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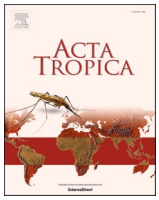

Efficacy and gametocyte carriage in *Plasmodium falciparum* cases treated with artemether-lumefantrine alone versus with single-dose primaquine in Ethiopia: A randomized controlled study

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ABSTRACT

This study evaluated the efficacy of adding primaquine (PQ) to artemether-lumefantrine (AL) for treating uncomplicated *Plasmodium falciparum* malaria in Ethiopia. Asexual parasite clearance was monitored with microscopy and molecular techniques, while gametocyte clearance was observed solely through microscopy. Genotyping to distinguish recrudescence from reinfection was performed using nested polymerase chain reaction (PCR). A parallel, two-arm, randomized controlled trial with a 1:1 allocation ratio was conducted involving 146 symptomatic patients, with 106 included in the final analysis. The study compared the outcomes of AL plus PQ with those of AL alone over 28 days. PQ treatment was initiated simultaneously with AL therapy. Both treatment groups showed no asexual parasites by day 2 when assessed by microscopy. However, PCR testing detected parasite DNA in 5.8 % (3/52) of the patients in the AL plus PQ group, compared to 18.5 % (10/54) in the AL alone group by day 7 ($P = 0.09$). PCR-uncorrected cure rate was 100 % for the AL alone and 98.1 % for the AL plus PQ groups ($P = 0.31$). Gametocyte clearance was faster in the AL plus PQ group, with 100 % clearance by day 7, compared to day 14 in the AL alone group. Notably, only 9.4 % (10/106) of cases had gametocytes at baseline, while this percentage increased to 11.3 % (12/106) by day 1 of post-treatment. The recrudescence rate was 2.8 % (3/106). Although AL alone was as effective as AL plus PQ in eliminating asexual parasites, adding PQ may accelerate gametocyte clearance. However, the low prevalence of gametocyte carriers among symptomatic cases at the study site, combined with the restriction of PQ administration to only those seeking treatment, may limit its overall impact on malaria transmission.

Trial registration number: PACTR202502642820967

1. Introduction

Artemisinin-based combination therapies (ACTs) effectively treat uncomplicated *Plasmodium falciparum* malaria (WHO, 2023) but have a limited impact on mature gametocytes, which are responsible for transmission to mosquitoes (Dondorp, 2013; Ngotho et al., 2019). Primaquine (PQ) targets the mature gametocyte (stage V), while AL mainly reduces the asexual stage and gametocyte production (Dondorp, 2013). The World Health Organization (WHO) recommends using PQ to limit malaria transmission in areas with low to moderate transmission (WHO, 2015), however, its efficacy may be reduced in high-transmission settings where few symptomatic individuals carry gametocytes (WHO, 2022). In Ethiopia, a single dose of PQ is prescribed along with AL to all *P. falciparum* patients, except for pregnant women, women in the first six months of breastfeeding, and children under six months of age, to curb transmission despite varying transmission levels (FMOH, 2018).

Several studies have been conducted in institutional settings to evaluate the efficacy of AL treatment for symptomatic cases of *P. falciparum*. These studies have found that most active cases did not show microscopic gametocytemia, and the prevalence was less than 10

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% in most cases (Abamecha et al., 2020; Daka et al., 2024; Eshetu et al., 2012; Gubae et al., 2023). While the benefits of reducing malaria transmission with PQ are substantial, these advantages can only be realized if most individuals receiving PQ carry gametocytes and there is no significant pool of asymptomatic parasite carriers (WHO, 2015). Additionally, the current increase in malaria cases raises important questions about treatment efficacy and overall malaria control strategies (WHO, 2024).

The emergence of partial artemisinin-resistant malaria parasites in several countries, including Ethiopia (Balikagala et al., 2021; Fola et al., 2023; Moser et al., 2022; Uwimana et al., 2020), is also a significant concern. Regular monitoring of the efficacy of ACT is essential for making timely decisions regarding treatment. This study aimed to investigate whether adding a single dose of PQ to AL improves the clearance of asexual *P. falciparum* parasites in symptomatic patients compared to AL alone. Additionally, we aimed to evaluate whether adding PQ enhances the clearance of gametocytes in symptomatic individuals who carry the gametocytes when combined with AL.

2. Materials and methods

2.1. Patient and public involvement

While this study did not involve patient participation during the trial design, execution, or reporting phases, it was carried out in close collaboration with the Regional Ministry of Health and the participating health center to ensure alignment with public health priorities and clinical practice.

2.2. Study design and endpoint assessment

The study was a two-arm, parallel-group, open-label, randomized controlled trial with a 1:1 allocation ratio. It included patients with symptoms and microscopically confirmed *P. falciparum* infection, aiming to evaluate the clearance of both asexual and sexual parasites over a 28-day follow-up period (WHO, 2009).

Based on the assessment results, patients were classified as either experiencing therapeutic failure or achieving an adequate response. The presence of gametocytes in symptomatic *P. falciparum* cases was evaluated to gain insight into the transmission-blocking benefits of adding a single dose of PQ to AL.

2.3. Trial setting

The trial took place from March to August 2023. Patients were recruited at the Woze Health Center in Arba Minch, located 505 km from Addis Ababa, the capital of Ethiopia. The altitude ranges from 1200 to 1300 m above sea level, with an average annual temperature of 29.7 °C and a total annual rainfall of 900 mm. The climate is characterized as hot and humid. Nearby water bodies, such as Lake Abaya and the Kulfo River, contribute to an environment conducive to malaria-carrying mosquitoes. The Kulfo River flows through the town and meets Lake Chamo. The Woze Health Center is situated close to the Kulfo River, which is an ideal site for mosquito breeding. *Plasmodium falciparum* is the dominant malaria parasite species (Getawen et al., 2018), and *An. arabiensis* is the principal malaria vector (Akirso et al., 2024; Getawen et al., 2018).

2.4. Eligibility criteria

The Health Center was purposely selected as many patients with malaria are treated there. The study was conducted following the WHO protocol for therapeutic efficacy studies, specifically the inclusion and exclusion criteria outlined in 2009 (WHO, 2009). Participants were eligible if they met the following criteria: (1) aged 15 years or older; (2) exhibited fever (body temperature of 37.5 °C or higher) or had a history of fever in the last 24 h; (3) had microscopically confirmed *P. falciparum* mono-infection; (4) had a parasitemia range from 1000 to 100,000 asexual forms per microliter; (5) were willing to comply with the study protocol; (6) could swallow oral treatment; (7) were prepared for follow-up visits and had provided written informed consent or assent prior to enrolment.

Participants were excluded from the study if they had: (1) severe malaria or danger signs; (2) a known allergy to AL or PQ; (3) been taking antimalarial drugs or completed such treatments within the past two weeks; (4) self-reported as pregnant or breastfeeding; (5) febrile conditions resulting from diseases other than malaria; (6) known serious or chronic medical conditions; and (7) were taking regular medications that might interfere with AL and PQ. Participation was based on the willingness to abide by the protocol.

2.5. Intervention and comparator

The intervention group received a standard three-day course of AL (manufactured by IPCA Laboratories Ltd, Batch number: HWE 112,232, and expiration date: 11/2025), plus a single low-dose PQ (0.25 mg/kg) (manufactured by Remedica Ltd., Cyprus, with lot number 92,728 and expiration date 02/2025). PQ treatment was initiated simultaneously with AL therapy. This is a treatment that follows the Ethiopian National Malaria Treatment guideline (FMOH, 2018). The comparator group received a standard three-day course of AL.The drugs were given under direct supervision at the start and during subsequent visits.

2.6. Outcomes

The primary outcome was the clearance of asexual parasitemia in the two treatment groups, as determined by microscopy and PCR. The secondary outcome was comparing gametocytemia between the two treatment groups during the follow-up period.

2.7. Classification of treatment outcomes

Antimalarial treatment outcomes were evaluated by assessing the parasitological and clinical results, following the guidelines (WHO, 2009). Consequently, patients were categorized into four groups: early treatment failure, late clinical failure, late parasitological failure, or an adequate clinical and parasitological response.

2.8. Harms

All patients were closely monitored, and no adverse effects were observed. However, since participants were not screened for glucose-6-phosphate dehydrogenase deficiency, the administration of PQ could pose a risk of drug-induced hemolysis in susceptible individuals.

2.9. Sample size determination

Based on a randomized controlled trial with parallel and equal allocation ratio, the sample size was determined using the following assumptions: a 95 % asexual parasite clearance rate at day 2 in both groups, with 80 % power, 95 % two-sided confidence interval and a 10 % non-inferiority margin (Sealed Envelope Ltd. 2012). The required sample size was 150 participants (75 per group). However, 146 study participants were included.

2.10. Randomization

After enrollment, patients were randomly assigned to receive either a single dose of PQ in combination with AL or AL alone. The drugs were labelled with even and odd numbers using a lottery method. Patients assigned odd numbers were placed in the AL treatment group, while those assigned even numbers received the single-dose PQ plus AL

treatment. Consistent procedures were followed in sequential order throughout the study period.

2.11. Study implementation

Treatment and follow-up: Participants received the standard three-day course of AL according to the Ethiopian National Malaria Treatment Guideline for treating *P. falciparum* (FMOH, 2018). Participants were all >15 years old, with a mean weight of 59.4 ± 9.7 years, and were classified into the four-tablet per dose weight band (24 tablets total). In the AL plus PQ group, a single low dose of PQ (0.25 mg/kg) was administered concurrently. No fatty foods or other foods were provided for the patients. A study nurse oversaw the administration of the first and subsequent visiting-time treatment doses, and the patients were monitored for 30 min. Vomiting once resulted in re-administration; vomiting more than once led to exclusion as a guideline. The evening doses were not supervised; however, patients were instructed to bring their drug capsules with them during their visits to the Health Center on days 1, 2, and 3.

Patients were asked to return for scheduled follow-up appointments on days 1, 2, 3, 7, 14, 21, and 28. They were also instructed to come in on additional days if the disease returned. An identification card was prepared and given to participants or their parents/guardians. Clinical and parasitological assessments were conducted at each visit. Axillary temperature was recorded on day zero and during follow-ups using a thermometer with 0.1 °C precision. Body weight was measured with a calibrated Salter scale on day zero to determine the appropriate tablet dosage.

Loss to follow-up occurs when a patient enrolled in a study misses scheduled visits and cannot be reached despite multiple attempts to contact them, making it impossible to ascertain their treatment outcomes. Patients were informed that they could withdraw from the study at any time without any negative impact on their future follow-up or access to treatment at the study site. Furthermore, patients who received a different antimalarial medication during the follow-up period or were diagnosed with a different species of malaria were discontinued from the study.

2.12. Blood sample collection for microscopy parasite detection

Patients suspected of having malaria were screened using microscopy. Two experienced laboratory technicians independently performed microscopic examinations (WHO, 2009). The results from the first reader were not disclosed to the second reader to ensure unbiased assessments. Only the cases confirmed as positive by both readers were included in our study.

Thick and thin blood films were prepared and examined under a magnification of X100 to identify the parasite species and density at the baseline and follow-up days (1, 2, 3, 7, 14, 21 and 28). Duplicate thick and thin blood smears were prepared from the finger prick at the first visit and subsequent follow-up days. A fresh Giemsa stain solution was prepared. The thin smear was fixed with 100 % methanol. The blood smears were stained for 10 min with 10 % Giemsa, air-dried, and then examined for parasite species, stage, and density (WHO, 2009).

Thin smears were used to identify parasite species, while thick smears quantified asexual parasites and gametocytes based on specific white blood cell counts (WHO, 2009). The two laboratory technicians independently assessed the sexual and asexual parasite density. Asexual parasite density (per μl) was calculated based on 200 white blood cells (WBCs) counted, whereas gametocyte density (per μl) was calculated using a count of 1000 WBCs.

2.13. Blood sample collection for molecular analysis

Four drops (20 μl each) of capillary blood samples were collected from each participant on day 0 and follow-up days. The samples were placed on Whatman™ 3 MM filter paper, labelled, and air-dried at room temperature. After drying, they were stored in zip-locked plastic bags with silica gel (Gee Jay Chemicals Ltd) for molecular analysis. The samples were transported daily to the Arba Minch University Advanced Medical Entomology and Vector Control Laboratory, where they were kept at −40 °C.

2.14. Parasite DNA extraction and confirmation

The confirmation of *P. falciparum* and the clearance of asexual parasites were performed using genomic DNA extraction from dried blood spots (DBS) with the 6 % Chelex method. The DBS samples were punched into 6 mm circles using a single-hole punch before processing (Baidjoe et al., 2013). For the genotyping of recurrent cases, DNA was extracted from the samples both the enrolment and recurrence day DBS using the Qiagen DNeasy® Blood and Tissue Kit (QIAGEN, Germany), following the manufacturer's instructions. The extracted DNA samples were stored at −20 °C until needed. Two steps of nested PCR (detection limit of approximately 0.5–5 parasites/μL) were performed to confirm the presence of *P. falciparum* by targeting the 18S rRNA (Snounou et al., 1993) using genus and species-specific primers. This was done in Advanced Medical Entomology and Vector Control laboratory at Arba Minch University.

2.15. Genotyping of recurrent cases

DNA samples were analyzed via allelic typing to identify variations in *P. falciparum MSP-1* (block 2) and *MSP-2* (block 3) genes using two rounds of PCR. The first round targeted the entire *MSP-1* and *MSP-2* loci, while the second round focused on specific alleles: *MSP-1* (K1, MAD20, RO33) and *MSP-2* (FC27, 3D7) (supplementary file). Genetic variations were classified as new infections if post-treatment samples differed by >20 bp from initial samples, or as recrudescence if alleles matched within 20 bp (WHO, 2007). The recrudescence indicates that the treatment did not eliminate the initial infection, and the same strain has reappeared. In contrast, reinfection occurs when a patient who was successfully treated is later infected again by a different strain from another source. Genotyping was conducted at Aklilu Lemma Institute of Pathobiology.

2.16. Data quality assurance

Before beginning data collection, we prepared all necessary materials and forms to ensure accuracy. Patients were randomly assigned to receive either AL plus PQ or AL alone, maintaining unbiased results. The microscopy assessors were blinded to the treatments, and a regional malaria expert reviewed the slides. We used PCR to confirm the parasite species and assess clearance. DBS samples were processed and stored following standard protocols. We maintained data quality at all stages: pre-collection, during collection, and post-collection.

2.17. Data handling and statistical analysis

In this study, we employed a per-protocol (PP) analysis. This method focuses on evaluating the outcomes of participants who strictly adhered to the study's protocol, including all specified procedures and treatment plans. The data was recorded in an Excel file and then cleaned, validated, and analyzed using R-software version 4.3.2 (2023–10–31 curt). Only participants with PCR-confirmed cases were considered for analysis, and those who were withdrawn were excluded. An independent sample *t*-test was conducted to compare means, while proportions were determined with 95 % confidence intervals (CI) and compared using either the Chi-square test or Fisher's test. Medians and geometric means were compared using the Mann–Whitney U test. A 2-sample test for equality of proportions was used to compare proportions. Generalized linear mixed models (GLMMs) with a binomial distribution were

employed to compare the PCR-tested parasite clearance rates between treatment groups. Statistical significance was established as $P < 0.05$.

3. Results

3.1. Characteristics of study participants

A total of 3116 suspected malaria cases were screened at the health facility during the study period. Out of these, 846 patients tested positive for malaria through microscopic examination, representing 27.2 % (95 % CI: 25.6–28.7 %) of all screened patients. Among those with positive results, 46.3 % (392/846) had *P. falciparum* infections, 35.6 % (301/846) had *P. vivax* infections, and 18.1 % (153/846) had mixed infections. Of the *P. falciparum*-positive cases, 146 patients who met the inclusion criteria were included in the study (Fig. 1). The remaining 246 were excluded based on age classification and parasite density cut-off criteria.

In the AL plus PQ group, 57.7 % (30/52) of the participants were male, while in the AL alone group, 51.9 % (28/54) were male. Additionally, in the AL plus PQ group, 76.9 % (40/52) of participants presented with a fever at baseline, whereas the AL group had a fever prevalence of 68.5 % (37/54). The remaining 23.1 % of patients in the AL plus PQ group and 31.5 % in the AL group had axillary temperatures below 37 °C at enrolment, but they had a recent history of fever within the last 24 h.

The geometric mean of asexual parasite load at baseline was 5202 in the AL plus PQ group and 4712 in the AL alone group. Out of 106 patients, only 9.4 % (10 patients; 5 in each group) had gametocytemia at baseline (Table 1).

3.2. Asexual parasite clearance rate by microscopy

The asexual parasite clearance rate by microscopy did not show significant difference between the two treatment groups ($P = 0.381$). On day 1 post-treatment, 30.8 % (16/52) of patients in the AL plus PQ group remained parasite positive compared to 22.2 % (12/54) of patients in the AL alone group. Both groups were parasitemia negative on day 2.

The initial median parasite density of the patients was 4271/µl (IQR: 2640–8850) in the AL plus PQ group and 4450/µl (IQR: 2160–9320) in the AL alone group, which did not differ statistically ($P = 0.61$). On day one, the parasite density decreased to 467/µl (IQR: 260–860) in the AL plus PQ group and 1120/µl (IQR: 330–2533) in the AL alone group, with no significant difference ($P = 0.23$). No parasites were found on day 2 in either treatment group (Fig. 2). A version of the figure with non-log-transformed y-axis labels is provided in the supplementary material for clarity (supplementary figure).

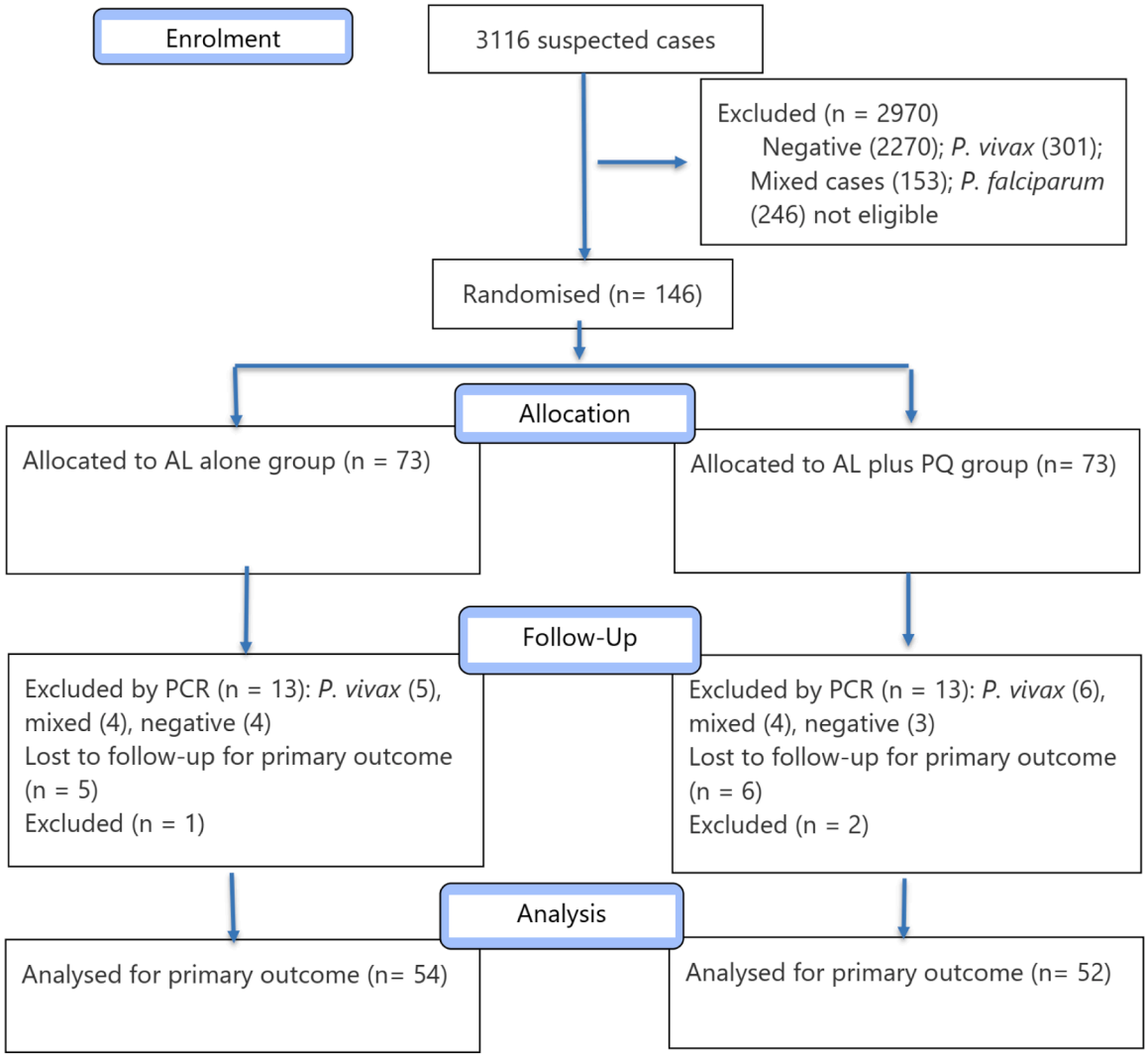

Fig. 1. The number of suspected and positive cases and study participants followed for 28 days in the two treatment groups in Arba Minch, Ethiopia.

Table 1
Baseline characteristics of the study participants in the AL plus PQ and AL alone groups in Arba Minch, Ethiopia.

Characteristics	AL plus PQ	AL	*P* value
Patients, n	52	54	-
Male/female, n	30/22	28/26	0.56
Age (year)			
Median (range)	26.0 (15–60)	22.0 (15–50)	0.1
Mean ± SD	26.7 ± 9.7	25.1 ± 9.4	0.1
Weight mean ± SD (kg)	59.4 ± 9.7	54.4 ± 7.5	0.19
Fever within 24hr, n (%)	12 (23.1)	17 (31.5)	0.39
Fever during enrolment, n (%)	40 (76.9)	37 (68.5)	0.39
Auxiliary temperature (°C)			
Mean ± SD	37.8 ± 0.95	37.8 ± 1.2	0.74
Parasite density (per µl)			
Geometric mean (range)	5202 (1000–79,555)	4712 (1040–34,400)	0.87
Gametocyte density (per µl)			
Geometric mean (range)	761.2 (40–12,000)	389.6 (120–2086)	0.22
Gametocyte carriage rate by microscopy, no. (%)	5 (9.6)	5 (9.3)	1.00

3.3. Treatment efficacy of AL plus PQ and AL alone

During the follow-ups, one patient in the AL plus PQ group experienced recurrent parasitemia with fever on day 21, suggesting a late clinical failure (1.9 %; 95 % CI: 0.0–10.3). Thus, the PCR-uncorrected cure rate for the AL alone group was 100 % (95 % CI: 93.4–100 %), as compared to 98.1 % (95 % CI: 89.7–99.9 %) in the AL plus PQ group.

3.4. Gametocyte carriage rate and clearance by microscopy

At baseline, only 9.4 % (10/106) of cases had detectable gametocytes, which slightly increased to 11.3 % (12/106) by day 1 post-treatment, as observed by microscopy. During follow-up, three new cases with gametocytes were detected on day 1 post-treatment. Notably, one baseline-positive participant had cleared gametocytes by this time point. Among the gametocyte carriers at baseline, 9.6 % (5/52; 95 % CI: 3.2–21) were in the AL+PQ group and 9.3 % (5/54; 95 % CI: 3.1–20.3) in the AL group. The PQ plus AL treatment group achieved a complete clearance rate by day 7, whereas the AL alone treatment group reached it by day 14, as confirmed by microscopy (Fig. 3).

The median gametocyte density before treatment was 1280/µl (IQR: 80–5200) in the AL plus PQ group and 320/µl (IQR: 200–560) in the AL alone group. On day 1, the gametocyte density was 1347/µl (IQR: 200–2260) in the AL plus PQ group and 680/µl (IQR: 320–1509) in the AL group, which was higher than the baseline density. By day 2, the median gametocyte density had decreased to 680/µl (IQR: 560–1840) in the AL plus PQ group and 400/µl (IQR: 120–600) in the AL alone group. There were no significant differences in the clearance of gametocytes between the groups on days 0 ($P = 0.9$) and 3 ($P = 0.3$). However, on day

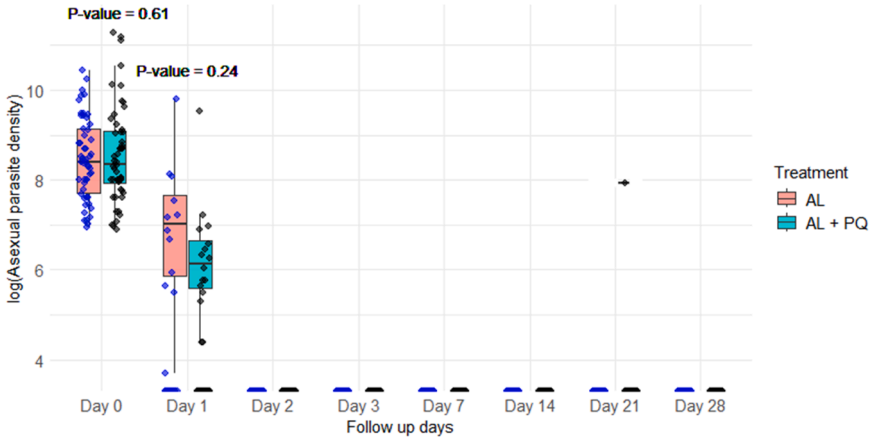

Fig. 2. Microscopically tested asexual parasite clearance between treatments on different follow-up days, Arba Minch, Ethiopia.

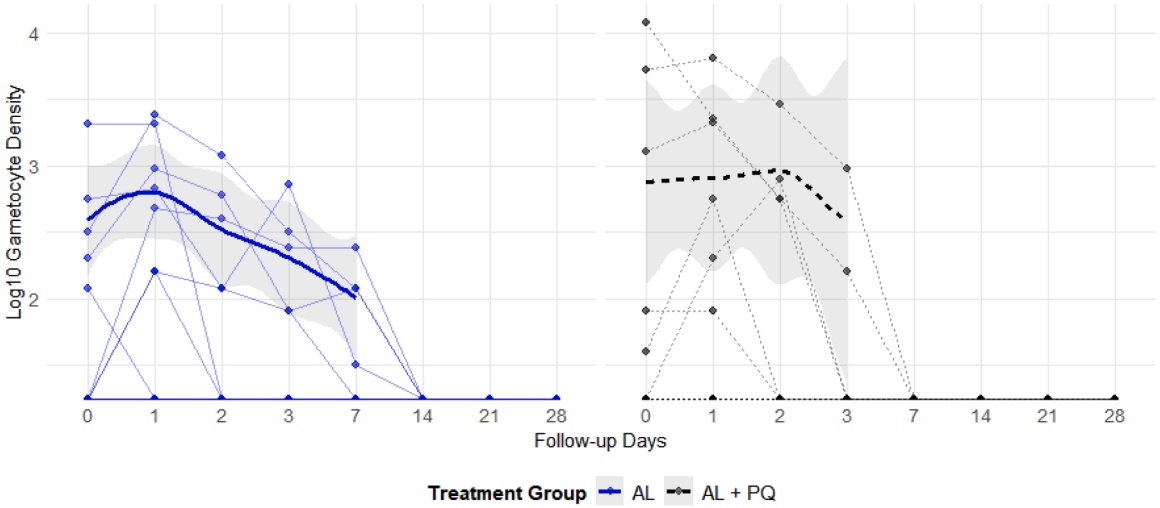

Fig. 3. The number of patients with microscopically detected gametocytes in the two-treatment groups, Arba Minch, Ethiopia.

7, there was borderline significance ($P = 0.05$).

3.5. Parasite clearance detected by PCR

Based on the PCR results, on day 1, 24.1 % (13/54) of patients in the AL group were free of parasite DNA, while 9.6 % (5/52) of patients in the AL plus PQ group were parasite DNA-free. However, by day 14, patients in both the AL plus PQ group and the AL alone group had completely cleared asexual parasites (Table 2).

There was no significant difference in the proportions of positive cases between treatments on follow-up days (Table 2).

PCR test results showed six positive cases (all on day 21) in both treatment groups: four in the AL plus PQ group and two in the AL alone group. Only one patient who was positive on day 21 was still positive on day 28.

3.6. Determination of recurrent infections by MSP genotyping

During the follow-up period, *P. falciparum* DNA was detected in six of the participants on day 21, as confirmed by PCR. Four were in the AL plus PQ group, and two were in the AL alone group. Only one patient in the AL plus PQ group had microscopic parasitemia and fever; this patient also tested positive by PCR (Table 3). Reinfection was not observed in either of the treatment groups; however, the overall recrudescence rate was 2.8 % (3/106), as determined by *MSP* genotyping. The other three cases of recurrence were not amplified for the *MSP1* and *MSP2* genotypes. Based on the genotyping results, the PCR-corrected cure rate was 98.1 % and 96.2 % for the AL alone and AL plus PQ groups, respectively ($P = 0.31$).

4. Discussion

Our study demonstrates that the antimalarial drug AL, used either alone or in combination with a single dose of PQ, effectively clears parasites by day 2 in cases of uncomplicated symptomatic *P. falciparum* malaria, as confirmed by microscopy. However, PCR results showed that detectable parasite DNA persisted until day 7 in both groups. On day 21, six participants tested positive by PCR—four from the AL plus PQ group and two from the AL alone group, while there was only one positive case by microscopy in the AL plus PQ group. Gametocyte carriage was notably low in symptomatic *P. falciparum* cases, as confirmed by microscopy. In cases with detectable gametocytes, complete clearance was achieved by day 7 in the AL plus PQ group, and by day 14 in the AL alone group.

AL plus PQ and AL alone effectively cleared asexual parasites by day 2, consistent with previous studies conducted in Ethiopia (Gubae et al., 2023; Teklemariam et al., 2017). However, PCR-detectable parasite DNA persisted until day 7 in both treatment groups, a finding consistent with a previous study in Tanzania (Mhamilawa et al., 2020). The PCR-corrected cure rate of AL was 100 %, which was consistent with previous studies conducted in Ethiopia (Abamecha et al., 2020; Daka et al., 2024; Eshetu et al., 2012; Getnet et al., 2015; Gubae et al., 2023; Mekonnen et al., 2015). The PCR-corrected cure rate of AL plus PQ was also comparable to that of studies in Tanzania (Mwaiswelo et al., 2016). However, there are controversies regarding the PCR detection of parasite DNA. Some support the idea that targeted amplification of parasites from blood samples provides evidence for the existence of live parasites. The other reported that no detectable parasite DNA was identified through PCR after 48 h following the injection of inactivated parasites into mice (Jarra and Snounou, 1998). The nuclear material from parasites that drug or immune responses have eliminated is quickly removed from the bloodstream and does not significantly contribute to amplification. On the other hand, the PCR tests based on DNA can yield positive results for several weeks even in the absence of viable sexual or asexual parasites, potentially leading to over diagnosis of recrudescence and reinfections (Haanshuus and Mørch, 2020). Interestingly, neither microscopy nor PCR can differentiate between viable, active parasites and those that are deceased or have been influenced by medication and impaired in their ability to infect (Beavogui et al., 2010).

In our study, all patients were PCR-negative on day 14, and recurrent cases were reported on days 21 and 28, suggesting that the detected parasites may have been alive and viable. Therefore, relying solely on microscopy to assess the efficacy of antimalarial drugs may lead to an overestimation of drug efficacy. Also, the recent detection of partial ACT resistance markers emphasizes the importance of closely monitoring the efficacy of ACT (Fola et al., 2023). Accurate diagnosis and appropriate treatment are essential, as the accuracy of microscopy results in our study and previous studies may have overestimated the efficacy of the drug.

The prevalence of gametocyte carriage among symptomatic *P. falciparum* cases seeking treatment at health facilities was very low, 9.4 % at baseline. Previous studies in Ethiopia have reported similarly low prevalence rates of gametocyte carriage, ranging from 3.4 % to 13.5 % (Abamecha et al., 2020; Daka et al., 2024; Gubae *et* al., 2023). Despite this low prevalence of *P. falciparum* gametocyte carriage among symptomatic cases, it remains the most common malaria parasite in the country. This raises several questions. First, how does *P. falciparum* continue to dominate even when individuals are promptly seeking treatment at nearby health facilities and receiving treatment that includes the gametocytocidal drug PQ? PQ is known to target mature gametocytes to block transmission (Eziefula et al., 2012; Lin et al., 2017). Controversies may arise due to the prevalence of asymptomatic carriers, as individuals can harbor gametocytes without displaying symptoms, often due to prolonged stays in their communities (Björkman and Morris, 2020; Bousema et al., 2014). Secondly, does the provision of PQ as a health facility-based preventive measure effectively reduce

Table 2
PCR-based asexual parasite clearance rate between the two treatment groups at 28 follow-up days in Arba Minch, Ethiopia.

Follow-up days	Treatment	Count positive	Total count	Proportion of positive case	Standard error	P-value
0	AL	54	54	1	0	
	AL + PQ	52	52	1	0	
1	AL	41	54	0.76	0.06	0.08
	AL + PQ	47	52	0.90	0.04	
2	AL	25	54	0.46	0.07	0.2
	AL + PQ	32	52	0.61	0.06	
3	AL	19	54	0.35	0.06	0.6
	AL + PQ	22	52	0.42	0.07	
7	AL	10	54	0.18	0.05	0.09
	AL +PQ	3	52	0.06	0.03	
14	AL	0	54	0	0	
	AL + PQ	0	52	0	0	
21	AL	2	54	0.04	0.03	0.6
	AL +PQ	4	52	0.08	0.04	
28	AL	0	54	0	0	
	AL + PQ	1	52	0.02	0.02	

Table 3
The MSP1 and MSP2 genotyping of microscopy and PCR test recurrent cases to assess the recrudescence and reinfection of P. falciparum malaria in the two groups, Arba Minch, Ethiopia.

Recurrent cases	AL group (%); $n = 54$	AL plus PQ group (%); $n = 52$
Microscopy testing parasite recurrence, n (%)	0 (0.0)	1 (1.9)
PCR testing parasite recurrence, n (%)	2 (3.7)	4 (7.7)
PCR confirmed recrudescence	1 (1.9)	2 (3.8)
PCR confirmed reinfection	No	No
MSP1 and *MSP2* genes not amplified	1 (1.9)	2 (3.8)

malaria transmission, considering the low prevalence of gametocyte carriage rates in symptomatic cases? Reducing the carriage of gametocytes is crucial for effectively controlling malaria transmission. Treating only symptomatic cases that visit healthcare facilities may not significantly impact transmission rates since these cases might represent only a small portion of the overall infection sources (Tine et al., 2017). The existence of asymptomatic cases in regions with high malaria transmission may diminishes the efficacy of administering PQ (WHO, 2022). The high levels of gametocytes found in asymptomatic individuals, particularly in those infected *P. falciparum*, are concerning. This highlights the significant role that asymptomatic carriers play in maintaining malaria transmission.

The treatment of *P. falciparum* infections with ACT plus PQ enhances the clearance of gametocytes. Patients receiving the AL plus PQ combination cleared gametocytes by day 7, while those without PQ only cleared the parasites by day 14. Several studies have reported similar findings (Gonçalves et al., 2016; Lin et al., 2017). A meta-analysis highlighted the benefits of adding PQ to artemisinin-based combination therapy (ACT) for reducing gametocyte carriage and transmission to mosquitoes when compared to ACT alone (Stepniewska et al., 2022). Antimalarial medications that effectively eliminate asexual parasites shorten the duration of gametocyte carriage by preventing the generation of gametocytes from asexual parasites. AL exhibits a more significant gametocytocidal effect than other ACTs (Stepniewska et al., 2022). Although adding PQ improves the rate of gametocyte clearance, complete clearance is not achieved until day 7, which may create a potential transmission window. However, PQ quickly sterilizes mature gametocytes and alters the ratio of male and female *P. falciparum* gametes, preventing the formation of zygotes in mosquitoes (White et al., 2012; White, 2017).

Notably, the proportion of gametocyte carriage increased on day 1 post treatment compared to the baseline. We observed three new cases (two in AL alone and one in AL plus PQ group) with gametocytes. An increase in gametocyte prevalence following treatment likely results from a combination of parasite survival mechanisms, adaptive responses to drug pressure, and the timing of gametocyte emergence from maturation sites like the bone marrow and spleen (Joice et al., 2014). A study conducted in Tanzania, which assessed the safety and efficacy of a single dose of 0.25 mg/kg of PQ, found that patients without baseline gametocytemia became infectious to mosquitoes by day seven post-treatment, and more patients tested positive for gametocytes following PQ administration (Mwaiswelo et al., 2016, 2022). The production of gametes is often seen as a response to parasite stress, allowing adaptation to harsher environments (Bousema and Drakeley, 2011). The efficacy of PQ also depends on the metabolism of the enzyme CYP2D6. A deficiency in this enzyme can reduce the efficacy of PQ (Stewart et al., 2021). Individuals with impaired CYP2D6 function may exhibit different drug metabolism and responses compared to those with normal enzyme activity (Camarda et al., 2019). Therefore, it is essential to recognize that variations or deficiencies in CYP2D6 activity can influence the metabolism of PQ, potentially affecting its overall efficacy (Stewart et al., 2021).

Three cases of *P. falciparum* malaria infections were identified as recrudescence, confirmed through genotyping. Recrudescence can occur due to factors such as drug resistance, poor adherence to treatment, or vomiting (WHO, 2023). The drug's efficacy depends on the parasites' susceptibility, the host's nutritional and immune status, and the drug's pharmacokinetics and pharmacodynamics (White, 2013). It is important to consider these factors to identify the causes of treatment failure. Additionally, three recurrent cases that remained undetermined underwent repeated genotyping. These tests consistently returned negative results for the *msp1* and *msp2* genes, despite the detection of parasite DNA through nested PCR. This discrepancy could be due to low parasite density. Moreover, the limitations related genotyping may contribute for this. The *MSP* genes are highly polymorphic, and genotyping may not accurately detect all strains present, as it is limited to the *msp-1* and *msp-2* loci (Mobegi et al., 2014). Therefore, there is a need for targeted amplicon sequencing of *Plasmodium* genomes to enhance the accuracy in detecting recrudescence and reinfection.

While the initial dose and the subsequent visits doses were directly observed, nighttime intake was unsupervised, which may have impacted adherence and treatment outcomes. The sample size was initially estimated using the single population proportion formula and adjusted for two groups. Although the randomized control trial formula was later applied, the final included sample size fell slightly short of the target, this may limit the robustness of the findings. While genotyping has limited accuracy in identifying cases of recrudescence and reinfection, the study did not assess *PfK13* gene mutations or other molecular markers, limiting insights into whether recrudescence was linked to drug resistance or reinfection. Enrollment was limited to participants aged $\geq$15 years, restricting generalizability to younger populations who may exhibit different treatment responses. Despite these limitations, the randomized allocation of cases and the public health relevance of the findings support the validity of the study.

5. Conclusions

In the study site, AL was found to be as effective for treating uncomplicated *P. falciparum* cases in patients aged 15 and older, whether used alone or in combination with PQ. The prevalence of gametocytes was low, and among the few positive cases, clearance was achieved by day 7 with the AL plus PQ treatment, while it took until day 14 with AL alone. These results suggest that adding a single dose of PQ could help reduce transmission; however, its overall impact may be limited due to the low number of gametocyte carriers. Further studies involving all age groups are necessary to better understand PQ's role in decreasing malaria transmission.

Ethical considerations

The study received ethical approval from the Institutional Review Board of Arba Minch University (IRB/1391/2023). Participants were informed about the study's objectives, potential benefits, risks, and alternatives. Written informed consent was obtained, and for minors under 18, assent was secured along with parental consent. Participants received immediate treatment at no cost and were reimbursed for transportation during seven follow-up visits to the health centre.

CRediT authorship contribution statement

Tinsae Kumsa: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Betelihem Jima:** Writing – review & editing, Supervision. **Girum Tamiru:** Writing – review & editing, Validation, Formal analysis. **Ribka Getu:** Writing – review & editing, Investigation. **Mesay Melaku:** Writing – review & editing, Investigation. **Yilikal Tesfaye:** Writing – review & editing, Visualization, Software, Formal analysis, Data curation. **Bernt Lindtjørn:** Writing – review & editing, Supervision, Software, Project administration, Funding acquisition, Conceptualization. **Fekadu Massebo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no competing interests, underscoring the integrity of their work.

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21/0162). The funders had no role in the study's design, data collection and analysis, decision to publish, or preparation of the manuscript. We would like to express our gratitude to the study participants for their active involvement and willingness to contribute to this research.

Data availability

Data will be made available on request.

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