in 2012 (Fig. 1). While they were among the townships with the highest malaria burden in Myanmar, the two eastern townships also had disproportionally high vivax malaria incidence. The study was performed at the local township hospitals, where febrile patients with axillary temperature   37.5 °C or history of fever within the last 24 h were diagnosed for malaria by microscopic examination of Giemsa-stained thick and thin blood smears. Inclusion criteria included patients with *P. vivax* mono-infection with a parasite density of   250 asexual parasites per microliter (method of calculation detailed below), aged   five years old, able to swallow oral medication, and able to fulfill the regular follow-up visits. Exclusion criteria included patients with underlying chronic diseases, patients with symptoms of severe malaria, those younger than five years, and pregnant or lactating women. For women of reproductive ages, pregnancy was diagnosed with a rapid human chorionic gonadotropin urine test. In addition, patients with a recent malaria history and receiving malaria treatment in the previous month were also excluded.

Study procedures

A general physical examination was performed at the enrollment, and a questionnaire was used to record the demographic and clinical information of the patients (e.g., age, sex, axillary temperature). Participants who met inclusion criteria and gave written consent/assent were given 3-day directly observed treatment with CQ at 25 mg base/kg, divided into 10, 10, and 5 mg base/kg on days 0, 1, and 2, respectively, according to the Myanmar National Malaria Control Program (NMCP) guidelines. CQ phosphate tables (150 mg chloroquine base/tablet, Chlorofos®), manufactured by Burma Pharmaceutical Industry, was administered to patients in three age groups as follow: 5–9 years (2 tablets each on days 0 and 1, and 1 tablet on day 2), 10–14 years (3 tablets each on days 0 and 1, and 1.5 tablets on day 2), and 15 years (4 tablets each on days 0 and 1, and 2 tablets on day 2). After drug administration, each patient was monitored for at least half an hour for vomiting, allergy to treatment, or any adverse reactions. If no adverse reaction occurred, patients were requested to return for the follow-up visits on days 1, 2, 3, 7, 14, 21, and 28, and at any time during the follow-up period when they felt ill. When a patient came for the follow-up or unscheduled visits, finger-prick blood was taken to prepare thick and thin blood smears for microscopic examination and blood spots on filter paper for molecular identification of *Plasmodium* species. In addition, a general physical examination was given, and adverse events were recorded on each visit. Because of PQ's schizonticidal activity, PQ treatment was postponed after completion of day 28 follow-up according to World Health Organization (WHO) 28-day protocol for determining CQ clinical efficacy [23]. The primary outcome of the study was the risk of *P. vivax* recurrence between day 7 and day 28 as a measure of adequate clinical and parasitological response and late parasitological failure. The secondary outcomes were the duration of parasite and fever clearance to identify cases of early treatment failure.

Microscopy examinations and species identification by PCR

Microscopic identification of *Plasmodium* species and parasite counting were done by two qualified microscopists independently at the field sites and confirmed by an expert whenever necessary. A blood smear with no asexual parasite detected after counting 1,000 white blood cells (WBCs) was considered negative. Parasite density, the number of asexual parasites per μl of blood, was determined as follows, assuming 6,000 WBCs/μl of blood: Parasite density = $\frac{\text{Number of asexual parasites counted}}{200 \text{ WBCs}} \times 6{,}000 \text{ WBCs/μl}$. The parasite densities measured by two microscopists were averaged. If they differed by >50%, a third microscopist was required, and the parasite density was deliberated by averaging the two most concordant parasite counts. For day 0 and recurrent parasitemia during the follow-up period, *Plasmodium* parasite species were confirmed by nested PCR of the 18S rRNA gene [24].

Data analysis

Statistical Package for the Social Sciences (SPSS, IBM Corp. 2015 for Macintosh, Version 23.0. Armonk, NY) was used for statistical analysis. The Mann-Whitney U test and Fisher's exact test were used to compare asexual parasite densities and patients with adequate clinical and parasitological response (ACPR) and patients with recurrent parasitemia. The risk of

recrudescence during the follow-up was estimated by the Log-rank Mantel-Cox test. The cumulative incidence of treatment failures and 95% CI were calculated as described earlier after correction for the dropouts [10].

Results

Baseline characteristics of the patients

A total of 62, 67, and 68 patients with *P. vivax* mono-infection diagnosed by microscopy were enrolled at the township hospitals of Kyauktaw, Thanbyuzayat, and Kawthoung, respectively (Fig. 2). All were *P. vivax* mono-infections except for a single *P.vivax/P. falciparum* mixed infection that was later confirmed by PCR (Fig. 2). The study population had 65.8% male patients, mainly due to the recruitment of male-biased mobile and migrant worker populations from a rubber plantation area of Thanbyuzayat Township (Table 1). There were also differences in the age of patients among the three study sites. According to the treatment regimen, the participants were divided into three age groups: 5–9, 10–14, and 15 years (Table 1). The 15 years age group accounted for 57.1% of the study population. This age group also had significantly higher asexual parasite density than the younger age groups ($P < 0.010$, Mann Whitney U Test) (Table 1). This difference was even more prominent at the Thanbyuzayat site, where the 15 years group had a mean parasite density of 11,861 asexual parasites/μl blood. At enrollment, 42.3% of the patients had detectable gametocytes by microscopy (Table 1). During the entire study, 12 participants were lost to follow-up, 8 of which occurred in the Thanbyuzayat Township, where many patients were migrant workers.

Fever and parasite clearance

There were differences in the treatment response and clinical efficacy of CQ among the three study sites. Parasite clearance was rapid at the Kyauktaw and Kawthoung townships, with 6% of patients remaining parasitemic on day 2 and all being aparasitemic on day 3. However, parasite clearance was slow in Thanbyuzayat, where adult patients had significantly higher parasitemia at enrollment, with ~51% and 15% remaining parasite positive on day 2 and day 3, respectively (Fig. 3A). At all sites, no patients had parasitemia on day 7. In addition, the dynamics of fever clearance were also different among the sites (Fig. 3B). In Thanbyuzayat Township, the proportion of febrile patients was sharply reduced from 94% to 9% within 24 h of CQ treatment. In Kawthoung, although only 46.3% of patients had fever at enrollment, 35.8% remained febrile 24 h after the treatment. Nevertheless, all patients cleared fever within 48 h of CQ treatment. No serious adverse events occurred.

Parasite recurrence within 28 days

Among the 197 total enrollments, 12 patients had recurrent parasitemia, all occurring after day 14 (4 on day 21 and 8 on day 28), which resulted in an overall cumulative incidence rate of recurrence of 4.7% on day 28 (95% CI: 1.5% – 7.8%) (Fig. 4A). According to the WHO definition, these recurrent infections between follow-up days 7 and 28 were regarded as late parasitological failures (LPF). Interestingly, despite the slow parasite clearance rate in Thanbyuzayat, no patients had recurrent parasitemia between day 7 and day 28. In

comparison, the day 28 cumulative incidence rates of parasite recurrence were 7.2% (95% CI 0.6%–13 .9%) in Kyauktaw and 6.9% (95% CI: 0.6%–13.2%) in Kawthoung. Next, the potential influences of demographic factors on CQ efficacy were compared between the LPF and ACPR cases (Table 2). Though the mean age of the LPF cases (15.9 years) was younger than that of the ACPR cases (20.5 years), the differences were not statistically significant ($P < 0.05$). Given the difference in the CQ regimens among the three age groups, a more detailed analysis of the cumulative hazard of recurrent parasitemia was performed. This analysis revealed that the 10–14 years age group had no recurrent parasitemia, whereas the younger and older groups had a similar cumulative hazard (Fig. 4B). However, the difference was not statistically different ($P = 0.229$, Log-rank Mantel-Cox test). Although the slower parasite clearance curve of the patients from Thanbyuzayat may imply the impact of parasitemia, a comparison of the initial parasitemia between LPF and ACPR patients did not reveal a significant difference (Table 2).

Discussion

The lack of molecular markers for detecting CQ-resistant *P. vivax* demands continuous monitoring of the clinical efficacy of CQ at sentinel sites [9]. This study compared the clinical efficacy of CQ for *P. vivax* in three sites located in the western and eastern borders of Myanmar. The overall efficacy of CQ for treating uncomplicated vivax malaria remained relatively high, with a cumulative incidence rate of recurrence within 28 days below 5%. However, for the intent-to-treat patient populations, CQ treatment showed 100% efficacy in Thanbyuzayat, 93.5% in Kyauktaw, and 88.1% in Kawthoung. Such a remarkable heterogeneity also reverberates with other CQ efficacy studies conducted across vivax-endemic areas of Myanmar [16, 17, 19]. In southern Myanmar (Tanintharyi Region), substantial evidence shows the deterioration of CQ efficacy and the potential emergence of CQ-resistant *P. vivax* parasites. While an earlier study in 2002–2003 in Dawei showed a worrisome 66% CQ efficacy [12], several subsequent surveys in the southern tip township Kawthoung in 2009–2012 consistently revealed lower than 90% CQ efficacy [17, 18]. These results suggest that the existence of hotspot areas in Myanmar where the deterioration of CQ efficacy was more substantial.

While the variations in CQ efficacy may reflect intrinsic genetic differences among the parasite isolates, demographic, immunological, and epidemiological settings may also contribute to these variations. Patients in Thanbyuzayat with a much higher proportion of adults had a significantly higher parasitemia than in other sites, which may be responsible for the observed delayed parasite clearance, with 10 out of 67 patients (14.9%) remaining parasitemic on day 3. The adult population in Thanbyuzayat was made up mostly of migratory workers at the rubber plantations. Delayed healthcare-seeking behavior, often associated with the mobile population [25], may account for the higher parasitemia in adult patients. In addition, since the mobile worker populations carried a significantly higher risk of repeated malaria infection [26, 27], which increases their immunity against malaria, it is also possible that malaria symptoms in these patients occurred at much higher parasitemia. Nevertheless, CQ treatment demonstrated 100% efficacy in the population from Thanbyuzayat, suggesting that delayed parasite clearance on day 3 may not be a useful surrogate marker of recurrence after CQ treatment [9, 28]. In contrast, the patient cohorts

from Kyauktaw and Kawthoung townships presented with recurrent *P. vivax* infections within 28 days of CQ treatment, even though no patients had persistent parasitemia on day 3. Regardless of whether the recurrent parasitemia may represent relapse, recrudescence, or reinfection, they may be CQ-resistant since the 28-day *in vivo* efficacy test presumes that an effective blood level of CQ persists for at least 28 days [22]. Alternatively, the recurrent parasitemia may be due to CQ blood levels falling below the minimum inhibitory concentration, since all recurrences occurred in weeks 3 and 4 after CQ treatment. In line with this assumption, a recent study conducted in Cambodia detected 60% of the recurrences within 28 days occurred when CQ blood concentrations fell below the therapeutic level [29]. Thus, the CQ level in the blood on the day of recurrence should be measured in the future to identify true CQ resistance.

Previous reports from other areas revealed that children had higher chances to have recurrent parasitemia of *P. vivax* malaria [30–33]. The underlying mechanism is ill-defined but might be due to the incomplete development of their immune system, limiting the ability to eliminate the residual parasites that lead to recurrence. CQ underdosing is especially prevalent in children and may also be associated with the increased incidence of recurrent parasitemia in children [34, 35]. In this study, patients with LPF were much younger than those with ACPR, although the difference was not statistically significant. More robust studies to consider bodyweight and blood CQ levels are needed to determine the factors associated with recurrent *P. vivax* infections.

To evaluate the clinical response of *P. vivax* to CQ treatment, PQ treatment was postponed after completion of day 28 follow-up, thus eliminating the complication from the additional schizonticide activity of PQ [36, 37]. There is clear evidence showing that PQ noticeably enhances the therapeutic efficacy of CQ in the treatment of *P. vivax* infections [9, 14, 38]. Thus, it is expected that CQ/PQ combination would result in lower recurrence rates. Considering this, two recent studies conducted at the northeastern border of Myanmar detected early recurrent *P. vivax* infections after CQ/PQ treatment [16, 19]. Together with the much earlier reports of sporadic CQ resistance in Myanmar [11–13], these studies suggested the decline of the CQ/PQ efficacy and the emergence of CQ-resistant strains in Myanmar, which warrants follow-up studies to ensure the efficacy of the frontline treatment of vivax malaria.

Conclusion

This study confirmed the relatively high clinical efficacy of CQ for the treatment of vivax malaria in the eastern and western borders of Myanmar and, at the same time, revealed geographical heterogeneity in CQ efficacy. The detection of recurrent *P. vivax* infections after CQ or CQ/PQ treatment in the eastern (Kawthoung and Laiza) and western (Kyauktaw) borders, together with other longitudinal monitoring efforts of CQ efficacy in Myanmar and neighboring regions, indicates the emergence of CQ-resistant *P. vivax*.

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Fig. 1.
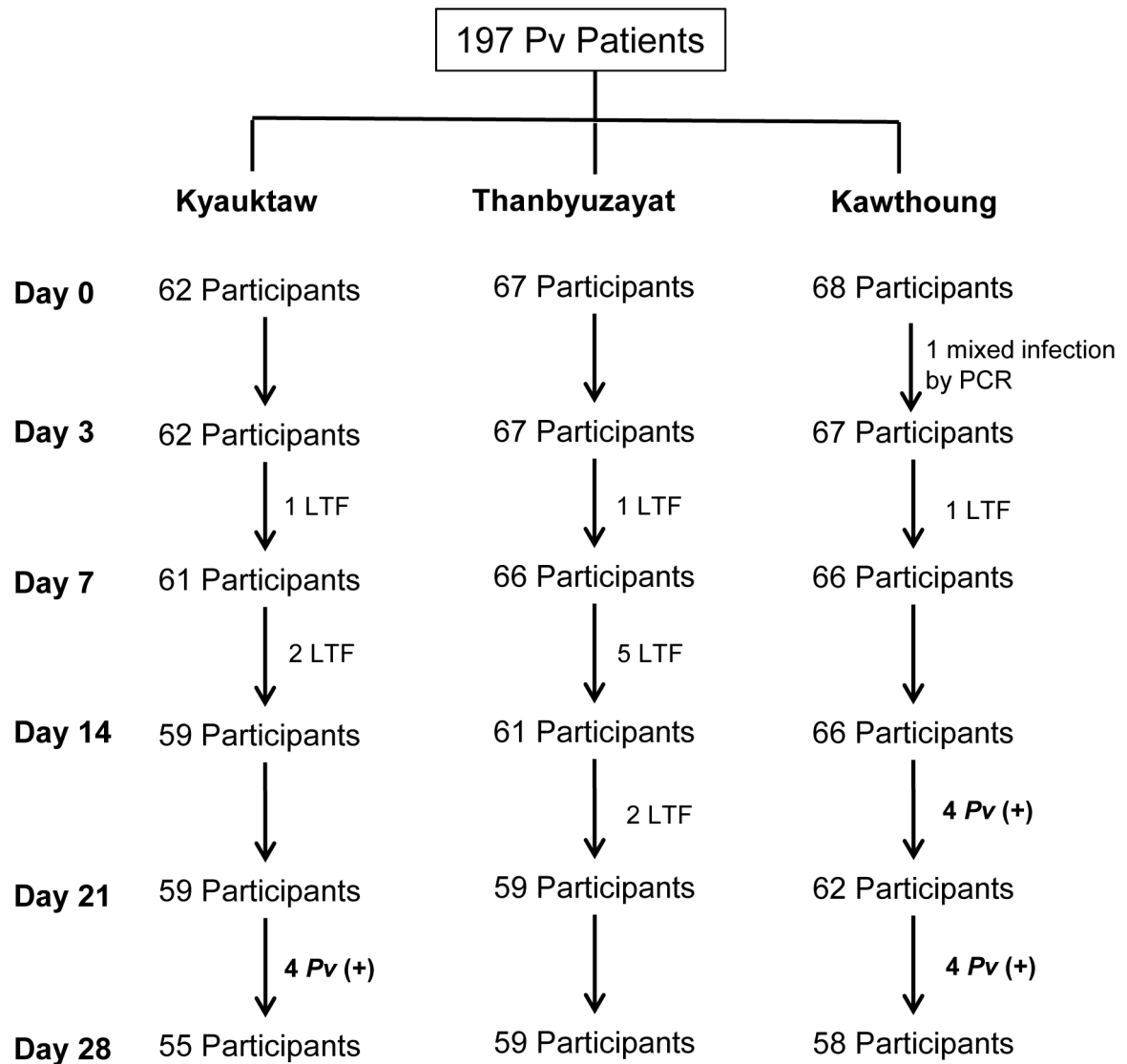Locations of the three study sites in Myanmar.

Fig. 2.
Flow chart of the study on the therapeutic efficacy of chloroquine for uncomplicated *P. vivax* malaria in three study sites of Myanmar. LTF, lost to follow-up; *Pv* (+), *P. vivax* recurrent parasitemia.
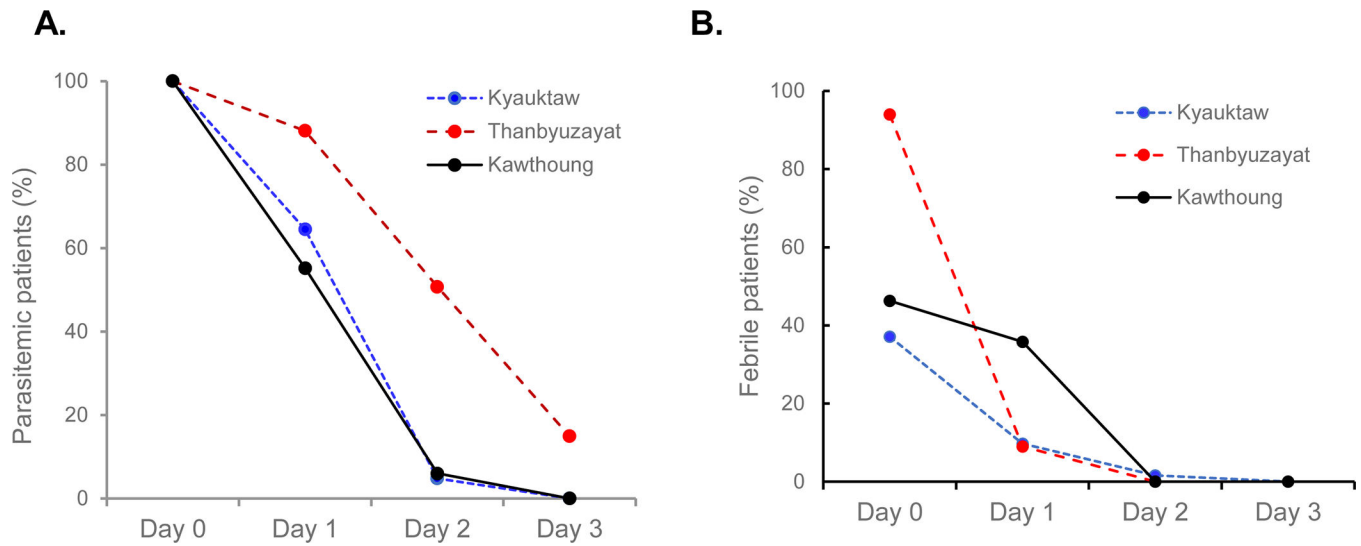
A.

B.

Fig. 3.
Percentages of patients with parasitemia (**A**) or fever (**B**) within the first three days of CQ treatment at the three study sites.
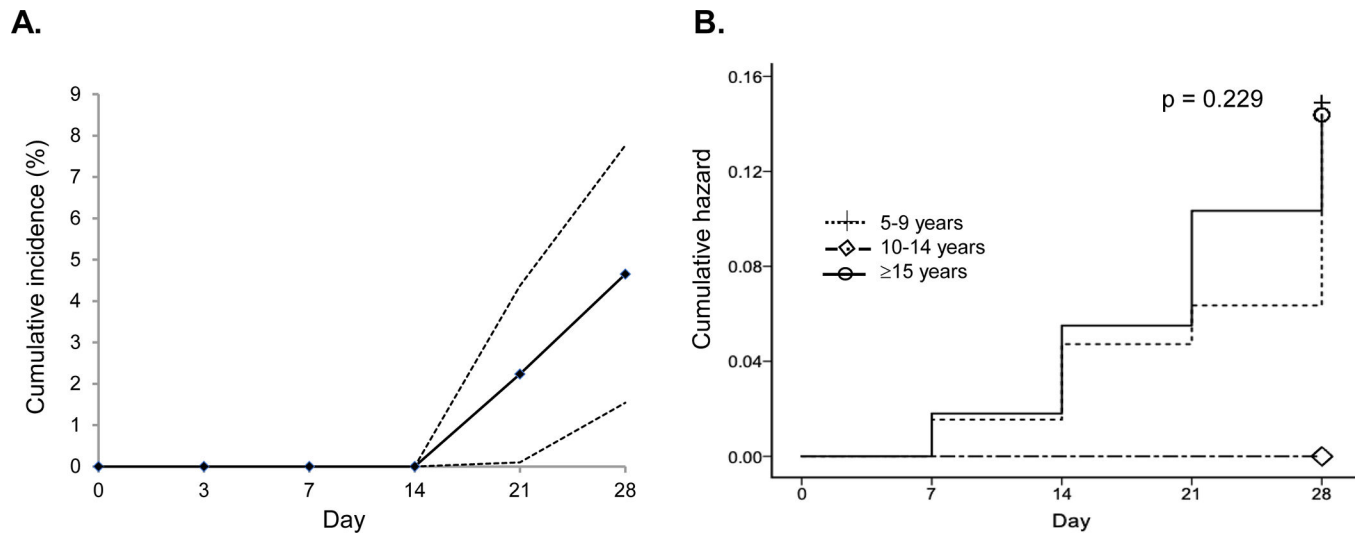
A.

B.

Fig. 4.
A. The overall cumulative incidence rate of recurrent *P. vivax* parasitemia during the 28-day follow-up after CQ monotherapy. **B.** The cumulative hazards of recurrent parasitemia among the three age groups.

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Table 1.

Demographic and clinical features of the *P. vivax* patients at enrollment

Characteristics	Kyauktaw	Thanbyuzayat	Kawthoung	Total[*]
Patient number (% of male)	62 (48.5%)	67 (85.0%)	67 (57.4%)	196 (65.8%)
Age in years [mean (range)]	9.3 (6–43)	28.6 (6.5–60)	21.9 (5–55)	20 (5–60)
Febrile patients (> 37.5 °C) [n (%)]	23 (37.1%)	63 (94.0%)	31 (46.3%)	117 (59.2%)
Axillary temperature, °C [mean (range)]	37.5 (36.5–40.6)	38.7 (36.8–40.0)	37.7 (36.1–41.2)	38.0 (36.1–41.2)
Asexual parasite density (/µl) [geometric mean (range)]				
5–9 years (n = 65)	5,341 (323–21,101)	5,733 (4,015–8,318)	2,535 (394–8,209)	4,810 (323–21,101)[a]
10–14 years (n = 20)	5,660 (515–18,712)	7,157 (488–17,619)	1,440 (500–4,860)	4,271 (488–18,712)[a]
15 years (n = 112)	4,141 (537–14,908)	11,861 (262–62,221)	6,955 (263–29,739)	9,320 (262–62,221)[b]
Patients with gametocytemia [n (%)]	37 (59.7%)	24 (35.8%)	22 (32.8%)	83 (42.3%)

[*] For parasite density, age groups marked with the different letters (a,b) were significantly different (*P* < 0.01, Mann-Whitney U test).

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Table 2.

Comparison between patients with recurrent parasitemia and the ACPR patients

Characteristics	Recurrent (n=12)	ACPR (n=173)	P value
Age (years): mean, median (range)	15.9, 12.5 (6–36)	20.5, 18 (5–60)	0.188[#]
Sex (% male)	7 (58.3%)	113 (65.3%)	0.419[*]
Asexual parasite density (/μl) [geometric mean (range)]	5,607 (263–27,029)	7,243 (261–62,221)	0.734[#]

[#]Mann-Whitney U test

[*]Fisher's exact test.