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Therapeutic Efficacy of Artemether-Lumefantrine for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Metehara Town, Central-east Ethiopia

By
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Contents

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Abbreviations

ACPR	adequate clinical and parasitological response
ACTs	artemisinin combination therapies
AL	artemether-lumefantrine
ART	artemisinin derivatives
AS-AQ	artesunate-amodiaquine
ASMQ	Artesunate plus mefloquine
CI	Confidence Interval
CQ	Chloroquine
DHA-PPQ	dihydroartemisinin-piperaquine
ETF	early treatment failure
FMoH	Federal Ministry of Health
IRS	indoor residual spraying
ITNs	insecticide-treated nets
LCF	late clinical failure
LPF	late parasitological failure
MOI	Multiplicity of infection
MSP1	merozoite surface protein 1
NMSP	national malaria strategic plan
PfKelch13	*Plasmodium falciparum* Kelch13
PQ	primaquine
SSA	sub-Saharan Africa
TES	therapeutic efficacy studies
WHO	World Health Organization

Abstract

Monitoring and identification of drug-resistant Plasmodium falciparum strains is paramount for the fight against malaria. Close surveillance of the emergence and distribution of artemisinin resistance is recommended to guide policy decisions. The efficacy of national first- and second-line anti-malarial treatments should be monitored at least once every 2 years, as recommended in the WHO standard protocol. In Ethiopia, a three-day regime of AL (artemether 20mg and lumefantrine 120mg in each tablet) is the first-line anti-malarial drug for the treatment of uncomplicated P. falciparum malaria since 2004. The objective of this study was assessing the therapeutic efficacy of AL for the treatment of uncomplicated P. falciparum malaria in Metehara, central-east Ethiopia. The study was conducted at Metehara town health center from November 26, 2020 to March 24, 2021. One-arm prospective evaluation was conducted on the clinical and parasitological responses to directly observed treatment for uncomplicated P. falciparum malaria. During the study regime, 80 patients were screened and 73(50 male and 30 female) participants completed the follow-up and among those 14 patients were <5 age, 25 between the age 5-14 and 34 were >14. The overall cure rate was 100% (73/73; 95% CI: 95.1-100.0) with no early treatment failure, late treatment failure, and late parasitological failure as in Kaplan–Meier analyses all participants completely recovered from parasitemia and fever on day (D) 3; the asexual parasite clearance rate was 100% and clinical symptoms resolved quickly. Gametocyte carriage was reduced from 8.4% on D0 to 1% on D3 and complete clearance was achieved on D7. There was no serious adverse event. In the study location, AL was effective for treating uncomplicated P. falciparum malaria.

Keywords: Artemether-Lumefantrine, *P. falciparum*, Therapeutic efficacy, Metehara

1. Introduction

Malaria is a disease of the most vulnerable, the very young and the poor. (WHO, 2022). In 2020, 1 year after the COVID-19 pandemic and service disruptions, the estimated number of malaria cases rose to 241 million cases and 627,000 deaths, an additional 14 million cases and 218,000 deaths compared with 2019 (WHO, 2021b). Globally in 2022, there were an estimated 249 million malaria cases and 608 000 malaria deaths in 85 countries(2023).WHO has launched the Global Technical Strategy for Malaria 2016-2030 with the aim of helping countries reduce malaria burden and others eliminate the disease malaria (WHO, 2015). However, the highest burden of malaria is in Africa, especially in sub-Saharan Africa (SSA) remains unabated although there is substantial progress towards malaria control and elimination.

Unemployment, low-income and poor construction of households which are suitable for Mosquito breeding are socio-economic triggering factors that hinder the effort taken to mitigate the suffering by malaria (Id *et al.,* 2019). In addition, urbanization and climate change are putting more people at the risk of malaria in sub-Saharan African countries (Caminade *et al.,* 2014). Urbanization causes deforestation for various land use purposes, such as construction of buildings to accommodate the urban growing population. These bring vector species distribution-related changes leading to predictable changes in temperature, humidity and precipitation which are expected to occur and will affect the vector's ecology and biology, and increase the risk of disease transmission (Kweka *et al.,* 2017). Effective vector control and case management are the pillars for the malaria preventive and control intervention. Effective vector control comprises instigating insecticide-treated nets (ITNs) and indoor residual spraying (IRS) (Okumu *et al.,* 2011). For case management, the first-line treatment regimen for *P. falciparum,* the deadliest malaria parasite, is artemisinin combination therapies (ACT) in nearly universally and the combination of two drugs artemether-lumefantrine (AL) is the most common ACT in use (Eastman *et al.,* 2009). The distinctive feature of ACT is its combination of longer-acting partner medications that eradicate residual parasites and stop recrudescence with fast-acting artemisinin derivatives that quickly reduce parasite biomass (Nsanzabana., 2019). By focusing on both the sexual forms that transmit the disease and the asexual blood stage parasites that cause clinical symptoms, this dual strategy efficiently stops the disease's progress and lowers the risk of medication resistance (Rasmussen *et al.,* 2023).

Anti-malarial medications are important instruments in the battle against malaria because they help cure the infection and prevent it from spreading. These medications are classified as first-line and second-line therapies, based on their efficacy, safety, and resistance trends. Globally, the selection of first- and second-line antimalarial medications is influenced by factors such as local malaria epidemiology, drug resistance trends, and healthcare infrastructure (White., 2008). Nevertheless, the threat of emergence and rapid spread of drug-resistant parasite strains is ever continuous. A resistance against AL has been established in Southeast Asia and in Africa recent reports from Rwanda and Uganda appear to follow suit although in SSA the treatment remains effective so far (Takala-Harrison *et al.,* 2015).

Over the past two decades, ACT has become the cornerstone of malaria treatment, recommended by the World Health Organization (WHO) as the first-line therapy for uncomplicated *falciparum* malaria in virtually all endemic areas. In areas where ACT has been widely used, significant improvements have been seen in lowering malaria-related morbidity and death (WHO, 2020). Despite this, certain obstacles endure, such as the emergence in resistance to artemisinin derivatives in Southeast Asia, highlighting the significance of sustained investigation and oversight initiatives to preserve the effectiveness of this important medicinal instrument (Hanboonkunupakarn *et al.,* 2022).

In general, close surveillance of the emergence and distribution of ACT resistance is recommended to guide policy decisions. The efficacy of national first- and second-line anti-malarial treatments should be monitored at least once every 2 years, as recommended in the WHO standard protocol for monitoring drug efficacy (WHO, 2009). A change in an anti-malarial medicine recommended in the national malaria treatment policy should be initiated as soon as possible in order to prevent the spread of multidrug-resistance. When treatment failure exceeds 10% policy intervention is necessitated.

To summarize, the global distribution of malaria incidence and deaths emphasizes the importance of targeted treatments adapted to the unique problems that various areas experience. Strengthening healthcare systems, boosting access to effective antimalarial medications and vector control measures, and encouraging international collaboration are critical for making steady progress toward malaria eradication.

1.1 Statement of the problem

Plasmodium resistance to antimalarial medicines is one of the key recurring challenges in the fight against malaria. Monitoring antimalarial drug efficacy supports early detection of changes in how well the recommended treatments work; this enables rapid action to mitigate any impact of resistance and prevent its spread (Vestergaard *et al.*, 2007) . Therapeutic efficacy studies (TESs) provide a measure of clinical and parasitological patient outcomes, and are the main source of data on which the national Malaria programs(NMPs) base their decisions regarding which treatment to recommend (FMoH, 2009) .

The World Health Organization (WHO) recommends that the efficacy of the first- and second- line antimalarial drugs be regularly assessed for early detection and prevention of spread of resistant parasite populations (Rovin., 2005) . For rapid and evidence-based revision of treatment policies malaria drug efficacy studies are vital. Failure to detect the emergence of anti-malarial drug resistance could lead to drug resistant malaria epidemics with drastic public health and economic consequences. Most malaria epidemics recorded in history were partially attributed to unrecognized resistance to the anti-malarial therapy being used at the time (WHO, 2009). There are different methods of detecting the emergence and spread of malaria drug resistance in vivo therapeutic efficacy study being the gold standard. Knowledge of malaria transmission intensity and factors that influence it is of paramount importance in identifying sentinel sites where antimalarial drug efficacy studies could be conducted (Nsanzabana *et al*., 2018) .

In line with the above global task, Ethiopia, which currently is the 15th in malaria case number from 43 African countries (WHO, 2021b), has been closely monitoring AL efficacy in nationwide sentinel sites since the introduction of the treatment in 2004. Ethiopia had experienced a devastating malaria epidemic in 1958, resulting in about three million cases and 150,000 deaths. Since then, malaria was considered as a main concern for the health sector. Although Ethiopia achieved an estimated 75% reduction in malaria cases from baseline of 2013 by 2020 and elimination for *P. falciparum* malaria in selected low transmission areas by 2020 is planned and underway (Alegana *et al,* 2020) malaria burden persists. The Ethiopian national malaria strategic plan (NMSP) 2021-2025 extended to 2030 is ongoing following a malaria programme review (MPR) to anticipate malaria elimination (FMoH, 2021).

Accordingly, the Ethiopian Federal Ministry of Health (FMoH) has established sentinel sites to conduct national antimalarial drug efficacy studies every two years. A number of studies have already been conducted in different parts of the country on AL efficacy in largely low malaria transmission (Gebreyohannes *et al.*, 2017) . Metehara is one of the sentinel sites, its estate irrigation by using the nearby rivers for the industrial cultivation of sugarcane, in turn, suits the condition for breeding of the Anopheles mosquito. Transmission of malaria in this area occurs year-round. The estate irrigation by using the nearby rivers for the industrial cultivation of sugarcane, in turn, suits the condition for breeding of the Anopheles mosquito (Nega *et al.*, 2016) . This study was an extension of the national AL therapeutic efficacy studies (TES) in one of the multiple sentinel sites in east-central Ethiopia.

1.2 Objective

1.2.1. General objective

The general objective of this study was to assess the therapeutic efficacy of AL for the treatment of uncomplicated *P. falciparum* malaria in Metehara, central-east Ethiopia.

1.2.2. Specific objective

The specific objectives of the study were the following:

1. To measure the clinical and parasitological efficacy of AL in uncomplicated *P. falciparum*-mono infected patients over 6 months by determining primary and secondary endpoints: early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) or adequate clinical and parasitological response (ACPR) as indicators of efficacy;
2. To evaluate the adverse events during the study period
3. To estimate fever clearance rate: proportion of patients who have fever cleared at day 1, 2 & 3
4. To assess gametocyte carriage during the study period.

2. Literature Review

2.1. Human Malaria

Malaria is a deadly illness transmitted to people by five species of mosquitoes, among those one species is zoonotic.Malaria is mostly transmitted to humans through the bites of infected female Anopheles mosquitoes. Tough, the illness is not transmitted from person to person. Blood transfusions and infected needles can potentially spread malaria (Elliott *et al.,* 2011).Malaria is mostly caused by *P. falciparum* and *P. vivax*. Both species together provide the greatest hazard, accounting for the vast majority of malaria-related illness and mortality. Among the two species, *P. falciparum* causes the majority of severe clinical malaria. As evaluated by clinical infection outcomes, it has been linked to parasite density, which is controlled by the capacity to attach to endothelial cells (Hayward *et al.*,1999).

2.1.1 Global Burden of Malaria

The target year for malaria goals was set in 2015 by the World Health Assembly to reduce malaria incidence and mortality and the launch of WHO's Global technical strategy for malaria 2016-2030 (WHO, 2015) . From the year 2018-2020, the estimated number of cases has been increasing repeatedly. The estimated number of cases in 2015 was 214 million which has increase in 2020 to 229 million. However, the estimated number of deaths to some extent has shown to decrease from 438,000 in 2015 to 409,000 in 2019 but unexpectedly show significant increase in 2020 to 627,000 due to the Covid pandemic disruption (WHO, 2015 , WHO, 2020) .

According to WHO data from 2019, the WHO African Region has the highest rate of malaria cases and deaths, followed by the WHO Eastern Mediterranean, WHO Western Pacific, WHO South East Asia, and WHO Region of the Americas. The WHO African Regions saw a halt in the decline in cases and death rates between 2015 and 2020, although they continued to lag behind the other Regions in terms of both numbers (WHO, 2020). According to WHO estimates,the WHO African Region accounted for 88% of malaria cases and 90% of deaths in 2015. In a similar vein, 90% of 88% estimated cases and 80% of 93% estimated deaths in 2016 and 2017 were confirmed, respectively (WHO, 2017). Furthermore, 93%, 94% of cases and 93%, 94% of deaths were recorded consecutively in 2018 and 2019 (WHO, 2019). It is

feasible to conclude that there have been more cases and deaths in the WHO African Region in 2019 based on the higher case death rate from the six-year documented percentages (WHO, 2020).

Cabo Verde and Sao Tome and Principe have reported zero malaria deaths since 2018, while theWHO African Region accounts for 95% of cases and 96% of deaths from malaria in 2020 (WHO, 2021b). Nigeria has the highest case rate (25%) among the WHO African regions, followed by the Democratic Republic of the Congo (12%), Uganda (5%), and Mozambique (4%). In a similar vein, in 2019, Nigeria has the highest death rate (24%), followed by the Democratic Republic of the Congo (11%), United Republic of Tanzania (5%), and Niger (4%) (WHO, 2020).

The second highest malaria burden was found in the WHO South East Asia Region, where case and fatality rates were 5.2% and 5.7%, respectively, in 2015 and 2016, indicating that the rates would likely stay similar in the years that followed (WHO, 2017). In addition, in 2017, 2018, and 2019, the case rate was 4.5%, 3.3%, 2.7%, and the death rate was 4.2%, 2.6%, and 2.2%, correspondingly. This has shown a consistent drop in the region's case and fatality rates, accounting for 2% of global malaria cases in 2020 (WHO, 2021b).

Between 2015 and 2019, the case and mortality rates in America, the West Pacific, the Eastern Mediterranean, and the remaining WHO areas are all somewhat around 3%. In countries like Sudan, Somalia, and Djibouti, the anticipated number of cases in the Eastern Mediterranean increased by 500,000 between 2019 and 2020 (WHO, 2021a). Additionally, the nations in this region have eradicated malaria. For instance, the countries of the Islamic Republic of Iran, Iraq, Oman, and the Syrian Arab Republic had not reported any instances of malaria in the Eastern Mediterranean region before to 2015.In the Americas and Western Pacific, China, Belize, and El-Salvador all reported having no malaria cases in 2019 (WHO, 2020).

2.1.2. Burden of malaria in East Africa

For centuries malaria has been a global burden of the world. Among parts of the world, the sub-saharan african countries account the highest case and death records. According to the recent report of the WHO in 2022, the WHO African Region was responsible for around 95.4% of fatalities and 93.6% (approximately, 223 million of cases) worldwide; among these

children under the age of five accounted for 78.1% of all deaths and nearly half of all cases worldwide were concentrated in four countries: Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), and Mozambique (4%) in this region (WHO, 2023).Between the year 2018-2022, the number of case and death has been increasing in WHO African regions, only on the year 2020 and 2021 number of case were stable and from the year 2018 to 2019 the number of death decrease.

In the western Kenyan highlands in 1918 and 1919, during the demobilization and resettlement of troops following World War I, the first malaria outbreak was recorded in the wake of the influenza pandemic. Rare reports of malaria outbreaks were made in the highlands of eastern Africa between the 1920s and the 1950s.Malaria had returned in the 1980s in the eastern part of the region and persists still (Himeidan, Y. E., *et al*, 2012).

East Africa has a tropical climate, diversified terrain, and outstanding biological factors that promote the development of malaria; this in turn is suitable for vector breeding and malarial parasite survival. Worldwide, there are over 3500 identified species of mosquitoes. In East Africa, the Anopheles genus of mosquitoes is responsible for the transmission of malaria. There are around 460 identified species in this genus, and between 60 and 100 Anopheles species have been linked to the spread of malaria parasites (Amimo, F. A. ,2023).

P. falciparum is the most lethal of the four human malaria parasites and causes the majority of cases in Africa. It is also because the most effective malaria vector, the mosquito Anopheles gambiae, is among the most prevalent in Africa and is the hardest to eliminate. Malaria kills an estimated hundred thousands of people in Africa annually, with the majority being children under the age of five following pregnant women(WHO,2003).. In sub-Saharan Africa, *P. falciparum* is more common and is associated with a higher prevalence of mortality from malaria. The precise geographic range of *P. falciparum* has the potential to influence both the intensity of infections and the local immune response (Robert, 2006).

Even though the highest burden of malaria continued in Africa, The northernmost countries Algeria, Egypt, Libyan, Jamahiriya, Morocco, and Tunisia had eliminated it since the disease was mostly caused by *P. vivax* and spread by mosquitoes that were simpler to manage than those in Africa south of the Sahara(WHO, 2019). Since 2018, Cabo Verde has reported no malaria-related fatalities. In 2022, there were no malaria deaths recorded for the first time in

the Comoros and Sao Tome and Principe; also, less than 10 deaths were reported in Botswana, Eritrea, and Eswatini (WHO, 2023).

The WHO's strategy to address antimalarial drug resistance in Africa was introduced in November 2022. This advocacy and technical paper has four pillars: improve surveillance of antimalarial medication effectiveness and resistance, Optimize and better control the use of diagnostics and therapies to decrease drug pressure through preventative approaches, Respond to resistance by restricting the spread of antimalarial drug-resistant parasites and Encourage research and innovation to improve existing tools and create new ones against resistance. (WHO, 2022).

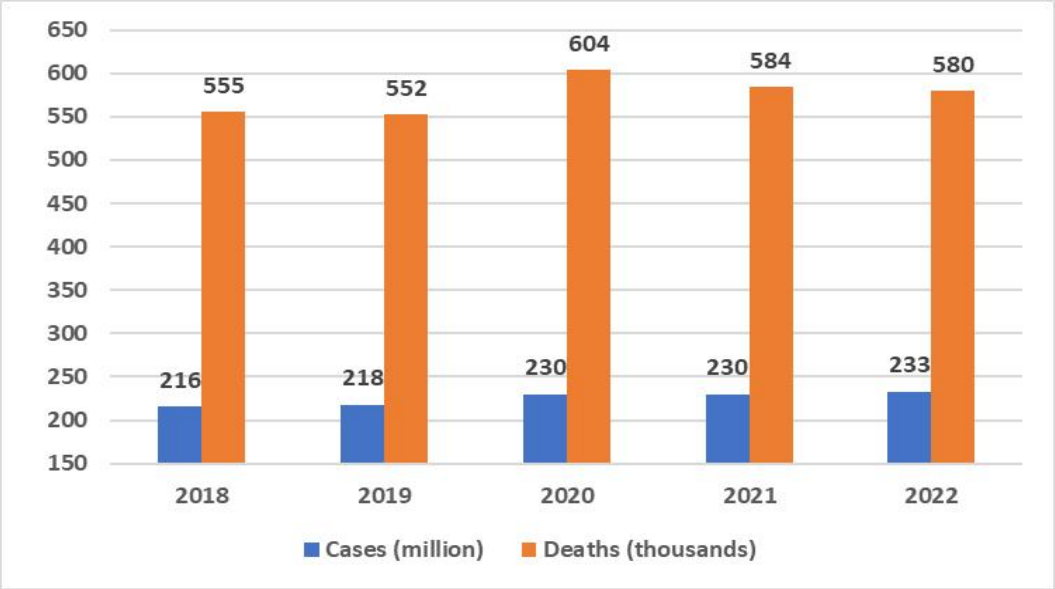


Figure 1. Global Malaria Cases and Deaths, 2018-220. Source: World Malaria Report.

The recent three years, 2020-2022, showed an increasing trend of malaria cases compared to the years 2018 and 2019. However, it appears that malaria deaths remained constant without much increase unlike the cases (Figure 1).

2.1.3. Burden of Malaria in Ethiopia

Ethiopia is located in the Horn of Africa, with latitudes 3-18°N and longitudes 33-48°E. The Great Rift Valley, which encompasses desert, semidesert, and savannah, separates the nation into the northwestern and southeastern highlands. The Dalol Depression in the Danakil Desert, located 125 metres below sea level on the northern border with Eritrea, is one of the

hottest locations on Earth (FMoH, 2014). Climatic is influenced by height, and altitude is used to define Ethiopia's three climatic zones: kola, or hot lowlands (<=1500 mASL), weyna dega (1500-2400 mASL; mean annual temperature 16-29o C), and dega, or cold highlands (>2400 mASL). Of the three climatic zones, the lowland and temperate regions are conducive to the survival of the malaria parasite and vector, which means that those residing in these areas are susceptible to the disease.

High malaria prevalence was estimated for the Great Rift Valley, the country's eastern and northwest regions (Humera, Metema, Sanja, Quara, and Kebridehar, Gode, respectively). On the other hand, the country's centre regions were expected to have a low malaria prevalence and the prevalence of malaria was found to be positively correlated with climatic factors, including annual mean temperature, annual mean precipitation. (Simane, 2016).

Since 2005, efforts have been undertaken to expand malaria interventions, including the distribution of insecticide-treated bed nets (ITN), indoor residual spraying (IRS), and the implementation of artemisinin-based combination treatment (ACT)(Initiative, U. P. S. M. ,2020). Despite the fact that effective vector control and case management were established, there were significant increases in predicted case numbers in Ethiopia (estimated 2.4 million) between 2019 and 2022; nonetheless, Ethiopia is now ranked 14th among African nations in terms of case numbers in 2023(WHO, 2023) The distribution of the malaria case within the two species is listed in table 1.

Table 1 Malaria case report by species in Ethiopia from 2018-2023: Source-World Malaria Report.

Year	**Case number with species**			
	P. falciparum	*P.vivax*	Mixed Species	Total cases
2018	859,675	102,412	-	962,087
2019	738,155	166,340	-	904,495
2020	1,340,869	263,877	30,051	1,743,755
2021	912,075	252,589	14,380	1,390,351**
2022	1,323,355	388,416	-	1,731,097**

2.1.4. Burden of malaria in Oromia region

Oromia is named for the Oromo people, who settled along the edge of the Ethiopian Highlands that form this Zone. Oromia Zone is bordered on the southwest by North Shewa Zone, on the northwest by South Wollo and Argobba special woreda, and on the east by the Afar Region. According to a study conducted in 2022, Data on malaria were collected from 643 georeferenced sites from every part of the nation and the Oromia region holds 0.3% of malaria prevalence from the 11 regions (Alene *et al.,* 2022).

Metehara is a sentinel location for malaria TES in the Oromia region. The town uses local waterways to irrigate sugarcane, vegetables, and fruits, which promotes Anopheles mosquito reproduction and exposes the populace to malaria. Malaria transmission in this location is year-round, however strength varies by season.The area is classified under semi desert with annual average rainfall 1.5mm, humidity 67% and daily temperature 32 o C and 19 o C on average(Desalegn *et al.,* 2016).

2.2. Life cycle of *P. falciparum*

The infection mechanism and the occurrence of clinical symptoms mainly count on the parasite life cycle inside the vector and in the host. The parasite life cycle begins when a female anopheles mosquito bites an infected person and takes a blood meal, containing gametocytes (sexual stage). In the mosquito midgut, gametogenesis (the union of male and female gametes) occurs, the zygotes will form and develop into ookinetes, and the ookinetes traverses the mosquito midgut wall and forms an oocyst. Finally, the oocysts undergo multiple rounds of cell division and produce thousands of sporozoites (Arthur *et al.,* 2004). When an infected mosquito bites a person, an infectious form of the parasite in humans (sporozoites) enters into the bloodstream infecting liver cells, multiplying and maturing into a form called merozoites (blood stage). In the bloodstream, merozoites invade red blood cells; undergo a series of developmental stages, leading to the release of more merozoites upon rupture of the infected red blood cells (Robert *et al.,* 2013).

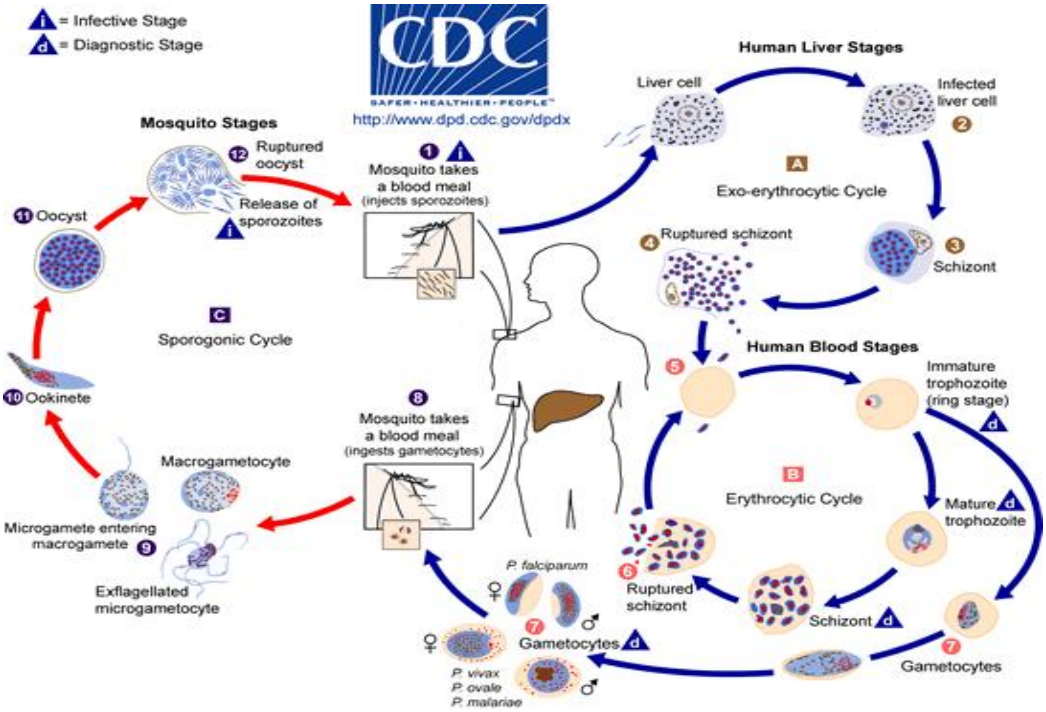


Figure 2 The Life cycle of *P. falciparum* in mosquito and human host.

2.2.1. Gametocytogenesis

P. falciparum is the most fatal protozoan parasite for humans. *P. falciparum* undergoes a puberty-like process in human blood, transitioning from an asexual to a sexually competent parasite. Gametocytogenesis is the process of producing male and female gametocytes (pre-gametes) for eventual fertilization in an invertebrate host (Dhawan *et al.,* 2016) and this is due to extreme environmental change (temperature, pH, salt composition) and also, there are pharmaceutical substances that either seem to start gametocytogenesis or raise the quantity of gametocytes. Red cell lysate, for instance, has been demonstrated to raise the rate of gametocytogenesis.place significantly impact gene expression patterns (Baker, 2010).

The exchange of parasites between primary and secondary hosts is crucial to maintain the life cycle. Exchanged parasite stages between hosts play a critical role in interrupting transmission (Dash *et al.,* 2022). There are two theories as to how gametocytogenesis, or sexual stage commitment, happens. Within the human host, a subset of merozoites is dedicated to growing into gametocytes, the sexual form, without going through the erythrocytic schizogony (Bancells *et al.,* 2019). The alternative path involves gametocyte commitment at the erythrocytic schizont, meaning that every committed schizont's

merozoites will eventually grow into one or both types of gametocytes, but never both (Silvestrini *et al.,* 2000).

In *P. falciparum* species gametocytes mature in five phases over ten to twelve days . Gametocytes in stage I and early stage II resemble trophozoites and are virtually round. Late stage II gametocytes can be differentiated from trophozoites by morphology. Late-stage III and IV gametocytes are lengthy and spindle-shaped. Stage V is crescent shaped (falciform in Latin) (Meibalan *et al.,* 2017). While in the case of *P. vivax* species because of its complexity, research into *P. vivax* gametocyte biology has been restricted to human infection (both natural and experimental) (Roobsoong *et al.,* 2015). Merozoites prefer reticulocytes, which are uncommon in peripheral blood. They also take longer to develop on culture media, making in-vitro studies challenging. Gametocytogenesis can begin with the initial generation of *P. vivax* merozoites (around 48 hours). Gametocytes can form within three days after encountering the first asexual parasites. Gametocytes leave the bloodstream within three days after development (Sinden, 2002).

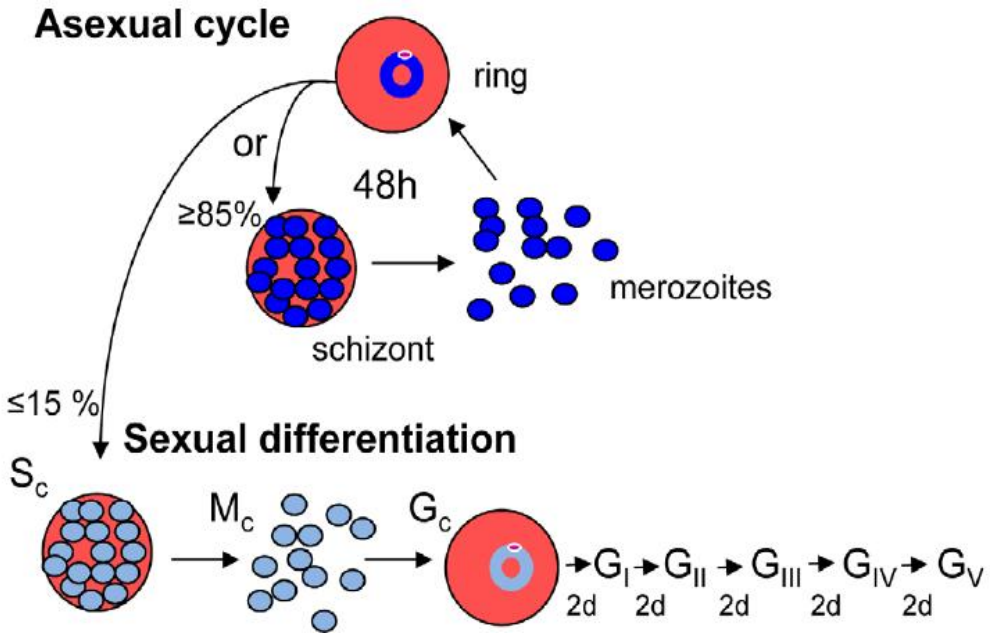


Figure 3 Gametocytogenesis in *P. falciparum* malaria.

2.3. Malaria Case Management

More than 600,000 people die each year from malaria, which is a curable disease. Malaria must be diagnosed and treated as soon as possible in order to manage the condition effectively. High-quality malaria diagnosis, whether by microscopy or rapid diagnostic

testing, is critical in all malaria-endemic areas. Artemisinin-based combination therapy (ACT) is the most effective treatment available, especially for *P. falciparum* malaria (WHO, 2007).

2.3.1. Diagnosis

The efficient therapy of malaria requires prompt and precise diagnosis. Malaria diagnosis is detecting malaria parasites or antigens in patient blood. Although this may appear to be a straightforward concept, diagnostic effectiveness is dependent on a variety of circumstances. Malaria diagnosis based on clinical, laboratory diagnosis and molecular diagnostic methods (Tangpukdee *et al*, 2009).

2.3.2.1. Clinical Diagnosis

This approach is the least costly and most extensively used. Clinical diagnosis is based on the patient's signs and symptoms, as well as physical findings during examination. Malaria's first symptoms are generic and diverse, including fever, headache, weakness, myalgia, chills, disorientation, stomach discomfort, diarrhoea, nausea, vomiting, anorexia, and pruritus (Gill *et al.*, 2011). Clinical diagnosis of malaria remains difficult due to the non-specific character of the signs and symptoms, which overlap significantly with other infectious diseases (Varo *et al.,* 2021). The overlapping of malaria symptoms with other tropical illnesses reduces diagnostic specificity, which can promote the indiscriminate use of antimalarials and undermine the quality of care for patients with non malarial fevers in endemic regions (Chipeta *et al.*, 2009). As a result, combining clinical and parasite-based data can significantly improve the accuracy of malaria diagnosis (Kyabayinze *et al.,* 2008).

2.3.2.2. Laboratory Diagnosis

Rapid and accurate malaria diagnosis not only relieves pain but also reduces community transmission. Malaria's unspecific clinical signs and symptoms may result in over-treatment or non-treatment of various diseases in malaria-endemic regions, as well as misdiagnosis in non-endemic areas (Bhandari *et al.,* 2008). Malaria is diagnosed in laboratories using a variety of approaches. Among the many techniques, standard microscopic diagnosis by staining thin and thick peripheral blood smears and fast diagnostic tests are the most popular (Wilson, 2013).

2.3.2.2.1. Microscopic Diagnosis

Microscopic detection and identification of Plasmodium species in Giemsa-stained thick blood films (for screening the presenting malaria parasite) and thin blood films (for species confirmation) remain the gold standard for laboratory diagnosis (Mathison *et al.,* 2017). Moreover, This technique's widespread acceptance by laboratories throughout the world may be ascribed to its simplicity, low cost, and ability to determine parasite presence, infecting species, and parasite density--all of which are relevant characteristics for malaria control (Chander *et al.,* 2001).

2.3.2.2.2. Rapid diagnostic test

Several antigen rapid detection tests (RDTs) are commercially available and are rapidly being utilized for malaria diagnosis globally.These techniques use lateral flow assays (cassette, dipstick, or card formats) with a nitrocellulose membrane containing parasite antigens(Wilson, 2013). Globally, between 2010 and 2022, manufacturers sold 3.9 billion rapid diagnostic tests (RDTs) for malaria, with Sub-Saharan African nations accounting for 82% of the total. During the same year, NMPs distributed 2.9 billion RDTs, with 90% in Sub-Saharan Africa(WHO, 2023).

Malaria RDTs are indirect tests that identify antigens and enzymes generated by the malaria parasite. RDTs can give a fast diagnosis, especially in non-endemic nations where it is difficult to maintain microscopic skills and there are few imported cases each year; hence, RDTs have been employed increasingly often for malaria diagnosis (Cunningham *et al.,* 2019). But , RDT sensitivity can be affected by parasite densities in blood circulation. It implies that the RDT kit's detection decreases with decreasing parasitemia density (Erdman *et al.,* 2008). The reported RDT sensitivity is typically good for *P. falciparum*, but only moderate for *P. vivax* (66.0-88.0%) (Wu *et al.*, 2023).

The efficiency of malaria parasite clearance interventions is highly dependent on the performance of diagnostic tools. Several additional RDTs assessed in the WHO trials had higher sensitivity rates for detecting high and low *P. falciparum* and *P. vivax* densities. New RDTs are still being developed, and they may offer further advantages over existing tests (WHO, 2015).

2.3.2.3. Molecular Diagnosis

In low transmission areas malaria infections have low parasite loads or are even asymptomatic, making infection identification in elimination settings more challenging. In order to eradicate the disease, malaria diagnosis must shift from passively identifying sick patients in medical facilities to actively identifying carriers in the community who may or may not exhibit clinical symptoms (Britton *et al.*, 2016).

PCR is a very sensitive molecular diagnostic in research and reference laboratory settings in malaria in endemic and non-endemic regions. It improved the detection limit for malaria infection to less than 2 parasites/μL (Erdman *et al.,* 2008). PCR uses genotyping technology in the laboratory because of its robust heat cycling approach, which is specific, simple, quick, and sensitive. PCR has enabled the development of very sensitive parasite detection methods, and the specificity of these approaches allows for the conclusive identification of the parasite genome at the species level (Fitri *et al.,* 2022). However, while these procedures are easily available in reference laboratories, they are not as accessible in resource-constrained settings due to the high cost of reagents, equipment, and technical specialists required (Tedla, 2019).

2.4. Treatment

2.4.1. Antimalarial activity of Artemisia annua (Asteraceae)

Artemisia annua (annual mugwort) is an annual herb native in Asia, especially in China. The name of the plant is qinghao. In the traditional Chinese medicine *A. annua* was used for over two millennia. There are over 500 species in the genus Artemisia (Astraceae), which are found all over the world. *Artemisiae annuae folium* and *Artemisiae annuae herba* are two of them that are used as therapeutic raw materials (Bora *et al.*, 2011).Aqueous preparations of the dried herb were applied against fever, malaria, skin diseases, jaundice and haemorrhoids (Klayman *et al.,* 1984). Besides a lot of other parasiticidal and antibacterial effects it was active with patients suffering from malaria infections with *P. falciparum* and *P. vivax,* especially such ones with chloroquine- resistant strains (Pizzorno, 2020).

B. *A. annuae herba* A. *A. annuae folium*

Figure 4 *Artemisiae annuae folium* and *Artemisiae annuae herba.*

Source-A.https://www.google.com/url?sa=1&url=https%3A%2F%2Fwww.magicgardenseeds.com%2FQing-Hao-Sweet-Wormwood-Artemisia-annua-organic-seed s&p B.,https://www.google.com/url?sa=i&url=https%3A%2F%2Flovepik.com%2Fimages%2

An infusion of *A. annua* herb in varying quantities was given to individuals with uncomplicated malaria caused by *P. falciparum* infection in an open, randomized clinical study. 74% of patients were healed after 7 days of treatment, while 91% of patients in the quinine-treated control group were cured (Mueller *et al.*, 2004).

Artemisia species are among Ethiopia's numerous traditional medicinal herbs, used to treat both infectious and non-infectious health issues (Nibret *et al.,* 2010). Historically, *A. annua* was introduced to Ethiopia through a religious institution named Kale Hiwot church in Chencha and is being farmed on a modest scale and traditional healer used it to treat mainly, malaria, haemorrhoids, and cancer and the demand for the species has grown during the COVID-19 rise in the research regions and also commercialise by local traders in the country at large (Gelgelo *et al.,* 2021).

2.4.2. The Development of Artemisinin Combination Therapies (ACTs)

The World Health Organization (WHO) initiated the Global Malaria Eradication campaign in 1955, employing chemotherapy techniques in conjunction with residual insecticides, which led to a significant and sustained decline in malaria cases (Das *et al.,* 2007). However, in many countries there has been resurgence in malaria resulted from the rise and spread of drug-resistant parasites, the evolution of insecticide-resistant mosquitoes (Cohen *et al.*, 2012), Increased population density (the world population has doubled since 1963), global warming

(which has permitted the spread of vectors into areas that were before outside their range), poverty, political instability and loss of productivity due to infectious diseases (Hartl, 2004).

Monotherapy, extensive access to low-quality drugs and misuse of anti-malarial led to the emergence and spread of anti-malarial drug resistance (Hanboonkunupakarn *et al.,* 2015). After the first use of effective drug combination for the treatment of malaria by the Chinese in the Vietnam war, the interest of developing novel anti-malarial drugs were increased and led to the development of additional combination therapies (Manuscript, 2010). Drug combinations have been proposed with two basic importance, first the rates of malaria parasites resistant to all the components are much reduced, such that the evolution of resistance is delayed compared with when its components are used alone. The second importance is that the resistant combinations will be broken down much more frequently during recombination in meiosis: the greater the number of genes required to encode resistance, the greater the rate of loss (D'Alessandro *et al.*, 2001). Combination therapy is now widely accepted as the way forward to slow the rapid emergence and spread of resistance to malaria, and to increase the useful therapeutic life of antimalarial drugs.

Artemisinin was first synthesised in 1971 by Tu Youyou from the plant Artemisia annua, a herb that has commonly been used in Chinese traditional medicine and did not become widely available outside China until the 1990s.(Tse *et al.,* 2019).Artemisinin is obtained from the Chinese herb sweet wormwood (Artemisia annua). The first report of the use of sweet wormwood dates from the year 168 BC, when the plant was used by the member of the Han dynasty for the treatment of 52 diseases; in the year 1086 it was suggested in a Chinese compendium of medicines for the treatment of fevers and chills (Efferth, 2007). Artemisinin derivatives have a more comprehensive activity than other antimalarial drugs, ranging from the young ring stage of parasite development to the early schizont and reduce gametocyte carriage, thereby limiting malaria transmission (Premji, 2009).

Artesunate plus mefloquine (ASMQ) was the first ACT to be introduced over 25 years ago in north-western Thailand along the border with Myanmar in a context where multidrug-resistant *falciparum* malaria had become difficult to treat (Nosten *et al.,* 1994). ACTs are currently the recommended first-line treatment for *P. falciparum* in all malaria endemic countries (WHO, 2020). Implementation of ACT has the greatest significance in accomplishing the effort taken to eliminate malaria (Eastman *et al.*, 2009). Some ACTs

recommended by the WHO are artemether/lumefantrine, artesunate/amodiaquine, artesunate/mefloquine, artesunate/pyronaridine (Cui *et al.,* 2015). More than 3.5 billion treatment courses of ACT were sold globally by manufacturers in 2010–2020. NMPs distributed 191 million ACTs in 2020, 96% of which were in sub-Saharan Africa. There were about 48 million fewer distributions in 2020 than in 2019 (WHO, 2021b).

Currently, ACTs are implemented as the first- and second-line anti-malarial drug for the treatment of uncomplicated *P. falciparum* malaria globally (WHO, 2023). ACT is a combination of a rapidly acting artemisinin derivative with a longer-acting (more slowly eliminated) partner drug. The artemisinin component rapidly clears parasites from the blood and the longer-acting partner drug eliminates the remaining parasites and offers protection against development of resistance to the artemisinin derivative (Eastman *et al.*, 2009). The first-line treatments used in most African countries for *P. falciparum* are AL and artesunate-amodiaquine (AS-AQ), with some countries' treatment policies also allowing for the use of dihydroartemisinin-piperaquine (DHA-PPQ) (WHO, 2019). Similarly, the southeast Asia, Latin America, Mediterranean and Western Pacific countries use ACT, such as DHA-PPQ, AS-AQ, AS-MQ as their first-line anti-malarial drug (WHO, 2020).

2.4.2.1. Artemether Lumefantrine

Coartem® (AL), which is one of several ACTs, has become a mainstay of malaria treatment worldwide. In 2004, Coartem® became the first fixed dose ACT to be pre-qualified by the WHO and in 2009 approved by the Food and Drug Administration in the US for treatment of uncomplicated malaria (Stover *et al.*, 2012). AL tablets have been included on the WHO model list of Essential Medicines since March 2002 and on the first WHO Model List of Essential Medicines for Children since October 2007 (WHO, 2019). Zambia was the first African country with nationwide endemic malaria to adopt AL as first-line therapy according to national malaria treatment guidelines (Barnes *et al.,* 2009). Ethiopia has adopted artemether–lumefantrine (AL), an ACT antimalarial, as the first-line drug for the treatment of uncomplicated *P. falciparum* malaria in July 2004 (FMoH, 2012).

One of the advantages of artemether–lumefantrine combination beside the efficiency of artemether, 100-100,000 enduring parasites when the drug is administered alone for a three-day treatment course, and after the three-day regime lumefantrine eliminate any remaining

parasites to prevent recrudescence (Premji, 2009). AL also has anti-gametocyte activity, which breaks the cycle of transmission between the mosquito vector and the human host. One study, using membrane-feeding Anopheles mosquitoes, has demonstrated a reduction in malaria transmission following the six-dose regimen of AL (Sutherland *et al.,* 2005).

The efficacy of AL combination is strongly influenced by the wide variation in the pharmacokinetics of lumefantrine among individuals. Food (especially dietary fat) improves the bioavailability of artemether and lumefantrine, though this effect is more significant for lumefantrine (White *et al.*, 1999). Administration of AL to healthy volunteers with the uptake of high-fat meal increases the bioavailability of artemether and lumefantrine by two-fold and 16-fold, respectively, compared with the fasted state (Ezzet, 2000).As its absorption is enhanced by concomitant intake of fatty foods, treatment failures with this combination might be due to insufficient absorption of lumefantrine. For instance, In Cambodia, studies of artemether–lumefantrine were conducted between 2001 to 2004. High treatment failure rates were observed in 2001 (26.1%) and 2002 (28.9%); however, in 2003, when treatment was given with fatty foods, the failure rate decreased to 13.5% (Denis *et al.,* 2006).

The other advantage of AL for patients which is not already resistant to one or more of the drugs, AL reduces the chance of survival by a resistant parasite, since their different sites of action mean that mutations resistant to both agents would be required for the parasite to survive (Hasting *et al.*, 2005). Moreover, paediatric formulation of AL tailored to the needs of children with *P. falciparum* malaria was developed by the collaboration of Novartis and the Medicines for Malaria Venture (MMV). By this, the previous standard of care such as crushing standard tablets of AL, which is problematic for caregivers and difficult for the treatment of sick children, while the crushing procedure risked loss of drug and the bitter taste intended that child might spit it out are overcome (Abdulla *et al.,* 2009).

2.4.3. Mode of Action of Artemisinin against *P. falciparum* Malaria

Artemisinin is a traditional Chinese herbal remedy for malarial fevers that has recently been discovered to have substantial effect against a variety of malarial species, including chloroquine-resistant *P. falciparum.* DHA is the active in vivo metabolite of all clinically applied ARTs. ARTs are extremely quick acting against intra-erythrocytic asexual blood-stage malaria parasites, resulting in parasite load decreases of up to 10,000-fold per 48 hours

(White, 2008). The exceedingly short in vivo half-lives of ARTs (~1 hr in humans) are an inherent drawback. Due to this, in ART-based combination treatments (ACTs), ARTs are co-administered with extended half-life partner medications including lumefantrine, amodiaquine, piperaquine, mefloquine, sulphadoxine-pyrimethamine, or pyronaridine (Dondorp *et al.*, 2009).

The antimalarial activity of artemisinin (and, by extension, other endoperoxides) is dependent on two sequential processes. The first step, activation, involves iron-mediated breakage of the endoperoxide bridge, resulting in an unstable organic free radical or other electrophilic molecule (Meshnick, 1994). The parasite may then be killed by a free radical intermediate that is produced by alkylating and poisoning one or more vital malarial protein(s) (Meshnick, 1998). The second phase, alkylation, includes the creation of covalent adducts between the medication and malarial proteins.Together, these proceed to oxidative stress, resulting in permanent parasite damage and parasite death (Cui *et al.,* 2009).

Artemisinin have been suggested to target a very broad array of parasite proteins and seem to disturb a number of organellar and cellular processes including haemoglobin endocytosis, glycolysis, protein synthesis and degradation, and cell cycle regulation. Similar to CQ and other aryl alcohol compounds, artemisinin derivatives mainly target the parasitic food vacuole, interfering with heme detoxification by inhibiting hemozoin polymerization leads to heme accumulation (Hyde, 2002).

As part of its life cycle, the parasite eats haemoglobin from red blood cells, which causes the release of iron-containing heme molecules. Heme is poisonous because of the reactivity of iron, thus the parasite has developed ways to detoxify it (Jani *et al.*, 2008). Drug research has focused on these detoxification pathways since inhibiting heme detoxification will eradicate the parasite (Weissbuch *et al.,* 2008). The creation of inert hemozoin crystals in the parasite's digestive vacuole is the main method of heme detoxification (Weissbuch *et al.,* 2008). Furthermore, it has been proposed that either heme crystallization is enhanced by lipid environment confinement or by protein catalysis (Nakatani *et al.,* 2014). Since haemoglobin breaks down more quickly than crystallization, excess heme will build up in the digestive vacuole and eventually kill the parasite (Kapishnikov *et al.,* 2013). Therefore, non-crystalline heme must either be retained inside the parasite or haemoglobin breakdown must be closely

linked to heme crystallization in order to prevent the release of extra heme (Kapishnikov *et al.,* 2017).

2.4.4. ACT Resistance Mechanism in *P. falciparum* Malaria

Resistance to the currently available anti-malarial drugs is an obstacle for malaria elimination programs in malaria endemic countries (WHO, 2019). Anti-malarial drugs mainly target the asexual erythrocytic stages of malarial parasites that are responsible for human infection (Cui *et al.*, 2015). *P. falciparum* is one of the *Plasmodium* species with high rate of morbidity and mortality (Phillips *et al.,* 2017).

The parasite uses various resistance mechanisms to deter the activity of anti-malarial drugs. The two main resistance mechanism in *p. falciparum* are first, due to the mutations in genes related with drug resistance or escalating in their gene copy number (Menard *et al.*, 2017), and the second is a change in the parasite target due to mutations in corresponding genes (Arya *et al.,* 2021). Mutation on the key enzymes or transporter has been identified as a factor for anti-malarial drug resistance in *P. falciparum* and *P. vivax* by using genetic, molecular and pharmacological methods. In *P. falciparum*, the drug resistance linked genes comprise *P. falciparum* chloroquine resistance transporter (*Pf*crt), *P. falciparum* multidrug resistance protein 1 (*Pfmdr1*), *P. falciparum* dihydrofolate reductase (*Pfdhfr*), *P. falciparum* dihydropteroate synthase (*Pfdhps*), *P. falciparum* cytochrome B (*Pf*cytb) and *P. falciparum* sodium–hydrogen exchanger gene (*Pf*nhe-1) (Patel *et al.*, 2017).

Recently, the efficacy of artemisinin derivatives (ART) is in threat due to point mutation in *P. falciparum* kelch-like protein which is a primary marker of artemisinin drugs (Kumar *et al.,* 2018). The first ART resistance was detected in Western Cambodia resulting from monotherapy and inappropriate administration of the artemisinin derivatives (Nsanzabana, 2019). Recently, clonal expansions of Pfkelch13 mutations detected in Rwanda and Uganda, evidence of artemisinin partial resistance emerging independently in the WHO African Region (WHO, 2021b). Parasitic quiescence (the convergence of an adaptive process to cope with an adverse environment and an active preparation to efficiently resume proliferation) resulting in delayed parasite clearance and altered temporal response were shown to be responsible for ART resistance (Ouji *et al.*, 2018).

In 2014, a molecular marker for artemisinin resistance was identified: Several mutations in the Pflkelch13 (k13) propeller domain were found to be associated with delayed parasite clearance in vitro and in vivo (WWARN, 2015). The Pfk13 gene is located on chromosome 13 with only one exon in the parasite endoplasmic reticulum (Arya *et al.*, 2021). Using *in vitro* ring-stage survival assays (RSAs) propeller domain of a *P. falciparum* kelch gene 13 (codons 441 to 726), 14 independent Pfk13 mutations have been associated with clinical delayed parasite clearance, and of these, only 5 have been confirmed by *in vivo* and *in vitro* experiments: N458Y, Y493H, R539T, I543T, and C580Y (Pacheco, 2019). K13 mutations are thought to mediate artemisinin resistance in rings stage mainly by over-expression of the unfolded protein response leading to rapid elimination of artemisinin and removal of artemisinin-damaged peptides (Wicht *et al.,* 2020).

2.4.5. Status of drug efficacy study of AL in Ethiopia

Therapeutic drug efficacy studies of antimalarial drugs are critical for determining treatment success, monitoring drug resistance, directing treatment policies, improving treatment tactics, and supporting continuing malaria research and development activities (Williams *et al.,* 2004). According to an efficacy research conducted by the Federal Ministry of Health in 2004, resistance to the antimalarial medicine Sulphadoxine-Pyrimethamine (SP) is unacceptably high, with an average therapeutic failure rate of 36% (Jima *et al.,* 2005a).

Estimated number of malaria case 6,260,168.00 was expected in 2004 between September to December in the country and in order to reduce the disease's impact at this crucial time,WHO and UNICEF were in the process of quickly mobilizing resources to support the FMoH with this critical and urgent drug policy adjustment and to administer the new recommended Artemesinin Combination Treatment (ACT) Artemether-Lumefantrine (Arowolo, 2010). In Ethiopia, a three-day regime of AL (artemether 20 mg and lumefantrine 120mg in each tablet) and single dose primaquine (PQ) is the first-line anti-malarial drug for the treatment of uncomplicated *P. falciparum* malaria (FMoH, 2018). Second-line treatment for uncomplicated malaria is used when the first-line treatment is unavailable or when the first-line medication fails or does not work. In Ethiopia, oral quinine is the second-line therapy for both *P. falciparum* and *P. vivax* (FMoH, 2012).

After the first ART, resistance was detected in Western Cambodia the efficacy of artemisinin derivatives and artemisinin combination therapy fell to question (Hanboonkunupakarn, 2015). To deter such resistance in the other malaria endemic countries the WHO recommended regular monitoring of Anti-malarial drugs through therapeutic efficacy study to assess the clinical and Parasitological response after therapy and to evaluate adverse events (WHO, 2009). The first anti-malarial drug efficacy of AL was conducted in 2005, a years after its introduction in Ethiopia. And according to the study, A therapeutic success rate of 99.1% (95% confidence interval [CI] 96.9, 99.8) was recorded, with no side effects or drug-related complaints requiring treatment termination or withdrawal from follow-up (Jima *et al.,* 2005b). And similar study was conducted in 2009, it shows achieving 100% adequate clinical and Parasitological response (ACPR) with no serious adverse reactions was seen (Kefyalew *et al.,* 2009).

A systematic review and meta-analysis of AL efficacy was conducted on ten prospective single-arm cohort studies on total 1179 patients that followed for 28–42 days and showed the cure rate with the use of AL was 98.2% (PCR corrected) and 97.01% (PCR uncorrected) (Ayalew, 2017). A similar systematic review and meta-analysis of twenty-one studies reported the treatment success in malaria patients with *P. falciparum* and treatment of AL, and shows a therapeutic efficacy rate 98.1% (97.0–99.2) (Gebreyohannes *et al.*, 2017). A study conducted in high transmission area, northwest Ethiopia in 2016, the overall cure rate was 98.8% with 95% confidence interval of 0.915-0.998 without polymerase chain reaction correction. The parasite clearance rate was high, and clinical symptoms were quickly resolved; 100% of the research subjects cleared Parasitemia and fever on day three (Teklemariam *et al.,* 2017). A research was conducted to evaluate the therapeutic efficiency of AL(Coartem®) in treating uncomplicated *falciparum* malaria after 9 years of use in Metehara, Eastern Ethiopia and its overall cure rate was PCR uncorrected, 97.6% (95%CI: 93.6–99.5) and PCR corrected, 98.8% (CI: 93.5–100%). On day three and beyond, no parasite was found. Day 3's fever clearance was greater than 91%. Among the research participants, there were no significant adverse medication responses (Nega *et al.,* 2016).

3. Materials and Methods

3.1. Study Site

The study was conducted in the Eastern Shewa zone health department, Metehara town Health Center (MHC), Ethiopia. Metehara is an administrative town of Fentale district in Oromia Regional state of Ethiopia. The area is located in the Great Rift Valley, about 210 km east of Addis Ababa, the country's capital. Its coordinate is 8°540' N39°550'E and average elevation is 947m. Rivers Awash and Germama, and Lake Basaka are important water bodies in the district. An estimated total population of 39,585 live in Metehara town (Metehara town health bureau) from the two *kebeles* (the lowest administrative unit). The area is classified under semi desert with annual average rainfall 1.5mm, humidity 67% and daily temperature 32^{o}C and 19^{o}C on average.

Metehara is one of the sentinel sites of malaria TES in central-east Ethiopia. The transmission level in the area is moderate. The town has estate irrigation by using the nearby rivers for the industrial cultivation of sugarcane and other vegetables and fruits, and this in turn, suits breeding of the Anopheles mosquito, exposing the community to malaria. Transmission of malaria in this area occurs year-round but the intensity fluctuates within the season(Desalegn *et al.,* 2016).

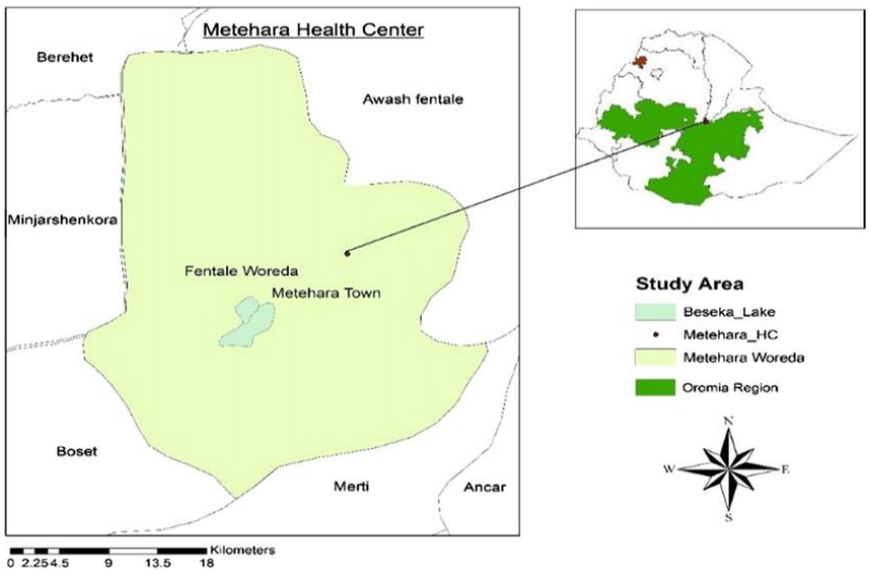


Figure 5 Geographical location of Metehara health center.

Source Reda, Abeba & Kebede, Alebachew & Mohamed, Hussen & Assefa, Ashenafi & Golassa, Lemu & Mamo, Hassen. (2022). Temporal dynamics of Plasmodium falciparum population in Metehara, east-central Ethiopia. Malaria Journal. 21. 10.1186/s12936-022-04277-5.

3.2. Study Design

This surveillance study was a one-arm prospective evaluation of clinical and parasitological responses to directly observed treatment for uncomplicated *P. falciparum* malaria. Patients with uncomplicated *P. falciparum* malaria, who met the study inclusion criteria were enrolled, treated with AL on site for 28 days. The follow-up consisted of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. Based on the results of these assessments, the patients were classified as having early treatment failure (ETF), late treatment failure (LTF) and adequate clinical and parasitological response (ACPR). The proportion of patients experiencing therapeutic failure during the follow-up period was used to estimate the efficacy of the study drug.

3.3. Sample size

According to the working WHO protocol for antimalarial TES with an expected failure rate of 5%, a minimum of 73 patients must be included at a confidence level of 95% and a precision around the estimate of 5%. With a 20% increase to allow loss to follow-up (LTFU) and withdrawals during the 28-day follow-up period.

3.4. Timing and duration of study

Transmission of malaria in this area occurs year-round. Thus, the study was conducted from November 2020 to March 2021.

3.5. Study Subjects, inclusion criteria, exclusion criteria

3.5.1. Study Subjects

The study population targeted febrile patients or those having history of fever within the previous 24 hour, visiting the outpatient department of the health center who fulfilled the inclusion criteria set by the WHO for the assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated *P. falciparum* malaria (WHO, 2009).

3.5.2. Inclusion criteria

The inclusion criteria were age ≥6 months, having *P. falciparum* mono-infection detected by microscopy, asexual parasitemia ≥1000/µl, axillary, armpit or oral temperature ≥37.5°C, or history of fever during the past 24h, ability to swallow oral medication, willingness to comply with the study protocol for the duration of the study, and to comply with the study visit schedule. Moreover, willingness to provide informed consent, and a negative pregnancy test or not breastfeeding.

3.5.3. Exclusion criteria

The exclusion criteria included presence of general danger signs in children aged under 5 years or of severe *falciparum* malaria according to the definitions of the WHO; mixed or mono-infection with *non-falciparum Plasmodium spp.* detected by microscopy. Febrile conditions due to diseases other than malaria like measles, acute lower respiratory tract infection or severe diarrhea with dehydration or other known underlying chronic or severe diseases such as cardiac, renal and hepatic diseases, HIV/AIDS (adult patients were tested for HIV with RDT for other CDC monitored HIV prevalence surveillance); having a regular medication, which may interfere with antimalarial pharmacokinetics; history of hypersensitivity reactions or contraindications to any of the medicine(s) being tested or used as alternative treatment(s) and a positive pregnancy test or breastfeeding were additional exclusion criteria.

3.4. Data collection and sampling technique

3.4.1. Recruiting of study participants

Patients who fulfilled the inclusion criteria were recruited and a brief explanation about the study was done.

3.4.2. Physical examination

A standard physical examination, medical history, demographic information and contact details were registered at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28 after treatment.

3.4.3. Laboratory examination

According to the WHO protocol 2009, capillary blood samples were collected from patients aseptically and labelled anonymously. Thick and thin blood films were prepared at day 0 (D0), stained with fresh 10% Giemsa stain and examined for the eligibility of inclusion criteria. If the patient was eligible, thick blood films were examined on day 2, 3, 7, 14, 21 and 28 or on any unscheduled day. Parasite count was done on the number of asexual parasites observed against 200 white blood cells (WBCs). Parasite density was calculated by multiplying average number of parasites count by 8000 WBCs and dividing average no of WBCs count (200 WBCs) Hemoglobin/hematocrit was determined on days 0 by using the Microhematocrit capillary (KIMBLE® Micro-Hematocrit Capillary Tube, Heparinized, 0.5 x 75 mm, Case of 1200, 60 mm).

3.4.5. Drug administration

Three days of drug was administered based on age/bodyweight under partial supervision of the clinical team. Before treatment, axillary temperature was measured and recorded. A participant was observed for 30-60 minutes after the first dose. When there is vomiting, the patient was retreated with another dose and observed for another 30 minutes. If vomiting had happened again, he/she was excluded from the study, if not they were given a second dose with extra (in case they vomited at home), and if they were febrile, antipyretics were provided.

3.5. Classification of treatment out come

Treatment endpoint was classified on the basis of an assessment of the parasitological and clinical outcome of antimalarial treatment according to the latest WHO guideline. Studyout come were divided into primary out come and secondary out come. While the primary out come was the day-28 adequate clinical and Parasitological response the additional out come

were clinical and parasitological evaluations (parasite, fever and gametocyte clearance rate and incidence of drug adverse events) (WHO. 2009).

3.6. Data analysis

Data were double entered into the WHO Excel spreadsheet designed for therapeutic efficacy data. Data were also entered into IBM SPSS (version 20) software to calculate descriptive statistics (mean, standard deviations, percentages). Paired sample t-test was used to check the presence or absence of statistically significant mean differences between the Parasitemia among age groups. All comparisons were performed at 95% confidence interval (CI) and significance level of 0.05.

3.7. Data quality control

The study were conducted on the clinical and Parasitological respond of patients treated with AL for three days. Information regarded with patients follow-up and their results were recorded by the health center public health officers (HOs) and the Parasitological diagnosis and evaluation (taking blood film, identifying species and parasite count) were done by the health center laboratory technician who had a theoretical and practical experience and knowledge. After the study completed the slides were taken to Adama Regional Laboratory for cross-check and examined by WHO certified experts.

3.8. Ethical Considerations

The study protocol was approved by the College of Natural and Computational Sciences Institutional Review Board (IRB), Addis Ababa University and by the Ethical Review Committee of Ethiopian Public Health Institution (EPHI), Ethiopia. Informed consent was obtained from adult participants and assent of parental/guardians were collected for minors. Data collected from each participant and laboratory results were kept confidential and used only for the research purpose.

4. Results

4.1. Study Participants

A total of 2332 (1187 male and 1145 female) malaria suspects visited MHC for malaria diagnosis from November 26, 2020 to March 24, 2021. Of this, 178(7.6%) were positive and the rest 2154(92.4%) were negative. Among 178 positives, 102(57.3%) were *P. falciparum* mono-infections and 76(42.6%) were *P. vivax*. Out of 102 *P. falciparum* positives, 94 were eligible and targeted for the study. But, 14 refused consent due to their mobile working nature and 80 patients were enrolled in the study. During the study period, 5 patients were LTFU, 2 found as protocol violation (one patient found with schizont stage *P. vivax* D21 and the other patient found with *P. vivax* infection at D28) so, excluded from the study and 73 has completed the follow up (fig1).

At baseline, the majority of the patients with *P. falciparum* mono-infection were male 50(62.5%), and the females were 30(37.5%). Twenty-nine (36.3%) patients were febrile at baseline and 69/80 (86.3%) had a self-reported history of fever within the previous 24 hours. Mean parasitemia were 15305.8±16765.6, and 3 patients had asexual parasitemia >50,000/µl blood. Mean body temperature was 37.3±1.3. Hemoglobin (Hb) was evaluated at baseline for each patient. During enrollment, 42(52.5%) were anemic, 15/80 (18.8%) had Hb between 6.30-8.0 g/dL, 19/80 (23.8%), 8.1-10.0 g/dL and 46 (57.4%), 10.3-14.0 g/dL and the mean baseline Hb was 10.4±2.1 g/dL, ranging 6.30-14.0 g/dL. The total mean bodyweight was 36.3±19.9kg. The bodyweight range was 0.8-72.5 kg. Baseline gametocyte carriage was 6.3% (5/80) with gametocyte numbers ranging from 640-5320/µL of blood. Thirty-four (42.5%) of the 80 participants reported a previous history of clinical malaria and 47(58.8%) confirmed the availability of a bed net but only 20(37.5%) reported utilization of the nets (Table 1).

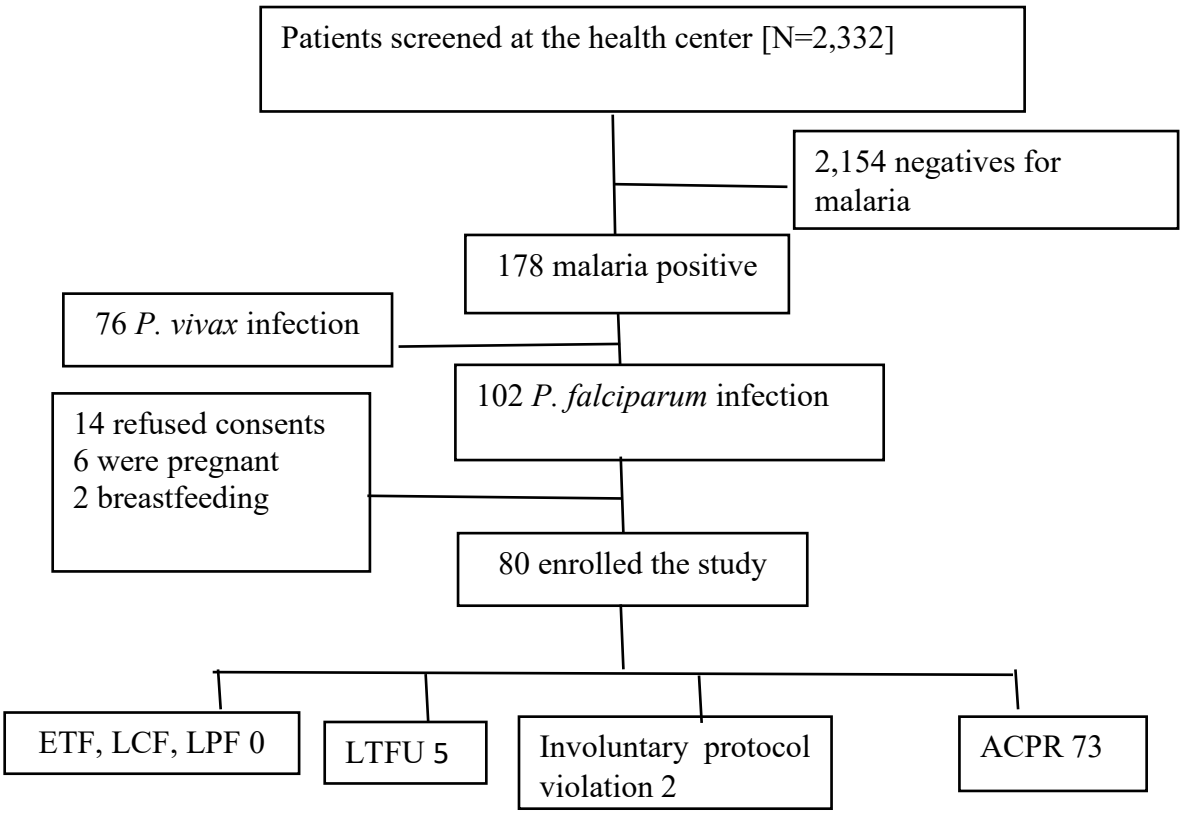


Figure 6 Screening, enrollment, follow-up and treatment outcomes of *P. falciparum* malaria patients in Metehara Health Center. [ACPR – Adequate Clinical and Parasitological Response, ETF – Early Treatment Failure, LCF – Late Clinical Failure, LPF – Late Parasitological Failure, LTFU- Lost to Follow-up].

Table 2. Baseline characteristics of the study participants at Metehara Health Center, East-Central Ethiopia, November 2020 – March 2021

Variables	**Age Category**			
	<5 (n = 15)	**5-14 (n=26)**	**>14 (n=39)**	**Overall**
Mean age (range)±SD, yr.	3(4) ±1.2	9(9) ±2.2	30(50) ±12.0	18(64) ±14.6
Sex Male, n (%)	8(53.3)	12(46.1)	30(76.9)	50(62.5)
Female, n (%)	7(46.7)	14(53.9)	9(23.1)	30(37.5)
Total, n (%)	15(18.8)	26(32.4)	39(48.8)	80(100)
Mean body tem. ±SD, ºC	37.7±1.3	37.7±1.3	35.8±5.9	37.3±1.3
Mean bodyweight ± SD	10.5±3.05	23.3±2.2	53.9±9.7	36.3±19.9
Mean Hb ±SD, g/dL	8.3±2.1	9.7±2.0	11.4±1.5	10.4±2.1
Mean parasitemia ±SD, per µl	11,844.9±12215.0	13,410.2±12295.3	17,902.7±20391.8	15,305.8±16765.7
Gametocyte presence, n(%)	1(6.7)	2(7.7)	2(5.1)	5(6.3)
Bed net utilization, n(%)				
Yes, n(%)	5(33.3)	7(26.9)	19(48.7)	30(37.5)
No, n(%)	10(66.7)	19(73.1)	20(51.3)	50(62.5)
Past malaria attack, n(%)				
Yes, n(%)	4(26.7)	9(34.6)	21(53.8)	34(42.5)
No, n(%)	11(73.3)	17(65.4)	18(46.2)	46(57.5)

4.2. Primary out come

Of the 80 patients treated, five were LTFU, two patients had protocol violation and were excluded from the study, and ACPR was recorded for the 73 participants. The ACPR was 100% (73/73, 95% CI: 95.1-100.0) (Table 2) with no ETF, LTF, LPF as in Kaplan–Meier analyses (Table 3, Fig. 2).

Table 3. Artemether-lumefantrine efficacy endpoints in uncomplicated P. falciparum malaria, Metehara Health Center, Central-east Ethiopia

Treatment outcome	Frequency in each age (year) category, n(%)			
	<5 (n= 15)	5-14 (n = 26)	>14 (n= 39)	Overall
ACPR	14(19.2)	25(34.2)	34(46.6)	73(100)
ETF	0(0)	0(0)	0(0)	0(0)
LTF	0(0)	0(0)	0(0)	0(0)
LPF	0(0)	0(0)	0(0)	0(0)
LTFU	0(0)	1(20.0)	4(80.0)	5(100)
PV	1	0(0)	1	2

ACPR - Adequate Clinical and Parasitological Response, ETF - Early Treatment Failure, LTF - Late Treatment Failure, LPF - Late Parasitological Failure, LTFU Loss-to-follow-up, PV- Protocol Violation

Table 4. Treatment outcomes based on Per Protocol analysis among patients treated with artemether-lumefantrine at Metehara Health Center, East-Central Ethiopia, November 2020 – March 2021

Efficacy endpoint	**n (%)**
ETF	0
LTF	0
LPF	0
ACPR	73
Total patients at baseline	80
PP PCR uncorrected cure rate	100% (95% CI: 95.1-100.0)
K-M PCR uncorrected cure rate	100% (95% CI: 95.1-100.0)

ETF - Early Treatment Failure, LTF - Late Treatment Failure, LPF - Late Parasitological Failure, ACPR - Adequate Clinical and Parasitological Response, PP - per protocol analysis, K-M - Kaplan-Meier

4.3. Secondary out come

4.3.1. Parasite Clearance

At the start of the study, 52.5% (42/80) of the participants had a high level of parasitic infection (>10,000 parasites/µl), while 71.2% (38/80) had a moderate level (1000–9999 parasites/µl). Among the 18 participants with a high level of infection, 58.9% (23/39) were over 14 years old, 46.2% (12/26) were between 5 and 14 years old, and 46.6% (7/15) were

under 5 years old. After three consecutive days of drug treatment, parasitic infection decreased to zero (0.0%) from the starting level on D2.

4.3.2. Fever Clearance

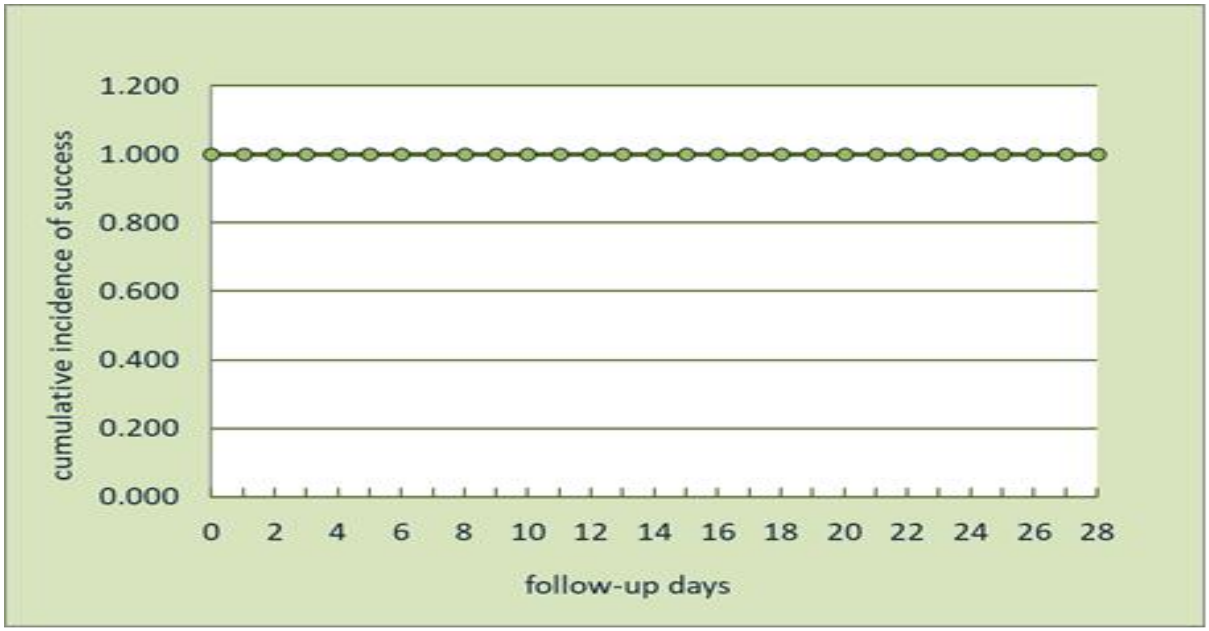
On the day of enrollment, 36.3% (39/80) of individuals had a fever of ≥37.5°C. This percentage decreased to 10% (8/80) on D1, 0.0% (0/80) on D2 and D3, 2.5% (2/80) on D7, 0.0% (0/80) on D14, 1.3% (1/80) on D21, and 3.8% (3/80) on D28. This means that fever clearance was 90% (72/80) on D1, 100% (0/80) on days 2, 3, and 14, but 97.5% (2/80) on D7, 98.8% (1/80) on D21, and 96.3% (3/80) on D28. Among severely parasitemic patients, 38.1% (16/42) had a fever and among moderately parasitemic patients, 34.2% (13/38) had a fever at baseline (Fig. 3).

4.3.3. Gametocytemia clearance

At baseline, gametocyte carriage was detected in 6.3% (5/80) of the participants. Among these 5 individuals with gametocyte carriage, 7.7% (2/26) were detected in the 5–14 year age group, 5.1% (2/39) were detected in the >14 year age group, and 6.7% (1/15) were detected in the <5 age group. Two patient had gametocyte carriage on days 2 and 3 and only one patient on day 7 but it cleared by D14. When considering only participants who completed follow-up, the proportion of gametocyte carriage declined from 6.3% (5/80) on D0 to 2.5% (2/80) on days 2 and 3, and 1.3% (1/80) on D7, completely disappearing on D14.

4.3.4. Advert events

At baseline, 48.9% (39/80) of the patients self-reported having a headache. During treatment with the drug, no adverse events were reported on days 1 and 2. Additionally, there were no reported adverse events on D3. However, one adverse event was reported on D7. Furthermore, two adverse events were reported on D14, and one adverse event was reported on both days 21 and 28.



The follow-up days were on day 0,1,2,3,7,14,21, and 28

Figure 7 Survival analysis of 28-day cure rate for artemether-lumefantrine against uncomplicated *P. falciparum* malaria at Metehara Health Center, central-east Ethiopia, November 2020 to March 2021

5. Discussion

During the consecutive 6-year (2018-2023), increased prevalence of malaria was recorded in the study area. Between the two species, *P. falciparum* was predominant and this showing that control measures were less effective for various reasons. This study presented the 28-day cure rate of a standard six-dose of AL 100% in treating uncomplicated *P. falciparum* malaria, with rapid clearance of fever and parasitemia within the first three days. For PP analysis, the PCR-uncorrected cure rate among the study participants was 100% showing the high efficacy of the drug.

The presence of Parasitemia on D3 is key indicator to suspect artemisinin resistance (WWWARN, 2015). However, in this study the baseline mean Parasitemia was 15,305.8±16765.7 declined to zero on D2. Since artemether is a potent anti-malarial that absorbed quickly which result in a fast reduction in parasite biomass, prompt symptomatic improvement and are eliminated rapidly (Stover et al, 2012), in this study the absence of ETF confirms non-existence of probable artemisinin-resistant *P. falciparum* strains. Similarly, during the study there were no LTF and LPF present. The adjusted cure rate at day-28 was 100% each for <5 children, 5-14 and >14 age groups. High levels of Parasitemia in the bloodstream can contribute to severe fever. A similar study conducted in 2009 shows achieving 100% adequate clinical and Parasitological response (ACPR) with no serious adverse reactions was seen (Kefyalew *et al.,* 2009). And the first efficacy study of AL also show similar finding with this study, therapeutic success rate of 99.1% (95% confidence interval [CI] 96.9, 99.8) with two cases of late treatment failure were noted on days 21 and 28, when measurements of fever and peripheral Parasitemia were made (Jima *et al.,* 2005b).

Fever is a response to the body's immune system attempting to fight off the infection and also indicate high replication and development of the parasite (Tripathi *et al.,* 2023). At baseline febrile individuals, ≥37.5°C were 36.3% (29/80), among 29 febrile patients 55.2% (16/29) patients has >10,000 Parasitemia and the rest 44.8% (13/29) patients has Parasitemia between 1000-9999. Although, high fever and Parasitemia was recorded at enrollment, after the administration of the drug the parasitemia and fever eliminated on D2. This demonstrates that AL has rapid clearance of parasites deterring the progression of the disease, fast resolution of symptoms and reduces the risk of complicated malaria. According to a study conducted by Michael and his colleagues, The parasite clearance rate was high, and clinical symptoms were

quickly resolved; 100% of the research subjects cleared Parasitemia and fever on day three (Teklemariam *et al.,* 2017) similar with the current study.

Primarily, AL is designed to decrease and clear the asexual stage of malaria parasite but also have gametocidal activity, which breaks the cycle of transmission between the mosquito vector and the human host. One study, using membrane-feeding Anopheles mosquitoes, has demonstrated a reduction in malaria transmission following the six-dose regimen of AL (Sutherland *et al.,* 2005). In this study from baseline, the gametocyte clear on day 3 but 1 case persisted up to D7 and completely disappeared on D14 and onwards.

Malaria can cause anemia, which is characterized by a decrease in Hb levels. However, the effect of different malaria treatments on Hb levels can vary. Some studies have shown that certain antimalarial treatments can lead to an improvement in Hb levels. For example, effective treatment of malaria with antimalarial drugs can help clear the infection, allowing the body to recover and restore Hb levels (Zwang *et al.,* 2017, Sagara *et al.,* 2014). This improvement in Hb levels is often seen in individuals who receive prompt and appropriate treatment. On the other hand, there have been reports of antimalarial drugs causing a decrease in Hb levels or exacerbating anemia in some individuals (De Nardo *et al.,* 2013, Corpolongo *et al.,* 2012). For example, certain medications, such as PQ, used for the treatment of *P. vivax* malaria and nowadays against *P. falciparum* gametocytes, may cause hemolysis, leading to a drop in Hb levels (Tylor *et al.,* 2019).

No serious adverse events were noted; the majority of the reactions were already recognized by the manufacturer as common adverse reactions and documented with the food and drug administration. At baseline, the majority reported Head ache. Except for days 1 and 2, adverse events were reported on the rest of the follow-up days (D3, 7, 14, 21 and 28). Although there were adverse events reported, there were no records of parasitemia for each patient who reported a symptom. This suggests that the symptoms may have been caused by other infections or illnesses. The results of other studies (Desalegn *et al.,* 2016, Michael *et al.,* 2017) are consistent with the absence of any major adverse events following AL treatment as in the current investigation.

Similar high efficacy findings were already reported in other countries of SSA (Derbie *et al.,* 2020). Meta-analysis in Anti-malarial treatment outcomes in Ethiopia, among 21 publications reported the average efficacy of 98.1%, which is almost similar to the present study finding (Gebreyohannes *et al.,* 2017). In contrast to results from Southeast Asian, nations where delayed fever clearance of AL, the rapid fever-resolving ability of AL is seen in other efficacy tests carried out in Ethiopia and elsewhere in SSA.

To evaluate the effect of the drug on the level of Hb, measurement of the level of Hb was mandatory but, due to shortage of logistics, we could measure Hb level only at baseline. Finally, this study illustrates the efficacy of AL in the study area during the study year and can be a fine source for further efficacy study of AL in the area.

6. Conclusions

In summary, the effectiveness of artemether-lumefantrine (AL) as a treatment for *P. falciparum* is a critical component of the worldwide effort to combat malaria. In patients with simple *falciparum* malaria, AL has regularly shown excellent rates of parasite clearance, fever resolution, and treatment efficacy via a number of clinical trials and real-world investigations. Lumefantrine's longer-lasting impact, which lowers the likelihood of recrudescence and transmission, complements its quick action against asexual blood-stage parasites, highlighting its efficacy as a first-line therapy.This therapeutic efficacy study highlights AL as an effective, safe, and well-tolerated treatment option for uncomplicated *falciparum* malaria, with a high cure rate and rapid parasite and fever clearance, with minimal adverse effects as reported.

Moreover, AL has demonstrated outstanding effectiveness in a variety of epidemiological contexts, such as areas with differing malaria endemic levels and medication resistance patterns. However, it is still critical monitoring and evaluating drug efficacy, along with the emergence of resistance in order to assure continued efficacy over time. Furthermore, addressing issues like as availability to therapy, adequate dose, and treatment compliance is critical for optimizing AL's influence on malaria control and eradication efforts.

7. **Recommendations**

Since parital artemesinin resistance had been seen in some studies, It is essential to comprehend the processes underlying partial resistance in order to guide clinical practice and public health initiatives. Going forward, further study efforts need to concentrate on clarifying the fundamental genetic pathways that propel partial resistance, in addition to investigating innovative therapeutic targets and treatment approaches. Even while AL is still a vital part of the worldwide effort to combat malaria, the emergence of partial resistance highlights the necessity of ongoing surveillance and strategy modifications. And this study recommends further molecular assessment of patient outcome to detect the presence and absence of resistance parasite strains.

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ANNEX

Annex I: Consent Form

Amharic and English version

1. Monitoring the efficacy of frontline antimalarial drugs at Metahara, Ethiopia, **2022/23**

የ አ ማር ኛ ስ ምምነ ት /መግ ባ ቢ ያ ሰ ነ ድ Consent/Assent form in Amharic

ር እ ስ

በ ኢ ት ዮ ጵ ያ በ 2015 ዓ.ም ወ ባ በ ሽ ታ ለ ማ ከ ም የ ሚ ሰ ጡ ተ ቀ ዳ ሚ መ ድ ኒ ቶ ች ያ ላ ቸ ዉ ን ዉ ጤ ታ ማ ነ ት ና ብ ግ ር ነ ት ማ ጥ ና ት ።

ይ ህ የ ስ ምምነ ት መ ግ ባ ቢ ያ ሰ ነ ድ ለ አ ዋ ቂ ዎ ች (ከ 18ዓ መ ት ላ ይ ለ ሆ ኑ) ለ ታ ዳ ጊ ዎ ች ከ 7-17 ዓ መ ት ,ለ ህ ፃ ና ት ከ 6 ወ ር እ ስ ከ 11 ዓ መ ት እ ድ ሜ ዉ ስ ጥ ላ ሉ በ ወ ላ ጅ /በ አ ሳ ዳ ጊ /በ ተ ን ባ ካ ቢ ያ ች ዉ አ ማ ካ ነ ኝ ነ ት የ ጥ ና ቱ ተ ሳ ታ ፊ መ ሆ ና ች ዉ የ ፍ ቃ ደ ኝ ነ ታ ች ዉ በ ቃ ል ና በ ፊ ር ማ ይ ገ ለ ፃ ል ፡ ፡

ማ ና ገ ር ቢ ያ ስ ፈ ል ግ ዎ ፡

በ ኩ ረ ጵ ዮ ን ግ ደ ይ ፡ የ ኢ ት ዮ ጵ ያ
ህ ብ ረ ተ ሰ ብ ጤ ና
ኢ ን ስ ቲ ት ዩ ት ሠ ራ ተ ኛ ና
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ሠ ራ ተ ኛ ና የ ጥ ና ቱ አ ስ ተ ባ ባ ሪ ኢ ብ ራ ሂ ም

ኪደር ፡ የ ኢትዮጵያ ህብረተሰብ ጤና

ኢንስቲትዩት

ሞባይል ቁጥር ፡ +251 910133453

የ ጥናቱ ዓላማ፡

የዚህ ክትትል ጥናት አላማ በሀገሪቱ ለወባ ህክምና
የ ሚውሉት የፀረ ወባ መድሀኒቶችን የመላመድ እድል
መኖሩንና የ ወባ ህክምና በኢትዮጵያም ምን ያህል እየሰራ
እንደሆነ ለማወቅ ነው። ከዚህ ጥናት የተገኘው መረጃ
በአገር አቀፍ የ ወባ የ ማጥፋት ፕሮግራም አስተዳዳሪዎች
መከሰትን ለመቋቋምና መላመድን ቅድሚያ ለማወቅና ተጨማሪ
ስርጭትን ለመከላከል ተገቢውን የ ጣልቃ ገብነት ስልቶችን
እንዲያደርጉ ይረዳላ ፡ ፡ ይህ ጥናት በ ጤና ሚኒሰትርና
በኢትዮጵያየ ህብረተሰብ ጤና ኢንስቲትዩት የ ተደገፈ ነው።
እርስዎ ወይም ልጆቻችሁ የ ወባ በሽታ ስለተገኘባችሁ በዚህ
ጥናት እንድትሳተፉ እንጋብዛችኻለን።

<u>የ ጥናትጊዜ ፡</u> ጥናቱ ለ 28 ቀናት የ ሚቆይ ሲሆን
ወደ ጤና ተቋም መምጣት ባለብዎት ቀናት ቀን
1፣ 2፣ 3፣ 7፣ 14፣ 21 እና 28ተኛ ቀናት በ ጤና
ተቋም ውስጥ መገኘት አለብዎት

<u>ሂደቶች ፡</u> ጥናቱ የ ሚካሄደው ለ 28 ቀናት ሲሆን ከነዚህም
ውስጥ ህክምና ው ለ 3 ቀናት የ ሚሰጥ ሲሆን እርሰዎ በ ቀጠዩ
ቀናት ጧት ጧት ወደ ጤና ጣቢያ ው እየ መጡ መድሐኒቱን
ይወስዳሉ ፡ ፡ በዚህም ወቅት

አደጋዎች፡ ጣት በ ሚወጉ በ ትኒዜ ት ን ሽህ መምሊሰ ማዎ ት ይችላል.
ህ መሞች በ አ ብዛ ኛ ውቀ ላ ል ና ቸ ውእ ና በ ቅር ቡ ይ ጠፉ ሉ.
የ ሚወሰ ዱ ት መድ ሐኒ ት በ ውል ተ ለ ይ ቶ ያ ል ታ ወቀ የ ጎ ን ዮ ሽ
ጉ ዳ ት ሊ ኖ ረ ው እ ን ደ ሚች ል ይ ገ መታ ል ፡ ፡ የ መድ ሐኒ ቱ ን
ተ ጽ እ ኖ በ ማጥ ና ት ሂ ደ ት ሳ ይ ሻ ለ ው የ ሚሄ ድ ታ ካ ሚ ከ ተ ገ ኘ
በ ወ ባ የ ህ ክ ምና መመ ሪ ያ መሰ ረ ት ህ ክ ምና በ ነ ጻ
ይ ደ ረ ጋ ል ፡ ፡

<u>የ ሚጠበ ቁ ጥቅ ሞች፡</u> በ ዚ ህ ጥ ና ት ውስ ጥ በ መሳ ተ ፍ የ ግ ል
(ቀ ጥታ) ጥቅ ማጥቅ ሞች ን ላ ያ ገ ኙ ይ ች ላ ሉ እ ና በ ጥ ና ቱ
በ መሳ ተ ፍ ክ ፍ ያ አ ያ ገ ኙ ም ነ ገ ር ግ ን የ ጥ ና ቱ ግ ኝ ቶ ች
ለ አ ገ ር አ ቀ ፍ የ ወ ባ የ ማጥ ፋ ት ፕ ሮ ግ ራ ም ፤ የ ወ ባ መ ዳ ኒ ቶ ች

የ መ ቋ ቋ ም መ ከ ሰ ት ን እ ና የ ወ ባ ህ ክ ምና ን ለ ማ ሻ ሻ ል
ተ ገ ቢ ው ን የ ጣ ል ቃ ገ ብ ስ ል ቶ ች ን እ ን ዲ ያ ደ ር ጉ
ይ ረ ዳ ል። ይ ህ እ ር ስ ዎ ን ወ ይ ም የ ሚ ያ ው ቁ ት ን ሰ ዎ ች ወ ደ ፊ ት
ይ ረ ዳ ል ፡ ፡

<u>ጉ ዳ ቶ ች፡</u> በ ዚ ህ ጥ ና ት ውስ ጥ
በ መሳ ተ ፍ ምክ ን ያ ት ለ ሚ ደ ር ስ ማ ን ኛ ው ም ጉ ዳ ት ፣ የ ሰ ራ ተ ኞ ች አ ባ
ላ ት ህ ክ ምና ን ለ ማግ ኘ ት ይ ረ ዱ ዎ ታ ል ፣ ይ ህ
ም እ ን ደ አ ስ ፈ ላ ጊ ነ ቱ የ ድ ን ገ ተ ኛ ህ ክ ምና ፣ የ ሆ ስ ፒ ታ ል እ ን ክ ብ
ካ ቤ እ ና ክ ት ት ል የ ሚ ደ ረ ግ በ ት እ ን ክ ብ ካ ቤ እ ና ክ ፍ ያ ይ ከ ፈ ላ ል

<u>ማ ካ ሻ፡</u> ለ ክ ት ት ል ወ ደ ጤ ና ተ ቋ ም ሲ መ ጡ የ ት ራ ን ስ ፖ ር ት ወ ጪ ዎ ት
የ ሚ ሸ ፈ ን 100 ብ ር የ ሚ ሰ ጥ ይ ሆ ና ል

<u>ተ ጨ ማ ሪ ወ ጪ፡</u> የ ለ ም፡

<u>ሚ ስ ጥ ራ ዊ ነ ት፡</u> ሕ ጉ በ ሚ ፈ ቅ ደ ው መ ጠ ን ስ ለ እ ር ስ ዎ ያ ለ ው ው ጤ ት
ወ ይ ም መ ረ ጃ በ ሚ ስ ጥ ር ይ ጠ በ ቃ ል።

ተ ሳ ት ፎ (በ ፈ ቃ ደ ኝ ነ ት)

በ ዚ ህ ጥ ና ት ውስ ጥ መሳ ተ ፍ የ እ ር ስ ዎ ምር ጫ ነ ው።

በ ማ ን ኛ ው ም ጊ ዜ በ ጥ ና ቱ ውስ ጥ ላ ለ መሳ ተ ፍ ወ ይ ም

ላለማቋረጥ መወሰን ይችላሉ. ምርጫዎ ለወባ የሚወስዱትን
ሕክምና አይጎዳውም. እንዲሁም፣ የሚቀበሉት የትኛውም
ህክምና አይጎዳም። ጥናቱን በማንኛውም ጊዜ መተው
ይችላሉ። ይህ በጤና አጠባበቅዎ ላይ ተጽእኖ አይኖረውም,
እና አሁንም የወባ ህክምናን በነጻ ያገኛሉ፡ ፡ የፍቃድ
መግለጫየ አውራ ጣት አሻራዬን በመፈረም ወይም ከታች
በማስቀመጥ አረጋግጣለሁ፡ –

ይህን ቅጽ አንብቤአለሁ፣ ወይምተነበበኝ፤ ስለሱ
ጥያቄዎችን ለመጠየቅ ችያለሁ,እናምለጥያቄዎቼ መልስ
አግኝተዋል.

ለአዋቂዎች፡ የእኔ ወይምየልጄ ተሳትፎ በፈቃደኝነት
እንደሆነ እና ጥናቴን በማንኛውም ጊዜ እንክብካቤዬን
ሳይነካኝ መተውእንደምችል ተረድቻለሁ።

የእኔተሳትፎ በፈቃደኝነት እንደሆነ እና ጥናቴን
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መተውእንደምችል ተረድቻለሁ.የመሳተፍ ውሳኔዬ
በወላጅ/አሳዳጊየተደገፈነውነገርግን
በእሱ/ሷአስገድዶ አይደለም።

ስምምነቱን የሚያስሞላውባለሚያ

ስም __ፊርማ_____ቀን _____

_ ምስክር፡ ስም_____ፊርማ_____ቀን __

_____ የተሳታፊ ስም_____ቀን _____

_____

የ ጣት አሻራ ወይም ፊር ማ_____

የ ተሳ ታፊ ወላ ጅ/አ ሳ ዳ ጊ ስ ም የ ጣት አ ሻ ራ _____ወይ ም ፊ ር ማ ____

ቀ ን __

1. Monitoring the efficacy of frontline antimalarial drugs at Metahera, Ethiopia 2022/23

Consent/Assent Form in English

Title:- Monitoring the efficacy of frontline antimalarial drugs 2019/20

Consent form for informing adults (>18) or consent form for parental permission for a child six months up to 11 years and assent form for children aged between 11-17 years old study participants enrolment form for malaria in the vivo efficacy study.

Contact Person:

Ashenafi Assefa: Ethiopian Public Health Institute (EPHI) staff and the PI of the project Mobile No: +251 931 57 18 38

Addis Ababa, Ethiopia

: EPHI staff and

EPHI-IRB director Mobile No: +251 911 95 71 61

Addis Ababa, Ethiopia

Participation Duration: 28 days

Anticipated Number of participants: 90-100 Voluntary Participants /site

Purpose: The purpose of this research study is to find out how well the treatment for malaria is working 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 diagnosis results show malaria parasites 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 days: 1, 2, 3, 7, 14, 21, and 28.

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, diarrhea, 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 treatments guideline policy till they recover.

Benefits

You may or may not get personal (direct) benefit from taking part in this study but you will not get paid for participating in the study. There are possible benefits of taking part in this study which follow: You will not have to pay fees for any of the clinic visits during this study including any visits for 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. You do not give up any of your legal rights by signing this consent form.

Compensation

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

Participation (voluntary)

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, then we will not continue to follow you. If you are removed from the study before the treatment is complete, or if the medicine does 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 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 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.

For children aged 12-17 years: Read the assent below for supplements.

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.

Person Obtaining Consent/Assent

Print Name:____Signature:____Date:__/_____/_____

Witness:

Print Name: _Signature:____Date:__/_____/_____

Parent or guardian

Print Name:____Signature:____Thumbprint:__Date:__/_____/_____

Annex II Sever malaria and danger sign

Severe manifestation of *P. falciparum* malaria in adults and children

Clinical manifestations

· prostration,

· impaired consciousness,

· respiratory distress (metabolic acidosis),

· multiple convulsions,

· circulatory collapse,

· pulmonary edema (radiological),

· abnormal bleeding,

· jaundice,

· hemoglobinuria.

Laboratory findings

· severe anaemia (haemoglobin < 5 g/dl, haematocrit < 15%),

· hypoglycaemia (blood glucose < 2.2 mmol/l or 40 mg/dl),

· acidosis (plasma bicarbonate < 15 mmol/l),

· hyperlactatemia (venous lactic acid > 5 mmol/l),

· hyper parasitemia (> 4% in non-immune patients),

· renal impairment (serum creatinine above the normal range for age).

Classification of severe malaria in children

Group 1: children at increased risk for death

· Respiratory distress.

· Prostration,

Group 2: children at risk for clinical deterioration

· Haemoglobin < 5 g/dl, haematocrit < 15%,

· Two or more convulsions within 24 hr.

Group 3**:** children with persistent vomiting

Annex III Microscopic blood film examination

· Prepare a 3% solution of Giemsa stain, and pour the stain into the trough while avoiding pouring directly onto the thick films

· Stain the smears for 30 minutes

· Rinse slides by gently immersing the staining trough/jar into a bowl of clean water, to float off the scum.

· Proceed to examine under the thick blood smear under a microscope to determine parasite species and density for *P. falciparum* density using the 100X objective.

· Determine the actual parasite density of the positive thick blood smear by counting the number of asexual parasites per 200 leukocytes.

· Examine the thin smear under the light microscope to determine whether or not it is a mono infection of *P. falciparum.*

Record results on the study Record Sheet

Pull the cuvette holder out to the loading position.

Remove the cuvette and discard it in a waste container

To test a second sample, wait until the meter is ready,

indicated by the appearance of dash lines LCD

Annex IV DBS sample collection

· Label with study identifier and date in pencil

· Perform finger prick

· Collect 2nd, 3rd& 4th drops onto filter paper

· Allow the blood drop to go down to the filter paper and DO NOT touch the filter paper with the finger

· Air-dry the filter paper, on a rack or leave it on a clean dry surface protected from flies, and dust (4-8 hrs).

· Place individual filter papers into separate zip-lock plastic bags using forceps.

· It is very important that the sample has dried completely(overnight), before placing it into the plastic bag

· Add 2-3 desiccant & humidity detector in plastic bag.

· Label the outside of the plastic bag in a marker pen with the patient's bar code number, Date of sample, and follow-up day (Day 0 or Day TF).

· Store at + 4o C

Annex V Drug dosage

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

Artemether-lumefantrine was administered twice daily for three days as tablets containing 20 mg of artemether plus 120 mg of lumefantrine in a fixed dose combination with a single dose of 7.5 mg of primaquine on the first day only.

AL 20mg /120 mg tabs						
Weight (kg)	Day 0		Day 1		Day 2	
	Am	8 hrs later	12 hrs later	12 hrs later	12 hrs later	12 hrs later

5-14	1	1	1	1	1	1
15-24	2	2	2	2	2	2
25-34	3	3	3	3	3	3
≥ 34	4	4	4	4	4	4

Weight per (Kg)		8-18	19-24	25-35	36-50	50+
Number of tablets (Primaquine)	7.5 mg tablet	½	¾	1	1 ½	2

Annex VI 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. falciparum/P. vivax* mono-infection asexual parasites/µl Pf 1000-100,000 /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 a 5-10 km radius of the health center 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. ' Pneumonia/RTI ' Measles ' Otitis Media ' UTI ' Gastroenteritis Other:	Yes:	No:

10.	Evidence of severe malaria/danger signs If "YES" indicates the criteria. If "NO", leave blank. ' Unarousable coma (if after convulsion, > 30 min) ' Repeated convulsions (> 2 within 24 h) ' Recent convulsions (1-2 within 24 h) ' Altered consciousness(confusion, delirium,, coma) ' Lethargy ' Unable to drink or breastfeed ' Vomiting everything ' Unable to stand/sit due to weakness ' Severe anemia (Hb < 8.0 g/dL) ' Respiratory distress (labored breathing at rest) ' Jaundice (yellow coloring of eyes)	Yes:	No:
11.	Known hypersensitivity to AL	Yes:	No:

Annex VII Enrolment form

Monitoring the efficacy of frontline antimalarial drugs 2019/20

ENROLMENT FORM

1. Age…….	2. Gender/Male……Female	3. Weight……

7. Patient 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 localizing features/Owners' name/Direction:
15.P.no
Previous malaria attack: Yes___No ___ Previous antimalarial intake: Yes_____No ___If yes, CQ____AL? Hold Bed net: Yes ___No___If yes, Bed net use Yes___No ___

Annex**VII** . **Patient Lab Request Sheet**

Patient Lab Request Sheet Date:____ Treatment Group_____ PIN. No._____ Name:_____ Follow up date: _____ Lab Results Parasite: Asexual_____Gametes_____ Hb:____DBS:_____ _____ HCG:

Annex VIII Case record form

Monitoring the efficacy of frontline antimalarial drugs 2022/23, _____

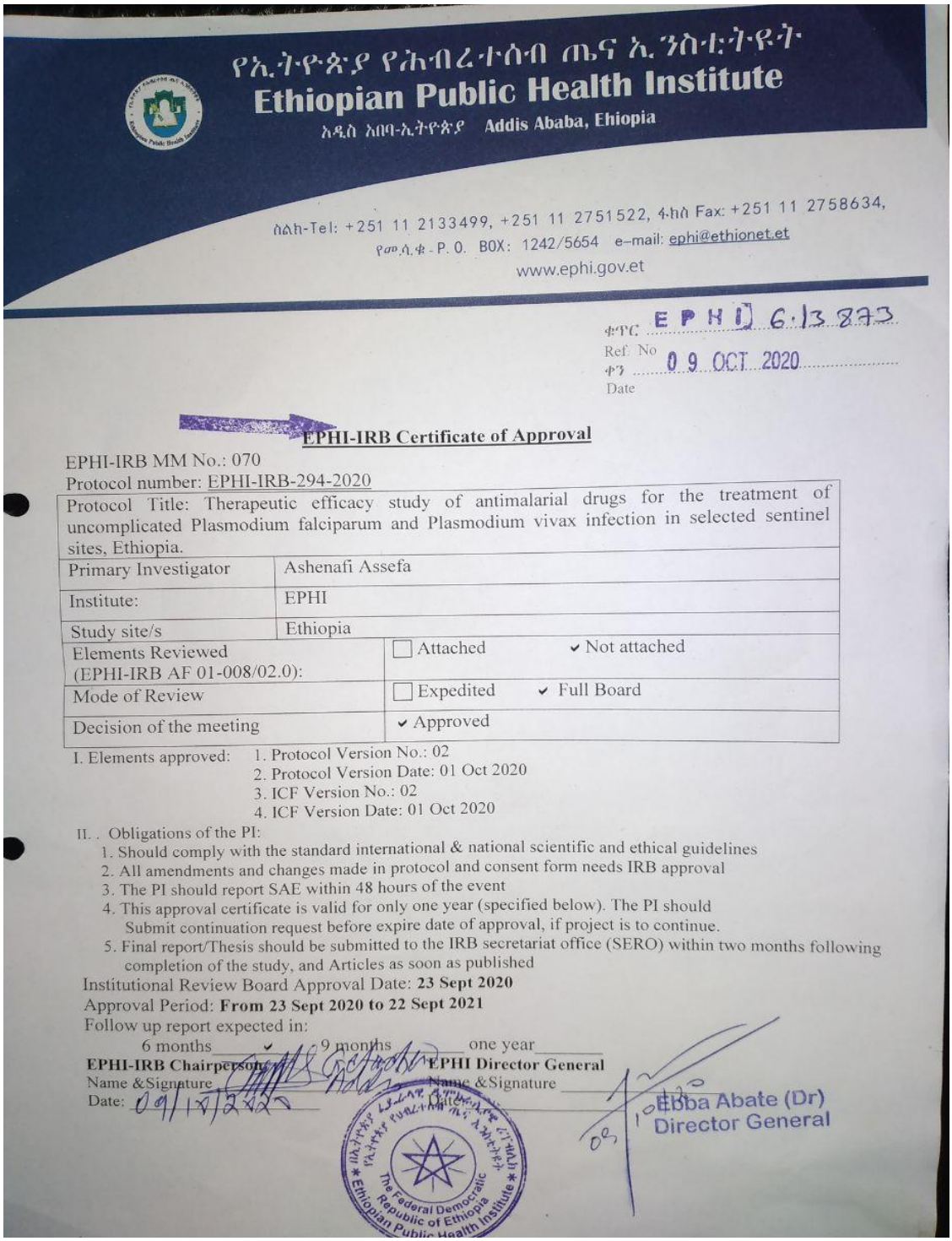
Study site: _____ Name_____ Pin No_____ No. of Tablets. _____

S.No	day 0	day 1	day 2	day 3	day 7	day 14	day 21	day 28	xtra DAY
1. Date (DD/MM/YY)									
2. Axillary T^{o} with ^{o}C									
3. Parasite asexual									
4. Gamet No.									
5. Hemoglobin									
6. DBS sample (Yes/No)									
7. Adverse events*									
8. Concomitant treatment									
9. Reasons for withdrawal									
10. Treatment outcome									
11. Remarks									

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

Annex IX Ethical consideration

EPHI-IRB Certificate Approval



የኢትዮጵያ የሕብረተሰብ ጤና ኢንስቲትዩት
Ethiopian Public Health Institute
አዲስ አበባ-ኢትዮጵያ Addis Ababa, Ethiopia

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www.ephi.gov.et

ቁጥር Ref. No EPHI 6.13.873
ቀን Date 09 OCT 2020

EPHI-IRB Certificate of Approval

EPHI-IRB MM No.: 070
Protocol number: EPHI-IRB-294-2020

Protocol Title: Therapeutic efficacy study of antimalarial drugs for the treatment of uncomplicated Plasmodium falciparum and Plasmodium vivax infection in selected sentinel sites, Ethiopia.		
Primary Investigator	Ashenafi Assefa	
Institute:	EPHI	
Study site/s	Ethiopia	
Elements Reviewed (EPHI-IRB AF 01-008/02.0):	☐ Attached	✔ Not attached
Mode of Review	☐ Expedited	✔ Full Board
Decision of the meeting	✔ Approved	

I. Elements approved:
1. Protocol Version No.: 02
2. Protocol Version Date: 01 Oct 2020
3. ICF Version No.: 02
4. ICF Version Date: 01 Oct 2020

II. . Obligations of the PI:
1. Should comply with the standard international & national scientific and ethical guidelines
2. All amendments and changes made in protocol and consent form needs IRB approval
3. The PI should report SAE within 48 hours of the event
4. This approval certificate is valid for only one year (specified below). The PI should Submit continuation request before expire date of approval, if project is to continue.
5. Final report/Thesis should be submitted to the IRB secretariat office (SERO) within two months following completion of the study, and Articles as soon as published

Institutional Review Board Approval Date: **23 Sept 2020**
Approval Period: **From 23 Sept 2020 to 22 Sept 2021**
Follow up report expected in:
6 months ✔ 9 months ____ one year ____

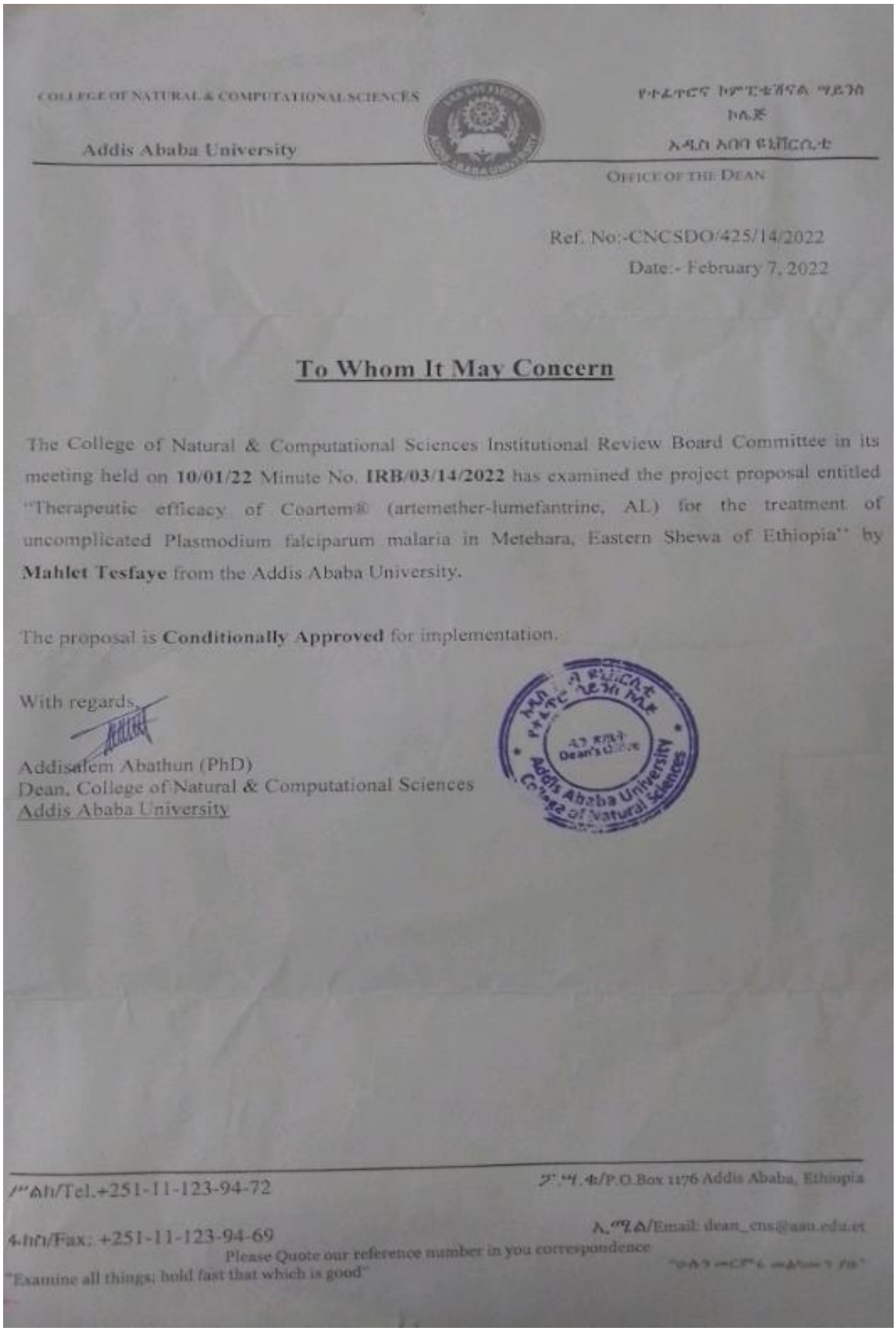
EPHI-IRB Chairperson Name &Signature ____ Date: 09/17/2020

EPHI Director General Name &Signature ____

The Federal Democratic Republic of Ethiopia Ethiopian Public Health Institute

Ebba Abate (Dr)
Director General

CNCS-IRB Certificate approval



COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES

Addis Ababa University

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OFFICE OF THE DEAN

Ref. No:-CNCSDO/425/14/2022

Date:- February 7, 2022

To Whom It May Concern

The College of Natural & Computational Sciences Institutional Review Board Committee in its meeting held on **10/01/22** Minute No. **IRB/03/14/2022** has examined the project proposal entitled "Therapeutic efficacy of Coartem® (artemether-lumefantrine, AL) for the treatment of uncomplicated Plasmodium falciparum malaria in Metehara, Eastern Shewa of Ethiopia" by **Mahlet Tesfaye** from the Addis Ababa University.

The proposal is **Conditionally Approved** for implementation.

With regards,

Addisalem Abathun (PhD)
Dean, College of Natural & Computational Sciences
Addis Ababa University

Addis Ababa University College of Natural Sciences Dean's Office

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Please Quote our reference number in you correspondence

"Examine all things; hold fast that which is good"

Annex IIX Classification of Treatment Outcomes

Classification of treatment outcomes **for *P.falciparum***

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 28 days 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 previously meet 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.