INSTITUTE OF HEALTH SCIENCES

DEPARTEMENT OF PHARMACY

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Background

Malaria is a life-threatening disease caused by a parasite called Plasmodium. It is transmitted to humans through the bite of infected female Anopheles mosquitoes. Once inside the human body, the parasites multiply in the liver and then infect red blood cells, leading to destruction of the cells and the release of more parasites into the bloodstream.

The symptoms of malaria include fever, headache, chills, and flu-like symptoms such as sweating, muscle aches, and fatigue. In severe cases, malaria can cause organ failure, seizures, coma, and death.

Malaria is a major health problem in many parts of the world, particularly in sub-Saharan Africa, where it is a leading cause of death among children under the age of five. However, it is preventable and treatable with the use of antimalarial drugs, insecticide-treated bed nets, and indoor spraying of insecticides.

Efforts to control and eliminate malaria have been ongoing for many years, with the goal of reducing the burden of the disease and ultimately eradicating it. These efforts include the development of new drugs and vaccines, as well as the implementation of prevention and control measures in affected areas.

Uncomplicated malaria is a type of malaria that is not severe and has not yet affected vital organs like the brain or kidneys. It is caused by the Plasmodium parasite and is transmitted through the bite of infected mosquitoes.

Plasmodium Vivax is a type of malaria parasite that causes uncomplicated malaria, which can be treated with anti-malaria drugs such as Chloroquine and Primaquine. However, there have been concerns about the emergence of drug-resistant strains of Plasmodium Vivax, which has led to the need for studies on the therapeutic efficacy of these drugs. Several clinical trials have been conducted to evaluate the effectiveness of Chloroquine and Primaquine in combination for treating uncomplicated Plasmodium Vivax malaria. These studies have shown that the combination therapy is highly effective in clearing the parasites from the blood and preventing

relapses. However, the optimal dosages and duration of treatment for this combination therapy are still being investigated to improve its therapeutic efficacy.

The treatment of uncomplicated malaria involves the use of antimalarial drugs such as Chloroquine, Artemisinin-based Combination Therapy (ACT), and Quinine-based regimens. The choice of drug and treatment regimen depends on the species of the malaria parasite, the severity of the infection, and the patient's age and weight.

Antimalarial drugs work by killing the parasites in the blood and preventing them from multiplying. They are usually administered orally, and the duration of treatment varies depending on the drug used and the severity of the infection.

In addition to drug treatment, supportive care such as hydration and management of symptoms like fever, headache, and nausea is also important in the treatment of uncomplicated malaria. Regular monitoring of patients is also necessary to ensure the effectiveness of treatment and detect any complications that may arise.

Monitoring the therapeutic efficacy of antimalarial drugs is crucial for ensuring effective treatment of uncomplicated malaria. It involves the regular evaluation of the clinical and parasitological response of patients to the drugs. This is done through clinical examination, laboratory tests, and follow-up visits to assess the patient's symptoms and the presence of malaria parasites in the blood.

The World Health Organization (WHO) has developed standardized protocols for monitoring the therapeutic efficacy of antimalarial drugs, which involve conducting clinical trials to evaluate the effectiveness of the drugs in different regions. These trials include evaluating the parasite clearance rate, the fever clearance time, and the rate of treatment failure.

Regular monitoring of therapeutic efficacy is essential in detecting the emergence of drug-resistant strains of malaria parasites and improving treatment regimens to ensure effective treatment and prevent the spread of drug resistance.

Statement of the Problem

Antimalarial drug resistance is a major barrier to the control and elimination of malaria. The emergence of drug-resistant strains of Plasmodium parasites has significantly impacted the effectiveness of antimalarial drugs in treating and preventing malaria.

The burden of antimalarial drug resistance is particularly high in sub-Saharan Africa, where the majority of malaria deaths occur. Resistance to Chloroquine, once the most widely used antimalarial drug, is now widespread in many parts of Africa and other regions. Resistance to other drugs such as Sulphadoxine-pyrimethamine, Amodiaquine, and Artemisinin-based Combination Therapy (ACT) has also been reported in some regions.

The emergence of drug resistance has increased the cost of malaria treatment and prevention, as alternative drugs and combination therapies must be used. It also poses a challenge to malaria control efforts, as it reduces the effectiveness of interventions such as insecticide-treated bed nets and indoor residual spraying.

Efforts to combat antimalarial drug resistance include the development of new drugs and combination therapies, as well as the strengthening of surveillance and monitoring systems to detect and respond to drug resistance.

Despite the widespread use of Chloroquine and Primaquine in combination for the treatment of uncomplicated Plasmodium Vivax malaria, there is still a need to investigate the optimal dosages and duration of treatment to improve therapeutic efficacy. The emergence of drug-resistant strains of *Plasmodium Vivax* also poses a challenge to the effectiveness of this combination therapy. Therefore, there is a need to conduct further studies to evaluate the therapeutic efficacy of Chloroquine and Primaquine in combination and to monitor the emergence of drug-resistant strains to improve the management of uncomplicated Plasmodium Vivax malaria.

METHODS

Study Area and Period

The study will be carried out from December, 2022 to March, 2023 in Shecha Health Center, Arbaminch Town, South West Ethiopia, 2022/23. Shecha Health Center is found in Arbaminch Town,Southern Nations, Nationalities and Peoples of Ethiopia regional state, South West Ethiopia.

Shecha Health Center is found in Arbaminch Town Administration which will be established in 1961 as a clinic and Upgraded to Health Centre in 2004.Currently the health center serving more than 31,434 populations. Among this, 15,654 were male and 15,780 were female. Shecha Health Centre offers a variety of service delivery units and clinics, including an emergency room, a unit for children under five, an adult outpatient unit, a unit for maternity and child health, a unit for young people, a laboratory unit, a pharmacy unit, a central medical store, an ART clinic, and a TB clinic.

Study Design

This Therapeutic Efficacy study is a prospective evaluation of clinical and parasitological responses to directly observed treatment for uncomplicated vivax malaria (51,52). People with uncomplicated plasmodium vivax malaria who meet the study inclusion criteria will be enrolled and treated on site with Chloroquine plus Primaquine for *p.vivax* infection and monitored up to 42 days.

The follow-up will consist of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the patients will be classified as having therapeutic failure (early or late) or an adequate response.

The proportion of patients experiencing therapeutic failure during the follow-up period will be used to estimate the efficacy of the study drugs. Molecular analyses will be used to distinguish between a true recrudescence due to treatment failure and episodes of reinfection as well as determine polymorphisms of known resistance markers. In addition, drug level testing may be conducted depending on the availability of resources. .

Population

Source Population

All Malaria Patients who visit Shecha Health Centre during the study period.

Study Population

Confirmed mono *p.vivax* malaria patients visiting Shecha Health Centre during the study period who fulfilled the inclusion criteria set by the WHO protocol for the assessment of the therapeutic efficacy of Chloroquine plus Primaquine.

Eligibility Criteria

Inclusion Criteria

- ♣ Age > 6 months
- ♣ Slide confirmed infection with P. vivax with > 250 asexual forms/µl
- ♣ Lives within 5 km of the enrolling health facility
- ♣ Weight ≥ 5.0 kg
- ♣ Ability to swallow oral medication
- ♣ Ability and willingness to comply with the protocol for the duration of the study and to comply with the study visit schedule
- ♣ Informed consent from patient or from a parent or guardian in the case of children

Exclusion Criteria

- ♣ Sever malaria with complication sign and symptoms (see Annex I)
- ♣ Signs or symptoms of severe malnutrition, defined as weight-for-age ≤ 3 standard deviations below the mean, symmetrical edema involving at least the feet, or mid-upper arm circumference <100 cm for children less than five years of age (NCHS/WHO normalized reference values; see Annex I)
- ♣ Mixed plasmodium infection
- ♣ Severe anemia, defined as hemoglobin (Hb) < 8 g/dl

- ♣ Presence of febrile conditions caused by diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhea with dehydration)
- ♣ Serious or chronic medical condition (e.g. cardiac, renal, hepatic diseases, sickle cell disease, HIV/AIDS)
- ♣ Positive pregnancy test or breastfeeding
- ♣ Unable or unwilling to take contraceptives for women of child-bearing age
- ♣ Children weighing less than 5 kilograms
- ♣ History of hypersensitivity reaction to any medication tested or used as an alternative treatment
- ♣ Participants with history of prolonged QT conditions
- ♣ Taking regular medication, which may interfere with antimalarial pharmacokinetics or efficacy (see Annex VII)?

Sample Size Determination and Sampling Technique

Sample Size Determination

The sample size will be determined according to the WHO (2009) protocol(11); by using the single population proportion formula and calculated assuming 5% margin of error, 95% confidence interval (CI), and treatment failure of 5% and 20% loss to follow-up rate. Accordingly, a minimum of 73 patients will be enrolled.

$$\text{Sample size (n)} = \frac{[Z\ a/2)^2\ p(1-p)}{d\text{^}2} \qquad \frac{(1.96)^2\ 0.05\ (1\text{-}0.05)}{(0.05)^2} = 73$$

Assuming an additional 20% loss to follow-up rate and withdrawal of consent (15 patients) during the study, at least 88 (73 + 15) patients will be required to bring about a representative sample size.n= (1+0.2) 73= 88 Where, n= sample size

P=Expected treatment failure (5%)

Z=confidence interval (95%)

d=margin of error (5%)

Sampling Technique

Convenient sampling technique will be used to collect the selected sample size. Samples will be taken from all the patients with positive plasmodium vivax who fulfill the inclusion criteria.

Study Variables

Independent Variables

- Age, Sex,Weight, Axillary temperature, leukocyte count, asexual and/ or sexual parasites count, Hemoglobin count

Dependent Variables

- Therapeutic Efficacy of CQ+PQ

Operational Definitions and Definition of Terms

Loss to Follow-Up

Loss to follow-up occurs when, despite all reasonable efforts, an enrolled patient does not attend the scheduled visits and cannot be found. No treatment outcome will be assigned to these patients. Every effort must be made to schedule a follow-up visit for patients who fail to return to the study site, especially during but also after administration of the study drug. These patients will be classified as lost to follow-up and censored or excluded from the analysis. Patients who are lost to follow-up but who subsequently return to the study site before day 42 will not be turned away and will be encouraged to return for check-up visits. The principal investigator will decide whether the patient is to be classified as lost to follow-up based on his or her history or is to be maintained for the analysis.

Patient Discontinuation or Protocol Violation

Patients meeting any of the following criteria will be withdrawn:

- Withdrawal of consent
- Failure to complete the treatment
    - Vomiting both initial and replacement doses at any single time in the treatment (i.e. If the patient vomits both attempts to administer the morning dose that would

require withdrawal; however, vomiting the initial morning dose but not the morning replacement dose would not require withdrawal), persistent vomiting
    - Severe side-effects necessitating hospitalization
    - Progression to severe malaria
    - Failure to attend the scheduled visit during the first 3 days
- Enrolment violation
    - Severe malaria on day 0
    - Erroneous inclusion of a patient outside of the inclusion/exclusion criteria
- Voluntary protocol violation: Antimalarial (or antibiotics with antimalarial activity) treatment administered by a third party or self-medication with antimalarial (or antibiotics with antimalarial activity) assessed by asking participants or their caretaker at follow-up visits (Annex VI)
- Involuntary protocol violation
    - Occurrence during the follow-up of concomitant disease that would interfere with a clear classification of the treatment outcome
    - Detection of a mono-infection with another malaria species during follow-up
    - Misclassification of a patient due to a laboratory error (parasitemia) leading to the administration of the rescue treatment

Treatment

Antimalarial Treatment

P.vivax patients will receive CQ+PQ. The correct drug doses will be determined by the dosing chart (Annex III). All drugs administered in the facility will be under supervision by the staff. Patients will be observed for 60 minutes after treatment for adverse reactions or vomiting. Those patients vomiting their medication within the first 30 minutes will receive a repeat full dose, while those vomiting from 30-60 minutes will receive half dose. Patients who vomit twice will be referred to higher level of care for management with parenteral artesunate therapy and withdrawn from the study. This is standard practice for anti-malarial treatment. All study drugs will be obtained from WHO Ethiopia office through Ethiopian Pharmaceutical Supply Agency.

Concomitant Treatment

Using standard clinic procedures, clinic personnel will administer supportive treatment to patients as necessary: antipyretics will be given for temperatures > 38˚C. All patients enrolled in the study will be given two additional doses of paracetamol for use at home. Patients/parents/guardians will be instructed in the use and application of tepid sponging and fanning.

Rescue Treatment

Patients failing in any arm will be treated with second line drug per National Guidelines. Whereas, patients failing with the second line will be administered as of severe malaria criteria that is an indication of treatment failure.

The following are the known efficacy and adverse effects and pharmacokinetics of treatment drugs that will be used in the study:

Chloroquine Plus Primaquine (CQ+PQ): Chloroquine Plus Primaquine is a WHO Pre-qualified anti-malarial and FDA approved; WHO certifies the quality, safety and efficacy of the medication, and verifies that the manufacturer complies with WHO Good Manufacturing Practices. CQ+PQ is currently the first line anti-malarial recommended by Ethiopia FMOH for the treatment of uncomplicated falciparum infection. Adverse events are generally mild, most commonly GI (vomiting and diarrhea) and hematologic (anemia and eosinophilia).Its safety will be demonstrated in studies from Ethiopia and Uganda. Chloroquine has a half-life of 30-60 hours (53,54), while primaquine has a elimination half-life of 5-7 hours (54).

Data Collection Instrument and Procedure

Data Collection: Data will be collected and recorded at the health facility by trained study staff members. All data entry will be checked for completeness. Laboratorians may complete brief reports on laboratory findings to share with clinicians who will transfer these results to the case report form of the appropriate patient, and the laboratory results will be entered into database.

Data Entry, Editing, Handling, Storage and Disposition: Some open-ended data including the names of specific drugs or diagnoses will be coded prior to data entry. A list of standard codes for these fields will be developed by hand once collection of data is complete. All forms will

then be coded and checked by two different team members.

Systematic routines will be developed to check for data entry discrepancies and range and consistency. If collected on paper, all discrepancies will be resolved by reference to the original checked data collection forms. The final data set will be transformed to statistical software for analysis.

Storage of Specimens: Specimens obtained in this study will be saved for the duration of specified testing. Specimens will not be tested for HIV.

Quality Control/ Assurance: Site supervisors will check data entry at the end of follow-up for completeness and accuracy of recording. Five percent of the slides will be read by WHO qualified microscopists for quality control.

Personnel Training: Three days TOT will be provided for the central team prior to the study. Study clinicians and laboratory personnel are trained and licensed in Ethiopia. They will also receive approximately 1 week additional training to review and familiarize with the study protocol. In addition to technical aspects of specific laboratory techniques and clinical assessments, training will stress the importance of confidentiality and appropriate techniques for obtaining informed consent. The Lab staff will participate in practical exercises. Continued appropriate behavior with regard to informed consent will be reinforced through observation by the supervisor. The staff will include 1-2 laboratory technicians for microscopy and 1-2 staff persons for enrollment and clinical assessments.

Bias in Data Collection, Measurement and Analysis: Some bias may be introduced through the fact that both treating clinicians and parents will be aware of which treatment a subject received; as much as possible we will rely on objective measures of improvement (fever resolution, malaria parasitemia) which will be unaffected by knowing the treatment allocation. In addition, the microscopists reading the slides will be blinded to the treatment allocation. Other biases in data collection will be minimized by use of standardized data collection instruments. The principal investigator will ensure that the study protocol is strictly adhered to and that all data are collected and recorded correctly on the case report form. Laboratory and clinical data will be recorded daily on the case report form designed for the study. Data derived from source

documents should be consistent with the source documents, or the discrepancies should be explained. Any change or correction to a case report form should be dated and explained and should not obscure the original entry. All case report forms will be checked for completeness.

After the study has been completed, data will be entered into a database and/or by double independent data entry. The trial data will be stored in a computer database and confidentiality will be maintained.

The principal investigator is responsible for keeping all screening forms, the case report form and the completed subject identification code list in a secure location.

Evaluation Criteria

Efficacy and Safety Evaluation

Classification of Treatment Outcomes

The primary outcome of this study will be to monitor the efficacy of AL for P. falciparum and CQ for P. vivax as a routine follow up study and develop baseline and complementary efficacy data for DHA-PPQ against P. falciparum and P. vivax malaria in Ethiopia.

The classification of treatment outcomes will be based on an assessment of the parasitological and clinical outcome of antimalarial treatment according to the latest guidelines from WHO. Accordingly, all P. falciparum and P. vivax patients will be classified as having an ETF, LCF, LPF, or ACPR (Annex I).

Safety endpoints

The incidence of any adverse event will be documented. All patients will be asked routinely about previous symptoms and about symptoms that have emerged since the previous follow-up visit. When clinically indicated, patients will be evaluated and treated appropriately. All adverse events will be recorded on the case report form. Serious adverse events will be reported to all relevant authorities.

Clinical Evaluation

All patients will be evaluated clinically as described below.

Physical Examination

A standard physical examination will be performed at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28, 35, and 42. A complete medical history, demographic information, and contact details will be recorded at baseline.

Body Weight

Body weight will be recorded on day 0 to the nearest kilogram on a scale or on a hanging scale for young children. The scales will be properly calibrated. Patients should not wear excessive clothing while being weighed as this can overestimate their true weight. All young children should only wear undergarments while being weighed. The screening weight will be used to satisfy the inclusion or exclusion criteria for weight and nutrition status as well as to calculate the dose (number of tablets) to be administered. The reliability of the scales will be verified before the study begins and checked at regular intervals.

The circumference of the left mid-upper arm will be measured, at the mid-point between the elbow and the shoulder and will be recorded to the nearest 0.2 cm. Oedema will be assessed by thumb pressure for 3 seconds on the dorsal surface of both feet.

Body Temperature

Axillary temperature will be measured at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28, 35, and 42. Temperature will be measured with a thermometer that has a precision of 0.1 °C. Temperature will also be measured as clinically indicated. If the result is < 36.0 °C, the measurement will be repeated. The same route will be used throughout the study. The quality of the temperature-taking technique and the thermometers will be assessed regularly.

Laboratory Examination

Microscopic blood examination: thick and thin blood smears will be taken from all participants and prepared on the same slide for detection of parasites at all time-points during the 42-day follow-up period. Two smears will be prepared in each case; the first will be stained rapidly with 10% Giemsa for 10–15 min for initial screening and examined by light microscopy immediately. On day 0, in order to rapidly confirm adherence to the lowest parasite density considered for enrollment, initial screening of patients will be made in 10% Giemsa-stained thick film after counting at least 1 parasite for every 6-8 WBCs, which corresponds to approximately 1000 parasites/µl.

The second one will be stained slowly with 3% Giemsa 45–60 min to be examined later by an experienced technician to provide definitive parasite count and speciation. The below formula will be used to determine parasite density.

$$\text{Parasite density (per } \mu\text{l)} = \frac{\text{number of parasites counted} \times (6000–8000)}{\text{Number of leukocytes counted} \approx 200}$$

Two experienced laboratory technicians will examine slides independently. Parasite densities will be calculated from thick blood smears by counting the number of asexual parasites against 200 WBC. If the count is <10 parasites/200 WBC, at least 500 WBC will be counted. A thick blood smear will be declared negative if no parasites are seen in 1000 WBCs and mixed infection has been excluded in 100 fields on thick film. Gametocytes will be detected and counted in thick film. Thin blood smears will be examined to determine parasite species. Gametocytes will be counted in thick blood films against 2000 WBCs.

Two qualified microscopists will read all the slides independently, and parasite densities will be calculated by averaging the two counts. Blood smears with discordant results (differences between the two microscopists in species diagnosis, in parasite density of > 50% or in the presence of parasites) will be re-examined by a third, independent microscopist, and parasite density will be calculated by averaging the two closest counts.

Hemoglobin: The hemoglobin concentrations will be measured using a portable spectrophotometer (HemoCue®, Anglom, Sweden), on days 0, 14, 28, and 42.

Blood sample collection: To minimize discomfort to the patient due to repeated finger pricks, all samples will be collected from a single prick. Two slides, hemoglobin and two to three drops of blood will be collected on Whatman filter paper and 100µl of blood using capillary tube will be collected during enrolment. Blood spots will be collected only on enrollment, on day 3, 7 and during treatment failure (Annex: VI)

Specimens will be labeled anonymously (study number, day of follow-up, date), kept in individual plastic bags with desiccant pouches and protected from light, humidity and extreme temperature until analyzed. Samples will be kept in freezers in the study sites and eventually transferred to EPHI, to be frozen at -20ºC.

Pregnancy Test: Female patients of child-bearing age, defined as those who menstruate or are aged over 18 years (depending on situations), will be asked to take a urine pregnancy test before enrolment in the study. They will also be asked to take a urine pregnancy test on day 28/42 or on

early withdrawal from the study. Female participants of child-bearing age who are sexually active to control pregnancy for the duration of the study.

Safety Assessment

Safety will be assessed by recording the nature and incidence of adverse events and serious adverse events. Adverse events will be assessed by direct questioning. An adverse event is defined as any unfavourable, unintended sign, symptom, syndrome or disease that develops or worsens with the use of a medicinal product, regardless of whether it is related to the medicinal product. All adverse events must be recorded on the case report form. A serious adverse event is defined as any untoward medical occurrence that at any dose:

- results in death, is life threatening
- requires hospitalization or prolongation of hospitalization
- results in a persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect.

'Life-threatening' means that the person will be at immediate risk for death; it does not refer to an adverse event that might have caused death if it were more severe. 'Persistent or significant disability or incapacity' means that a person's ability to carry out normal life functions is substantially disrupted. All serious adverse events occurring during the study must be recorded and reported by the principal investigator to the sponsor, regardless of whether the principal investigator considers the events to be related to the investigated medicine. The investigator will collect information on all people who become pregnant while participating in this study and will record the information on the appropriate form. The person will also be followed to determine the outcome of the pregnancy.

Emergency Care

Any patient with treatment failure or recrudescence will receive second line drugs. This will be given orally unless the patient has persistent vomiting, in which case it will be given Artesunate IM/IV until the vomiting resolves and then will be switched to oral therapy.

Patients with signs of severe malaria (altered mental status, coma, convulsions, hemoglobin <5g/dl, respiratory distress, circulatory collapse, or abnormal bleeding), persistent vomiting or

severe side effects will be hospitalized and receive parenteral therapy with artesunate and relevant supportive treatments, according to National Guidelines.

We will counsel patients and parents to return immediately if they or their child develops a raised rash (urticaria) or difficulty breathing suggestive of a severe allergic reaction. These patients will be treated with diphenhydramine or chlorpheniramine and will be withdrawn from the study. In addition, patient and or their parents, caretakers and family members will be counseled that the patient should not receive additional doses of the antimalarial they were given. These patients will be treated with oral quinine for seven days as per the rescue protocol.

Adult patients and parents with children in the study will be encouraged to return to the clinic for any fever or other signs of illness on any day during the 42-day follow-up period, allowing for early detection and treatment of illness before it becomes severe. Any hospitalization which occurs during the 42-day follow-up period will be paid by the study team.

Response to unexpected incidents

If sufficient numbers of malaria cases are presenting to the clinic but enrolment is insufficient, the supervisor will need to determine the problem and take needed actions to rectify the problem. In case of low patient recruitment due to unusually low transmission of malaria, necessary steps will be taken to either rectify the problem or to find a new site.

Screening and Enrollment Procedures

Screening

Screening for eligible patients at the study sites will be done in the following fashion:

1. All patients over 6 months with temperature $\geq 37.5^{o}C$ or a history of fever in the last 48 hours will have screening blood smear performed as per the national diagnosis and treatment guidelines at the health centers. Patients with microscopy confirmed P. falciparum or P. vivax, age greater than 6 months, and living within 5 km of the health center will be screened.
2. For all facilities, if the patient meets the screening criteria and the patient or the parent or guardian consents to enrollment, they will be assigned a consecutive, unique number that will become the patient's study number and will be used to identify all forms and blood samples from that patient. Enrollment will be conducted by a staff member not involved in

the clinical assessment of the participant to ensure that consent is freely given.

3. A screening form containing demographic, clinical, and laboratory information will be started on each smear positive patient, thus allowing for appropriate tracking of each patient through the screening procedure.
4. The patient's body weight will be measured to the nearest kilogram, using a hanging scale for weighing young children, or a floor scale for large children/ adults and recorded on the demographic form. The circumference of the left upper mid-upper arm will be measured at the mid-point between the elbow and shoulder, and will be recorded to the nearest 0.2 cm. Edema will be assessed by thumb pressure for 3 seconds on the dorsal surface of both feet.

Enrollment and Follow-up

If a patient meets the initial screening criteria and permission to participate in the study is obtained, the patient will go through the enrolling process.

1. The patient will be evaluated by clinical staff for signs of febrile illness other than malaria, (including but not limited to pneumonia, otitis media, tonsillitis, measles, chicken pox, abscesses) and signs of severe disease/danger signs, as presence of any of these will exclude the patient from participating in the study. Patients found to have any of these conditions will be treated appropriately per National Guidelines, and referred to the referral hospital as necessary. A standardized form listing demographic and clinical information will be completed on each patient.
2. Urine pregnancy test will be conducted on all women of reproductive age. Anyone testing positive will be excluded.
3. Those enrolled will have a second finger prick performed to assess for the following:
    - Thin smear will be used to verify parasite species and to conduct a formal parasite count. Rapid test will also be done for better speciation. Two microscopists will independently read each smear; if the 2 readings are >50% discordant, a third expert microscopist will examine the slide and make the final decision.
    - Hemoglobin measurement (Hemocue™ Hb301+; HemoCue, Angelholm, Sweden) will be carried out and those with hemoglobin <5g/dL will be excluded.
    - Filter paper will be obtained for molecular testing and drug levels.
4. Participants testing positive for P. Vivax will receive CQ+PQ. A Convenient sampling technique will be used. The study will not be blinded.

5. After assignment, on day 0, each patient will receive the dose of treatment medication under direct observation and will be monitored for vomiting. Each patient will return on days 1 and 2 for subsequent doses. Clinical reassessments will be made on days 1- 3, 7, 14, 21, 28, 35, and 42 (see Annex VI). Participants will be given 100 Ethiopian Birr for each visit to cover their travel expenses (this is a compensation for a round-trip transportation to the clinic). Patients will be advised to return on *any* day during the follow-up period if symptoms return and not to wait for scheduled visit days. Patients who do not present to the facility for follow-up visits will be followed at home. This is standard for *in vivo* studies based on the WHO protocol, but is not the standard for routine care. Typically, for patients not enrolled in a study, the parent would be given all the doses of the medicine to administer at home, and no follow-up would be scheduled. The parent would be told to return if they did not improve.
6. Blood films (thick and thin) for parasite count and dried blood spot will be obtained and examined as described above. All samples will have the study ID number and date of collection, but no other personal identifiers. The logbook that links the study ID number to the patient will be kept at the study site locked in a cabinet when not in use during the study enrollment period. After the completion of the study, this logbook will be stored at EPHI.
7. If patients do not appear for scheduled follow-up, the study team will attempt to reach them at home. After day 3, patients who fail to return on their scheduled day but return one day early or one day late may still be included in the analysis.
8. A case report form will be used to record the general information and clinical observations on each patient enrolled into the study. The appointment schedule will be clearly explained, and a follow-up card with a personal identification number will be provided. The study schedule (Annex VI) and follow-up schedule is summarized in (Annex VII).

Storage of specimens: Specimens obtained in this study will be saved for the duration of specified testing. Specimens will not be tested for HIV.

Data Management and Monitoring

Enrollment and case record forms will be completed for each study subject. Their initials and study code on the case record form will be used to identify the participants. The principal investigator will be responsible to complete case record forms at each visit. All corrections will

be made on the case record form by striking through the incorrect entry with a single line and entering the correct information adjacent to it.

All clinical data will be recorded onto standardized case record forms by the principal investigator. Laboratory data will be recorded in a laboratory record book by the laboratory technician and then transferred to the case record forms by the principal investigator. Data will be transferred from the case record forms into a computerized database (IBM SPSS Version 26, WHO excel sheet and plain excel spread sheet). The WHO excel sheet is specially designed for the analysis of efficacy study data and it only performs the analysis when double entry is assured to verify accuracy of entry. Two backup files and the database will be stored on compact discs after each data entry session.

Members of the central team (co-advisor) from EPHI will supervise the overall study progress of the study and reviewed all data records at the mid of the study period. All study record forms will be checked for completeness and accuracy. In addition, daily communication will be made with the members of the central team (co-advisor, other study team members).

Data Quality Control

The health personnel's (Medical Doctor, Health Officer, laboratory technician, and nurse (tracer)) involved in the study will be oriented on the use of the study procedure; following the in vivo study protocol designed by the World Health Organization WHO). Prior to the study, the laboratory technician will have a refresher training to ensure proper preparation of blood smear, correct identification of parasites and accurate parasite counting. Giemsa stock solution and working solution will be prepared at EPHI laboratory.

Microscopic results will be assessed following the procedure that emphasizes reproducibility of final outcome classification over reproducibility of exact parasite count s by crosschecking 10% of the total slides. To avoid errors in recording data, the study supervisors on a regular basis will review all case report forms during the assessment for completeness and accuracy.

Data will be entered into compute double entry using WHO data analysis excel sheets and SPSS version 26 and a random sample of 10% of computerized records will be selected and compared to hard copy case report forms for confirmation of consistency.

Statistical Analysis

Every data from recruited patients will be imported into the WHO Excel sheet (double entry) which is designed for analysis of therapeutic efficacy study data. Data will be also entered in to IBM SPSS (version-26) software to calculate descriptive statistics (mean, median, standard deviations, range). Independent sample t-test will be used to compare baseline temperature and parasitemia between children and adults; mean blood Hb level at D0, D14, D28 and D42 between patients with parasitemia ≥ 10,000 and < 10,000/µl blood. Non-parametric (median) test for independent sample will be used to compare D1 parasitemia between children and adults and paired sample t-test will be used to compare mean Hb level between D0 and D14, D0 and D28, D14 and D28,D0 and D42,D28 and D42. All comparisons will be performed at 95% CI and significance level of 0.05.

Kaplan Meier (K-M) survival analysis and per protocol (PP) analysis will be used for estimation of primary outcomes and PP analysis method will be used to analyze secondary outcomes. The K-M survival analysis method provides better approximation of cure rates as it incorporates probabilities for censored data (incomplete observations due to LFU and withdrawals) in to the analysis. The WHO excel sheet is especially designed for estimation of cure rate based on K-M survival estimator analysis method.

Ethical Considerations

All consented participants will be part of the study. Written informed consents will be obtained from all study participants (parents or caregivers for children under the age of twelve) after thorough information on the study will be provided in the local language. Name of study participants will not be mentioned and incorporated in to the final dataset.

Medical services will be provided for volunteer study participants free of charge during the follow-up period. At all times, patient well-being will be given priority over his/her continuation in the study. In addition, round trip transport fare (100.00 Ethiopia Birr) will be provided for every patient every scheduled visit. The study protocol will be approved by Institute of Health Sciences Institutional Ethics Review Committee,Wallaga University and Ethiopian Public Health Institute Institutional Ethics Review Board.

ANNEXES

Annex 1. Patient Screening Form

Patient Screening Form

STUDY SITE CODE: _____ TREATMENT GROUP: _____

1. Names:	**2. Date:** ***(dd/mm/yy)*** _____/_____/	**3.Weight (kg):**
4. Age*: years_____ months. _____	**5. Gender: M _____ F_____**	

1.	Patient aged > 6 months /Both sex	Yes:	No:
2.	***P. vivax*** mono-infection asexual parasites/µl **Pv** 250-100,000/µl	Yes:	No:
3.	Body weight > 5 kg	Yes:	No:
4.	Patient with fever or history of fever in the previous 24 hours	Yes:	No:
5.	Non-pregnant or breast-feeding female	Yes:	No:
6.	Ability to swallow oral medication	Yes:	No:
7.	Residents living within 5 km radius of the health centre and agree to return for all scheduled follow up visits	Yes:	
8.	Willing to give informed consent	Yes:	No:
9.	Evidence of concomitant febrile illness If "YES", indicate illness. If "NO", leave blank. ə Pneumonia/RTI ə Measles ə Otitis Media ə UTI ə Gastroenteritis Other:	Yes:	No:
10.	Evidence of severe malaria / danger signs If "YES" indicate criteria. If "NO", leave blank. ə Unarousable coma (if after convulsion, > 30 min) ə Repeated convulsions (> 2 within 24 h) ə Recent convulsions (1-2 within 24 h) ə Altered consciousness(confusion, delerium,, coma)	Yes:	No:

	ɜ Lethargy ɜ Unable to drink or breast feed ɜ Vomiting everything ɜ Unable to stand/sit due to weakness ɜ Severe anaemia (Hb < 5.0 g/dL) ɜ Respiratory distress (laboured breathing at rest) ɜ Jaundice (yellow colouring of eyes)		
11.	Known hypersensitivity to AL/CQ/DP	Yes:	No:

If any of the responses fall into the shaded area, exclude the patient from the study

Annex 2. Enrolment Form

Enrolment Form

STUDY SITE CODE: _____ TREATMENT GROUP: _ _____

1. Age……..	2. Weight……	3. Gender/Male……Female……
4. PIN/Study Number:……	5. Number of tablets……	6. Start Date: (dd/mm/yy)

7. Patients Full name:
8. Family head:
9. Mother's/Wife's (if married) name:
10. Caregiver's name and relationship:
11.Kebele/Street:
12. Home parish:
13 Village:
14. Home address and localising features/Owners name/Direction:
15. Phone number (s) and the owner(s):

16. Previous malaria attack: Yes _____No _____

17. Previous antimalarial intake: Yes _____No _____ If yes, CQ____ AL_____?

18. Hold Bed net: Yes ___No___ If yes, Bed net use Yes _____ No _____

Annex 3. Case Record Form

CASE RECROD FORM

Study site _____Treatment Group_____Name_____Pin No. _____ No. of Tablets_____.

Barcode no on it. Fill all follow-up days on the first day to trace

	day 0	**day 1**	**day 2**	**day 3**	**day 7**	**day 14**	**day 21**	**day 28**	**day 35**	**day 42**	**Extra DAY**
1. Date											
2.Axillary To C											

3. Parasite asexual											
4.Gametocyte No											
5.Hemoglobin											
7.DBS/PCR											
8.Adverse events*	It is not drug related adverse event										
9.Concomitant treatment											
10.Reasons for withdrawal											
11.Remarks/ Rare Event	Complete Annex 27										
12.Treatment outcome											
Completed by (Initials)											

***1. Headache 2. Anorexia 3. Nausea 4. Vomiting 5. Abdominal pain 6. Diarrhea 7. Cough 8 .Behavioral change 9. Dizziness 10. Skin rash 11. Mouth ulcer 12. Joint pain 13. Weakness 14. Other specify_____**

ANNEX 4: Classification of Treatment Outcomes (WHO, 2009)

Classification of treatment outcomes for plasmodium vivax malaria WHO

Early Treatment Failure (ETF)

- Danger signs or severe malaria on day 1, day 2 or day 3 in the presence of parasitemia;
- Parasitemia on day 2 higher than on day 0, irrespective of axillary temperature;
- Parasitemia on day 3 with axillary temperature ≥37.5 ºC;
- Parasitemia on day 3 ≥25% of count on day 0.

Late Treatment Failure (LTF)

Late Clinical Failure **(LCF)**

- Danger signs or severe malaria in the presence of parasitemia on any day between day 4 and 42 in patients who did not previously meet any of the criteria of Early Treatment Failure;
- Presence of parasitemia on any day between 4 and day 42 with axillary temperature ≥37.5 ºC (or history of fever) in patients who did not previously meet any of the criteria of Early Treatment Failure.

Late Parasitological Failure **(LPF)**

- Presence of parasitemia on any day between day 7 and day 42 and axillary temperature <37.5 ºC in patients who did not previous meeting any of the criteria of Early Treatment Failure or Late Clinical Failure.

Adequate Clinical and Parasitological Response (ACPR)

- Absence of parasitemia on day 42 irrespective of axillary temperature, in patients who did not previously meet any of the criteria of Early Treatment Failure, Late Clinical Failure, or Late Parasitological Failure.

Definition of Treatment Outcomes for P. vivax infections, WHO 2009

Treatment success:

the absence of parasitaemia on day 28/42, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of treatment failure.

Treatment Failure (TF)

Clinical deterioration due to *P. vivax* illness requiring hospitalization in the presence of parasitemia

Presence of parasitemia and axillary temperature ≥37.5°C on any day between day 3 and day 28/42

Presence of parasitemia on any day between day 7 and day 28/42, irrespective of clinical condition

ANNEX 5: Drug Dosing and Regimens

All patients will be weighed to determine the accurate weight-based dose for all drugs.

- **Chloroquine:** Total of 25mg base per kg over 3 days (10 mg base/kg on Days 1 and 2, and 5 mg base/kg on Day 3). 500 mg tablets. We will use the syrup (10mg/ml) formulation for children < 4 years of age, if available.

Weight (kg)	**Age**	**Day 1**	**Day 2**	**Day 3**
5–6	<4 mo	¼ tablet (5 ml syrup)	⅛ tablet (5 ml syrup)	⅛ tablet (2.5 ml syrup)
7–10	4–11 mo	¼ tablet (7.5 ml syrup)	¼ tablet (7.5 ml syrup)	¼tablet (5 ml syrup)
11–14	1–2 yrs	½ tablet (12.5 ml syrup)	½ tablet (12.5 ml syrup)	¼ tablet (7.5 ml syrup)
15–18	3–4 yrs	½ tablet (15 ml syrup)	½ tablet (15 ml syrup)	½ tablet (15 ml syrup)
19–24	5–7 yrs	¾ tablet (20 ml syrup)	¾ tablet (20 ml syrup)	½ tablet (15 ml syrup)
25–35	8–10 yrs	1¼ tablet	1¼ tablet	½ tablet
36–50	11–13 yrs	1½ tablet	1½ tablet	1 tablet
> 50	14+ yrs	2 tablet	2 tablet	1 tablet

Primaquine: 7.5 mg base tablet. Medication given as 0.25mg/kg daily for 14 days in people with normal G6PD levels. In people with G6PD deficiency, 0.75 mg/kg (45 mg) once a week for 8 weeks.

Body Weight (kg)	Single dose of primaquine (mg base)
5 to 10	Weight-based using extemporaneous preparation as outlined by Sanofi
10 to < 25	3.75
25 to <50	7.5
50 to 100	15

ANNEX 6: Definitions of Severe Malaria

Impaired consciousness: A Glasgow Coma Score <11 in adults or Blantyre coma score <3 in children

Acidosis: A base deficit of >8 meq/l or, if unavailable, a plasma bicarbonate of <15 mM or venous plasma lactate >5mM. Severe acidosis manifests clinically as **respiratory distress** – rapid, deep and laboured breathing

Hypoglycaemia: Blood or plasma glucose <2.2mM (<40mg/dl)

Severe malarial anaemia: A haemoglobin concentration <5g/dl or a hematocrit of <15%in children <12 years of age (<7g/dl and <20%, respectively, in adults) together with parasite count of > 10,000 µ/l

Renal impairment (acute kidney injury): Plasma or serum creatinine>265 µM (3 mg/dl) or blood urea >20 mM

Jaundice: Plasma or serum bilirubin >50 lM (3 mg/dl) together with a parasite count >100,000 µ/l

Pulmonary oedema: Radiologically confirmed, or oxygen saturation <92% on room air with respiratory rate >30/min, often with chest indrawing and crepitations on auscultation

Significant bleeding: Including recurrent or prolonged bleeding from nose, gums, or venipuncture sites; haematemesis or melaena

Shock: Compensated shock is defined as capillary refill ≥3 seconds or temperature gradient on leg (mid to proximal limb), but no hypotension. Decompensated shock is defined as systolic blood pressure < 70mm Hg in children or <80 mm HG in adults with evidence of impaired perfusion (cool peripheries of prolonged capillary refill)

Hyperparasitemia: *P. vivax*>2%

Annex 7. Medications with Antimalarial Activity that Should not be Used During the Study Period

- Amtimalarials:-Chloroquine, Amodiaquine; Quinine, quinidine;Mefloquine, halofantrine, lumefantrine; Artemisinin and its derivatives (artemether, arteether, artesunate, dihydroartemisinin); Proguanil, chlorproguanil, pyrimethamine;Sulfadoxine, sulfalene, sulfamethoxazole, dapsone;Primaquine; Aovaquone;
- **Antibiotics:**Tetracycline*,Doxycycline,Erythromycin,Azythromycin,Clindamycin ,Rifampicin ,Trimethoprim,Pentamidine
- Tetracycline eye ointments can be used.

ANNEX 8: Study Schedule

Day 0:

Screening

- Clinical assessment including measurement of weight and height — referral in case of severe malaria/danger signs
- Measurement of axillary temperature
- Parasitological assessment
- Informed consent

- Pregnancy test, if indicated

Enrollment

- Treatment, first dose
- Blood sampling for blood smears, hemoglobin and filter paper

Day 1:

- Clinical assessment — referral in case of severe malaria/danger signs
- Measurement of axillary temperature
- Blood sampling for blood smears and filter paper
- Treatment, second dose or alternative treatment in case of early treatment failure

Day 2:

- Clinical assessment — referral in case of severe malaria/danger signs
- Measurement of axillary temperature
- Blood sampling for blood smears and filter paper
- Treatment, third dose or alternative treatment in case of early treatment failure

Day 3, Day 7, Day 14, Day 21, Day 28, Day 35, and Day 42:

- Clinical assessment — referral in case of severe malaria/danger signs
- Measurement of axillary temperature
- Blood sampling for blood smears and filter paper
- Alternative treatment in case of treatment failure
- Hemoglobin/hematocrit (Day 0, Day 14, Day 28, Day 42)

Any other day:

- Clinical assessment — referral in case of severe malaria/danger signs

- Measurement of axillary temperature
- Blood sampling for blood smears and filter paper
- Hemoglobin/hematocrit if indicated clinically
- Alternative treatment in case of treatment failure

ANNEX 9: Schedule of Follow-Up Activities

	day 0	day 1	day 2	day 3	day 7	day 14	day 21	ay 28	ay 35	ay 42	ny other day
PROCEDURES											
Clinical assessment	X	X	X	X	X	X	X	X	X	X	X
Temperature	X	X	X	X	X	X	X	X	X	X	X
Blood slide for parasites count	X	X	X	X	X	X	X	X	X	X	X
Hemoglobin	X					X		X		X	X
Filter paper	X			X	X	(X)	(X)	(X)	(X)	(X)	X
Drug adverse event monitoring	X	X	X	X	X	X	X	X	X	X	X
TREATMENT											
Drug to be given	X	X	X								(X)
Rescue treatment		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

NOTES: Parentheses denote conditional or optional activities. Rescue treatment could be given on any day, provided that the patient meets the criteria for treatment failure. Extra days are any days other than regularly scheduled follow-up days when the patient returns to the facility because of recurrence of symptoms.

Annex 10: Consent Procedure

Categories of consent forms

I. for children **6 months-11 years** old parental/guardian permission will be obtained

II. For patients **12 -17 years old** assent from the patient and parental/guardian permission will be obtained

III. For patients age **18 yrs. and older** adult consent will be obtained

Procedure

1. Explain that he has P.vivax malaria infection and proceed with the consent process,
2. Read and Explain study objectives, procedures, the benefits and risks of taking part in the study.
3. If the person/guardian can read, provide a copy of the consent form in their preferred language and ask them to read the entire document. If they state that they cannot read, review the entire consent form with them using their stated preferred language version (Amharic/Afan Oromo). When finished, ask if they have questions.
4. Consenting participants and or their caretakers will be advised that they are free to decline any question or procedure and that they may terminate their participation at any time without loss of any benefits
5. If they agree to join the study get those to sign the consent form **with a witness (Obtain consent)**. Health worker/home visitor will act as a witness.
6. If the participant/guardian is illiterate, make a thumbprint using the left thumb by rolling the thumb from side to side on an inkpad and again on the consent form.
7. Once the consent form is completed, send it to the clinician to proceed

ANNEX 11: CONSENT/ASSENT FORMS

Consent/Assent Form in English

Title: - Monitoring the efficacy of frontline antimalarial drugs in Ethiopia, 2022/23

Consent form for informing adults (>18) or consent form for parental permission for child six month up to 11 years and assent form for children aged in between 11-17 years old study participants enrolment form for malaria in vivo efficacy study (Flesch-Kincaid Reading Level 9.0)

Contact Person:

Dinka Dugassa: Wallaga University, Institute of Health Sciences and the PI of the Research

Mobile Number:+251911176148,Nekemte, Ethiopia

Ibrahim Keder: EPHI staff and EPHI-IRB director

Mobile Number: +251 911 95 71 61 Addis Ababa, Ethiopia

Anticipated Number of Subjects: *100 Voluntary Participants*

Purpose: This research study aims to find out how well malaria treatment works in Ethiopia and will assist National Malaria Elimination Program (NMEP) managers to make appropriate intervention strategies for tackling the emergence of resistance and improving the treatment of malaria. This may help you or someone you know in the future. Therefore, we are asking you or your child to be part of this study because your/your child's diagnoses result shows a malaria parasite in your blood. This study is supported by the Ethiopia Federal Ministry of Health (FMoH) and EPHI.

Study duration: The study will take place over 42 days. During the study period, you will be asked to come to the health facility or to bring your child back to the clinic on scheduled s: 1st day, 2nd, 3rd, 7th, 14th, 21st, 28th,35th and 42 days.

Procedures: During each follow-up visit, we would like to obtain finger prick blood samples from you or your child by qualified lab personnel that would be used for blood hemoglobin level determination and malaria diagnoses to detect the presence of drug resistance markers, thus to see the outcome of treatment.

Risks There is very minimal risk in participating but you or your child may experience a small pain during finger pricking. The pain should disappear within a day. The drugs can cause an upset stomach, vomiting, diarrhoea, headache, dizziness, mild skin rash, and itching. But these are mostly mild and soon go away. Patients showing deterioration in their clinical status will be followed and immediately admitted to the clinic free of charge for appropriate treatment according to the national treatment guidelines until they recover.

Benefits You may or may not get personal (direct) benefits from participating in this study but will not get paid for participating. There are possible benefits of participating in this study: You will not have to pay fees for any clinic visits during this study, including any other illnesses during the 28/42 days of follow-up. You or your child will be closely followed for the next 28/42 days to see how well the administered treatments are working.

Injuries: Staff members will assist you in obtaining medical treatment, including emergency treatment, hospital care, and follow-up care as needed. Any hospital stays which occur during the 28/42 days follow-up period will be paid for. Signing this consent form does not give up any of your legal rights.

Compensation: You will receive 100 Ethiopian Birr for each visit to pay for your travel to the clinic

Participation: Taking part in this study is your choice. You can decide not to take part in or stop being in the study at any time. Your choice will not affect the treatment you receive for malaria. Also, none of the treatments you receive will be affected. You may leave the study at any time. This will not affect your health care, and you will still receive malaria treatment for free. If a staff member needs to take you out of the study for any valid reason, we will not continue following you. If you are removed from the study before the treatment is complete, or if the medicine did not make you better, then you will be referred to the clinic and treated with another treatment as noted in Ethiopia's malaria treatment guidelines.

Statement of Consent

By signing or placing my thumbprint below, I am saying that:

I have read this form, or it has been read to me; I have been able to ask questions about it, and my questions have been answered.

1. Children 6 months-11 years: parental/guardian permission: my child's participation is voluntary and that I can leave the study at any time without it affecting my care.

2 Children aged 12-17 years: I understand that my participation is voluntary and that I can leave the study at any time without it affecting my care. My decision to participate is supported by my parent/ guardian (parental/guardian permission will be obtained) but not forced by him/her.

3 For adults (18 years & older): I understand that my or my child's participation is voluntary and that I can leave the study at any time without it affecting my care.

I agree to enroll in this study. I agree to report any unexpected or unusual symptoms.

I have received a copy of this form. Signing this form does not waive any of my legal rights.

Circle the category of consent/assent below:

1. 6 months-11 years 2. 12-17 years of age 3. 18 years & older

Person Obtaining Consent/Assent

Print Name: _____ *Signature:* _____*Date:* ___/____/____

Participant

Print Name: _____ *Signature:* _____ Thumbprint: _____ *Date:* __/____/____

Parent or guardian

Print Name: _____ *Signature:* _____Thumbprint: _____ *Date:* ___/____/____

Witness:

Print Name: _____ *Signature:* _____*Date:* ___/____/____