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Efficacy And Saftey of Artemether-Lumefantrine with Single Dose Primaquine for Treatment Of Uncomplicated *Plasmodium Falciparum* Malaria In Arbaminch, Southwest Ethiopia.

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A thesis Submitted to Addis Ababa University, College of Health Sciences, Center for Innovative Drug Development and Therapeutic Trials for Africa, in Partial Fulfillment of the Requirements for the degree of Master of Science in Clinical Trials.

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Efficacy and Safety of Artemether-Lumefantrine with single dose Primaquine for treatment of uncomplicated *Plasmodium falciparum* Malaria in Arbaminch, Southwest Ethiopia

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DECLARATION

ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES, CENTER FOR INNOVATIVE DRUG DEVELOPMENT AND THERAPEUTIC TRIAL FOR AFRICA (CDT-AFRICA)

EFFICACY AND SAFTEY OF ARTEMETHER-LUMEFANTRINE WITH SINGLE DOSE PRIMAQUINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN ARBAMINCH, SOUTHWEST ETHIOPIA

I the undersigned have declared that this thesis entitled: EFFICACY AND SAFTEY OF ARTEMETHER-LUMEFANTRINE WITH SINGLE DOSE PRIMAQUINE FOR TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN ARBAMINCH, SOUTHWEST ETHIOPIA is my original work and has not been presented for a degree in any university. Furthermore, all the sources of information and materials have been acknowledged through referencing.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACPR= Adequate Clinical and Parasitological Response

ACT =Artemisinin-based Combination Therapy

AL = Artemether-Lumefantrine

BF = Blood Films

CRF=case record form

DBS = Dried Blood Spot

D0=Day 0

DHA=dihydroartemisinin

DHA-PPQ= dihydroartemisinin–piperaquine

EPHI = Ethiopian Public Health Institute

ETF =Early Treatment Failures

FMOH = Federal Ministry of Health

LCF= Late Clinical Failures

LPF= Late Parasitological Failures

NMESP= National Malaria elimination Strategic Plan

PCR = Polymerase Chain Reaction

Pf=*Plasmodium falciparum*

PADH=Post artemisinin delayed hemolysis

Pfcrt=Plasmodium falciparum chloroquine resistant transporter gene

Pfmdr1=Plasmodium falciparum multi drug resistance gene1

RDT=Rapid Diagnostic Test

SP= Sulphadoxine-pyrimethamine

WHO = World Health Organization

1. Abstract

Background: In Ethiopia, 60% of the population resides in areas prone to malaria infection. The first-line treatment for uncomplicated *Plasmodium falciparum* (*P. falciparum*) infection in Ethiopia is artemether-lumefantrine (AL), as outlined in the national malaria diagnosis and treatment guideline. The World Health Organization (WHO) recommends monitoring the efficacy of first-line antimalarial drugs at least once every two years post-implementations. Such information is crucial for assessing resistance levels and implementing timely interventions.

Objective: To assess the therapeutic efficacy and safety of AL for the treatment of uncomplicated *P. falciparum* based on clinical, parasitological and hematological parameters.

Methods: A prospective single-arm efficacy study was conducted among adults and children ≥ 6 months confirmed with uncomplicated *P. falciparum* and attending outpatient care at Shecha Health Center, Arbaminch, Southwest Ethiopia. The study took place from October 2023 to January 2024, during which the participants were enrolled and followed up for 28 days to evaluate clinical and parasitological responses. Enrolled participants were treated with the standard artemether 20mg + lumefantrine 120mg tablet, administered twice daily for three consecutive days and single dose primaquine given as 0.25mg/kg based on the national malaria treatment guideline of Ethiopia and the revised WHO protocol. Baseline assessments, including standard physical examinations and recording of medical history, were conducted on day 0 before dosing. Subsequent assessments were performed on days 1, 2, 3, 7, 14, 21, and 28. Thick and thin blood smears were taken at all time-points during the 28-day follow-up to detect parasites. Hemoglobin levels were measured from finger-prick blood samples on days 0, 14, and 28 to monitor changes and classify anemia severity. *P. falciparum* samples underwent polymerase chain reaction (PCR) testing for merozoite surface protein-1 (msp1), merozoite surface protein 2 (msp2), and glutamate-rich protein (glurp) to compare genotypic profiles pre- and post-parasite strains, distinguishing recrudescence from newly acquired infection. The primary outcome was the cure rate of AL measured by the adequate clinical and parasitological response (ACPR) on day 28. Secondary outcomes were parasite and gametocyte clearance, fever clearance, change in hemoglobin, and incidence of drug adverse events. Kaplan-Meier survival analysis and Per-protocol analysis were used to estimate primary and secondary outcomes, with statistical analysis conducted using SPSS version 25. One-Way ANOVA was used to compare baseline parasitemia between age groups, and a paired sample t-test was used to compare mean hemoglobin levels across follow-up dates. All comparisons were made at a 95% confidence interval (CI), with a significance level set at 0.05.

Results: Out of 3415 patients suspected of malaria, 272 (8%) tested positive, with 112 (41.2%) of these being identified as cases with *P. falciparum* infection. Among these, 93 (83%) met the inclusion criteria and enrolled, with an average age of 22.9 years, 63 (68%) adults, and 65 (70%) males. During the 28-day follow-up period, 88 (95%) participants completed the study, and 85 (96%) completed with ACPR. Before PCR, the overall cure rate of AL was 96.6% (95% CI 90.4-99.3), with three LPFs observed, all classified as reinfections after PCR correction. Thus, the PCR-corrected cure rate reached 100% (95% CI 95.8-100). The ACPR rate was high, accompanied by fast resolution of
clinical symptoms, with 83% and 96.6% of the participants clearing their parasitemia and fever on day 3, respectively. There was a slight drop in mean hemoglobin on day 28 compared to the baseline. No serious adverse events were reported during the 28-day follow-up period.
Conclusion: This study reaffirmed the efficacy of AL in treating uncomplicated *P. falciparum* malaria with acceptable safety profile. This supports the ongoing use of AL as the primary treatment option for uncomplicated *P. falciparum* infections in the study region. Regular efficacy assessments are advised for early detection of any emergence of drug resistance in the study setting and the broader country context.

Keywords: Malaria; Treatment; Efficacy; Safety; Artemether-Lumefantrine; *P. falciparum*; Ethiopia

1. INTRODUCTION

1.1 Background

Malaria is a potentially fatal disease caused by infection with *Plasmodium* protozoan parasites, which is transmitted to humans by the bites of infected female Anopheles mosquitos, and it remained the leading cause of death (1).The six *Plasmodium* species that cause malaria in humans are, *P. falciparum*, *P. vivax*, two morphologically identical sympatric species of *P. ovale* (curtisi and wallikeri), *P. malariae*, and the monkey malaria parasite *P. knowlesi* in Southeast Asia (2). *P falciparum* and *P. vivax* are responsible for the most serious threats worldwide (3). *P. falciparum* is the most lethal malaria parasite and the most common in Africa (2,4).

According to World Health Organization (WHO), there were around 247 million cases of malaria and 619,000 deaths worldwide in 2021. In the same year, malaria cases in Asia were estimated to be 5.4 million. Malaria cases had declined by 60% (from 1.5 million to 0.6 million) between 2000 and 2021 in the WHO regions of the America. In 2021, there were 234 million cases and 593,000 deaths in Africa, being responsible for 95% of cases and 96% of deaths globally (4).

In sub-Saharan Africa, malaria cases have worsen since 2015 especially during Covid 19 pandemic because of service interruptions(5). Between 2019 and 2021, estimated number of malaria cases increased significantly in Nigeria (4.0 million), Madagascar (2.8 million), Uganda (1.7 million), the Democratic Republic of the Congo (1.6 million), and Angola (1.4 million) (4). Similarly, out of global malaria mortality in the year 2021, only four African countries, Nigeria, Democratic Republic of Congo, Niger and Tanzania, contributed to more than half of deaths (4).

Malaria is largely unstable in Ethiopia, mainly due to its epidemic tendency. The major malaria transmission in Ethiopia happens from September to December shortly after the main rainy season. In addition, another minor season of transmission occurs from April to June, following a brief rainy season between February and March.(6). Because of the low and unstable malaria

transmission patterns in many parts of Ethiopia, people of all ages are at risk for significant clinical malaria illness (7).

Malaria transmission is speeding up due to human migration, urbanization, and agricultural development (8).

A patient with malaria symptoms and a positive test for parasites, using a microscope or rapid diagnostic test, with no symptoms of severe malaria, is called uncomplicated malaria. (9). Uncomplicated falciparum malaria has the potential to progress to severe case rapidly, especially in people with immunodeficiency (9). Early diagnosis and prompt treatment of malaria with a potent drug is crucial for disease control (7). The main objective of Ethiopian National Malaria Elimination Strategic Plan (NMESP-2021-2025) is diagnosing and treating all suspected cases of malaria within 24 hours (10).

Ethiopia has endorsed AL as first line treatment for uncomplicated cases of *P.falciparum* following multiple report of drug resistance against the old first line regimen in 2004 (11). Hence, artemisinin based combination therapy (ACT) is accepted as the treatment of choice for treatment of uncomplicated falciparum malaria (12).

Apart from human factors, the emergence of drug-resistant *plasmodium* parasites and the presence of vectors that are resistant to insecticide pose a great hinderance in controlling malaria not only in Ethiopia, but also at a global level.(13). Drug resistance remained a challenge to effective malaria control and eradication around the world. WHO strongly recommends and encourages that continuous and regular monitoring of first and second-line antimalarial drug efficacy study should be conducted at least in 24 months interval in areas with high burden of the disease to enable early detection of resistant parasite and help in tackling the transmission in the community (12). Therefore, conducting continuous drug efficacy studies in malaria-endemic site is important in order to inform antimalarial interventions on whether to continue the first line drug being used or recommend its revision.

1.2 Statement of the Problem

Malaria poses a significant concern on public health, affecting large number of people in Ethiopia. The commonest malaria causing species being *P. falciparum* and *P. vivax* and according to (HMIS 2015-2019) the former accounts for 82% of cases in the country (10). The expected number of fatalities from severe malaria due to *P. falciparum* is around 33% in children under the age of 12 and 10% in hospitalized adults. (14). The emergence of artemisinin resistance in Southeast Asia recently poses a significant challenge to global malaria eradication efforts.(15) .The advancing artemisinin resistance globally is also a current threat that requires increased attention (16).

In malaria endemic countries the increased access to ACT has impacted significantly on the reduction of global burden of malaria over the past 15 years (17). However, one of the obstacles to controlling malaria is the emergence of resistance to antimalarial drugs and There is growing evidence that *P. falciparum*, which accounts for the majority of malaria moralities worldwide, has developed resistance to ACT (4,18,19). Unrecognized resistance to already existing anti-malarial drugs in use were one of the reasons for the occurrence of malaria outbreaks in the past years (20). In order to prevent the spread of potential parasites that are resistant to major antimalarial medications, WHO recommends routine surveillance for early detection.

Based on the recommendation from WHO, for the conduct of efficacy studies at a regular interval of two years, the FMOH has identified sentinel sites. Consequently, multiple efficacy studies were done in different sites of the country (21–26). A recent study conducted 2 years ago at secha, Arbaminch showed 98.6% efficacy of AL against uncomplicated *P. falciparum* malaria (27). Even though the result showed a greater efficacy of AL and no indication to change AL as a first-line treatment, the authors of the previous papers recommended further efficacy studies for

early detection of resistance. The aim of the present study was, therefore to evaluate the efficacy and safety of AL in the treatment of uncomplicated *P. falciparum* malaria.

1.3 Significance of the study

Continued surveillance of ACT resistance is crucial to track the emergency spread of resistance and change the recommended drugs when it is appropriate. The WHO forwards updated treatment protocol based on scientific recommendations that arise from surveillance studies conducted in affected countries. For this reason, the WHO also recommends that the efficacy of first and second-line drugs be tested at regular interval of 24 months at all selected sites. Ethiopia is one of the high malaria burden countries where assessing the efficacy of the first-line drug is critical. So, this study provides scientific information about the efficacy and safety of AL.

This study also helps the MOH decide on whether the first-line treatment of the national guideline is still effective or not; which enables to change the guideline of antimalarial treatment when necessary. The community can also get an important advantage from this study by getting effective treatment. This is crucial to reduce deaths, illnesses, hospitalization, and economic crises due to drug resistance and ineffective treatment. It also gives evidence for clinicians on the clearance of clinical signs and symptoms after treatment with AL, and adverse events that occur after treatment.

2 LITERATURE REVIEW

2.1 Overview of anti-malarial drugs modes of action and resistance mechanism

Malaria treatment has been developed since 1940, and it is based on natural, semisynthetic, and synthetic compounds (28). The current antimalarial drugs are classified into three main groups: quinolone–derivatives, antifolates, and artemisinin derivatives. Emphasis is put on the ACT (which is the combination of artemisinin derivative with a partner drug), Artemether-Lumefantrine which Currently serves as a first-line treatment for *P. falciparum* malaria in most African countries including Ethiopia(9).

AL Is one of the ACTs in which artemether, a semisynthetic derivative of artemisinin extracted from the herb *Artemisia annua* is combined with Lumefantrine and it is purely synthetic. It is available as a 20mg Artemether and 120mg Lumefantrine fixed dose combination tablet. (12). The proposed mechanism of action of artemether is through producing free radicals that can kill the malaria parasite and by inhibiting protein synthesis. The mechanism of action of lumefantrine is not clearly understood, but it is assumed to interfere with B-hematin formation which is a critical detoxification pathway for the malaria parasite (29). Fatty meal increases the absorption and bioavailability of AL (30,31).

Primaquine belongs to the class of 8-aminoquinoline and it was in use since 1950. It is widely used as a radical cure therapy in *p.vivax* and *p.ovale* infections. Whereas, in *P.falciparum* it is administered as a single dose for gametocidal effect and block transmissions. The common side effect of primaquine is hemolytic anemia which is dose dependent and especially occurs in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency (32).

The two drugs act through different mechanism of action at distinct stages of parasite life cycle making their effect complementary. Artemether has rapid absorption and metabolism with short half-life of only two hours. The active metabolite of artemether dihydroartemisinin (DHA), rapidly kills most circulating malaria parasites resulting in rapid parasite clearance and clinical

improvement. On the other hand, lumefantrine has slower absorption and longer half of about 3-4 days allowing it to kill the remaining parasites and prevent recrudescence (30,31).

It has been demonstrated in different countries that AL is highly effective against *P.falciparum* parasites that are multidrug resistant (33,34). Thus it is the first drug in the class to be granted approval by WHO for treating uncomplicated malaria since 2001(9). AL tablets were incorporated in to the WHO "Essential Medicines" list in 2002(11) and later it has been approved in more than 80 countries globally(35). It is, therefore, the treatment of choice for uncomplicated malaria in several countries(9). AL has been shown to be well tolerated in clinical studies even with repeated doses Adverse events are generally mild and the most common ones are gastrointestinal(abdominal pain, anorexia, vomiting, diarrhea) and central nervous system such as (headache and dizziness) (36,37)

Drug resistance is defined as the "ability of a parasite to survive and multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject" (12). An effective malaria treatment requires the use of efficacious anti-malarial drugs which will eradicate the parasite, alleviate clinical symptoms and prevent recurrent infections (38).

The main reason for anti-malarial drug resistance is spontaneous mutations in the parasite in a way that makes the drugs less effective (29). For resistance to occur, some drugs only need single point mutations, whereas, others need multiple mutations.

It has been implicated from invitro susceptibility studies that growing number of copies of Pfmdr1 gene is highly linked with reduced efficacy of lumefantrine (39–41). Similarly, it has been demonstrated in a recent meta-analysis that, in patients who completed treatment with AL, point mutation and a higher copy number of the pfmdr1 gene were notable risk factors for parasite recurrence (42).

2.2. Factors associated with the emergence and spread of malaria drug resistance

2.2.1 Parasite related factors

Drug selectivity can happen when some parasites are resistant to treatment or when some parasites with mutations are exposed to drugs that are not completely eliminated from the blood (43). In the first case, more parasites will be affected by selective pressure than in the second case. This means there is a better chance for resistant mutants to appear and spread (44).

2.2.2 Drug related Factors

Poor quality drugs

One of the major challenges contributing to the emergence and spread of antimalarial drug resistance is, the use of preparations with subtherapeutic amount drugs which are available in the market. Using antimalarial drugs in low doses that are not enough to fully treat the infection can make drug resistance worse. Medicines might not have enough of the needed ingredients because they were made poorly or not stored properly. Falsified drugs with suboptimal amount of active ingredient can kill some sensitive parasites but leave behind others which are relatively resistant to replicate (45). According to a survey from 21 Sub-Saharan African countries, in which they reported rate of poor-quality drugs in the category of failed chemical analysis, failed packaging analysis and falsified drugs were found in 796 out of 2297 (35%), 28 out of 77(36%) and 79 out of 389(20%) respectively (46).

Monotherapies

Single drug preparations were widely utilized in numerous malaria endemic areas due to misconceptions that they are more affordable and tolerable as compared to ACT (12). As a consequence, exposure to a monotherapy facilitates the development of drug resistance easier than the use of combination drugs (47). Whereas, when a regimen containing multiple drugs with differing mechanism of action are utilized, it is more likely to result in parasite clearance even when the parasite is resistant to one of the component drugs. In addition, the use of two active ingredients will clear the parasite well before it gets the time to develop resistance against both drugs (44).

2.2.3 Human Related Factors

Irrational use of anti-malarial drugs

Among various other factors, irrational use of antimalarial drugs is a major contributor to anti-malarial drug resistance. This could be from irrational prescribing, dispensing, or consumption of antimalarial drugs. According to a study done in Gimbi referral hospital as high as 25% of antimalarial drug prescriptions were done either lower or higher than the recommended doses of AL which is the most commonly prescribed antimalarial medication in the hospital(48).

In addition, Poor dispensing practice plays a significant role in the development of anti-malarial drug resistance. In a study done in Tanzania, 84.6% of the drug dispensers did not have adequate knowledge about basic patient information, and majority of the dispensers were found to have insufficient knowledge about the proper dosage of AL(49).

Empirical treatment of suspected cases of malaria in the community may facilitate the emergence of antimalarial drug resistance. According to report from WHO, resistance rates were less common in rural as compared urban areas, where access to antimalarial drugs s easier (17).

This is a major concern in some regions where large number of patients are unnecessarily treated with antimalarial drugs, particularly sub-saharan Africa and South East Asia where the health system is underdeveloped (44).

Poor compliance with dosing regimen

Patients may not take complete dose of prescribed drug due to various reasons. This may include intolerable side effect and cost of prolonged or complicated treatment (50). Taking incomplete course of treatment will allow the survival of resistant parasites while the sensitive ones are cleared(17). The main reason for this behavior is lack of knowledge and reluctance of patients about rational use of the drug and its consequences.

2.3 Emergence of drug resistance *P. falciparum*

In Ethiopia, *P. falciparum* resistance to chloroquine was first seen among Ethiopian people who lives near the borders of Kenya, Somali and Sudan in 1986. The study showed that chloroquine had a treatment failure of 22% (51). Chloroquine resistance was also reported in the early 1990s

and it posed a significant challenge in preventing and controlling malaria (52). In the late 1990s, it was reported that chloroquine had a failure rate of 85% which resulted in the revision of the first-line anti-malarial drug to sulphadoxine-pyrimethamine (SP) in 1998 (53,54). A study done on SP in 2003 revealed a 72% failure rate of SP on 28 days follow up (55).

After an extensive malaria outbreak in 2003(56) and the subsequent identification of widespread resistance to SP (55,57–59) Ethiopia's Federal Ministry of Health (FMOH) changed AL as the first line anti-malarial drug for treating uncomplicated *P. falciparum* in 2004 (11).

2.3.1 Artemether Resistance

Therapeutic efficacy studies (TES) done in most countries worldwide have reported AL to be highly effective drug. According to a study done in South America, Colombia, the therapeutic efficacy of AL was 100% after polymerase chain reaction (PCR) correction (genotyping which is done to differentiate reinfection and recrudescence) (60). A descriptive analysis study from the USA showed a 91.5% cure rate of AL at day 7 and 96.9% at day 28 follow up period (61). Another study conducted in four malaria endemic states of India reported a 99.4% cure rate of AL (62). PCR corrected cure rates from studies conducted in different African countries have also been shown to be high with cure rates ranging from (93.9–100%) showing greater efficacy of AL (63–66).

In Ethiopia, when AL was introduced as a first line treatment in 2004, the efficacy was 99.1% (67). since then, several AL efficacy studies for *P. falciparum have been* conducted at different sentinel sites. Two studies from Oromia region done in Metehara and Jimma showed that the PCR corrected cure rates were 98.8% and 97.8%, respectively (21,26). An observational cohort study conducted in Bahirdar, Amhara region showed that AL has still been highly efficacious and well tolerated with a cure rate of 98.5% (68). From a recently published study conducted in Arbaminch, Southern region, the efficacy of AL was 98.6% (27). According to the prospective study undertaken in Alamata district, Tigari region the observed cure rate of AL was 97.2% at 28 days follow up period (69).

Although AL remained efficacious, the first case of artemisinin resistance was reported in Southeast Asia (70). This study was conducted in western Cambodia and northwestern Thailand and it demonstrated delayed parasite clearance and higher rates of recrudescence. Recent

resistance was also reported from an open-label, multi-center, multi country trial that included 7 countries in Asia and 3 countries in Africa (19). The study showed slower parasite clearance in patients enrolled from Thailand-Cambodian border.

In Africa, there are only a few reports regarding artemisinin resistance. According to a study done in Nigeria that evaluated treatment response of children, there was a decline in parasitological response to AL over the past 10 years(71). Another case report study done in Nigeria showed 3 treatment failure cases who had persistent parasitemia after taking a full dose of AL (72).

A study conducted on the Kenyan coast that evaluated treatment response of AL in 474 children under 5 years of age showed, declining in parasitological response over time perhaps because of emergence of drug resistant parasites (73). A case report study from Portugal reported two *-treatment failure cases among travelers coming from Angola and Mozambique (74). According to the study after the patients had been treated with a full dose of AL they presented to the hospital with signs of late clinical failure. Both cases have an adequate response after being treated with non-ACT anti-malarial drugs.

An efficacy study conducted in three sentinel sites in Angola to assess the efficacy of AL in children with acute uncomplicated *P. falciparum* showed a treatment failure rate of 12.4% in one of the sites (75). In another efficacy study conducted in five sites in Democratic republic of Congo treatment failure rate of 14% was reported in one of the sites (76). Reports from both studies were way below the 90% efficacy set by WHO to consider a revision in the first-line treatment (17).

In Ethiopia, almost all studies conducted on AL showed a greater efficacy and reports are lacking regarding resistance. However, there are few reports of late parasitological failure. A prospective study conducted at Kola Diba Health Center, Dembia district reported 8% late parasitological failures(25). A study conducted in the Ethio Sudan border demonstrated PCR detected trace parasitemia that were not cleared completely and continued to be seen in the blood up to day 28 follow up period in seven out of fifty patients. This may be an epidemiologically significant indicator of transmission of resistant parasites (77). Even though the efficacy of AL from this

study was 96% the presence of sub microscopic parasitemia might affect the efficacy and needs strict monitoring.

In spite of the fact that there was no report of failure alarm of AL in Ethiopia, studies showing late parasitological failure need continuous monitoring based on the WHO recommendations, and further studies are needed to support such reports. Therefore, this study is aimed to assess the efficacy and safety of AL against *P. falciparum*, to get updated information about the state of AL treatment on *P. falciparum* in Secha Health Center.

3 OBJECTIVES

3.1 General Objective

The aim of this study is to assess the efficacy and safety of AL for treatment of uncomplicated *P. falciparum* malaria based on parasitological, clinical, and hematological parameters.

3.2 Specific objective

Primary objective

- To evaluate the clinical and parasitological response (cure rate) of AL in patients with uncomplicated *P. falciparum* in 28 days follow up period starting from day 0.

Secondary objectives

- To determine parasite clearance rate, fever clearance rate
- To determine incidence of adverse events following AL treatment
- To determine mean hemoglobin level before and after treatment

4 MATERIALS AND METHODS

4.1 Study Area

This study was conducted in Secha Health Center, Arbaminch, which. is located about 500 kilometers south of Addis Ababa in the Gamo Gofa Zone of the South Ethiopia Regional State. The town has 800-1000 mm of rain per year and an average annual temperature of 29.0 °C. Arbaminch is divided into four kifle-ketemas (sub-city): Secha, Sikella, Abaya, and Nechsar. The four kifle-ketemas are divided into to11 kebeles which are all malarial areas.

According to the Central Statistics Agency report in the year 2021, the total population of Arbaminch was 192,043. The town has one general hospital, one primary hospital, two health centers which are currently functioning, one referral and teaching hospital, and one primary hospital which is under construction.

Secha Health Center is one of the two health centers in Arbaminch. this health center was established in 1961 as a clinic and upgraded to a Health Centre in 2004.Currently, the health center provides services for more than 31,945 population.

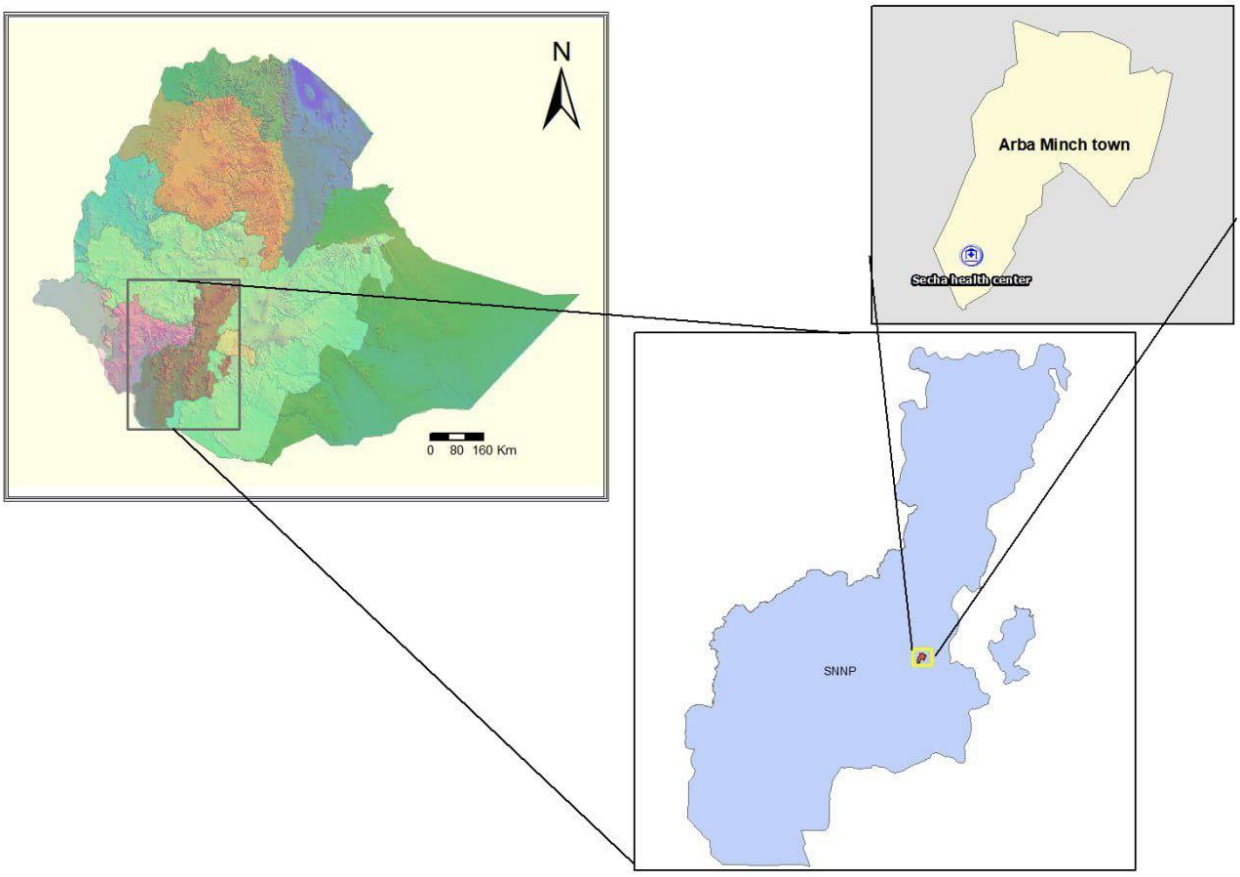


***Figure** 1: Study area Map*

4.2 Study Design

The efficacy study was a single-arm prospective evaluation of clinical and parasitological response to the treatment of AL for uncomplicated falciparum malaria.

4.3 Study Period

The study was conducted from October 2023- to January 2024.

4.4 Source Population

All malaria suspected individuals attending Secha Health Center during the study period were the source population.

4.5 Study Population

Patients with confirmed uncomplicated *P. falciparum* mono infection attending the outpatient

department (OPD) of Secha health center and who fulfill the inclusion criteria.

4.6 Sample size and sampling strategies

4.6.1 Sample size

The sample size was estimated following the WHO (2009) protocol; the assumptions were as follows: 5% margin of error, 95% confidence with the estimate and treatment failure of 5% and 20% drop out rate. With these assumptions, minimum of 73 patients will be required.

Sample size (n) $= \frac{Z\ a/2^2\ \ p\ (1\ -p)}{d^2}$

$(1.96/0.05)^2\ 0.05\ (1\text{-}0.05) = 73$

Assuming an additional 20% loss to follow-up rate and withdrawal of consent (15 patients) during the study, at least 88 (73 + 15) patients should 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%)

4.6.2 Sampling Technique

About 93 study participants confirmed with *P. falciparum* mono infection on blood film examination and who fulfilled the inclusion criteria by WHO were recruited by purposive sampling technique.

4.7 The study variables

4.7.1 Dependent Variable

Efficacy of AL for the treatment of uncomplicated *P. falciparum*

4.7.2 Independent Variables

- Age
- Sex
- Weight
- Clinical characteristics
- Hemoglobin
- Parasite density

4.8 Eligibility criteria

4.8.1 Inclusion criteria

- Age > 6 months
- Body weight >5kg
- Fever (axillary temperature ≥37.5 ^{0}C) or history of fever within the previous 48 hours
- Mono infection with *P. fa*lciparum confirmed by microscopic blood smear with asexual parasitemia >1000/µl of blood
- Patients living within the health center catchment area (10 km radius of the health center)
- 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.
- Willingness to give informed consent from patient or from a parent or guardian in the case of children under 12 years old and assent for children 12-17 years old. and agreed to return for all

scheduled visits (Annex X)

4.8.2 Exclusion criteria

- Severe malaria with complication signs and symptoms. (Annex VI)
- Intake of antimalarial drug within 2 weeks before enrolment.
- 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.
- Mixed or mono infection other than *P. falciparum* species.
- Severe anemia, defined as hemoglobin (Hb) < 5 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
- History of hypersensitivity reaction to any medication tested or used as an alternative treatment
- Taking regular medication, which may interfere with antimalarial pharmacokinetics or efficacy (see Annex VII).

4.9 Follow up procedures and evaluation

On D0 (enrollment day), each patient was treated with the first dose and given an appointment card with patient's name, PIN, and next scheduled visit date written on the front of it and the evening dose to be taken at home. Patients were then asked and advised to come back for treatment the following two days and for 28 follow up days according to scheduled visits on D3, D7, D14, D21, and D28 (Table1); and on any unscheduled day that the patient felt sick. For overall study schedule see (Annex VIII**).**

Table 1. Schedule of follow up activities for study participants treated with AL at Shecha Health Center.

Days	0	1	2	3	7	14	21	28
Clinical examination	X	X	X	X	X	X	X	X
Treatment with AL	X	X	X					
Blood film for Parasitemia	X		X	X	X	X	X	X
Hemoglobin	X					X		X

4.9.1 Loss to follow-up patient discontinuation or protocol violation

Loss to follow up

Loss to follow-up occurs when an enrolled patient does not attend scheduled visits and cannot be located despite all reasonable efforts. No treatment outcome was 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, particularly during but also after the study drug administration. These patients were labeled as unfollowed and either censored or excluded from the analysis.

Patient discontinuation or protocol violation

Patients meeting any of the following criteria were 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

- Enrollment violation
- Erroneous inclusion of a patient outside of the inclusion/exclusion criteria

- Voluntary protocol violation:
  - Self- or third-party administration of the antimalarial drug (or antibiotics with antimalarial activity) was assessed by asking participants or their caretakers at follow-up visits (Annex VII)

- 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.

4.10 Treatment and dosing procedures/

4.10.1 Antimalarial Treatment

The dosing was made as per Ethiopian national malaria treatment guideline(7) and the revised WHO protocol (Weight-based guideline) (Annex V). Accordingly, the study participants were treated with artemether 20mg + lumefantrine 120mg tablet (Manufacturer Ipca laboratories, India, batch number HWE 111327, expiry date Novemebr-2024) which was administered twice

daily for total duration of three days. The initial dose of the drug on the day of enrollment and the morning doses on following days were given in the health center under direct observation of the study personnel. The night dose of D0 was given 8 hours after the first dose while the evening dose of D1 and D2 were given 12 hours after administration of the morning dose. A single dose of 0.25mg/kg primaquine was administered with AL on the day of enrollment. The study participants or their guardians were advised to take the drug with fatty meal to facilitate adequate absorption. The study drug was given to young children in the form of suspension after crushing and mixing it with glass of water. In the case of older children and adults, it was given as tablets. Patients were monitored for 30 minutes after treatment for vomiting, for which repeat dose is recommended. If vomiting occurs more than once, patients will be referred to a higher level of care for management with parenteral artesunate therapy and withdrawn from the study.

4.10.2 Concomitant Treatment

Febrile patients with objective temperature $\geq 38^0$C were treated with conventional dose of paracetamol (12). Patient with new diagnosis other than malaria during the study period will be given free treatment at the health center.

4.10.3 Rescue Treatment

Patients with treatment failure will be treated with second line drug as per National Guidelines. Whereas, patients failing with the second line treatment will be administered as per the severe malaria criteria for treatment failure.

4.11 Evaluation Criteria

4.11.1 Efficacy and safety evaluation

Classification of treatment outcomes

The primary outcome of this study was monitoring the efficacy of AL for *P. falciparum* malaria as a routine follow up study in Ethiopia. The classification of treatment outcomes was based on an assessment of the parasitological and clinical outcome of antimalarial treatment according to the latest guidelines of WHO. Accordingly, all *P. falciparum* patients will be classified as having

early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or adequate clinical and parasitological response (ACPR) (Annex IV).

Safety Assessment

Safety was assessed by recording the incidence of adverse events. Study participants were asked directly about new symptoms that have occurred since the start of treatment. Participants will get appropriate examination and treatment when clinically indicated. All adverse events were documented on the case report form.

4.12 Clinical Evaluation

All patients were evaluated clinically as described below.

4.12.1 Physical Examination

Physical examination was done at baseline of the study and routinely on the following days 1, 2, 3, 7, 14, 21, and 28. A complete medical history, including prior and concomitant medication, demographic information, and contact details were recorded at baseline.

4.12.2 Body Weight

Body weight was recorded on day 0 to the nearest kilogram on a scale or on a hanging scale for young children. The scales were properly calibrated. Patients were checked that they were not overdressed while weighed on the scale. All young children wore only undergarments while put on the scale. The screening weight was used to satisfy the eligibility criteria for weight and nutrition status as well as to calculate the dose (number of tablets) to be administered. The reliability of the scales was verified before the study begins and checked at regular intervals. Oedema was assessed by thumb pressure for 3 seconds on the dorsal surface of both feet.

4.12.3 Body Temperature

Axillary temperature was measured at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, and 28. Temperature was measured with a thermometer with a precision of 0.1 °C. Temperature was measured as clinically indicated. The measurement was repeated if the

temperature was < 36.0 °C. The same route was used throughout the study. The thermometers were assessed regularly.

4.13 Laboratory Procedures

4.13.1 Microscopic Blood Examination

Thick and thin blood smears was taken from all participants and prepared on the same slide for detection of parasites at all time-points during the 28-days follow-up period. All the slides were read by two qualified laboratory technicians independently, and parasite densities were 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) were re-examined by a third, independent microscopist, and parasite density was calculated by averaging the two closest counts.

4.13.2 Hemoglobin Measurement

Hemoglobin level was measured from finger-prick blood samples and it was determined using portable spectrophotometer (HemoCue®, Anglom, Sweden) on days 0, 14, and 28 to observe change in blood Hgb level after treatment and classify the level of anemia. Anemia was defined according to the WHO classification: Hgb 7-9.9 g/dl for < 5 and 8-10.9 g/dl for 5-15 and >15 of age indicates moderate anemia. Hgb 10-10.9 g/dl, 11-11.9g/dl indicates mild anemia for <5, 5-15, respectively and Hgb 11-12.9g/dl also indicates mild anemia for non-pregnant women and adult male. Hgb >11g/dl for <5 year,>11.5g/dl for 5-15 years and non-pregnant women and >13g/dl for adult male indicates normal. Hb≤5.0 g/dl was considered severe anemia and an exclusion criterion (12).

4.13.3 Pregnancy Test

Female patients of child-bearing age defined as those who menstruate or aged over 18 years (depending on situations), were asked to take a urine pregnancy test before enrolment in the study.

4.13.4. Molecular genotyping of malaria parasites and molecular marker for antimalarial drug resistance

To differentiate a recrudescence (same parasite strain) from a newly acquired infection (different parasite strain), a genotype analysis was conducted based on the extensive genetic diversity among the malaria parasite. Using PCR, *P. falciparum* samples were tested for merozoite surface protein-1 (*msp1),* merozoite surface protein 2 (*msp2),* and glutamate-rich protein (*glurp).* The genotypic profiles of pre- and post-parasite strains was compared.

4.14. Data Management and analysis

Data collection and recording on the case report form (CRF) was done according to the study protocol. Laboratory and clinical data were recorded daily on the case record form designed for the study. Any modification to the CRF was dated and explained. All CRFs were assessed to make sure they were complete.

All data from recruited patients were imported into the WHO Excel sheet (double entry) designed for analysis of therapeutic efficacy study data. Data were also entered into IBM SPSS (version-26) software to calculate descriptive statistics (mean, median, standard deviations, range). One-Way ANOVA was used to compare baseline parasitemia between age groups, and paired sample t-test was used to compare mean Hgb level between D0 and D14, D0 and D28, D14 and D28. All comparisons were performed at 95% CI and a significance level of 0.05.

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

4.15 Data Quality control

The data entry was checked at the end of the study to ensure accurate and complete recording. Five percent of the slides were read by WHO qualified microscopists for quality control and the

correctness of the sample collection, data entry, and the correct completeness of consent/assent form were checked by supervisors from EPHI.

4.17 Ethical Consideration

Ethical clearance for this study was obtained from the Scientific and Ethics Review Committee of the Center for Innovative Drug Development and Therapeutic Trials for Africa (CDT-Africa), College of Health Sciences, Addis Ababa University (Protocol number:1851/23) and the Ethiopian Public Health Institute (EPHI) (protocol number:294/2022) before its initiation. Permission was sought from Shecha Health Centre. Written informed consent was obtained from adult patients while for children under 12 years, it was secured from their parents or guardians. Assent was taken for children at the age of 12-17 years. Information regarding details of the study along with its benefits and possible hazards was explained to study participants before they were enrolled in the study. Confidentiality was maintained, and patients were informed that they had the right to withdraw from the study at any time. (Annex X).

4.18 Dissemination of Results

The results of this study will be submitted to Addis Ababa University, College of Health Sciences CDT-AFRICA. The report will be shared to the FMOH through EPHI to inform the current status of antimalarial drug efficacy and guide the development of future updated management protocol. Furthermore, it will be presented at scientific conferences and the manuscript will be submitted to peer-reviewed journals for publication.

4.19 Operational Definitions

Adequate clinical and parasitological response: "Absence of parasitemia on day 28, irrespective of auxiliary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure, or late parasitological failure."

Adverse event: "An adverse event is defined as any unfavorable, unintended sign, symptom, or disease that develops or worsens with the use of a medicinal product, regardless of whether it is related to the medicinal product or not."

Early treatment failure: "Danger signs or severe malaria occurred on days1, 2, or 3 in the presence of parasitemia, parasitemia on day 2 higher than day 0, irrespective of auxiliary

temperature, parasitemia on day 3 with auxiliary temperature ≥ 37.5ºC and parasitemia on day 3 ≥ 25% of count on day 0."

Late clinical failure: "Danger signs or severe malaria in the presence of parasitemia on any day between days 4 and 28 in patients who did not previously meet any of the criteria of early treatment failure and presence of parasitemia on any day between days 4 and 28 with auxiliary temperature ≥ 37.5 ºC in patients who did not previously meet any of the criteria of early treatment failure."

Late Parasitological Failure: "Presence of parasitemia on any day between days 7 and 28, and axillary temperature: < 37.5 ºC in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure."

Per protocol analysis: "participants who couldn't complete a 28 day follow up period (withdrawnand lost to follow-up) were removed from the denominator."

Primary outcome: "The 28 days treatment outcome of the participants whether treatment failure (ETF, LCF, and LPF) or adequate clinical and parasitological response (ACPR). "

Secondary outcome: "The clinical and parasitological outcomes following AL treatment (parasite, fever and gametocyte clearance rate, incidence of drug adverse events)"

Serious adverse event: "Is defined as any untoward medical occurrence that at any dose; results in death/ life-threatening, requires hospitalization or prolongation of hospitalization and results in a persistent or significant disability."

Uncomplicated *Plasmodium falciparum*: "A patient who presents with symptoms of malaria and positive parasitological test, but with no features of severe malaria."

5 Results

5.1Characteristics of study participants

5.1.1 Socio demographic characteristics

A total of 3415 clinically suspected patients were screened for malaria. Among the suspected cases, 272 patients (7.9%) were microscopically confirmed to be positive for malaria. *P. falciparum* accounted for 112 (41.2%) of the cases from the PF mono infection, 93 patients (83%) who met the inclusion criteria were enrolled in the study.

From the total participants, majority were males, with a male to female ratio of 65:28. The mean age of the study participant was 22.9 with a range of 1-71 years. Among the study participants, 63(67.7%) were adults, 24 (25.8%) were 5-15 years old, and the remaining 6 (6.5%) were under five children. The mean body weight of the participant was 47kg with a range of 10.2-87kg.

Forty-nine (52.7%) patients from the participants had access to bed net of whom, 33(35.5%) properly used the bed net. Among the study participants, 74(79.6%) had previous history of malaria attack of whom, 72 (97%) were treated with antimalarial drugs. Among the 93 study participants, 48 (51.6%) took AL and 16 participants (17.2%) were treated with CQ. From the total enrolled cases, 88 participants (94.6%) completed the 28 days follow up. Five (5.4%) participants didn't complete the 28 days follow up, and they were censored. Three participants were withdrawn due to protocol violation (1 patient repeated vomiting of the evening dose on day 0, one patient took medication with anti-malarial activity on day 2, and one patient was found to have mixed infection on day 24). Two participants were lost to follow up on days 1 and 21 respectively, (figure 2). There was no hypersensitivity reaction during and after antimalarial treatment.

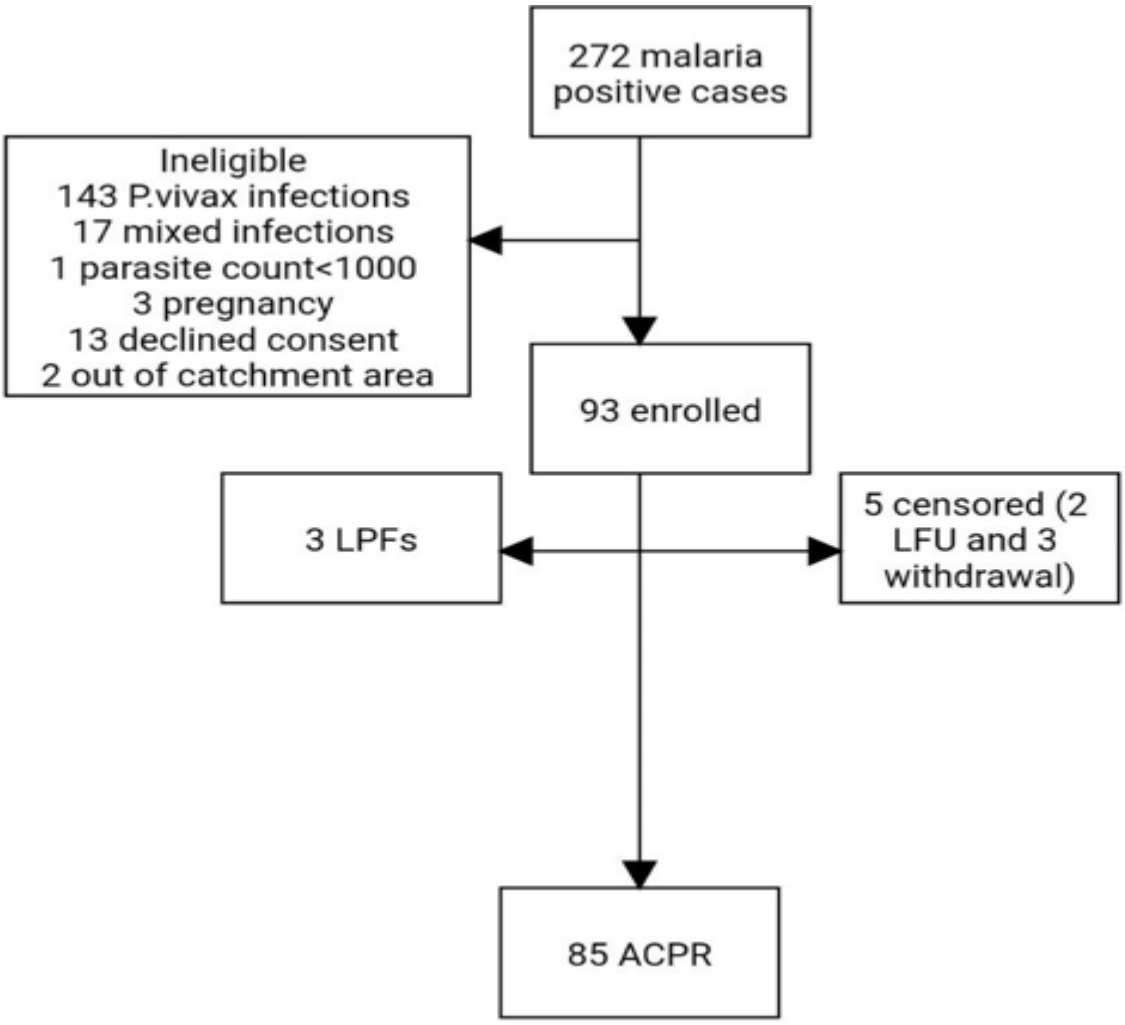


Figure 2: *Flow chart illustrating the follow up and outcomes of study participants*

5.1.2 Baseline clinical characteristics

Out of the total 93 participants enrolled in this study, 83.8% had history of fever both at the time of enrollment and in the last 48 hrs. The remaining 16 % of participants had history of fever only in the last 48 hours. The mean axillary body temperature at baseline was 38.2 with a range of 36.1-40.5^{0}C. Headache (90.3), joint pain (9.7%), vomiting (8.6%), and nausea (7.5) were the major clinical signs and symptoms reported on the first day (day 0).

At baseline of the study, the mean hemoglobin was 12.96g/dl with a range of (9.7-17.9g/dl) and the prevalence of anemia was 33.3% (26.8% mild and 6.5% moderate). The mean parasitemia at baseline was 5526 with a range of 1032-45120 with no significant difference between all age groups (p=0.489), and only 3 participants had gametocytes at baseline (Table2).

Table:2. Baseline characteristics of study participants treated with Artemether-Lumefantrine in Secha health center, Arbaminch, Ethiopia, from October 2023 to January 2024.

Variable		Age category			Total
		Under 5	5-15	Adult	
No (%)		6 (6.5%)	24 (26%)	63(67.7%)	93 (100%)
Mean Age (Range)		3.4(1-4.6)	9.9(5-14)	29.3(15-71)	22.9(1-71)
Gender	Male n (%)	6(100)	13(54.2)	46(73%)	65(69.9%)
	Female n (%)	0	11(45.8)	17(27%)	28(30.1%)
Mean T		38.6	38	38.2	38.2
Mean Weight(kg)		13.9	28.4	57.4	47
Mean Hgb(g/dl)		11.85	12.26	13.6	12.96
Anemia Status (%)	Mild	2	4	19	25(26.8)
	Moderate	0	4	2	6(6.5)

	Total	2	8	21	31(33.33)
Mean (Geo) asexual parasite(µl)		6288.33	10645.41	8568.13	5526
Gametocyte carriage n (%)		1	0	2	3

5.2 Primary outcome

5.2.1 Cure rate of AL

Based on per protocol analysis from the total of 88 participants who completed their follow-up period, 85 were classified under ACPR. Three cases of LPFs on days 7, 21 and 28 were observed. Based on per protocol analysis, the PCR uncorrected cure rate of AL was 96.6% (95 % CI 90.4-99.3%). (Table 3)

Table 3: Summary of treatment outcomes based on per protocol analysis stratified by age category.

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)	3(12.5)	0(0)	2(3.3)	1(3.7)	3(3.5)
ACPR n(%)	6(100)	21(87.5)	58(100)	59(96.7)	26 (96.)	85(96.6)
Total analyzed n(%)	**6(100)**	**24(100)**	**58(100)**	**61(100)**	**27(100)**	**88(100)**

According to PCR uncorrected Kaplan Meier survival analysis, on 28 days follow up period, the cumulative incidence of success rate of AL was 96.6% (95% CI: 89.9%-98.9%) with failure rate of 3.4 % (95% CI: 1.1 % - 10%) **(figure3).** After PCR correction all the three LPF cases observed were found to be reinfection thus, they were excluded from the PCR corrected analysis. Accordingly, the PCR corrected cure rate of AL in this study was 100% (95% CI 95.8-100%)

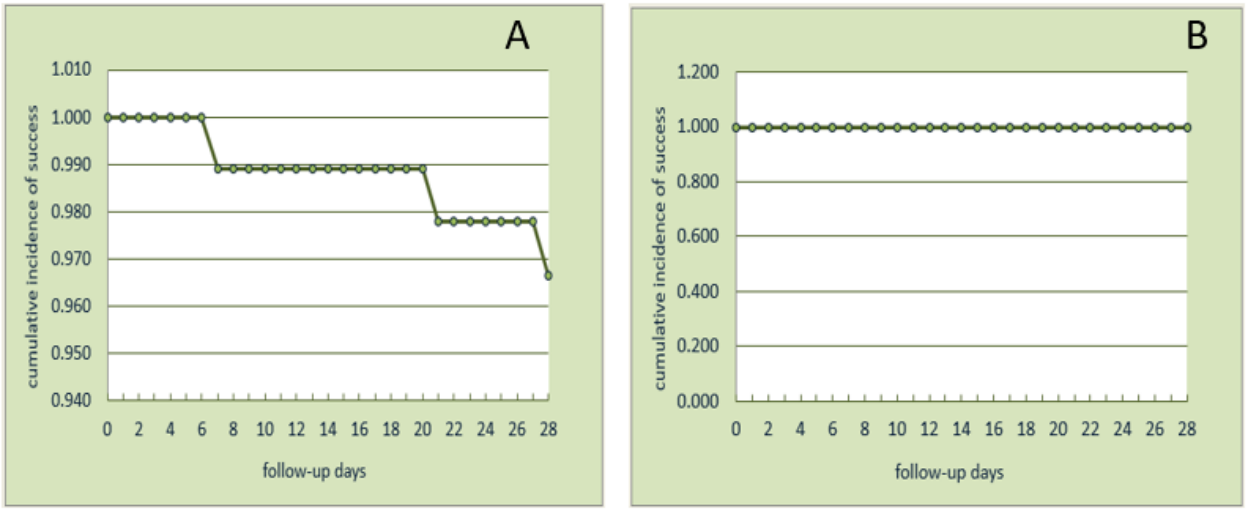


Figure 3. Kaplan Meier survival curve: A) PCR uncorrected, B) PCR corrected.

5.3 Secondary Outcome

5.3.1 Parasite clearance

Based on per protocol analysis, about 83 % of the study participants had cleared their parasitemia on day 3 follow up period. The number of participants with parasitemia decreased from 93 on day 0 (baseline) to 90 on day 1, 55 on day 2 and 15 on day 3. All participants cleared their parasites by day 7 except for three cases of LPF on days 7, 21 and 28. Gametocytes were detected only in three participants at baseline of the study, and all the gametocytes were cleared on day 3 follow up period. The rate of gametocytemia decreased from 3.2% on day 0 to 1% and 0% on days 2 and 3 respectively (Figure4).

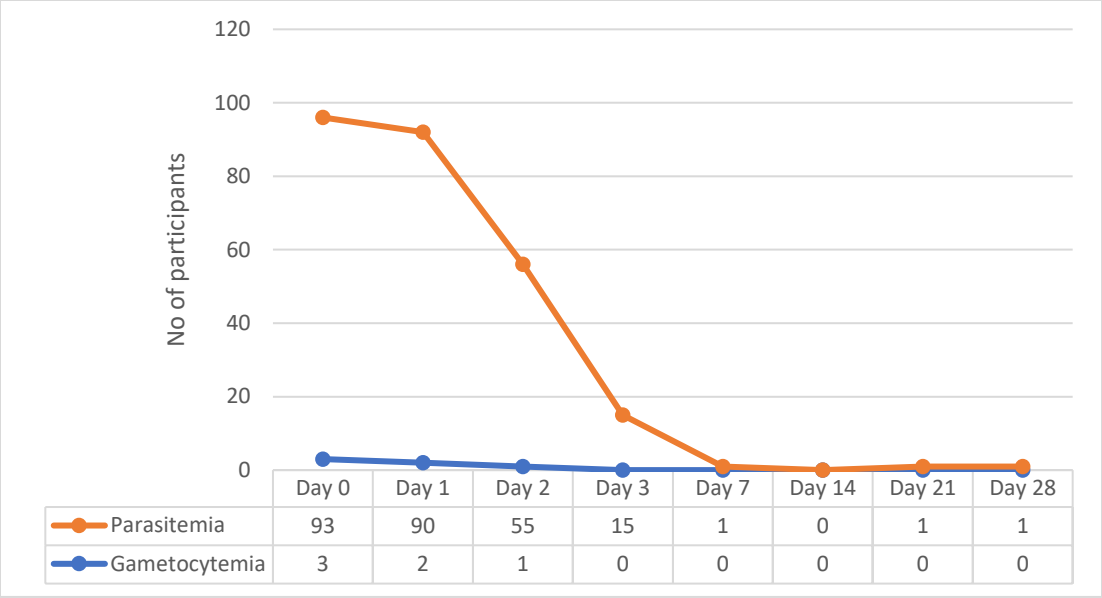
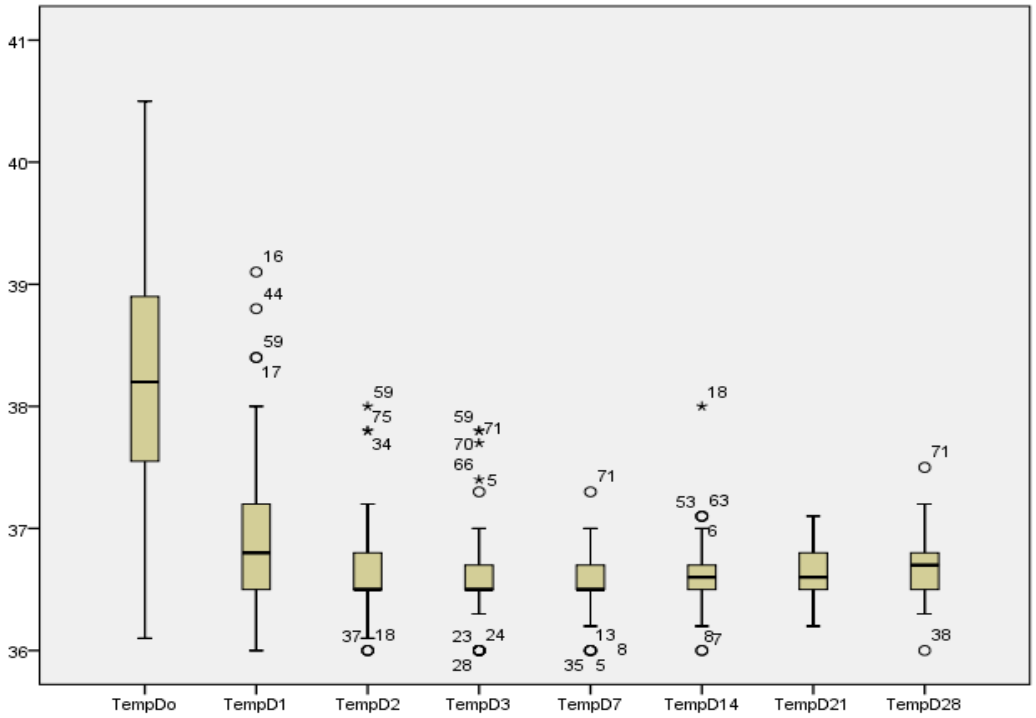


***Figure 4**:* Pattern of parasite and gametocyte clearance

5.3.2 Fever clearance

The mean body temperature declined from 38.2ºC on day 0 to 36.9 º C on day 1, 36.6 º C on day 2 and 36.5 º C on day 3 (figure 5) with 80.9% of the participants clearing their fever on day 1, 95.5% on day 2 and 96.6% on day 3 (figure5).



Statistic	Temp Day 0	Temp Day 1	Temp Day 2	Temp Day 3	Temp Day 7	Temp Day 14	Temp Day 21	Temp Day 28
Mean	38.22	36.96	36.62	36.58	36.55	36.62	36.64	36.69
Lower 95% Confidence	37.98	36.82	36.54	36.50	36.49	36.56	36.60	36.64
Upper 95% Confidence	38.45	37.10	36.70	36.66	36.60	36.68	36.67	36.74
Median	38.20	36.80	36.50	36.50	36.50	36.60	36.60	36.70
Std. Deviation	1.04	0.62	0.35	0.36	0.24	0.26	0.17	0.22
Minimum	36.10	36.00	36.00	36.00	36.00	36.00	36.20	36.00
Maximum	40.50	39.10	38.00	37.80	37.30	38.00	37.10	37.50
Interquartile Range	1.40	0.70	0.30	0.20	0.20	0.20	0.30	0.30

Figure 5: Mean body temperature on day 0-28 follow up days

5.3.3 Change in haemoglobin status

The hemoglobin level didn't show much improvement after treatment. Though the total number of anemic patients decreased from 31 at baseline of the study to 25 on day 28 with only one patient showing moderate anemia, there was a drop in the mean hemoglobin level. Accordingly, the mean hemoglobin level showed slight decline from the baseline (12.96g/dl ± 1.47) to 12.68g/dl ± 1.2 and 12.85gdl ± 1.23 on day 14 and 28, respectively. Even though there was a drop in the mean hemoglobin level, comparison of mean hemoglobin between days 0 and 14, days 0 and 28 and days 14 and 28 showed no significant difference (Table 4).

Table 4: Anemia status among study participants following treatment with AL.

Variables				Follow up days		
				Day 0	Day 14	Day 28
Age category	Under 5 (n)	Mean hgb(g/dl)		11.85	12.03	11.65
		Mild anemia		2 (6)	1 (6)	1 (6)
		Moderate anemia		0 (6)	0 (6)	0 (6)
	5-15 (n)	Mean hgb (g/dl)		12.26	12.20	12.08
		Mild anemia		4 (24)	6 (22)	8 (21)
		Moderate anemia		4 (24)	2 (22)	1 (21)
	>15 (n)	Mean hgb (g/dl)		13.36	12.95	13.33
		Mild		19 (63)	24 (55)	15 (50)
		Moderate		2 (63)	3 (55)	0 (50)
Sex	Male (n)	Mean hgb (g/dl)		13.05 (65)	12.85 (59)	12.84 (53)
		Mild		18 (65)	22 (59)	19
		Moderate		4(65)	2 (59)	1
	Female (n)	Mean hgb (g/dl)		12.78 (28)	12.30 (24)	12.9 (24)
		Mild		7 (28)	9 (24)	5 (24)
		Moderate		2 (28)	2 (24)	0 (24)
Total		Mean Hgb (g/dl)		12.96	12.68	12.85
		Mild		25	31	24
		Moderate		6	5	1
		Total anemia		31	36	25
Mean d/c of Hgb		D0 and D14	D0 and D 28	D14 and D 28		
	Significance	P= 0.132	P= 0.856	P= 0.232		

5.4 Clinical signs and symptoms and Adverse events following AL treatment

At the baseline of the study, headache was the most frequently reported symptom accounting for 90.3% followed by joint pain (9.7%), vomiting (8.6%), nausea (7.5%) and cough (6.5%). During follow up, most of the symptoms disappeared with clearance of parasitemia. Adverse events like Anorexia (1.1%), Abdominal pain (1.1%), dizziness (1.1%), and cough (1.1%) were observed following treatment completion (Table 5).

Table 5: Common malaria clinical signs and symptoms and adverse events following AL treatment at Secha Health Center.

Adverse	**Follow up days**							
events/symptoms	**D 0**	**D 1**	**D 2**	**D 3**	**D7**	**D 14**	**D21**	**D 28**
Headache n(%)	84(90.3)	23(24.7)	6(6.5)	8(8.6)	1(1.1)	0(0)	1(1.1)	0(0)
Anorexia n(%)	3(3.2)	0 (0)	2 (2.2)	0 (0)	0 (0)	0 (0)	1 (1.1)	0(0)
Nausea n(%)	7(7.5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Vomiting n(%)	8(8.6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Abdominal pain n(%)	3(3.2)	0(0)	0(0)	0(0)	1(1.1)	0(0)	0(0)	0(0)
Diarrhea n(%)	1 (1.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Cough n(%)	6(6.5)	1(1.10	2(2.2)	1(1.1)	0(0)	0(0)	0(0)	1(1.1)
Dizziness n(%)	4(4.3)	3(3.2)	0(0)	0(0)	1(1.1)	0(0)	0(0)	1(1.1)
Joint pain n(%)	9(9.7)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Itchin*g* n(%)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

6. Discussion

Early diagnosis and treatment of malaria remains an effective strategy to control and prevent drug resistance. The development of resistance to anti-malarial drug was a major challenge in controlling and preventing malaria. AL is a drug of choice for treating uncomplicated *p.falciparum* malaria and it has been widely used as a first line treatment in Ethiopia. Though it remained efficacious in Ethiopia, resistance to the drug has been reported from different areas of the world.

In the present study, the PCR uncorrected cure rate of AL at the end of 28 days follow-up period was 96.6%. This finding is consistent with studies done in other parts of Ethiopia; Metehara (97.6%), Alamata (97.2%), and Kersa 96.3 %. (21,69,78). Another study conducted at Arbaminch also showed an efficacy of 97.2% (27). This result is also in line with studies conducted in different countries other than Ethiopia, Benin 96.7% (79), Nepal 95.8% (80). The PCR-corrected cure rate of AL in the present study is comparable with the findings of other studies conducted in Ethiopia showing an efficacy of 99.4-100% (81–83). A study from Thailand and Colombia had a similar result reporting a 100% PCR corrected cure rate at 28 days follow up (60,84). Recent studies from Africa also support this finding. A study done in Somalia shows 100% clinical and parasitological response (85). Another study conducted in Tanzania also shows a 100% PCR-corrected cure rate of AL in two study sites (86).

The age-stratified result from this study demonstrates a higher efficacy of AL with a 100% cure rate for children less than 5 years of age. This finding is further supported by a study done in Kersa, southwest Ethiopia (78). another study conducted in Benin also showed 100% ACPR for children under 5 years. The higher efficacy rate in this age group can be explained by the presence of the increased amount of milk/fat in their diet which facilitates the absorption, bioavailability, and efficacy of AL (87).

There were no ETF cases observed in the present study. However, 3 LPF cases were detected on days 7, 21 and 28. The same result was observed from a study conducted in Gonder which showed three LPF cases (24). A study done on the same site 2 years back also showed two LPF cases (27). All the three LPF cases were in the age group of 5-15 years. This finding agrees with that of a study done in India in which all cases of LPF were for children aged 5-15 years (88).

This study demonstrated a relatively lower number of LPF cases compared to that of a study done in Dembia which showed 6 LPF cases (25). Another study from South Tanzania showed 11 LPF cases which was much higher than what is observed in the current study (89). All three LPF cases were later reclassified as reinfections after PCR correction and were excluded from analysis.

According to WHO one of the major criteria to suspect artemisinin resistance is the occurrence of parasitemia on day 3 (9). The absence of ETF cases in this study implies higher efficacy of AL in the study site, and this finding is in line with those of studies done in different areas of Ethiopia (21,26,68). However, one study done in Gonder showed 2 cases of ETF, and another study done in Nigeria showed 3 cases of ETF after treatment with AL (24,72).

There was a significant reduction and rapid parasite clearance in the study participants after taking AL for two days. This can be explained by the fast metabolism of artemether to its active ingredient DHA which is fast-acting with rapid absorption resulting in quick parasite clearance (20). Even though AL showed a significant reduction in parasite density, 15 participants (16.7%) were still found to have parasites on day 3. In Therapeutic Efficacy Studies (TES) the persistence of parasitemia in more than 10 % of patients on day 3 after starting treatment is considered as suspected artemisinin partial resistance in the area (17). The parasite clearance rate of AL demonstrated in the present study is relatively lower than earlier studies conducted in different parts of Ethiopia (22,24,26,27,69). A slower parasite clearance rate was associated with artemether resistance in the greater Mekong region (90). Recent reports from Africa also showed cases of slow clearing of *P.falciparum* infections (91–93). Another multi-center study in Rwanda demonstrated the first evidence of artemisinin partial resistance (94). However, the proportions of patients with day 3 parasitemia shown in the present study was considerably lower than the 21.9% patients found to be parasitemic on day 3 from a trial conducted in western Cambodia (95). Factors that determine parasite clearance rate include host immunity and splenic function (96), initial parasitemia, and nutritional status of the host (97).

The gametocyte clearance rate achieved in the present study is 100% on day 3. Other studies have demonstrated similar rate of gametocyte clearance on day 3. For instance, studies from Secha and Metehara have reported that all participants had cleared their gametocytes on day 3

(21,27). This is partly due to the effect of gametocidal drug, primaquine, which was administered in combination with AL. Multiple studies have shown, when used in combination with ACT, primaquine results in effective gametocyte clearance which has a significant public health importance in blocking transmission (98–100).

Quick parasite clearance results in subsequent relief of fever and other malaria symptoms. In the present study, fever clearance rate was 96.6% on day 3, which is consistent with that of a study done in Metehara (21) and Chewaka (23). However, The fever clearance rate observed in the present study is relatively lower than that of other efficacy studies conducted in Ethiopia that showed a 100 % fever clearance rate on day 3 (26,27,69). Another study from Kenya also demonstrated a 100% day 3 fever clearance rate (101). A delay in fever clearance is also an important indicator of artemisinin resistance.

Contrary to what is expected, there was a drop in the mean hemoglobin level of the study participants on days 14 and 28 compared to the baseline. Efficacy Studies from Ethiopia and other countries in Africa showed a slight improvement in the mean hemoglobin levels after treatment with AL (21,27,102,103). However, a study done in northwest Ethiopia showed a significant drop in the mean hemoglobin levels on days 14 and 28 compared to that of the baseline (104).

The hemoglobin drop after malaria treatment might be attributed to hemolysis from malaria itself or parasitized and non-parasitized red cells by the immune system (105). The treatment itself might also contribute to the change in hemoglobin levels. According to surveillance done in the US to evaluate the effectiveness and safety of AL in uncomplicated cases of *Plasmodium falciparum*, anemia was reported as severe AE of AL in two out of 108 patients, one of whom was readmitted for hemolytic anemia, the other one was treated outpatient and received a transfusion for ongoing hemolysis (61).

The review done on eleven published articles by Rehman *et.al* reported that delayed anemia is a common consequence following treatment of complicated severe malaria (106). In this situation, the most incriminated cause of anemia is post-artemisinin-delayed hemolysis (PADH), which typically occurs two weeks after starting treatment with artemisinin derivatives, leading to a significant decline in hemoglobin level. Among cases reported, the majority were treated with

parenteral therapy (107), whereas two cases of reported PADH were treated by oral AL (106). The mechanism behind PADH is likely related to the rapid clearance of ring forms of intraerythrocytic parasites, leading to the pitting of the red cells in the spleen. These pitted red cells have a reduced half-life when they return to circulation (108). While the concept of PADH could be a possible mechanism behind paradoxical drop in hemoglobin despite successful parasitic clearance, it is mostly reported in patients with severe malaria as opposed to patients included in this study, all of whom are cases of uncomplicated malaria. Therefore, further studies are warranted to confirm if the same mechanism would explain the finding in a population similar to this study.

Most of the AEs observed in this study were similar to the signs and symptoms of malaria itself. Some of the AE reported after treatment were headache, anorexia, dizziness, abdominal pain, and cough. The AEs reported in this study were almost similar to those observed in other studies conducted in different parts of Ethiopia (22,23,27,68). Mouth ulcer was commonly reported in different efficacy studies conducted in Ethiopia (22,68,78) however, it was not observed in the current study. All of the reported adverse events were mild and disappeared spontaneously and there was no serious adverse event reported up to the end of the 28-day follow-up period.

7. Conclusion

The result of this study demonstrated that the cure rate of AL remains high in the treatment of uncomplicated *P. falciparum*. AL is shown to be highly efficacious even after two decades of its implementation as first line drug in Ethiopia, with fast resolution of clinical symptoms as well as rapid parasite and gametocyte clearance. Eventhough a rise in hemoglobin level was expected after adequate parasite clearance, the result of this study demonstrated a slight drop of Hb on days 14 and 28; indicating the possible occurrence of AL induced delayed hemolytic anemia. Otherwise, there was no serious adverse event observed during the study period.

8. Recommendation

Based on the findings we recommend that AL should still be used as a first-line drug for the treatment of uncomplicated *P. falciparum* malaria in the study area. The possible causes for the drop in the mean hemoglobin level need further studies. Large scale periodic surveillance studies should however, be done for timely detection and institution of effective strategies to prevent emergence and control spread of drug resistance both in the study area and the country at large.

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ANNEXES

Annex I: Patient Screening Form

1.	Patient aged > 6 months /Both sex	Yes:	No:
2.	***P. falciparum/P. vivax*** mono-infection asexual parasites/µl **Pf** 500-100,000 /µl and **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 5-10 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, delirium,, coma) ə Lethargy ə Unable to drink or breast feed ə Vomiting everything ə Unable to stand/sit due to weakness ə Severe anaemia (Hb < 5.0 g/dL) ə Respiratory distress (laboured breathing at rest) ə Jaundice (yellow colouring of eyes)	Yes:	No:
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 II: 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 III: Case Record 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**	**Extra DAY**
1. Date									
2.Axillary To C									
3. Parasite asexual									
4.Gametocyte No									

5.Hemoglobin									
7.DBS/PCR									
8.Adverse events*									
9.Concomitant treatment									
10.Reasons for withdrawal									
11.Remarks/ Rare Event									
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 IV: 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 28 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 28 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 28 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 28 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.

V: Drug Dosing and Regimens

Artemether-lumefantrine (Coartem) will be administered twice daily for three days as tablets containing 20 mg of artemether plus 120 mg of lumefantrine in a fixed dose combination at a dosage.

Weight (kg)	**Day 1**		**Day 2**		**Day 3**	
	Morning	Evening	Morning	Evening	Morning	Evening

5–14	1	1	1	1	1	1
15–24	2	2	2	2	2	2
25–34	3	3	3	3	3	3
> 35	4	4	4	4	4	4

Annex VI: 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 labored breathing

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

Severe malarial anemia: A hemoglobin 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 edema: 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; hematemesis 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. falciparum*>2%

Annex VII: Medications with Antimalarial Activity that Should not be Used During the Study Period

- Antimalarial: -Chloroquine, Amodiaquine; Quinine, quinidine; Mefloquine, halofantrine, lumefantrine; Artemisinin and its derivatives (Artemether, arteether, artesunate, dihydroartemisinin); Proguanil, chlorproguanil, pyrimethamine; Sulfadoxine, sulfalene, sulfamethoxazole, dapsone; Primaquine; Avaquone;
- **Antibiotics:** Tetracycline*, Doxycycline, Erythromycin, Azithromycin, Clindamycin ,Rifampicin ,Trimethoprim, Pentamidine

- Tetracycline eye ointments can be used.

ANNEX VIII: 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 IV: Schedule of Follow-Up Activities

	day 0	day 1	day 2	day 3	day 7	day 14	day 21	day 28	Any other day
PROCEDURES									
Clinical assessment	X	X	X	X	X	X	X	X	X
Temperature	X	X	X	X	X	X	X	X	X
Blood slide for parasites count	X	X	X	X	X	X	X	X	X
Hemoglobin	X					X		X	X
Filter paper	X			X	X	(X)	(X)	(X)	X
Drug adverse event monitoring	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)

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 X: Consent Procedure and consent forms

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 PF 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/Gamo). 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
8. Clinician will start a patient folder and assign the next consecutive study ID number.

Consent/Assent Form in English

Title: - Monitoring the efficacy of frontline antimalarial drugs in Ethiopia, EPHI, 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:

Bontu Abate: Addis Ababa University College of Health Sciences, Department of Clinical Trial (CDT AFRICA) and PI of the Research

Mobile Number: +251911891545, Addis Ababa

Ibrahim Keder: EPHI staff and EPHI-IRB director

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

Anticipated Number of Subjects: *90-100 Voluntary Participants /site*

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 Federal Ministry of Health (FMoH) and EPHI.

Study duration: The study will take place over 28 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, and 28th 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 haemoglobin level determination and malaria diagnosis 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 days of follow-up. You or your child will be closely followed for the next 28 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 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 he/she can leave the study at any time without affecting his/her 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 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 participation is voluntary and that I can leave the study at any time without 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:* ___/____/____

Monitoring the Efficacy of Frontline Antimalarial Drugs in Ethiopia, 2022/23

የአማርኛ ስምምነት/መግባቢያ ሰነድ Consent/Assent form in Amharic

ርእስ

በኢትዮጵያ በ2015 ዓ.ም ወባ በሽታ ለማከም የሚሰጡ ተቀዳሚ መድኒቶች ያላቸዉን ውጤታማነትና ብግርነት ጥናት ማካሄድ።

ይህ የስምምነት መግባቢያ ሰነድ ለአዋቂዎች (ከ18 ዓመት ላይ ለሆኑ), ለታዳጊዎች ከ7 እስከ 17 ዓመትና ለህፃናት ከ6 ወር እስከ 11 ዓመት እድሜ ውስጥ ላሉ በወላጅ/በአሳዳጊ/ በተንባካቢያቸው አማካነኝነት የጥናቱ ተሳታፊ ለመሆናቸው ፍቃደኝነታቸውን በቃልና በፊርማ የሚገልፁበትና የሚያረጋግጡበት ቅፅ ነው።

ማናገር ቢያስፈልግዎ፡

ቦንቱ አባተ:

ሞባይል ቁጥር፡ +251 911891545 አዲስ አበባ፣ ኢትዮጵያ።

ኢብራሂም ከድር: የኢትዮጵያ ህብረተሰብ ጤና ኢንስቲትዩት ሠራተኛ (*EPHI-IRB SERO director)*

ሞባይል ቁጥር፡ +251 911 95 71 61 አዲስ አበባ፣ ኢትዮጵያ።

የጥናቱ ዓላማ፡የዚህ ክትትል ጥናት አላማ በሀገሪቱ ለወባ ህክምና የሚውሉት የፀረ ወባ መድሀኒቶችን የመላመድ እድል መኖር አለመኖሩንና የወባ ህክምና በኢትዮጵያም ምን ያህል እየሰራ እንደሆነ በጥናት ለማረጋገጥ ነው። ከዚህ ጥናት የተገኘው መረጃ በአገር አቀፍ የወባ የማጥፋት ፕሮግራም ለማስፈፀምና ለመከታተልና ያለውን ለውጥ የማዳን ብቃት የመድኃኒቱን መቋቋምና መላመድን ቅድሚያ ለማወቅና ተጨማሪ ስርጭትን ለመከላከል ተገቢውን የጣልቃ ገብነት ስልቶችን ለመተግበር ይረዳል። ይህ ጥናት በጤና ሚኒሰትርና በኢትዮጵያየ ህብረተሰብ ጤና ኢንስቲትዩት የተደገፈ ነው። ከላይ የተገለፀውን የምርምር ጥናት ለማከናውን እርስዎ ወይም ልጆቻችሁ የወባ በሽታ በረምዎ ውስጥ መብርመራ መኖሩ ስለተረጋገጠ በዚህ ጥናት እንድትሳተፉ እንጋብዛችኃለን።

የጥናት ጊዜ፡ ጥናቱ ለ28 ቀናት የሚቆይ ሲሆን ወደ ጤና ተቋም መምጣት ያለብዎት ቀናት፡ በ1ኛው ቀን ፣በ2ኛው ፣በ3ኛው ፣በ7ኛው ፣በ14ኛው ፣በ21ኛው እና በ28ተኛ ቀናት በጤና ተቋም ውስጥ መምጣትና መገኘት አለብዎት።

ሂደቶች፡ ጥናቱ የሚካሄደው ለ28 ቀናት ሲሆን ከነዚህም ቀናት ውስጥ ለህክምናው ሦስት ቀናት የሚሰጥ ሲሆን እርሰዎ በቀጠይ ባሉት ቀናት ጥዋት ጧት ወደ ጤና ጣቢያው እየመጡ መድሐኒቱን ይወስዳሉ። በዚህም ወቅት

አደጋዎች፡ ከጣታችሁ ላይ ደም በሚወሰድበት ጊዜ ጣት ላይ ሲወጉ በጊዜው ትንሽ ህመም ሊሰማዎት ይችላል ህመሞቹም በአብዛኛው ቀላል ናቸው ህመሙም ወድያው ይጠፋል የሚወሰዱት መድሐኒትም በውል ተለይቶ ያልታወቀ የጎንዮሽ ጉዳት ሊኖረው እንደሚችል ይገመታል። የመድሐኒቱን ተጽእኖ በማጥናት ሂደት ሳይሻለው የሚሄድ ታካሚ ከተገኘ በወባ የህክምና አሰጣጥ መመሪያ መሰረት ህክምና በነጻ ይደረግሎታል።

የሚጠበቁ ጥቅሞች፡ በዚህ ጥናት ውስጥ በመሳተፍ የግል (ቀጥታ) ጥቅማጥቅሞችን ላያገኙ ይችላሉ ግን በጥናቱ በመሳተፍ ክፍያ አያገኙም ነገር ግን የጥናቱ ግኝቶች ለአገር አቀፍ የወባ የማጥፋት ፕሮግራም ፤የወባ መዳኒቶች የመቋቋም መከሰትን ጥናትና የወባ ህክምናን ለማሻሻል ተገቢውን የጣልቃገብ እርመትና መስተካከያ ስልቶችን እንዲያደርጉ ይረዳል።ይህ እርስዎን ወይም የሚያውቁትን ሰዎች የጥናቱ ውጤት ተጠቃሚ ያደርጋል።

ጉዳቶች፡ በዚህ ጥናት ውስጥ በመሳተፍ ምክንያት ለሚደርስ ማንኛውም ጉዳት በህክምና ተቐማቱ ሰራተኞችና በጥናቱ አባላት ህክምናን ለማግኘት ይረዱዎታል ይህም እንደ አስፈላጊነቱ የድንገተኛ ህክምና የሆስፒታል እንክብካቤና ክትትል የሚደረግበት እንክብካቤና በወቅቱ ለተከሰተው ህክምና ወጪዎ ክፍያ በኛ ይከፈላል።

ማካካሻ፡ ለክትትል ወደ ጤና ተቋም ሲመጡ የትራንስፖርት ወጪዎት የሚሸፍን መቶ ብር የሚሰጥዎ ይሆናል።

ተጨማሪ ወጪ፡ ለትራንስፖርት ማካካሻ ከሚሰጠው ክፍያ ውጪ የተለየ ክፍያ የለም።

ሚስጥራዊነት፡ ሕጉ በሚፈቅደው መጠን ስለእርስዎ ያለው ውጤት ወይም መረጃ በሚስጥር ይጠበቃል።

ተሳትፎ (በፈቃደኝነት)

በዚህ ጥናት ውስጥ መሳተፍ የእርስዎ በግል ፍላጎት ላይ የተመሰረተ ምርጫ ነው። በማንኛውም ጊዜ በጥናቱ ውስጥ ላለመሳተፍ ወይም ለማቋረጥ መወሰን ይችላሉ የሚወስዱት ምርጫ ለወባ የሚወስዱትን ሕክምና አይጎዳውም እንዲሁም በህክምና ተቐሙ የሚያገኙት ወይም የሚቀበሉት የትኛውም ህክምና አይጎዳም ተፅእኖም አይኖረውም። ጥናቱን በማንኛውም ጊዜ መተው ይችላሉ። ይህ በጤና አጠባበቅዎ ላይ ተጽእኖ አይኖረውም በቀጣይም የወባ ህክምናን በነጻ ያገኛሉ። ተስማምቼ በዚህ ጥናት በፍቃደኝነት ለመሳተፍ የፍቃደኝነት ማረጋገጫዬን በተለመደው ፊርማዬ ወይም በአውራ ጣት አሻራዬ ከታች በተዘጋጀው ቦታ በማስቀመጥ ወይም በመፈረም በጥናቱ ለመሳተፍ ፍቃደኝነቴን አረጋግጣለሁ።

ይህን ቅጽ አንብቤአለሁ፣ ወይም ተነቦልኛል፤ ስለ አሰራሩና ጥናቱ ያልገቡኝን ጥያቄዎች ለመጠየቅ ችያለሁ ከዚያም ለጥያቄዎቼ ተገቢውን ማብራሪያና መልስ አግኝቻለው።

እኔም አዋቂ፤ ልጄ ወይም እኔ የምንከባከበው በጥናቱ ለመሳተፍ በስምምነትና በፈቃደኝነት እንደሆነ እና የጥናቱንም ተሳትፎ በማንኛውም ጊዜ እንክብካቤዬን ሳይነካኝ መተው እንደምችል ተረድቻለሁ።

<u>ተሳትፎ ለታዳጊ ልጆች፡</u> የእኔ ተሳትፎ በፈቃደኝነት እንደሆነ እና ጥናቴን በማንኛውም ጊዜ እንክብካቤዬን ሳይነካ መተው እንደምችል ተረድቻለሁ የመሳተፍ ውሳኔዬ በወላጅ ወይም አሳዳጊዬ የተደገፈ ነው ነገር ግን በእሱ/ሷ አስገዳጅነት አይደለም።

የፍቃደኛ ተሳታፊውን የሚያመላክተውን ተራ ቁጥር ይከበብ

1. **ከ6 ወር እስከ 11 ዓመት** ለሆኑ ህፃናት የተሰናዳ የስምምነት ቅጽ
2. **ከ12 እስከ 17 ዓመት** ለሆኑ ታዳጊዎች የተሰናዳ የስምምነት ቅጽ
3. **ከ18 ዓመት** በላይ ለሆኑ **አዋቂዎች** የተሰናዳ የስምምነት ቅጽ

ስምምነቱን የሚያስሞላው ባለሙያ

ስም _____ ፊርማ _____ ቀን _____

<u>የተሳታፊ ስም</u> _____ፊርማ _____የጣትአሻራ _____ቀን _____

<u>ወላጅ/አሳዳጊ ስም</u> _____ፊርማ ___ የጣትአሻራ _____ቀን _____

ምስክር ስም _____ ፊርማ _____ ቀን _____