Malaria Journal

RESEARCH

Open Access

In vivo efficacy of chloroquine plus primaquine combination therapy against uncomplicated *Plasmodium vivax* malaria in Limu Kossa District, Jimma Zone, Southwest Ethiopia

Wakgari Asfaw[1], Temesgen Bekele[1], Geleta Geshere[1], Eba Alemayehu Simma[1], Chernet Tuge Deressa[2] and Tsige Ketema[1*]

Abstract

Background *Plasmodium vivax* is the second most common malaria parasite in Ethiopia. It has been treated with chloroquine (CQ) for the past seven decades. However, the emergence of CQ-resistant strains in the nation urged the Federal Ministry of Health of Ethiopia to review its national malaria treatment guideline in 2018. In the revised guideline, the first-line treatment for uncomplicated *P. vivax* infection is a combination of CQ and primaquine (PQ). Thus, the present study was designed to evaluate the in vivo efficacy of CQ and PQ combination therapy against clinical *P. vivax* mono-infection in one of the malaria-endemic areas of Ethiopia.

Methods An open-label prospective clinical trial was conducted in the Limmu Kossa District, Jimma zone, Southwest Ethiopia, from September 2023 to March 2024. A total of 108 patients were recruited for the study. All participants received treatment with CQ at a dosage of 25 mg/kg over three days, followed by PQ at 0.25 mg/kg for 14 consecutive days. Patients were monitored for 42 days for any signs of treatment failure and malaria clinical symptoms, as per the World Health Organization (WHO) guidelines for anti-malarial drug evaluation. Additionally, haemoglobin (Hb) levels, body temperature, any adverse events, and signs of haemolysis were assessed. Data was analysed using R-software (version 4.0.0) and a significant level was considered at $p < 0.05$.

Results The median age of the patients was 23 years, ranging from 2.5 to 62 years. Of the 108 patients initially recruited, 100 completed the 42-day follow-up period. The combination therapy of CQ and PQ for uncomplicated clinical *P. vivax* malaria demonstrated excellent therapeutic efficacy, with a 100% cure rate observed at both day 28 and day 42. Additionally, the recommended low dose of PQ (0.25 mg/kg) was well-tolerated, with no signs of. Additionally, most common malaria symptoms were disappeared early in the follow-up period.

Conclusion The combination of CQ plus PQ has exhibited excellent efficacy against uncomplicated *P. vivax* malaria mono-infections. To preserve this efficacy, it is critical to ensure patients adhere to the full course of PQ treatment,

*Correspondence:
Tsige Ketema
tsigeketema@gmail.com
Full list of author information is available at the end of the article

despite its extended duration. Therefore, health authorities should put emphasis on the boosting of the public on the importance of finishing the prescribed medication regimen.

Keywords Chloroquine, Efficacy, Ethiopia, Limmu Kossa, Jimma, Primaquine

Background

Plasmodium vivax is the most widely distributed malaria parasite globally, with approximately 7 million clinical cases [1]. While it is commonly known that *P. vivax* has African origins [2], its existence on the continent is rare. For many decades, it was considered a benign malaria parasite [3]. However, the parasite is predominantly prevalent and steadily causing significant clinical disease in countries in the Horn of Africa including Ethiopia, Eritrea, Sudan, Djibouti, and Madagascar [4, 5]. *Plasmodium vivax* accounts for 3% of malaria cases worldwide, with about 12% of the global cases and deaths attributed to this parasite [1]. According to the Federal Ministry of Health of Ethiopia's Malaria Strategic Plan and a comprehensive systematic review and meta-analysis by Ketema et al. [6, 7], *P. vivax* accounts for nearly 31% of malaria infections in Ethiopia. In recent years, the prevalence of *P. vivax* has been steadily increasing, particularly in the central west region, and gradually spreading to the western part of the country [7, 8]. Despite significant efforts over many years to eradicate malaria in endemic areas, the disease remains the most pressing health concern in Ethiopia [9, 10].

In Ethiopia, chloroquine (CQ) was the first-line treatment for uncomplicated *Plasmodium falciparum* and *P. vivax* malaria. In 1998, following the widespread of chloroquine (CQ) resistant *P. falciparum*, CQ was replaced by sulfadoxine-pyrimethamine (SP) and subsequently, in 2004, SP was replaced artemether-lumefantrine (AL) for the treatment of uncomplicated falciparum malaria in Ethiopia [11, 12]. However, uncomplicated *P. vivax* infection was treated with CQ alone until recently. Then, the emergence of chloroquine-resistant *P. vivax* strains was reported in most vivax-endemic areas, as reviewed by Ketema et al. [13], the national malaria treatment guideline was revised in 2018 [14]. The updated treatment regimen recommends the use of a combination of CQ (25 mg/kg for three consecutive days) and primaquine (PQ, 0.25 mg/kg for 14 consecutive days) for uncomplicated *P. vivax* infections [14]. This combination therapy is advised for patients in endemic areas, as well as for those in endemic regions in specific districts targeted for malaria elimination and patients outside malaria-endemic areas. In the same guideline the second line drug in case of treatment failure or patients failed to respond to CQ and PQ, a 7-day quinine or artesunate was recommended [14]. The Ethiopian National Malaria Control Programme (NMCP) has also developed a strategic plan for malaria elimination [15].

An imperative challenge with this combination treatment is poor patient adherence to the 14-day PQ regimen, which may lead to the emergence of resistance due to subtherapeutic dosing [16]. Although the use of CQ plus PQ in combination for uncomplicated vivax malaria is relatively recent, studies from high-transmission settings in Northern and Southern Ethiopia have reported significant late treatment failures, with rates of 7.4% and 10.9% [17, 18]. Therefore, this study aimed to evaluate the in vivo efficacy of CQ plus PQ against uncomplicated clinical *P. vivax* infections in low vivax malaria transmission settings in Ethiopia.

Methods

Description of study area

The study was conducted at Laku Chime Health Centre located in Limu Kossa district, Jimma Zone, Oromia Regional State, Southwest Ethiopia (Fig. 1). The District is located 420 km southwest of Addis Ababa, the capital city of Ethiopia and lies between latitude of 7°50′ and 8°36′ North and longitude of 36°44′ and 37° 29′ East. The district's elevation was 1761 m. The area is characterized by several perennial rivers, intermittent streams, springs, and notable landmarks including Cheleleki Lake and Bolo Caves (data from the Limu Kosa District Agricultural and Rural Development Office). According to the 2007 national census [19], the District has a population of 161,338 with 81,462 men and 79,876 women. The majority of the population (72.6%) are Muslims, and 14,842 people, or 9.2%, are urban dwellers. The district is served by one hospital, seven health centres, 14 clinics of various levels, and 36 health posts. Laku Chime Health Centre is one of the district's health centres. Malaria is a significant health issue in the zone and district. Although there was no comprehensive studies on the malaria prevalence and effect of seasonal variability on the disease burden, the Serological study on the aetiologies of human malaria exposure showed a frequent response was against *P. falciparum* and *P. vivax* [20]

Study design and source population

From September 2023 and March 2024, an open-label prospective clinical trial was carried out. It involved patients with uncomplicated *P. vivax* mono-infection. Inclusion criteria: patients with *P. vivax* mono-infection,

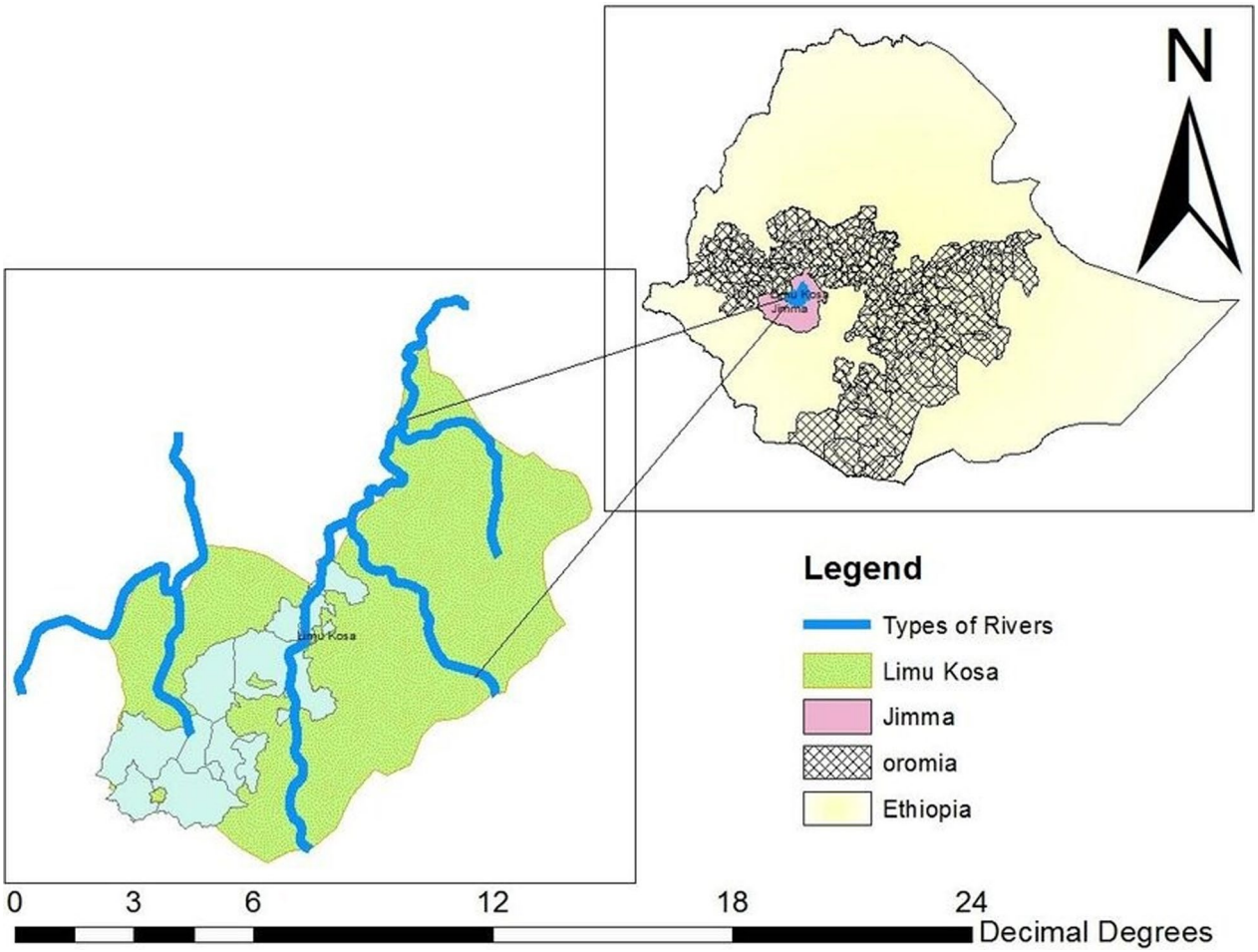

Fig. 1 The map of study site

over six months of age, experiencing symptoms of uncomplicated infection with a parasite load of greater than 250 parasite/μl, no known allergies to CQ and PQ, not-pregnant or breastfeeding, residing near the health facility, informed consent from the patient or from a parent or guardian in the case of children, no severe malnutrition as per the World Health Organization (WHO) child growth standards guideline, absence of regular medication, which might interfere with anti-malarial pharmacokinetics, and required commitment to attending all scheduled visits.

Exclusion criteria: severe malaria symptoms or complications, known contraindications to CQ plus PQ, comorbidities or chronic infections, inability to participate due to physical or mental health limitations, infections other plasmodium species, severe malnutrition as defined by WHO, haemoglobin level below 5 mg/dl in children and below 7 mg/dl in adults, inability to take oral PQ medication, and receipt of anti-malarial drugs within two weeks before the enrolment date [1].

Sample size determinations

The sample size for this study was determined based on an anticipated 5% rate of treatment failure with CQ plus PQ in a low transmission setting. The calculation aimed for a ± 5% precision and a 5% significance level. Additionally, a 20% loss to follow-up rate over the 42-day study period was expected. As a result, 108 study participants were included. The participants were purposely recruited according to the study's inclusion criteria.

Data collection procedures

The socio-demographics, and clinical data for patients recruited for efficacy testing (n = 108) were collected by health professionals at the health facility using a predesigned data collection format. Blood samples were taken from each patient via a lancet-pricked finger. The sample was used to prepare blood films and measure haemoglobin (Hb) levels. Thin and thick smears were prepared on a clean glass slide, air air-dried, and the thin film was fixed with 70% methanol. One of the smears was

stained with 10% Giemsa and examined under a 100X oil immersion objective. The other blood film used for the parasite load counting was stained with 3% Giemsa. Slides were deemed positive for specific *Plasmodium* species or mixed infection if any parasite stage was observed, and negative if no malaria parasites were seen under all fields of view. Parasite density (asexual stage) per microlitre (µl) of the blood was determined by counting the number of parasites per 200 white blood cells, assuming a standard human leukocyte count of 8000/µl). Gametocyte density was quantified against 1000 leukocytes and converted to the number of gametocytes/µl) of blood, assuming a mean leukocyte count of 8000/µl). As additional information to demonstrate pieces of evidence on the malaria positivity rate in the study area, retrospective data was collected from patient's medical records and the district health bureau from 2019 to 2023.

Haemoglobin (Hb) measurement

A lancet was used to prick the patient's finger and collect a single drop of blood in a cuvette. This blood sample was then used to determine the patients blood Hb level at enrollment (Day 0), Day 14, and Day 42 using a Hemocue machine [HemoCue® Hb 301 System (haemoglobinometer, Angelholm, Sweden)] following the manufacturer's instructions.

Patient treatment and follow-up

A 42-day in vivo drug efficacy test was conducted following WHO recommended methods [18]. This test involved treating symptomatic and parasitaemic *P. vivax* patients with a known dose of CQ and PQ. The CQ (chloroquine phosphate 250 mg, batch number 33342, Addis Pharmaceutical Factory PLC) was administered for 3 days, with a total dose of 25 mg per kg under professional supervision. Following this, PQ (Primaquine 7.5 mg film-coated tablets, batch number: 110701, Remedica Ltd., Aharnon Str., Limassol Industrial Estate, 3056 Limassol, Cyprus) was given for 14 days at a dose of 0.25 mg/kg per day. To ensure the correct administration of each dose, the patients were instructed to take all their medication under the supervision of a health professional only in the health centre. Those patients who missed any of their doses were excluded from the study and were not considered in the analysis.

Patients were monitored for parasitological and clinical responses over 42 days. All drug doses were administered under the supervision of professionals. Any physiological complaints were recorded during each visit. Patients were observed vomiting for 30 min after drug ingestion; those who vomited the first dose and expelled the entire drug were re-treated with an identical dose.

The axillary temperature and other clinical symptoms of the vivax malaria patients were assessed by an experienced nurse at the health centre and recorded in a specific format. Subjects who vomited the medication twice were excluded from the study. The participants were instructed not to take any other drugs except paracetamol if their axillary temperature was > 37.5 °C. Patients were scheduled for a follow-up examination on Days 1, 2, 3, 7, 14, 21, 28, 35, and 42 including temperature monitoring, and were advised to return at any time if they experienced malaria-like symptoms [21]. Blood smears were taken at all follow-up visits except on day 1. Patients who missed their follow-up schedules were visited at home. Additionally, haemolysis was assessed vissualy using urine colour estimation. A small portion of the patients urine was placed in a clear test tube and held up against a white piece of paper. Then colour was compared against the Hillmen Colour Chart for any evidence of haemoglobinuria on each patient from Day 1 to 14.

Treatment outcomes and endpoint classification

Treatment outcomes were assessed based on the parasitological and clinical results and classified according to the WHO [21] protocol as follows: clinical deterioration due to *P. vivax* illness requiring hospitalization in the presence of parasitaemia, and axillary temperature ≥ 37.5 °C on any day between days 3 and 28, and presence of parasitaemia on any day between days 7 and 28, irrespective of clinical condition was considered as treatment failure. Treatment success or adequate clinical and parasitological response (ACPR): was defined as the absence of parasitaemia on day 28 and beyond, regardless of axillary temperature., in patients who did not previously meet any of the criteria of treatment failure. In case of treatment failure detected, recrudescence from reinfections will be distinguished using molecular technique.

Quality management

Every data collector received training on adherence to the study protocol, and every day, all of the recorded data was verified. Before collecting data, all equipment and devices utilized in the process, including weight balances, haemoglobin meters, and thermometers, were calibrated. To guarantee precision, a certified medical laboratory technologist from Jimma University blindly reexamined 10% of all positive and negative blood smears. The Ethiopian Pharmaceuticals Supply Service provided all of the drugs necessary for the patient's treatment, and their quality was guaranteed.

Data analysis

The data was entered into a Microsoft Excel sheet and checked for completeness. It was then exported to R

software (version 4.0) for analysis. Descriptive and inferential statistics were used to compute some baseline data such as age, sex, temperature, parasite load and others. The tools employed included the Pearson correlation, which was to examine associations between variables, between variables, and the Mann–Whitney U test, which analysed Hb recovery in patients with adequate clinical and parasitological responses. Kaplan–Meier survival probability analysis was conducted to evaluate treatment outcomes during the follow-up period. A significance level of 95% confidence interval was used for all statistical tests.

Results

Characteristics of the study participants

During the study period, 1426 individuals suspected of having malaria were tested microscopically. Of these, 19.8% (n = 282/1426) tested positive for malaria, with 51.4% (n = 145/282) attributed to *P. vivax*. A total of 108 individuals (74.5%) infected solely with *P. vivax* from the rural areas met the inclusion criteria and were recruited for the study. In the initial follow-up phase (days 1 to 14), eight participants were excluded: one due to an adverse drug reaction, and seven due to loss to follow-up (Fig. 2).

At baseline, 55.6% (n = 60/108) of participants were male, with a median age of 23 years (± 15.2 years, range 2.5 to 62 years). Children under five accounted 8.3% (n = 9/108) of the total participants. Approximately, 62.04% (n = 67/108) of the patients had access (availability of at least one bed net) to abed net, and 36.11% (n = 39/108) use it regularly (every night). Among the 108 participants, 72.2% (n = 78) had experienced previous malaria episodes, all of which were treated with CQ plus PQ combination therapy (Table 1). The mean body temperature was 38.6 °C (range 37.4–39.5 C). The geometric mean parasite count was 5,135/parasites/µl (range 550–69,817 parasites/µl), with significant variation in baseline mean parasitaemia ($p < 0.001$). The overall gametocyte carriage rate was 93.5% (n = 100/108). The mean Hb level at enrollment was 12 g/dL. A total of 40.7% (n = 44/108) of the participants had varying levels of anaemia, though none of them had severe anaemia (Table 1).

There was a significant negative corelation between age and parasitaemia, with older individuals showing a notable reduction in parasite load ($r = -0.304$, $p = 0.0021$). Children exhibited a higher baseline parasite burden compared to adults. The geometric mean parasite load for nearly all patients was below 35,000 parasites/µl, except for with the exception of one outlier, which had a a parasite load of 69,800 parasites/µl (Fig. 3).

Efficacy of CQ plus PQ against uncomplicated *P. vivax* infection

The treatment of the *P. vivax* mono-infected case with CQ plus PQ demonstrated exceptional efficacy. None of the patients who completed their follow-up period experienced malaria symptoms or parasitaemia between days 7 and 42, resulting in a 100% cure rate and an adequate clinical and parasitological response rate (ACPR) (Table 2).

Rate of parasite and fever clearance post-treatment of CQ plus PQ

During the follow-up period, gametocytes and all asexual stages began to clear by day two. Initially, most of participants presented with fever, which resolved within the first few days. At the baseline, the average body temperature was 38.58 °C. Following treatment with CQ plus PQ, the average body temperature decreased to 37.69 °C on day two, 37.33 °C on day three, 37.22 °C on day four, and remained below 37 °C for the rest of the follow-up period. By the end of the follow-up period, 69% of the patients had recovered from fever by day one, 87% by day two, and 98% by day three. Nearly all patients had recovered from fever by day seven of the follow-up period (Fig. 4).

Hb levels and recovery

At enrollment (day 0), approximately 22% of participants had moderate anaemia and 19% had mild anaemia. Of the 41 anaemic individuals on day 0, only 84.5% had a history of malaria episodes. By the end of the follow-up period, these patients had not fully recovered their Hb levels, 7% still had moderate anaemia and 11% had mild anaemia. Only a small subset of the anaemic patients showed partial Hb recovery at the end of the follow-up. Among those with a history of malaria, 89% were able to normalize their Hb levels, while, the remaining 11% did not show improvement by day 42. Overall, the number of anaemic patients decreased from 41% at enrollment to 21% on day 14, and further 11% on day 28 following CQ plus PQ treatment (Table 3).

During the follow-up days, there was a progressive increment on the mean Hb level; on days 0, 14, and 42. It was 11.4, 12.1, and 12.9 g/dl, respectively. The individuals' Hb levels were positively impacted by the CQ plus PQ medication (Fig. 5).

Clinical signs observed during the follow-up period

At enrollment, the mean baseline body temperature was 38 ± 1 °C, with males at 38 ± 0.3 °C and females at 38 ± 0.4 °C. In the early days following treatment, many patients experienced various side effects from the medication or the disease symptoms, but most of

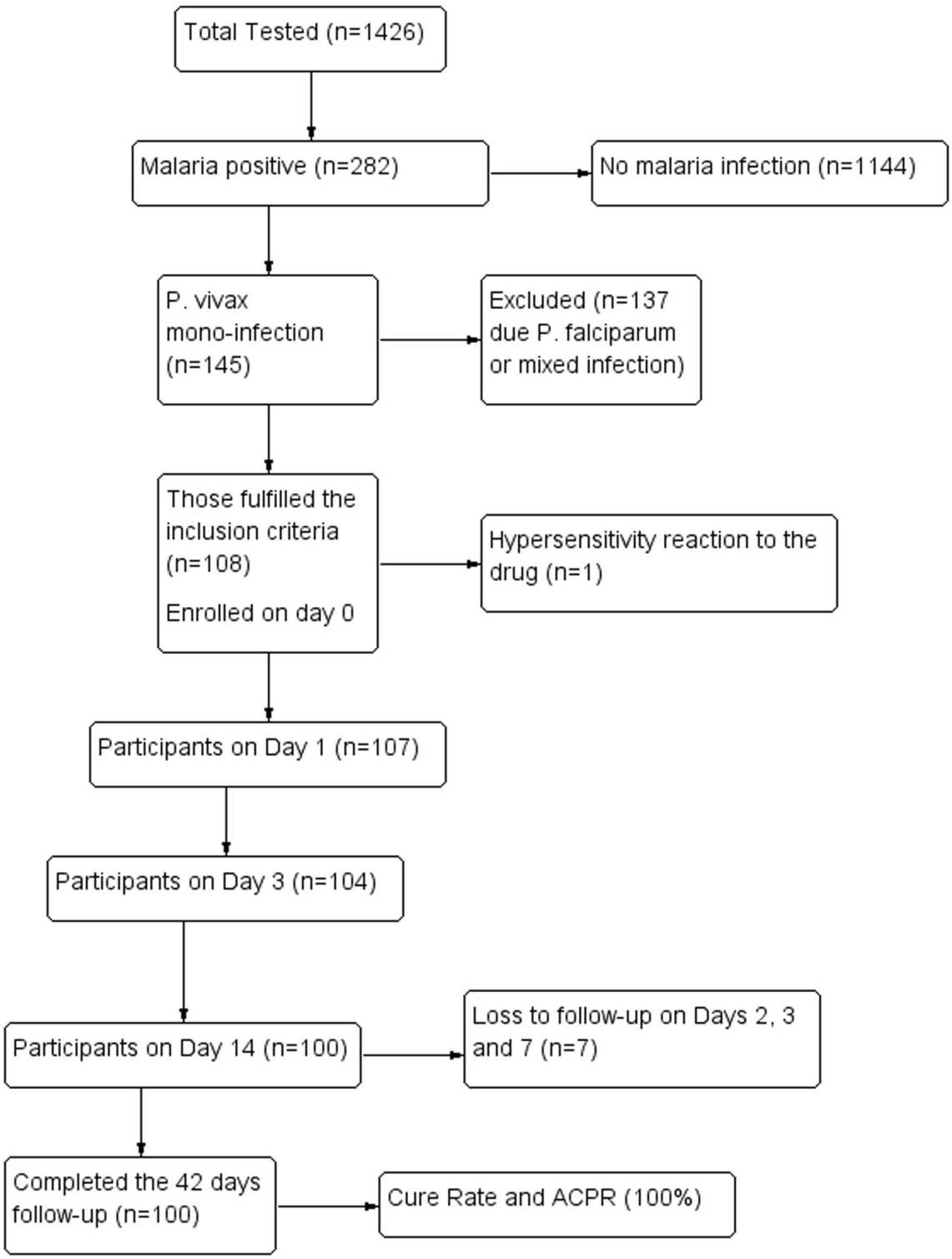

Fig. 2 The study flow diagram

these symptoms resolved within the first week of follow-up. The most common early symptoms were fever (91%) and headache (82%), followed by weakness (41%), and nausea (39%). Less common but notable symptoms such as anorexia, cough, and abdominal pain also resolved within the first week. Throughout the 42-day

Table 1 Baseline characteristics of the study participants in Laku Chime health center, Southwest Ethiopia

Variables	Total (%)
< 5 years n (%)	9 (8.3)
5 to 15 years	22 (20.4)
> 15 years	77 (71.3)
Median Age ± SD (year)	23 ± 15.2 (2.5 to 62)
Male patients (%)	60 (55.6)
Female patients (%)	48 (44.4)
Mean Temp (˚C)	38.58
Mean Hgb (g/dl)	12.15
Anaemic status (%)	44 (40.7)
Moderate (%)	23 (21.3)
Mild (%)	21 (19.4)
Geometric mean parasite count/µl (range)	5135 (550–69,817)
Gametocyte carriage (%)	100 (92.6)
Body mass index (BMI) kg/m^2	21.73
Availability of bed net n (%)	67 (62.04)
Utilize bed net every night n (%)	39 (36.11)
Previous malaria attack (%)	78 (72.2)

follow-up period, the majority of patients demonstrated good tolerability to the medication, with only a few exhibiting residual symptoms by Day 7 (Table 4).

Discussion

In this open-labeled prospective clinical trial, the combination therapy of CQ and PQ proven outstanding efficacy (100%) against clinical *P. vivax* malaria patients in a 42-day observation period. The study confirmed that CQ plus PQ treatment was safe, effective, and potent in easing common malaria symptoms within the first few days of the treatment start. One of the key challenges in managing, controlling, and preventing malaria is the emergence of drug-resistant strains of the *Plasmodium* parasites. In Ethiopia, CQ was the first-line treatment for uncomplicated *P. vivax* malaria for many decades. However, due to the rise of CQ-resistant strains of *P. vivax* [22–25], the Federal Ministry of Health of Ethiopia revised its malaria treatment guideline in 2018 [14]. The updated guidelines incorporated CQ plus PQ as the first line of treatment for uncomplicated vivax malaria to ensure radical parasite clearance and accelerate the achievement of the malaria elimination target [6, 14, 26].

Despite the importance of this decision, patients' compliance with the prolonged 14-day PQ treatment is a concern since poor adherence may result in the emergence of PQ-resistant strains and recurrence [27]. *Plasmodium vivax* infections accounted for 4.08% of the 14% five-year malaria-positivity rate reported in the study area (Supplementary Table 1 and Supplementary Fig. 1). The vivax malaria-positivity rate at this study site was relatively low (< 5%) given the current state of the nation [10, 28].

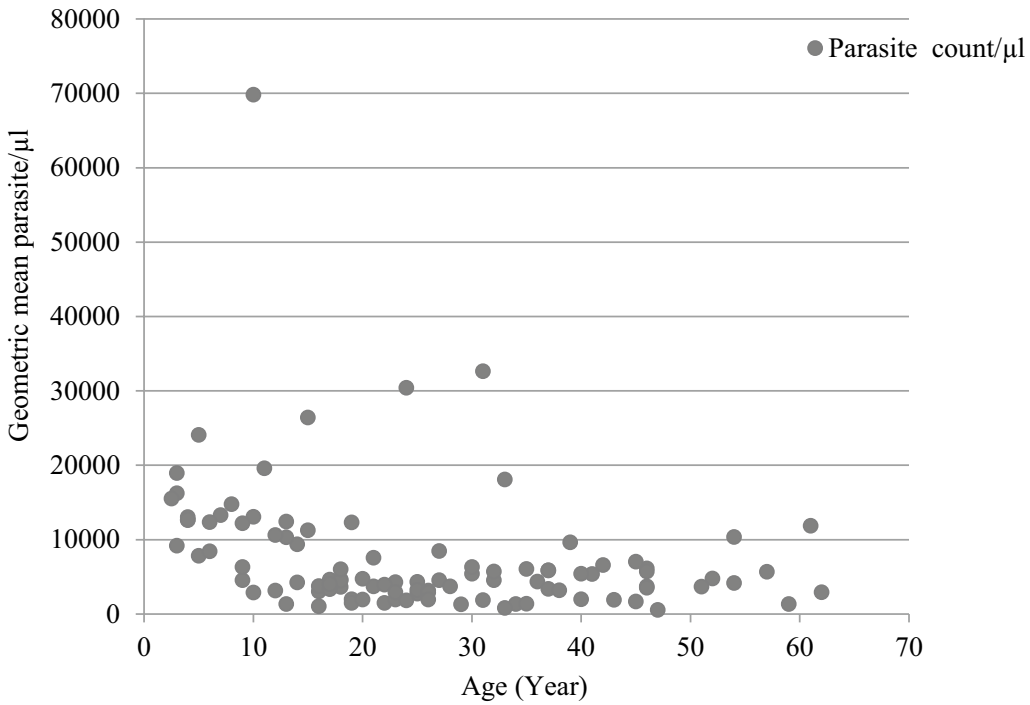

Fig. 3 Comparion of age of the patients and parasite load at enrollment in Laku Chime health center, Southwest Ethiopia

Table 2 Cure rate of CQ plus PQ against clinical uncomplicated *P.vivax* malaria infection after 42 days of follow-up period in Laku Chime health center, Southwest Ethiopia

Outcome	< 5	5–15	> 15	Sex		Total
				Male	Female	
ETF n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LCF n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LPF n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ACPR n (%)	7 (100)	22 (100)	71 (100)	56 (100)	44 (100)	100 (100)
Total analyzed n (%)	7 (100)	22 (100)	71(100)	56 (100)	44 (100)	100 (100)

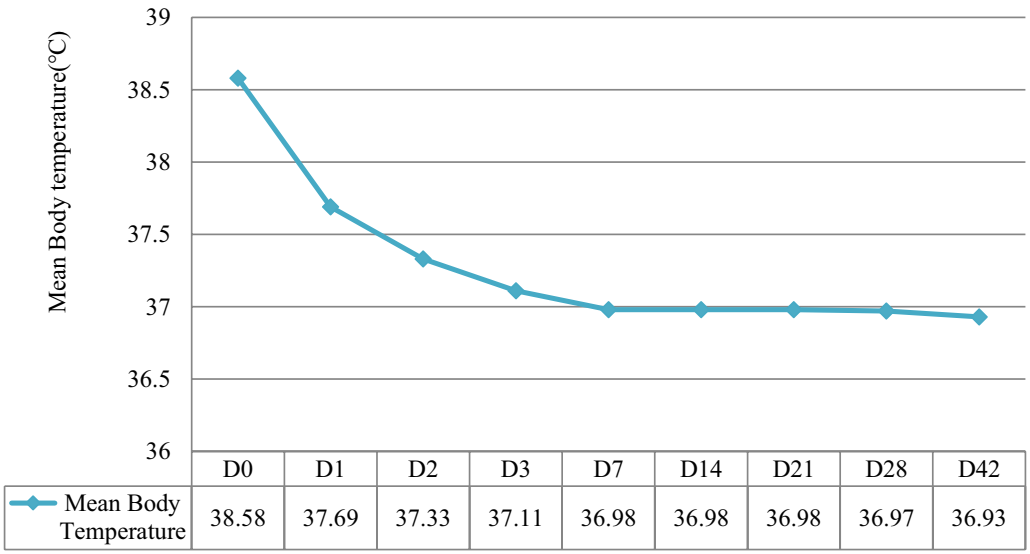

Fig. 4 Mean axillary temperature clearance during the follow-up periods in Laku Chime health center, Southwest Ethiopia

Table 3 Anaemia condition and Hb recovery among patients treated with CQ plus PQ in Laku Chime health centre, Southwest Ethiopia

Follow up days	Anaemia status	Frequency	Percent
Day 0	Moderate (7.0–9.9 g/dl)	19	19.0
	Mild (10.0–10^9 g/dl)	22	22.0
	No anaemia (> 11.0 g/dl)	59	59.0
	Total	100	100.0
Day 14	Moderate (7.0–9.9 g/dl)	11	11.0
	Mild (10.0–10^9 g/dl)	10	10.0
	No anaemia (> 11.0 g/dl)	79	79.0
	Total	100	100.0
Day 42	Moderate (7.0–9.9 g/dl)	7	7.0
	Mild (10.0–10^9 g/dl)	4	4.0
	No anaemia (> 11.0 g/dl)	89	89.0
	Total	100	100.0

It indicates a low-vivax malaria transmission scenario according to the WHO classifications for transmission conditions [29]. In such instances, anti-malarial therapy seems appropriate and a wider reach of interventional measures has the potential to halt the spread of malaria and lower malaria-related mortality [29]. Contrary to the high transmission settings, there is little chance that patients would re-infect with the same or a different species of *Plasmodium* since PQ eliminates the latent stage that causes relapse. Therefore, the reappearance of the parasitaemia could be the cause of any actual therapeutic failure found in these conditions. Nevertheless, none of the study's subjects who finished their 42-day follow-up exhibited any signs of treatment failure. Every patient responded to the combined therapy with a 100% adequate clinical and parasitological response (ACPR). This result is consistent with reports from various regions

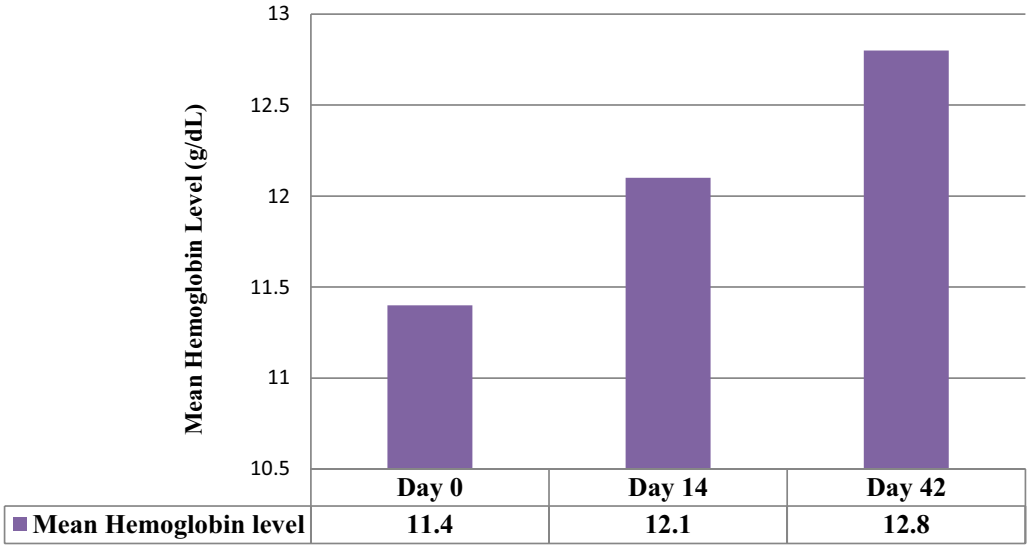

Fig. 5 Mean hemoglobin level on different follow-up days post CQ and PQ treatment in Laku Chime health center, Southwest Ethiopia

Table 4 Malaria clinical symptoms and their prognosis after CQ-PQ treatment in Laku Chime health centre, Southwest Ethiopia

Clinical symptoms/Adverse events	Follow up day									
	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Fever n (%)	91 (91)	31(31)	13(13)	2(2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Parasitaemia n (%)	100%		32 (32)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Headache n (%)	82 (82)	32 (32)	15 (15)	7 (7)	1 (1)	0 (0)	0 (0)	0 (0)	0(0)	0(0)
Cough n (%)	12 (12)	7(7)	4 (4)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)
Weakness n (%)	41(41)	28 (28)	22 (22)	6(6)	2(2)	0(0)	0(0)	0(0)	0(0)	0(0)
Vomiting n (%)	19 (19)	8(8)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)
Abdominal pain n (%)	5 (5)	11 (11)	8 (8)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)
Anorexia n (%)	27 (27)	12 (12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)
Nausea n (%)	39 (39)	17 (17)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)
Diarrhoea n (%)	4 (4)	3(3)	1 (1)	0 (0)	0(0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)
Behavioural change n (%)	0 (0)	1(2)	0(0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)

of the country, such as research done in Debre Zeit and Nazarate in East Shoa 14 years ago, as well as a study from Arba Minch in 2023, which found that a low dose of PQ (0.25 mg/kg per day for 14 days) did not result in any significant early or late treatment failures until day 28 [17, 30]. Furthermore, our results align with the outstanding effectiveness (100%) seen in Colombia and Pakistan some years ago [31, 32]. It conflicts with a recent Ethiopian study reported from Hamusit, which showed early treatment failures although did not provide genomic proof [18]. Additionally, despite knowing the exact G6PD enzyme levels before treatment, the lack of side effects, such as haemolysis, linked to PQ treatment in this trial indicates good tolerance to the low dose of PQ [33]. Thus, this study offers compelling evidence that treating vivax malaria in low-transmission conditions with the CQ plus PQ regimen now in use in Ethiopia is successful.

Higher treatment failure rates for PQ have been reported worldwide, especially in Western Thailand and the Northwest Frontier Province of Pakistan, where failure rates have reached as high as 7.7% [34, 35], this is in contrast to the current findings. PQ's failure to offer an effective treatment for *P. vivax* malaria has impeded efforts to prevent and control the disease in other areas [34, 35]. The explanations for these differences might be due to factors such as the duration of PQ that has been used to treat vivax or other illnesses (whether long or short), the level of drug supervision

(whether supervised or unsupervised), the timing of the primary outcomes assessments (14, 28 or 42), and the patient adherence levels (ranging from poor to good) [17, 18, 30, 35].

Three patients quit their treatment during the follow-up days, which resulted in their exclusion from the study. However, because of a recurrence of malaria symptoms, these patients were brought back to the health centre. In the health facility, they received a full dose of both PQ and CQ for treatment. This incident raises a concern about the need for patients to strictly adhere to their treatment plans and for healthcare workers at the health posts to provide appropriate guidance [16]. Such actions could be a contributing factor in the future establishment of drug-resistant strains of the parasite, since the sub-therapeutic dose may allow residual parasites to survive in deep tissues or blood [36, 37].

After a few days of CQ plus PQ administration, the patient's parasite density rapidly decreased. On day three, all patients cleared their parasitaemia, and there was no variation in the average time required for parasite clearance between the regimens. It is almost identical to the study conducted in Debre Zeit and in East Shoa of Ethiopia, where all the study participants cleared their parasites on day 3 [30]. This could be due to the drug's quick and full absorption in the digestive system after oral treatment and its quick metabolism [37]. PQ reaches its peaks in plasma levels within two to three hours and then quickly decreases, with a terminal phase of elimination half-life of 7.1 ± 1.6 h [38]. As one of the criteria for tracking and categorizing responses to PQ treatment, fever clearance time is critical for assessing the therapeutic efficacy of PQ in the treatment of *P. vivax*. On CQ plus PQ treatment, 98% of the participants cleared fever within three days of the follow-up period, which further supports the fact that PQ acts fast and quickly clears such symptoms. This finding supports similar reports from Ethiopia by Mekonned et al. [28], where fast fever clearance was reported.

In this study, one of the typical clinical manifestations (50%) of the patients was different levels of anaemia (mild and moderate). Treatment with CQ plus PQ brought a significant Hb recovery between the baseline and day 42, which aligns with a similar report from Hosanna, Ethiopia [25]. However, a small number of anaemic patients who had a previous malaria episode were unable to exhibit improvement in their Hb level, even on day 42. Since repeated malaria episodes and frequent relapse could cause impairment of Hb levels [39], poor malaria control is often related to weak interventional activities and a high prevalence of other infectious diseases and nutritional deficiencies, all of which contribute to the incidence of anaemia [40].

Limitation of the study

In this study the representation of infants < 2.5 years were limited as there was not patients encountered during recruitment. Thus, this findings might not be generalized to the whole population in the study site. In addition, as this study was conducted in low malaria transmission settings of Ethiopia, it might not be generalized to other settings in the Ethiopia such as low land rift vally areas commonly identified as vivax endmic areas in Ethiopia.

Conclusion

The study demonstrated a 100% cure rate for CQ (25 mg/kg) combined with PQ (0.25 mg/kg) treating uncomplicated clinical vivax malaria. The treatment resulted in rapid and effective clearance of parasites and fever and significant improvement in common malaria symptoms during the early follow-up period. Furthermore, the low dose of PQ (0.25 mg/kg for 14 days) was safe, with no patients showing signs of haemolysis. To preserve the effectiveness of this combination therapy, ensuring complete patient adherence to the full PQ regimen is crucial. Future studies should focus on evaluating the efficacy of CQ plus PQ in vivax malaria-endemic areas. Moreover, local, and national health authorities should implement community sensitization efforts to promote adherence to PQ and maintain the drug's effectiveness.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-024-05124-5.

Additional file 1

Additional file 2

Acknowledgements

The authors of the study would like to thank Jimma Zonal Health Department, and Limu Kossa District Health Bureau for permitting to conduct this study in the study area. We also would like to extend our gratitude to the study participants for their willingness to take part in the study and their genuine response, Jimma University, College of Natural Sciences, and the Department of Biology for facilitation of the study and unreserved support during the data collection. Jimma University for financial support

Author contributions

WA: involved in conceptualization, method design, data collection and curation, data analysis and interpretation, and write-up of the draft manuscript. TK: participated in the supervision of the study, conceptualization, methodology design, data analysis and interpretation, write-up of the draft manuscript, review, and editing. EA, GG, CT and TB: participated in the conceptualization of the study, methodology design, manuscript review, and editing.

Funding

This study was financially supported by Jimma University through the Staff Research Mega Project (Grant number: NS/II/24/02).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was ethically approved by Jimma University Institutional Review Board (IRB) (RSG/149/2024) prior to data collection. Written informed consent was obtained from each patient or guardian for patients younger than 18 years. Detailed ethical procedure and in depth study protocol were explained to each patient before data collection started. Only those volunteer and the fulfilled the inclusion criteria were included in the study.

Informed consent

All participants in the study provided written informed consent.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Biology, Jimma University, College of Natural Sciences, Jimma, Ethiopia. [2]Department of Mathematics, Jimma University, College of Natural Sciences, Jimma, Ethiopia.

Received: 5 August 2024 Accepted: 1 October 2024
Published online: 08 October 2024

References

1. WHO. World Malaria Report 2023. Geneva, World Health Organization, 2023. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023
2. Liu W, Li Y, Shaw KS, Learn GH, Plenderleith LJ, Malenke JA, et al. African origin of the malaria parasite *Plasmodium vivax*. Nat Commun. 2014;5:3346.
3. Villamil-Gómez WE, Eyes-Escalante M, Franco-Paredes C. Severe and complicated malaria due to *Plasmodium vivax.* In: Current topics in malaria. Rodriguez-Morales AJ (ed.). InTech Open. 2016.
4. Ménard D, Barnadas C, Bouchier C, Henry-Halldin C, Gray LR, Ratsimbasoa A, et al. Plasmodium vivax clinical malaria is commonly observed in Duffy-negative Malagasy people. Proc Natl Acad Sci USA. 2010;107:5967–71.
5. Twohig KA, Pfeffer DA, Baird JK, Price RN, Zimmerman PA, Hay SI, et al. Growing evidence of *Plasmodium vivax* across malaria-endemic Africa. PLoS Negl Trop Dis. 2019;13: e0007140.
6. Federal Ministry of Health. National Malaria Elimination Strategic Plan: 2021–2025 towards a malaria-free Ethiopia. Addis Ababa; 2020.
7. Ketema T, Bacha K, Getahun K, Portillo HAD, Bassat Q. *Plasmodium vivax* epidemiology in Ethiopia 2000–2020: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2021;15: e0009781.
8. Belay H, Alemu M, Hailu T, Mohammed H, Sime H, Hailegeorgies H, et al. Therapeutic efficacy of chloroquine for the treatment of uncomplicated *Plasmodium vivax* infection in Shewa Robit, Northeast Ethiopia. PLoS ONE. 2023;18: e0277362.
9. Teka H, Golassa L, Medhin G, Balkew M, Sisay C, Gadisa E, et al. Trend analysis of malaria in urban settings in Ethiopia from 2014 to 2019. Malar J. 2023;22:235.
10. Mandefro A, Tadele G, Mekonen B, Golassa L. Analysing the six-year malaria trends at Metehara Health Centre in Central Ethiopia: the impact of resurgence on the 2030 elimination goals. Malar J. 2024;23:32.
11. Golassa L, Kamugisha E, Ishengoma DS, Baraka V, Shayo A, Baliraine FN, et al. Identification of large variation in Pfcrt, Pfmdr-1 and Pfubp-1 markers in *Plasmodium falciparum* isolates from Ethiopia and Tanzania. Malar J. 2015;14:264.
12. Hailemeskel E, Menberu T, Shumie G, Behaksra S, Chali W, Keffale M, et al. Prevalence of *Plasmodium falciparum* Pfcrt and Pfmdr1 alleles in settings with different levels of *Plasmodium vivax* co-endemicity in Ethiopia. Int J Parasitol Drugs Drug Resist. 2019;11:8–12.
13. Ketema T, Bacha K, Getahun K, Bassat Q. In vivo efficacy of anti-malarial drugs against clinical *Plasmodium vivax* malaria in Ethiopia: a systematic review and meta-analysis. Malar J. 2021;20:483.
14. Federal Ministry of Health (FMoH). National malaria guideline, 4th Edn. Ethiopia, Addis Ababa, 2018.
15. U.S. President Malaria Initiative (PMI). Malaria Operational Plan FY; 2017.
16. Mehdipour P, Rajasekhar M, Dini S, Zaloumis S, Abreha T, Adam I, et al. Effect of adherence to primaquine on the risk of *Plasmodium vivax* recurrence: a WorldWide Antimalarial Resistance Network systematic review and individual patient data meta-analysis. Malar J. 2023;22:306.
17. Mekonnen DA, Abadura GS, Behaksra SW, Taffese HS, Bayissa GA, Bulto MG, et al. Treatment of uncomplicated *Plasmodium vivax* with chloroquine plus radical cure with primaquine without G6PDd testing is safe in Arba Minch, Ethiopia: assessment of clinical and parasitological response. Malar J. 2023;22:135.
18. Gebrie H, Yimer M, Ayehu A, Mohammed H, Hailgiorgis H, Wuletaw Y, et al. Efficacy and safety of chloroquine plus primaquine for the treatment of *Plasmodium vivax* malaria in Hamusit site, Northwestern Ethiopia. Malar J. 2024;23:202.
19. Central Statistical Agency (CSA) 2007 Population and Housing Census of Ethiopia: Results for Oromia Region, Vol. 1. ethiopianreview.com/pdf/001/Cen2007_firstdraft(1).pdf
20. Feleke SM, Brhane BG, Mamo H, Assefa A, Woyessa A, Ogawa GM, et al. Sero-identification of the aetiologies of human malaria exposure (*Plasmodium* spp.) in the Limu Kossa District of Jimma Zone, South western Ethiopia. Malar J. 2019;18:292.
21. WHO. Methods for surveillance of antimalarial drug efficacy. Geneva, World Health Organization, 2009.
22. Teka H, Petros B, Yamuah L, Tesfaye G, Elhassan I, Muchohi S, et al. Chloroquine-resistant *Plasmodium vivax* malaria in Debre Zeit, Ethiopia. Malar J. 2008;7:220.
23. Ketema T, Getahun K, Bacha K. Therapeutic efficacy of chloroquine for treatment of *Plasmodium vivax* malaria cases in Halaba district, South Ethiopia. Parasit Vectors. 2011;4:46.
24. Hwang J, Alemayehu BH, Reithinger R, Tekleyohannes SG, Teshi T, Birhanu SG, et al. In vivo efficacy of artemether-lumefantrine and chloroquine against *Plasmodium vivax:* a randomized open label trial in central Ethiopia. PLoS ONE. 2013;8: e63433.
25. Assefa M, Eshetu T, Biruksew A. Therapeutic efficacy of chloroquine for the treatment of *Plasmodium vivax* malaria among outpatients at Hossana Health Care Centre, southern Ethiopia. Malar J. 2015;14:458.
26. Thriemer K, Degaga TS, Christian M, Alam MS, Rajasekhar M, Ley B, et al. Primaquine radical cure in patients with *Plasmodium falciparum* malaria in areas co-endemic for *Plasmodium falciparum* and *Plasmodium vivax* (PRIMA): a multicentre, open-label, superiority randomised controlled trial. Lancet. 2023;402:2101–10.
27. Win KM, Aung PL, Ring Z, Linn NYY, Kyaw MP, Nguitragool W, et al. Interventions for promoting patients' adherence to 14-day primaquine treatment in a highly malaria-endemic township in Myanmar: a qualitative study among key stakeholders. Malar J. 2023;22:302.
28. Moltot T, Bekele G, Gebreegziabher ZA, Lemma T, Sisay M, Silesh M, et al. A five years malaria surveillance data analysis of North Shewa zone, Amhara region, Ethiopia: July 2018 to June 2023. Malar J. 2024;23:187.
29. WHO. A framework for malaria elimination. Geneva, World Health Organization, 2017.
30. Yeshiwondim AK, Tekle AH, Dengela DO, Yohannes AM, Teklehaimanot A. Therapeutic efficacy of chloroquine and chloroquine plus primaquine for the treatment of *Plasmodium vivax* in Ethiopia. Acta Trop. 2010;113:105–13.
31. Rowland M, Durrani N. Randomized controlled trials of 5- and 14-days primaquine therapy against relapses of vivax malaria in an Afghan refugee settlement in Pakistan. Trans R Soc Trop Med Hyg. 1999;93:641–3.
32. Alvarez G, Pineros JG, Tobón A, Ríos A, Maestre A, Blair S, et al. Efficacy of three chloroquine-primaquine regimens for treatment of *Plasmodium vivax* malaria in Colombia. Am J Trop Med Hyg. 2006;75:605–9.
33. Recht J, Ashley EA, White NJ. Use of primaquine and glucose-6-phosphate dehydrogenase deficiency testing: divergent policies and practices in malaria endemic countries. PLoS Negl Trop Dis. 2018;12: e0006230.
34. Leslie T, Mayan I, Mohammed N, Erasmus P, Kolaczinski J, Whitty CJ, et al. A randomised trial of an eight-week, once weekly primaquine regimen to prevent relapse of *Plasmodium vivax* in Northwest Frontier Province, Pakistan. PLoS ONE. 2008;3: e2861.

35. Longley RJ, Sripoorote P, Chobson P, Saeseu T, Sukasem C, Phuanukoonnon S, et al. High efficacy of primaquine treatment for *Plasmodium vivax* in Western Thailand. Am J Trop Med Hyg. 2016;95:1086–9.
36. Commons RJ, Rajasekhar M, Edler P, Abreha T, Awab GR, Baird JK, WorldWide Antimalarial Resistance Network (WWARN) Vivax Primaquine Dosing Efficacy, Tolerability and Safety Study Group, et al. Effect of primaquine dose on the risk of recurrence in patients with uncomplicated *Plasmodium vivax*: a systematic review and individual patient data meta-analysis. Lancet Infect Dis. 2024;24:172–83.
37. Smith ER, Klein-Schwartz W. Are 1–2 dangerous? Chloroquine and hydroxychloroquine exposure in toddlers. J Emerg Med. 2005;28:437–43.
38. Popovici J, Tebben K, Witkowski B, Serre D. Primaquine for *Plasmodium vivax* radical cure: what we do not know and why it matters. Int J Parasitol Drugs Drug Resist. 2021;15:36–42.
39. Kotepui M, Punsawad C, Kotepui KU, Somsak V, Phiwklam N, PhunPhuech B. Prevalence of malarial recurrence and hematological alteration following the initial drug regimen: a retrospective study in Western Thailand. BMC Public Health. 2019;19:1294.
40. White NJ. Anaemia and malaria. Malar J. 2018;17:371.

Publisher's Note