Therapeutic efficacy of artemeter-lumefantrine against uncomplicated *Plasmodium falciparum* malaria in Setit Humera Health Center, Northwest Ethiopia: Nine years after its approval as first-line drug.

By

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A Thesis Submitted to the Department of Microbial, Cellular and Molecular Biology

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Biomedical Sciences)

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Therapeutic efficacy of artemether-lumefantrine against uncomplicated *Plasmodium falciparum* malaria in Setit Humera Health Center, northwest Ethiopia: Nine years after its approval as first-line Drug

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BSTRACT

Malaria still remains a major public health problem in Ethiopia. Early diagnosis and prompt treatment of malaria cases is a primary malaria control strategy in the country. However, this control strategy is severely challenged by the evolution and rapid spread of resistant *P. falciparum* strains. Artemether-lumefantine (Art-L) is the first-line antimalarial drug against uncomplicated *P. falciparum* malaria in the country since 2004. Regular monitoring of first- and second-line antimalarial drugs is recommended by the World Health Organization (WHO) to help early detection of drug resistance and search for more effective options. However, there is no data regarding the efficacy status of AL in Setit Humera, northwest Ethiopia. This study was, therefore, conducted to fill in this gap. One arm prospective study of a 28-day follow-up was conducted from October 2014 to January 2015 according to the revised WHO 2009 efficacy study protocol. Study outcomes were classified into primary and secondary. The primary study outcome was the day 28 adequate clinical and parasitological response. The secondary study outcomes were clinical and parasitological evaluations (parasite, fever and gametocyte clearance rate, incidence of drug adverse events) and the relative increment in hemoglobin level from baseline to day (D) 14 and D28. A total of 92 patients were enrolled and 80 had completed the 28-day follow-up period. The overall cure rate was 98.8% with 95% confidence interval (0.915-0.998) without polymerase chain reaction correction. The parasite clearance rate was high with fast resolution of clinical symptoms; 100% of the study participants cleared parasitaemia and fever on D3. Gametocyte carriage was reduced from 7% on D0 to 1% on D3 and complete clearance was achieved on D21. Mean hemoglobin concentration has significantly increased on D28 compared to that of D14. There was no serious adverse event. Overall, AL was efficacious and safe in the study population for treatment of uncomplicated falciparum malaria.

Keywords*:* artemether-lumefantine, artemisinin combination therapy, drug-resistant malaria, *Plasmodium falciparum*, therapeutic efficacy, treatment failure

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TABLE OF CONTENTS

List of figures **Page**

List of tables **Pages**

LIST OF ABBREVIATIONS/ACRONYMS

ACT/s	Artemisinin-based combination therapy/therapies
AE/s	Adverse event/s
Art-L	Artemether-lumefantrine
APCR	Adequate parasitological and clinical response
CI	Confidence interval
CQ	Chloroquine
D	Day
DHPF	Dihydropholate reductase
DHPS	Dihydropteroate synthase
DHA	Dihydroartemisinin
EPHI	Ethiopian public health institute
ETF	Early treatment failure
FMoH	Federal ministry of health
g/dl	Gram per deciliter
Hb	Hemoglobin
IPT	Intermittent preventive treatment in pregnancy
IRS	Indoor residual spray
ITNs	Insecticide treated bed nets
K-M	Kaplan-Meier
LCF	Late clinical failure

LFU	Lost to followup
LLINs	Long lasting insecticidal nets
LPF	Late parasitological failure
LTF	Late treatment failure
PADH	Postartemisinin delayed hemolysis
Pfcrt	*P. falciparum* chloroquine resistance transporter gene
Pfmdr	*P. falciparum* multi-drug resistant gene
PMI	President's malaria intiative
PP	Per protocol
RBC	Red blood cells
SHHC	Setit humera health center
TF	Treatment failure
SP	Sulfadoxine-Pyrimethamine
U5	Under the age of five
WBC	White blood cells
WHO	World Health Organization

1. INTRODUCTION

Malaria, which is caused by apicomplexan protozoa belonging to the genus Plasmodium (described by Marchiafava and Celli in 1885) and transmitted by anopheline mosquitoes, remains a major public health burden. It is estimated that 214 million cases (uncertainty range: 149–303 million) and 438,000 deaths (uncertainty range: 236 000–635 000) occurred due to malaria in 2013 although malaria case incidence and mortality rates decreased 30% and 47% respectively since 2000 (WHO 2015). According to this same World Health Organization (WHO) source, 90% of the deaths occur in sub-Saharan Africa among children under the age of five (U5). In Ethiopia, malaria transmission follows unstable and seasonal pattern making the country prone to recurrent outbreaks and epidemics (Adugna 2014). It is estimated that about 5-6 million clinical and above 600,000 confirmed malaria cases reported each year (non-epidemic year) (FMoH 2012). Climatic and ecological changes, population movements, global warming and expansion of developmental schemes coupled with inefficiency in existing control interventions are likely to put more people at-risk of malaria than before (Edward and Michael 2010; Darlymple *et al*. 2015).

1.1. The parasite and its vector

Currently human malaria is caused by at least five plasmodium species: *Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi.* Of these *P. falciparum* is the most notable species in both distribution and gravity. It is well-known that most malaria-related deaths (90%) in tropical Africa are due to this species. *P. vivax* is the second in significance with wider geographical distribution including in many temperate areas. Malaria is transmitted by mosquitoes of the genus *Anopheles* which was first named by Johann Wilhelm Meigen (1818) and there are about 465 species and 50 unnamed species complex which belong to the genus *Anopheles* (Harbach 2013). Of these about 70 species can transmit human malaria (Warrel and Gilles 2002) and 41 have been considered as the dominant and most efficient vector species of malaria (Levine *et al*. 2004; Sinka *et al*. 2010).

The biology and epidemiology of this highly important pathogen is extensively covered in most parasitology literature including texts books (reviewed in Schmidt and Roberts 2009). Here a brief description of a generalized plasmodium life cycle may suffice. An infected female

Anopheles mosquito inoculates sporozoites into a human host during blood meal feeding. Shortly after inoculation the sporozoites disappear from the circulation and invade hepatocytes initiating exoerythrocytic schizogony. This process generates merozoites which leave the liver, return to the circulation and infect erythrocytes inducing erythrocytic schizogony that produces second generation merozoites. This merozoitic blood-stage cycle repeats and at a certain point certain merozoites are differentiated into gametocytes. The gametocytes are to be ingested by the mosquito. The sexual reproduction taking place in the mosquito producing sporozoites ready for further inoculation into a human host perpetuating the cycle unless interrupted (Figure1).

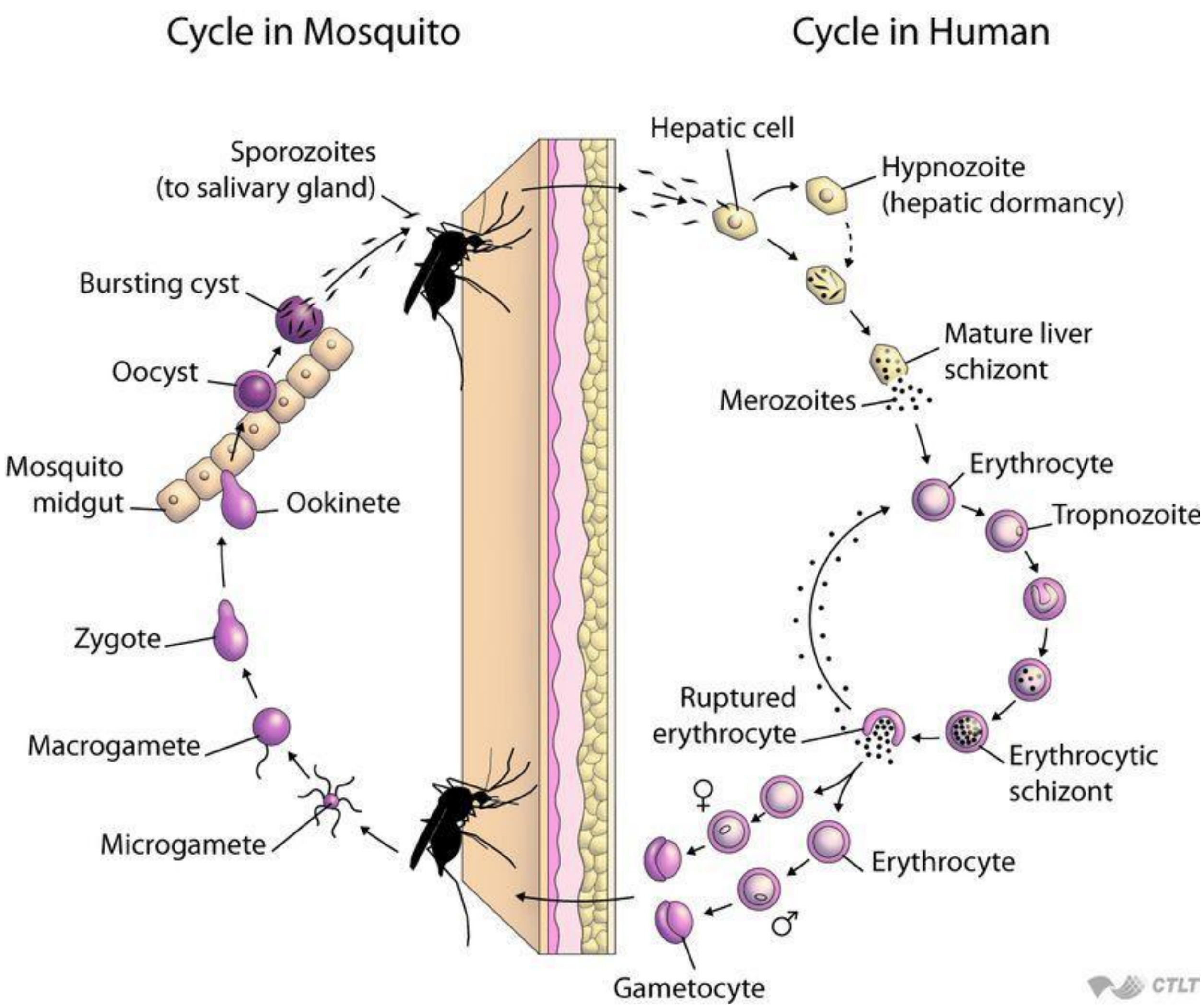

Figure 1: A generalized life cycle of the malaria parasites (Source: http://ocw.jhsph.edu/)

1.2. Malaria control interventions

Proven malaria preventive strategies currently in use are insecticide-treated nets, indoor residual spraying and larval source management. As a physical barrier from the mosquito nuisance, the use of bed nets has been practiced for many years. The impregnation of a bed net with insecticides made it effective as a result of its dual actions of repelling and/or killing mosquitoes. The efficacy of this tool in preventing malaria is documented in several endemic settings (Bermejo and Veeken 1992). Indoor residual spraying which has resulted in malaria eradication in North America and Europe in the early 1960s is the most important tool nowadays next to bed net (Biscoe *et al.* 2004). However, rapidly developing insecticide-resistant vectors to available insecticides is jeopardizing the effectiveness of both of these strategies (WHO 2010).

Larval habitat manipulation and modification, and chemical and biological larviciding have been used as larval source management strategies in certain countries. Despite the success of this strategy to some extent it is not taken as a core strategy to prevent malaria for the reason that a larval habitat should be well-defined for its implementation (Fillinger and Lindsay 2011).

Preventive chemotherapy and case treatment are integrated into the above malaria control interventions. The WHO recommends preventive chemotherapy for pregnant women and infants in areas with moderate-to-high malaria transmission. In highly seasonal malaria transmission settings seasonal malaria chemoprevention is also prescribed for children 3-59 months old (WHO 2012). Intermittent preventive treatment in pregnancy (IPTi) is the administration of the appropriate antimalaria drug at each scheduled ante-natal care visit (each dose to be given at least one month apart) starting early in the second trimester (WHO 2009). Studies have implied that this intervention measure reduces severe maternal anemia, low-birth weight and perinatal mortality (reviewed in Yohannes 2012). IPT in infants (IPTi) is the mass administration of amodiaquine plus sulfadoxine-pyrimethamine (SP) for children aged 3-59 months (WHO 2010). This intervention measure works by maintaining therapeutic antimalarial drug concentrations in the blood during periods of greatest malaria risk. In some African countries including Ethiopia preventive chemotherapy is not included in the package of malaria prevention because of unstable malaria epidemiology and high level of SP resistance by *P. falciparum* (FMoH 2012).

Early case detection and prompt treatment is the mainstay to minimize malaria-related morbidity and mortality. Microscopy remains the gold standard for definitive malaria diagnosis in most endemic areas (Tangpukdee *et al.* 2009). Rapid diagnostic techniques are being widely employed to detect malaria parasites especially in rural settings where microscopy is lacking (WHO 2015). In the absence of these methods clinical diagnosis is the only way out. Although various versions of molecular diagnostic methods exist and are superior to microscopy and the rapid technique, in both sensitivity and specificity (WHO 2012), the question of affordability and relative technical complexity make these tools less applicable in resource-limited settings.

2. LITERATURE REVIEW

2.1. Drugs for malaria treatment

The list of malaria drugs is long and includes quinine, chloroquine (CQ), amodiaquine, pyrimethamine, proguanil (chloroguanide), SP, mefloquine, primaquine, halofantrine, doxycycline, clindamycin and artemisinin and its derivatives. The history, structure and chemistry, mode of action, safety (dosage) and plasmodium resistance to these drugs is extensively reviewed by the WHO (WHO 2001, Gopalkrishnan and Pannicker 2014). While some of these drugs are used in combinations, others are monotherapis. Here, a brief account of the most widely known malaria drugs (quinine, CQ, SP, mefloquine, primaquine, and artemisinin and its derivatives) is provided. Emphasis is on artemisinin and its derivatives.

Quinine which is an alkaloid isolated from the bark of cinchona tree (a traditional medicinal plant used in Peru and Bolivia) first appeared in the 17th century and remained as drug of choice for malaria until the 1940s (Rosenthal P 2003, Achan *et al.* 2011). Despite its poor patient tolerance, low compliance, high toxicity and complex dose regimen; quinine continued to be used for the treatment of complicated *P. falciparum* malaria in some countries (WHO 2015).

CQ, which is a 4-aminoquinolone alkaloid, was made widely available in the early 1950s although it was introduced as early as 1934 (Cooper and Magwere 2008). It has been the most widely used malaria drug because of its safety, price related factors and it is also considered as the safest of malaria drugs to use during pregnancy (WHO 2009). It has additional anti-pyretic and anti-inflammatory properties which increases its importance (O'Neill *et al*. 2012). Although CQ is replaced by more effective drugs against *P. falciparum* in most settings nowadays it remains the drug of choice in most African countries for *P. vivax* treatment (WHO 2015).

When CQ-resistant *P. falciparum* strains emerged pyrimethamine combined with sulfadoxine (Fansidar) was deployed. The target of this drug is mainly the schizont stage of the parasite during the blood-stage cycle and the component drugs of the combination exert synergistic effects (Aguiar *et al*. 2012).

During the Vietnam War a chemically related antimalarial drug to quinine was produced. This drug was mefloquine with the intention to protect American soldiers from multi-drug resistant *P. falciparum* strain (Muller and Hyde 2010). But, the WHO recommends mefloquine (usually combined with artesunate) for the treatment of drug-resistant *P. falciparum* strains only. The drug is recommended for malaria treatment as well as prophylaxis. It is now strictly used for resistant strains including during pregnancy (CDC 2013).

Primaquine is an 8-aminoquinolone known for its activity against the gametocytes of *P. falciparum* and hypnozoites of *P. vivax or P. ovale.* Thus, the drug is capable of blocking-transmission and radical cure thereby preventing both relapsing malaria and management of acute cases. Hemolytic anemia may be triggered following the intake of this drug among glucose-6 phosphate dehydrogenous deficient individuals (Aguiar *et al*. 2012).

The tradition of using the herb *Artemisia annua* for fever and malaria treatment in China goes back to over 2000 years (van Agtmael *et al*. 1999). The chemical artemisinin which is a sesquiterpene lactone having a rare peroxide bridge linkage was isolated first in 1972 (Djimde and Lefevre 2009). The compound was unique in structure among malaria drugs when determined in 1979 (Cui and Su 2009). It is the most rapid antimalarial (clearing parasitaemia within 1-3 days) and effective against all stages/forms of multidrug resistant *P. falciparum* (Golsner *et al*. 2006). It is not only effective against malaria; artemisinins also act against other parasites such as leishmania (Yang and Liew 1993) and schistosomes (Xiao *et al*. 2000) as well as tumor cells (Singh and Lai 2004; Maria *et al*. 2012, Das 2015).

2.2. Antimalarial drugs: mode of action and resistance mechanism

Drug resistance is defined as the ability of a parasite to survive and multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within the limits of tolerance of the subject (WHO 2009).The successful treatment of malaria requires the administration of an effective anti-malarial regimen that results in the clearance of all the parasites from the blood, resolution of symptoms of acute disease and prevention of recrudescent infections (Rosenthal 2003).

Understanding the mode of action of antimalaria drugs is particularly important to detect and prevent the emergence of antimalaria drug resistance. Resistance to antimalaria drugs is mainly due to spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs (Golenser *et al*. 2006). For some drugs, only a single point mutation is required to confer resistance, while for other drugs, multiple mutations appear to be required. Provided the mutations are not deleterious to the survival or reproduction of the parasite, drug pressure will remove susceptible parasites while resistant parasites survive.

Single malaria isolates have been found to be made up of heterogeneous populations of parasites that can have widely varying drug response characteristics, from highly resistant to completely sensitive (Muller and Hyde 2010). Similarly, within a geographical area, malaria infections demonstrate a range of drug susceptibility. Over time, resistance becomes established in the population and can be very stable; persisting long after specific drug pressure is removed (WHO 2010). Brief description on the mode of action and resistance mechanism of the commonly known groups of antimalarial drugs is presented below.

2.2.1. Antifolates

Antifolate combination drugs, such as SP, act through sequential and synergistic blockade of two key enzymes involved with folate synthesis (Rosenthal 2003). Pyrimethamine and related compounds inhibit the step mediated by dihydrofolate reductase (DHFR) while sulfones and sulfonamides inhibit the step mediated by dihydropteroate synthase (DHPS) (Muller and Hyde 2010).

Resistance to the dihydrofolate inhibitors (pyrimethamine and biguanides) and dihydropteroate inhibitors (sulphonamides) is mediated by single or double mutations in genes encoding DHFR and DHPS, respectively (Hailemeskel *et al*. 2013). Specific combinations of these mutations have been associated with varying degrees of resistance to antifolate combination drugs.

2.2.2. Aminoquinolines

This class of antimalarial drugs includes CQ and amodiaquine. Despite many studies conducted to elucidate the parasitocidal activity of this class of antimalarial drugs, the exact modes of action remain largely unclear. However, it is suggested that CQ works by inhibiting the detoxification of heme in to hemozoin (Cooper and Magwere 2008). As the malaria parasite digests haemoglobin, large amounts of a toxic by-product are formed. The parasite polymerizes this by-product in its food vacuole, producing non-toxic haemozoin (malaria pigment). Antimalarial drugs in this group inhibit this detoxification process leading to accumulation of toxic heme inside the parasite's food vacuole which eventually leads to death of the parasites (O'Neill *et al*. 2012).

It is believed that resistance of *P. falciparum* to CQ is related to an increased capacity for the parasite to expel CQ at a rate that does not allow it to reach levels required for inhibition of hemozoin polymerization and this CQ efflux occurs at a rate of 40 to 50 times faster among resistant parasites than sensitive ones (Muller and Hyde 2010). The molecular bases of CQ resistance have been clearly associated with multiple mutations in genes encoding for membrane transporters located in the digestive vacuole, namely the CQ resistance transporter *Pfcrt* and the multi-drug resistance transporter 1 *Pfmdr*1 (Hastings 2004).

Initial emergence of CQ-resistant *P. falciparum* malaria was reported from Southeast Asia in 1957 and then was spread rapidly to East Africa in 1977 (reviewed in Payne 1987). In sub-Saharan Africa, the first report on *P. falciparum* resistance to CQ was in 1977 in expatriates in Kenya (Nuwaha 2001).

2.2.3. Quinoline-4-methanols

These are group of structurally simalar antimalarial drugs including quinine, mefloquine, lumefantrine and halofantrine. The exact mode action of these quinolines is not known. However, studies implied that mefloquine and quinine are known to inhibit hemoglobin ingestion/endocytosis, digestion and membrane recycling which may eventually lead to parasite death (Hoppe *et al.* 2004).

The inverse effects of 4-aminoquinolines and quinoline-4-methanol on parasite morphological abnormalities are consistent with observations made on the role of the two membrane transport genes on resistance to these antimalarial drugs (Muller and Hyde 2010). Resistance to mefloquine and halofantrine is mainly limited to South East Asia and appears to be related in part to both mutations and amplification of the *pfmdr1* gene like CQ (Golsner *et al*. 2006).

2.2.4. Artemisinins

The exact mode of action of artemisinins/its derivatives is not determined yet. However, it has been suggested that it interferes with parasite transport proteins, disrupts parasite mitochondrial function, modulate host immune function (Golenser *et al.* 2006). PfATP6, a transporter protein found on membranous structures within the parasite cytoplasm, is another suggested target of artemisinin containing drugs (Gopalakrishnan and Panicker 2014). Artemisinins interact and selectively inhibit PfATPase, the only sarco/endoplasmic reticulum Ca^{2+}-ATPase/ (SERCA) type Ca^{2+} ATPase in the *P. falciparum* genome (Cui and Su 2009). Artemisinins are considered as prodrugs with endoperoxide-bridge which is believed to be essential for their anti-malarial activity. Peroxides are known sources of reactive species and it is suggested that artemisinins modulate parasite oxidative stress and reduce the levels of antioxidants and glutathione in the parasite (Aguiar *et al*. 2012).

Artemisinin reduces to a least stable and most effective artemisinin metabolite dihydroartemisinin (DHA) which has a strong blood schizonticidal action and reduces gametocyte transmission (Djimde and Lefevre 2009, Biamonte *et al*. 2013). Artesunate and artemether are other derivative of artemisinin apart from DHA. These semi-synthetic artemisinin derivatives are easier to use than the parent compound and are converted in the body rapidly once to the active compound DHA (Rosenthal 2003). Artemether is a methyl ether and has been shown to be less effective against *P. vivax/ovale* hypnozoites but significantly active against gametocytes (White *et al*. 1999). Artesunate which is currently the most frequently used of all the artemesinin-type malaria therapies is a hemisuccinate derivative of DHA (Cui and Su 2009, Biamonte *et al*. 2013).

Artemisinin eliminates almost all asexual forms of the parasite in its intraerythrocytic stage and it is also active against immature gametocytes; important in transmission blocking which makes it preferable drug both for control and possible interventions (Biamonte *et al*. 2013). The mechanisms that underlie artemisinin resistance are not fully understood. However, studies in Southeast Asia implied that mutation in the *P. falciparum* K13-propeller domain was associated with artemisinin resistance (Ariey *et al*. 2014; Ashley *et al*. 2014). However, studies in malaria endemic countries of Africa showed that mutation in the K13-propeller region was not found

(Ashley *et al*. 2014; Conrad *et al*. 2014; Kamau *et al*. 2014; Taylor *et al*. 2014) as well as there was no association between K13 polymorphisms and parasite clearance rates (Ashley *et al*. 2014).

2.2.5. Artemisinin-based combination therapies

It is well known that *P. falciparum*, the most deadly and prevalent *Plasmodium* species in the tropical Africa has developed resistance to all aforadable and safe antimalarial drugs in disposal. Due to the wide spread resistance to SP and CQ monotherapies, the WHO recommended the use of ACTs for the treatment of uncomplicated falciparum malaria throughout malaria endemic areas. There are many combinations of ACTs and the choice for a particular combination therapy depends on the efficacy profile of the long acting partner drug in the specific area (WHO 2009).

ACTs are highly potent anti-malarial drugs and are also active against early stage gametocytes (White *et al*. 1999). Despite the recent reports of resistance to ACTs from the Great Mekong region in which the use of artemisinin monotherapies were intense and ACTs were deployed early, there is no report of resistance to ACTs in other malaria endemic areas of the world (WHO 2015). Artemisinin and its derivatives have short half-lives and inherent capacity of rapidly clearing parasite mass and the long acting partner drug has longer half-life and acts slowly and clears the remaining fewer parasites (Hastings 2004).

2.2.6. Arthemether-Lumefantrine (Art-L)

Art-L having trade names *Coartem*, *Riamet* and *Falcynate-LF*, is one of a number of ACTs. In Art-L the semi-synthetic artemisinin derivative artemether is combined with lumefantrine which is purely synthetic. The drug manufactured in tablet form using 20mg artemether and 120mg lumefantrine is marketed as Coartem® by Novartis (WHO 2009).

The two component drugs act independently but complementary manner at different stages of the parasite life cycle (Rosenthal 2003). Detailed pharmacokinetics of Art-L is found elsewhere (White *et al*. 1999; Djimde and Lefevre 2009). Artemether and its active metabolite, DHA, rapidly kill most circulating malaria parasites, while lumefantrine clears the remainder more slowly. Artemether is rapidly absorbed and metabolized, with a half-life of about two hours, whereas lumefantrine is absorbed more slowly and has a half-life of 3-4 days in malaria patients (Cui and Su 2009). The probability of selecting parasites resistant to the partner drug, lumefantrine, is theoretically reduced due to the small parasite load remaining following activity of artemether.

Art-L has been shown to be highly efficacious against multi-drug-resistant *P. falciparum* in different areas (Nosten *et al*. 1994; Vugt *et al*. 1999; Makanga *et al*. 2006; Mueller *et al*. 2006; Jima *et al*. 2005; Asefa *et al*. 2010; Ebstie *et al*. 2015) and thus has become the first fixed-dose ACT to be approved by the WHO for the treatment of uncomplicated *P. falciparum* malaria in 2001 (WHO 2015). Art-L tablets were included in the WHO "Essential Medicines" list in 2002 (FMoH 2004) and later is approved in over 80 countries worldwide (WHO 2010). It is currently recommended as first-line treatment for uncomplicated malaria in several countries (WHO 2015).

Studies in Africa have implied that Art-L selects for parasites with single nucleotide polymorphisms in *P. falciparum* chloroquine resistance transporter gene (pfcrt) and *P. falciparum* multidrug resistance gene 1 (pfmdr1) (Sisowath *et al*. 2007; Humphreys *et al*. 2007). Furthermore, in vitro susceptibility studies have been implied that reduced susceptibility to Lumefantrine was associated with the increase in the copy number of Pfmdr1 gene (Lim *et al*. 2009; Gadalla *et al*. 2011; Malmberg *et al*. 2013). As reviewed in recent meta-analysis of clinical trials, point mutation and an increased copy number of the pfmdr1 gene were significant risk factors for recurrence of parasites in patients treated with Art-L (Venkatesan *et al*. 2014).

2.3. Factors that contribute to the emergence and spread malaria drug resistance

2.3.1. Parasite related factors

Evolution of malaria parasites from drug sensitive to resistant is a complex and dynamic process involving many factors; including the size of the infecting biomass, the immunity of the host, the pharmacokinetic profile of the drug, the inherent drug susceptibility of the parasite and fitness cost of the mutants (WHO 2010). Selective pressure of drugs can occur either when a primary infection consists of resistant parasites capable of surviving treatment or when a subset of parasites with spontaneous mutations encounter residual concentrations of a slowly eliminated antimalarial drug (Sibley and Price 2012). The number of parasites exposed to selective pressure will be far greater in the first case than the second and thus will provide the greater opportunity for resistant mutants to arise and spread (Hastings 2004).

2.3.2. Drug related factors

Poor quality drugs

The availability of subtheraputic antimalarial drugs in the market is one of the risk factors for the emergence and spread of malaria drug resistance. Widespread use of subtherapeutic antimalarial regimens is also likely to play a major role in facilitating the emergence of drug resistance. Drugs may not contain sufficient amount of the required active ingredients due to poor manufacturing practices, intentional counterfeiting, or deterioration due to inadequate handling and storage. Fake drugs with inadequate amounts of active ingredient may kill off some susceptible parasites, but leave those more likely to develop tolerance to multiply (WHO 2014).

Monotherapies

Due to the wrong perception that monotherapies have fewer side effects and the fact that they are cheaper than ACTs, monotherapies were widely used in many malaria endemic areas (WHO 2009). However, it is highly likely and relatively easy to develop resistance to monotherapies than combination therapies as it is easier to adapt and evolve to one drug (Neodl *et al.* 2003). If a treatment involving two or more drugs is used, it is likely to kill the parasite even if it has developed resistance, to one of the drugs. It is far more complicated for a parasite to develop resistance to effective ingredients in both drugs (Hastings 2004).

2.3.3. Human related factors

Unnecessary use of antimalaria drugs

Reliance on presumptive treatment and mass antimalarial drug administration may facilitate the development of antimalarial drug resistance. As implied in the WHO report on drug resistance, resistance rates are higher in urban and peri-urban areas than rural communities, where access to and use of drug is greater (WHO 2010). Lack of proper dosing regimen has been described as an important factor in the development of drug resistance. It is important to note that both overdosing and under-dosing regimens would enhance development of drug resistance.

In many malaria endemic areas presumptive treatment for malaria is a common practice and has the potential for facilitating resistance by greatly increasing the number of people who are treated unnecessarily. In some areas and at some times of the year, the number of patients being treated unnecessarily for malaria can be very large especially in sub-Saharan Africa and Southeast Asia where public health infrastructures are not well established (Hastings 2004).

Poor compliance to dosing regimen

Patients often stop therapy as soon as their acute symptoms have resolved. This habit of poor compliance may arise because of the occurrence of adverse side effects, the cost of medication or because therapies are prolonged and complicated (Sibley and Price 2012). Failure to take the full course of the drug means that while some susceptible parasites are killed, resilient ones live on, leading to resistance, to the drugs to which they were initially exposed (WHO 2010). Most of the times, this occurs because of the luck of awareness and negligence of patients, regarding the use of the drug and the consequences of misusing it.

Spontaneous de novo mutation is the base of all malaria drug resistances. However, the establishment and spread of drug resistance is governed by different interacting factors. The frequency of mutation, degree of resitance conferred, fitness cost of the mutation, drug pressure, transmission intensity/ or level of infection, type of antimalarial drug used etc determines the speed of emergence and spread of malaria drug resistance (Figure 2).

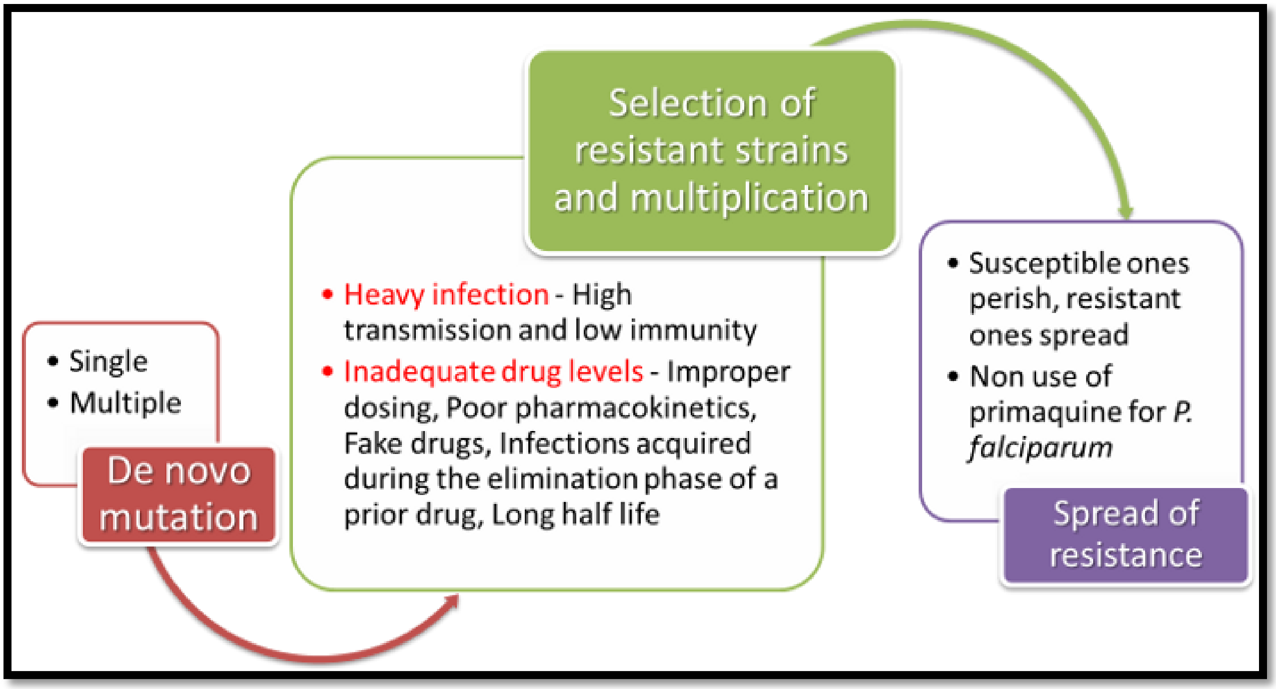

Figure 2: Factors for the emergence and spread of malaria drug resistance (Source: *http://www.malariasite.com/drug-resistance/*

2.4. Malaria drug resistance prevention strategies

In the absence of preventive vaccines and other preventive and curative tools which results in elimination of malaria parasites, the use antimalaria drug to control malaria would continue long in to the future. The intense dependence on antimalaria drug to prevent and treat infections is one and most important risk factor for the emergence and spread of drug resistant malaria parasite populations (Talisuna *et al*. 2012). It is well established fact that malaria parasites have been developed resistance to widely used drugs. Development of resistance by *P.falciparum* to many antimalaria drugs has been resulted in dramatic increase in morbidity and mortality (WHO 2015). Currently, small numbers of effective antimalaria drugs are in the market and it has been observed that the development of new drugs appears to be taking longer than development of parasitological resistance as witnessed in the Southeast Asian countries (WHO 2009).

Detail knowledge on the risk factors that increase the likelihood of emergence and spread of malaria drug resistance is crucial to tackle the problem. It is well known that reduction of overall malaria transmission have an indirect impact on development of drug resistance by reducing the number of infections needing to be treated and therefore, overall drug pressure and by reducing the likelihood that resistant parasites are successfully transmitted to new hosts. This can be achieved by routine use of protective measures like use of ITNs, IRS, environmental control (mosquito breeding site or “source” reduction), other personal protection measures (e.g. use of repellent soap or screening windows) and chemoprophylaxis in malaria endemic areas (Talisuna *et al*. 2012).

Another measure which mainly focuses on reducing overall drug pressure through more selective use of drugs; improving the way drugs are used through improved prescribing, follow-up practices, and patient compliance; or using drugs or drug combinations which are inherently less likely to foster resistance or have properties that do not facilitate development or spread of resistant parasites (Cui and Su 2009).

Following more restrictive use of antimalarial drugs and prescription practices would be helpful for limiting the emergence, spread, and intensification of drug resistance (WHO 2010). The greatest decrease in antimalarial drug use could be achieved through improving the diagnosis of malaria. Basing treatment on the results of a diagnostic test, such as microscopy or a rapid antigen test would result in the greatest reduction of unnecessary malaria treatments and decrease the probability that parasites are exposed to sub-therapeutic blood levels of drug (WHO 2014).

Generally, enhancing rational use of antimalarial drugs; improve access to effective antimalaria drugs for those who need to be treated while at the same time reducing the inappropriate use of the drugs (avoiding presumptive treatment) are crucial intervention measures to prevent malaria drug resistance.

2.5. Methods of detecting antimalaria drug resistance

2.5.1. In vitro susceptibility tests

In vitro parasite sensitivity test is exposure of parasites in culture medium to antimalarial drugs with a range of varying strengths (WHO 2009). Parasite growth and development to schizont stage in the presence of antimalarial drugs in the culture medium is the bases for determining the inherent resistance capacity of the parasite. Growth and development to a schizont stage of the parasite indicates that the amount of test drug in the respective test well is tolerated by the parasite.

2.5.2. Therapeutic efficacy study

According to the WHO (2009) revised guideline, therapeutic efficacy is defined as the clinical and parasitological efficacy of antimalarial drugs on plasmodium infections through defined follow-up criteria. It is measured using parasite density count in blood films and assessment of malaria clinical sign and symptoms assessed through physical and clinical assessment. The decision criteria for therapeutic efficacy are based on complete clearance of parasitemia and clinical sign and symptoms of the disease.

Outcomes of therapeutic efficacy studies can be expressed as treatment failure or cure rate. The WHO (2009) defines antimalarial treatment failure as "inability to clear malarial parasitaemia or resolve clinical symptoms despite administration of an antimalarial medicine." The presence/absence of parasitemia and clinical sign and symptoms of malaria following completion of a full course of treatment is the bases for determination of efficacy study treatment outcomes.

Treatment failure does not confirm emergence of drug resistance as it could occur due factors related to the incorrect dosage, lack of patient compliance to the prescribed quantity and duration of treatment, poor drug quality, low drug absorption and interaction with another drug concomitantly administered and rapid elimination due to poor metabolism of the drug (Neodl *et al*. 2003, Laufer 2009). To ensure that enough drug concentration is absorbed and available in the blood circulation, the WHO recommends the integration of drug pharmacokinetic studies in to efficacy study protocols. Drug concentration is an estimate of the concentration of the active chemical component and or the metabolite of an antimalarial drug in whole blood, plasma or serum (Neodl 2003). This method intends to determine the amount of antimalarial drug in the blood as it would affect the treatment outcome. The presence of parasites in the blood sample with drug concentration above minimum effective concentration for the specific antimalaria drug is an indicator for parasite resistance.

2.5.3. Surveillance of resistance molecular markers

Polymorphic genes that are responsible for modifying the response of the parasite to a given antimalarial drug can be used as markers or indicator for resistance (Sibley and Price 2012). To locate the presence mutations encoding biological resistance to antimalarial drugs, genetic markers are analyzed through polymerase chain reaction and enzyme digestion of the parasite dioxyribose nucleic acid regions containing the genes of interest (Neodl *et al*. 2003). These methods are in the process of being developed and validated, but offer promising advantages to the other methods. Theoretically, the frequency of occurrence of specific gene mutations within a sample of parasites obtained from patients from a given area could provide an indication of the frequency of drug resistance in that area analogous to information derived from in vitro methods (Laufer 2009).

Advantages of this method include the need for only small amounts of genetic material as opposed to live parasites, independence from host and environmental factors, and the ability to conduct large numbers of tests in a relatively short period of time (Cui and Su 2009). The main draw back of this method the requirement for sophisticated equipment and training, and the fact that gene mutations that confer antimalarial drug resistance are currently known or debated for only a limited number of drugs primarily for dihydrofolate reductase inhibitors (pyrimethamine), dihydropteroate synthase inhibitors (sulfadoxine and chloroquine) (Neodl *et al*. 2003). Confirmation of the association between given mutations and actual drug resistance is difficult, especially when resistance involves more than one gene locus and multiple mutations (Muller and Hyde 2010). If these complexities can be resolved, molecular techniques may become an extremely valuable surveillance tool for monitoring the occurrence, spread, or intensification of drug resistance.

2.6. Mitigating artemisinin resistance

Following the reports of resistance to ACTs from the Thai-Cambodian border, the use of artemisinin containing drugs is strictly controlled under WHO guidelines. The WHO has also launched a program known as “Global plan for containment of artemisinin resistance” aimed to eliminate artemisinin resistant parasite populations and prevent the spread of these parasites to other regions (WHO 2014). One of the pillars of the WHO global plan for artemisinin resistance containment is to increase monitoring and surveillance (WHO 2015). Mapping the geographic extent of resistance is essential for planning containment and elimination strategies.

To mitigate the threat of artemisinin resistance in Africa, improvement of drug-resistance surveillance and response systems is urgently required improving peripheral health system in malaria endemic areas; ensuring antimalarial drug qualities and proper use of them are equally important to reduce the likelihood of emergence and spread of malaria drug resistance in Africa (Talisuna *et al*. 2012).

2.7. Malaria treatment and drug resistance in Ethiopia

In Ethiopia about 75% of the country's total landmass is malarious and over 60% of the population is at-risk of malaria (Adugna 2014). Due to the unstable seasonal transmission pattern, the country has witnessed ruccurent epidemics. In 1953 severe malaria outbreak in Dembia district claimed the lives of 7000 people; other wide spread malaria epidemic occurred in 1998 in many areas and around 1 million cases were reported from all over the country (FMoH 2004). The recent epidemic that occured in 2003/2004 was severe with elevated number of cases and deaths (15 million cases and 114,000 deaths) (Adugna 2014). According to the FMoH estimation, about 5-6 million clinical and above 600, 000 confirmed malaria cases occur each non epidemic year (FMoH 2012).

Malaria is not only public health problem but also a major obstacle to the country's socio-economic development since its peak transmission coincides with the major farming activities of the people. The peak malaria transmission season in Ethiopia is generally from September to December following the major rainy season from June to September and a shorter transmission season also occurs from February to March following the short rainy season from April to May (Adugna 2014).

Although there are seasonal and focal variations in the relative frequency of *P. falciparum* and *P. vivax* in Ethiopia, these two species accounts for about 60% and 40% respectively (Adugna 2014). While *An. arabiensis* is the major malaria transmitter in the country *An. pharonesis*, *An. funestus* and *An. nili* are considered as secondary vectors (FMoH 2004). Following the dramatic scale-up of malaria preventive interventions and improvement of knowledge, attitude and practice of the public substantial decline in malaria-related morbidity and mortality is achieved nationwide although major shortcomings and gaps still remain (Adugna 2014). The FMoH has planned to shrink the malaria map and achieve elimination in some selected low transmission localities by 2020 (FMoH 2014).

In Ethiopia, CQ has been widely used for many years before resistance against it has been developed. It has been in use for the treatment of all forms of malaria for more than three decades (FMoH 1998). The first CQ-resistance by *P. falciparum* was detected among Ethiopians residing along the borders with Kenya, Somalia and Sudan (Teklehaimanot 1986). In that study, treatment failure of 22% was reported. Another study also reported CQ resistant *P. falciparum* case in a staff member of the Ethiopian National Research Institute of Health who visited the town of Gambella, Western Ethiopia bordering the Sudan in 1997 (FMoH 1998). Later widespread resistance (up to 65% therapeutic failure) was reported from almost all over the country (Alene and Beneett 1996; Tulu *et al*. 1996; Tedros and Desta 1998; Medhin 2012). Based on those reports, a nation wide study on the efficacy of CQ for the treatment of *P. falciparum* was conducted in 18 sites by the FMoH from 1997-1998 (FMoH 1998) and confirmed the prevous reports (FMoH 1998). The finding led to the decision of introducing SP in 1998 to replace the failing CQ as the first-line antimalarial drug for the treatment of uncomplicated *P. falciparum* malaria (FMoH 1998).

Following this the Ethiopian national malaria drug policy was shifted from CQ to SP as a first-line for the treatment of uncomplicated *P. falciparum* malaria in 1998 although the SP itself had a treatment failure of 7.7% at the time of its introduction (FMoH 1998). Shortly after its introduction, the failure of SP for the treatment of uncomplicated *P. falciparum* had reached 35.6% (Kassa *et al*. 2005) leading to its replacement by Art-L in 2004 (FMoH 2004; Jima *et al.* 2005). Quinine and CQ maintained as secondline drug for *P. falciparum* and the treatment of *P. vivax* malaria respectively; mefloquine and artemether injection were approved for prophylaxis and pre-referral treatment of severe malaria respectively (FMoH 2004).

The existing malaria treatment guideline (FMoH 2012) states that ACTs should be available at all public health facilities to treat all *P. falciparum* malaria cases, whereas CQ continues to be first-line treatment for *P. vivax* infections. Quinine tablet remains the treatment of choice for uncomplicated *P. falciparum* malaria for pregnant mothers during the first trimester of pregnancy, children under 5 kilograms body weight, and as second-line for treatment failures (FMoH 2012). Rectal artesunate is recommended to be available at rural health posts and intravenous artesunate or intramuscular injection and intramuscular artemether (alternative) at health centers and hospitals for the treatment of severe malaria. The introduction of DHA-piperaquine as second-line treatment for non-complicated *P. falciparum* and possibly to target

mobile populations is being considered. The policy promotes primaquine for radical cure of *P. vivax* and single dose treatment for gametocytocidal activity against *P. falciparum*, where appropriate. While mixed infections of *P. falciparum* and *P. vivax* are treated by AL, MQ and atovaquone-proguanil are the recommended chemo-prophylactic antimalaria drugs in Ethiopia.
On the other hand, limited number of studies (Tulu *et al*. 1996; Yeshiwondim *et al*. 2010) reported the occurrence of CQ-resistant *P. vivax* malaria cases. Apart from these, CQ efficacy against *P. vivax* malaria has not been widely explored in Ethiopia and the FMoH maintained CQ as first-line drug for the treatment *P. vivax* malaria (FMoH 2012).

The WHO recommends the introduction of ACTs in all endemic areas to treat uncomplicated falciparum malaria. In Ethiopia, Art-L was introduced as a first-line drug against uncomplicated falciparum malaria in 2004 followed by the high treatment failure of SP (FMoH 2004). However, the recent resistance (Ashley *et al*. 2014) to artemisinin reported from the global drug resistance epicenter (Thai-Cambodian border) and the spread to the Great Mekong region poses yet another threat in malaria control efforts.

Wide-scale molecular epidemiology investigation conducted on African parasite populations found no mutations in the parasite's K13-propeller gene which is associated with artemisinin resistance in Southeast Asia (Taylor *et al*. 2014). A recent study that analyzed *P. falciparum* isolates from 12 sub-Saharan African countries including Ethiopia also could not detect the K13-propeller mutations previously reported in Southeast Asia although the study has reported 22 unique mutations (Heuchert *et al*. 2015; Kamau *et al*. 2015).

2.8. Statement of the problem

Development of drug resistance to almost available antimalarial drugs is the main challenge in the control and prevention of falciparum malaria in all endemic areas. The recent report of artemisinin resistance in the Great Mekong sub-Region is posing a great threat in the global fight against malaria. To tackle this problem, the WHO strongly recommends the continuous and regular monitoring of first and second line antimalarial drugs in all endemic areas as it would help early detection and prevention of spread of resistant parasite populations. Failure to early detect the emergence and spread of malaria drug resistance could lead to a drug-resistant malaria epidemic, which could have 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 that time (WHO 2014).

In line with the WHO recommendations, the FMoH has established sentinel sites where efficacy studies should be conducted every tow years. Accordingly, many studies have been conducted in different sentinel sites (Jima *et* al. 2005; Assefa *et al*. 2010; Lemma 2012; Hamde 2011; Ebstie *et al*. 2015; Getnet *et al*. 2015; Mekonnen *et al*. 2015). However in Setit Humera town, which is one of the malaria endemic areas in Northwest Ethiopia fulfilling the criteria for sentinel site, such a study is lacking. This study was, therefore, conducted to assess the therapeutic efficacy of Art-L against uncomplicated *P. falciparum* malaria infection in malaria patients visiting Setit Humera Health Center.

3. OBJECTIVES

3.1. General Objective

The general objective of this study was to assess the therapeutic efficacy of AL (Coartem®) for the treatment of uncomplicated *P. falciparum* malaria in patients visiting Setit Humera Health Center.

3.2. Specific Objectives

The specific objectives of the study were to:

3.2.1. Determine the proportions of early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) and adequate clinical and parasitological response (ACPR)

3.2.2. Measure the clinical and parasitological efficacy of AL (Coartem®) in patients who fulfilled the inclusion criteria

3.2.3. Evaluate the incidence of adverse events (AE) following AL treatment

3.2.4. Compare baseline (D0), D14 and D28 blood hemoglobin (Hb) level

4. MATERIALS AND METHODS

4.1. The study site

Setit Humera is a town found in Western Zone of Tigray region, Northwest Ethiopia at a distance of 983 kilometers from Addis Ababa. It is located at latitude of 14°17′N and longitude 036°39′E with an altitude of 637 meters above sea level (Figure 3).

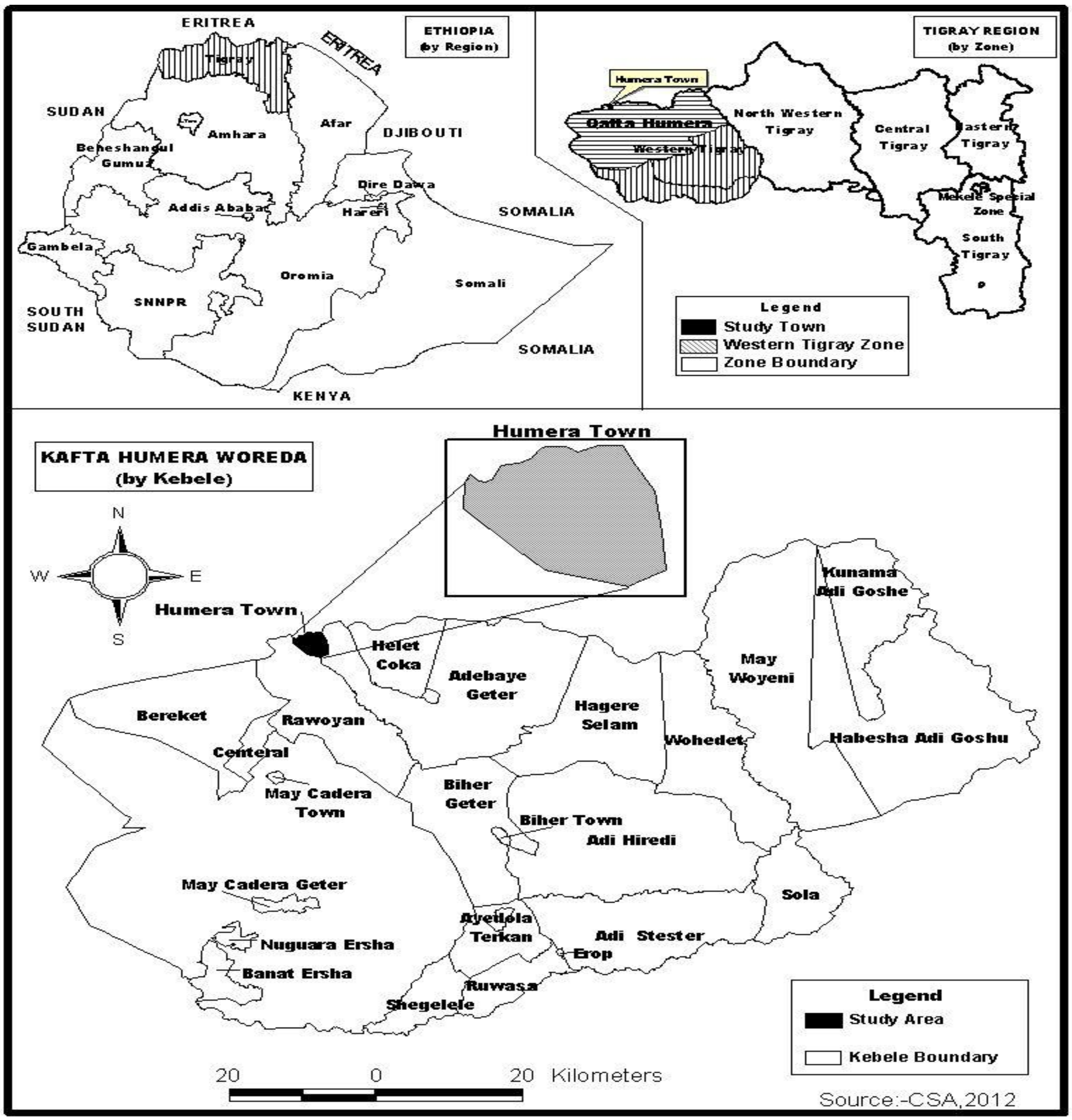

Figure 3: Map of the study site

According to The District Bureau of Agriculture and Rural Development, the total area is 153.03 square kilometers, and temperature and rainfall shows great variation throughout the year with average annual temperature and rainfall, 27.6 ^{0}C and 611mm respectively. Rain mostly starts in June and ends in September and reaches highest in August. May is the hottest month of the year with an average temperature of 33.0 ^{0}C and January is the coldest with 24.2 ^{0}C. Sesame and barley are the main agricultural crops in the area. According the 2014 national population projection, the population size of Humera town is about 30918 (16887 male, 14031 female) which shows an increase of 8205 over the 2007 national census (Central statistics agency 2014). Majority of the residents belongs to the Tigray ethnic group of which many are repatriates from Sudan refugee camps and others came from different localities of the region during resettlement programmes.

Humera is a low land area found in Tekezze river basin with suitable micro and macro habitats suitable for vector and parasite reproduction particularly during the rainy season and the subsequent three months (Wozam 1994; Mengesha *et al*. 2000; Gidena 2015). Malaria transmission is high from September to December especially during times when rainfall amount is high and the duration becomes prolonged (Local health office). The nature of malaria transmission is seasonal and unstable with high risk of outbreaks and mesoendemic level (Mengesha *et al*. 2000; Gidena 2015).

The huge influx of non immune labor workers to and from the malaria endemic area every year makes malaria epidemiology more complex. Unpublished reports from the zonal administration office show that close to half a million people are mobilized during the major labor demanding seasons every year. These migrant workers mostly originating from the highland areas are immunologically naïve to malaria which increases the likelihood of introducing malaria to previously malaria free areas posing great challenge in the national fight against malaria.

4.2. Study design

The study was conducted between October 28, 2014 and January 9, 2015 in Setit Humera Health Center. The efficacy study was a one-arm prospective evaluation of clinical and parasitological responses to partially supervised treatment of Art-L for uncomplicated falciparum malaria. Patients with uncomplicated malaria who meet the study inclusion criteria were enrolled, treated with Art-L (partially supervised), and monitoring and follow-up were carried out for 28 days.

The follow-up was based on fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the patients were classified as having therapeutic failure (early or late) or an adequate parasitological and clinical response. The proportions of patients experiencing therapeutic failure during the follow-up period were used to estimate the efficacy of the study drug.

4.3. Sample size determination

The sample size was determined according to the WHO (2009) protocol; sample size was determined using the single population proportion formula and calculated assuming 5% margin of erroor, 95% confidence interval (CI), treatment failure of 5% and 20% dropout rate. Accordingly, minimum sample size of 88 was calculated and 92 patients were enrolled in this study which is above the calculated minimum.

$n > (z/d)^2 P (1-P)$

$> (1.96/0.05)^2 0.05 (1-0.05) = 73$; $n= (1+0.2) 73= 88$

Where, n= sample size

P = the expected treatmet failure (5%)

z = confidence interval (95%)

d = margin of error (5%)

The study participants were selected from malaria suspected symptomatic patients who visited the health center. All adult and children (above 6 months) who fulfilled the inclusion criteria and signed an informed consent were eligible for the study.

4.4. Inclusion criteria

The study was conducted according to the WHO revised protocol (WHO 2009) for malaria drug therapeutic efficacy study. On admission attending patients with signs/symptoms of malaria were screened for the following selection criteria:

- Both sexes ≥6 months of age
- Body weight >5 kg
- Fever (axillary temperature ≥ 37.5 ^{0}C) or history of fever with in the previous 24 hours
- Microscopically confirmed *P. falciparum* mono-infection, with asexual parasitemia between 1000 and 200,000 asexual parasites/µl of blood

- Non-pregnant or breast-feeding women
- Patients living within the health center catchment area (i.e. 5-10 km radius of the health center)
- Informed consent by patient (Annex 8) or by caregivers for children under 12 years old (Annex 9) and agreed to return for all scheduled visits
- Ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule.

4.5. Exclusion criteria

- Mixed or mono-infection with another plasmodium species (besides *P. falciparum)*
- Hb ≤ 5.0 g/dl
- Intake of AL with in the 2 weeks prior to enrollment
- Unable to take oral medication or continuous vomiting
- Known hypersensitivity to Art-L
- Evidence of severe malaria or other danger signs according to WHO definition for severe malaria (not able to drink or breast-feed, vomiting (i.e. more than twice in past 24 hours), recent history of convulsions (i.e. more than once in past 24 hours), unconscious state, unable to sit or stand);
- Presence of severe malnutrition (defined as a child who had a mid-upper arm circumference < 110 mm)
- Presence of febrile conditions due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhoea with dehydration) or other known underlying chronic or severe diseases (e.g. cardiac, renal and hepatic diseases, HIV/AIDS); and
- Regular medication which may interfere with Art-L pharmacokinetics.

4.6. Laboratory procedures

4.6.1. Microscopic blood examination

Thick and thin blood smears were taken from all study participants and prepared on the same slide for detection, identification and quantification of parasites at all scheduled and unscheduled visits during the 28-day followup period. New frosted edge glass slides were used to prepare blood smears through out the study period. Sterile automatic lancets were used to prick the fourth finger for blood collection. Two smears were prepared in each case; the first was stained rapidly with 10% Giemsa for 10-15 minutes for initial screening and examined by light microscopy immediately (WHO 2009). The second one was stained according to the standard protocol (stained slowly with 3% Giemsa for 45-60 minutes) and examined later to provide definitive parasite count and plasmodium species identification.

On D0, in order to rapidly confirm adherence to the lowest parasite density considered for enrollment, initial screening of patients were made in 10% Giemsa-stained thick film after counting at least 1 in every 6-8 WBCs, which corresponds to approximately 1000 parasites/μl (WHO 2009).

Slides were examined by one experienced microscopist; parasite densities and gametocyte carriage were calculated according to the WHO revised protocol (WHO 2009). Accordingly, asexual parasite density was estimated from thick blood smears by counting the number of asexual parasites against 200 WBC or against 500 WBC (if the count was <10 parasites/200 WBC) assuming an average WBC count of 8,000/μl blood. A thick blood smear was declared negative if no parasite was detected in 100 high power fields. Gametocytes were detected and counted in thick film against 500 WBCs. Thin blood smears were examined to determine parasite species. All slides stained and fixed following the standard protocol were wrapped with soft tissue paper, collected and transported to EPHI for quality control. Quality control was conducted on 82 slides by experienced malaria microscopists. Slide and staining quality was recognized as “excellent” and “good” respectively.

4.6.2. Measurement of blood hemoglobin level

Hb level was measured from finger-prick blood sample. Finger-prick blood samples collected on D0, D14 and D28 using hemocue microcuvvets were measured with a portable spectrophotometer (HemoCue, Ängelhom, Sweden) to observe improvements in blood Hb level after treatment. Anemia was defined according to the WHO classification (Hb <12.0 g/dl for women and children, <13.0 g/dl for adult men; Hb=10.0-11.9 and 11.0-12.9 g/dl, mildely anemic for children and/or women and adult men respectively; Hb<10 and <11, moderately anemic for children women, and adult men respectively; Hb≤5.0 g/dl was considered as severe anaemia and an exclusion criteria) (WHO 2009).

4.7. Baseline evaluation

Potential participants were assessed further for adherence to the rest of inclusion criteria. Base-line physical, clinical examinations with particular attention to any danger signs or symptoms associated with severe malaria were thoroughly assessed by clinical study team. Febrile patients were treated with appropriate dose of antipyretics/paracetamol. History and demographic data were taken, axilary temperature and body weight were measured. Patients who met the selection criteria at this stage were assigned Patient Identification Number and referred to the laboratory again for:

- Determination of Hb to exclude severe anemia (Hb ≤ 5.0 g/dl);
- Collection of finger-prick blood sample for repeated thick and thin blood smears, before the patient was enrolled and treated with Art-L.
- Collection of blood spots (DBS) on Whattman filter paper for late molecular genotyping in case treatment failure >5% occurred.

Patients with Hb level ≤5.0 g/dl and/or positive for mixed infection were excluded from the study and were referred immediately to the outpatient department for appropriate care. Patients satisfying all the inclusion criteria were enrolled to the study and started partially supervised treatment with Art-L.

4.8. Treatment and dosing procedures

4.8.1. Study medication and dosing procedures

The same batch (Batch No.1070103) of AL (Coartem®) drugs (i.e. 20 mg Artemether/120mg Lumefantrine tablet), produced by Novartis and obtained from a quality assured source (PHARMID, FMoH) were used throughout the study.

Drug dosage was detrmined according to the revised WHO protocol (Weight-based guideline) (Annex 5). Accordingly, enrolled patients were treated with the standard six-dose regimen of Art-L given twice daily for three consecutive days. The first dose of the medication and all morning doses were administered in the health center under direct supervision of the study team.

The evening doses of D0, D1 and D2 medication were taken respectively 8 and 12 hours after the administration of the morning dose. Every patient or parent were well instructed how and when to take the drug as well as advised to consume fatty meals before or after drug intake to ensure proper absorption of the drug.

4.8.2. Supervised treatment administration

Dosing was administered by the study clinical team according to weight-based guidelines (Annex 5). Study medications given to young children was crushed, mixed with water, and administered as suspension. Study medications administered to older children and adults were given as tablets to be taken orally with a glass of water. Patients were given some peanuts or cream biscuits before/after each dose. At each supervised drug administration the patient was observed for 30 minutes. If vomiting occurred before 30 minutes, the dose was repeated and observed for an additional 30 minutes. Patient vomitted more than once were withdrawn from the study and was referred immediately to the OPD for rescue treatment with intramuscular or intravenous quinine.

4.8.3. Treatment administration at home

The evening doses of the drug with replacement were given to the patient or caregiver responsible to administer the medications at home with proper and clear verbal instruction when and how to take the medication. They were advised to administer the drugs with some fatty meals. The patients or caregiver were instructed to come back to health center if the patient vomited the study medication administered at home. One on duty nurse was assigned to take care of study patients at the health center at night/off-duty hours.

4.8.4. Other medications

Patients with admission fever $\geq 37.5^{0}$C were treated with a standard dose of 10 mg per kilogram paracetamol tablets every 6 hours until the symptoms subsided (WHO 2009). Patients who encountered illnesses other than malaria during standard 28-day followup were received standard care and treatment in the clinic free of charge. Routine use of non-study medications with antimalarial activity, including antifolate, co-trimoxazole, tetracycline and doxycycline, were avoided. Patient with TF was treated according to the national antimalarial treatment guidelines with oral quinine given 10mg/kg three times a day for seven days (FMoH 2012). Patients detected with *P. vivax* infection were treated with standard dose of CQ.

4.9. Follow-up procedures and evaluations

On D0 (enrollment day), each patient who was successfully treated with the first dose, had given an appointment card with patient name, PIN and next scheduled visit date written on the front of it and the evening dose to be taken at home. Patients were then asked and advised to come back for treatment the following two days and for a 28 followup days according to scheduled visits on D3, D7, D14, D21, and D28; and on any unscheduled day that the patient felt sick. The over all study conduct flow chart is presented in figure 4.

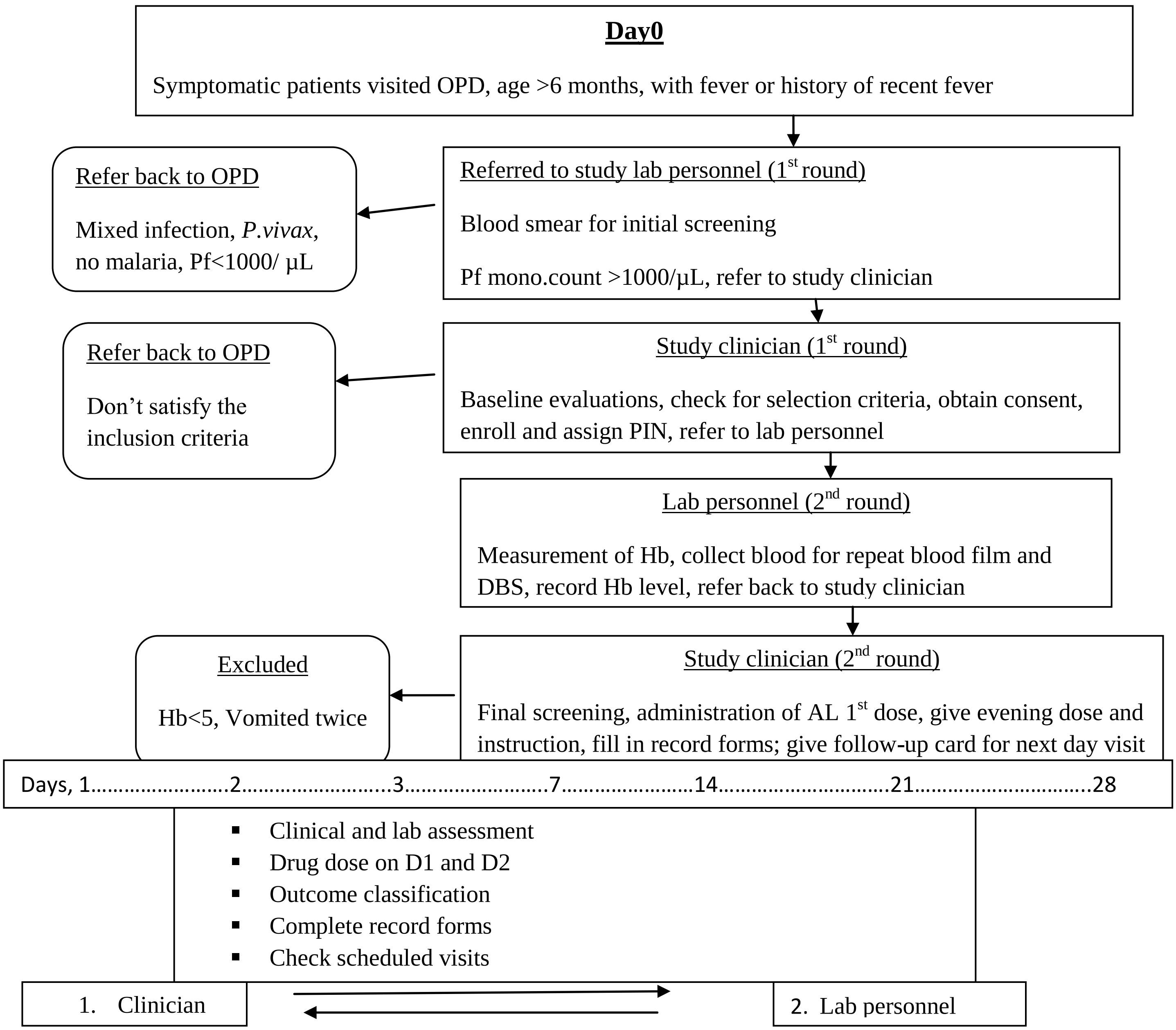

Figure 4: Study conduct flow chart with responsible personnel

4.9.1. Withdrawals after enrollment

Patients were excluded from the study after enrollment if:

1. D0 repeat parasite count <1000/µl
2. Missed the evening dose on the first three followup days
3. Vomited any study dose twice and missed any treatment dose
4. Evidence of severe and complicated malaria
5. Experience of serious AE that forced discontinuation of treatment
6. Intake of other drugs with antimalarial properties (Annex 12)
7. Detection of other malaria species during the followup period
8. Development of a febrile illness (pneumonia, dysentery, measles) that interferes with outcome classification.
9. Withdrawal of consent.

4.9.2. Loss follow-up (LFU), patient discontinuation/protocol violation

Study participants were classified as LFU when they did not attend the scheduled visits and can not be found despite maximum effort. Data of LFU patients were censored during data analysis.

Study patients who met the following criteria were withdrawn from the study at any time:

a) Withdrawal of consent- a patient may withdrew consent at any time, without prejudice for further followup or treatment at the health center.

b) Failure to complete treatment due to:
   - Persistent vomiting of the treatment- a patient who vomitted the study medication twice or more was withdrawn from the study and given rescue treatment.

c) Failure to attend the scheduled visits.

d) Serious AE necessitating termination of treatment before the full course was completed.

e) Enrolment violation:

- severe malaria on D0;
- erroneous inclusion of a patient who did not meet the inclusion criteria;

f) Voluntary protocol violation:

- self or third-party administration of antimalarial drug or antibiotics with antimalarial activity (Annex 12);

g) Involuntary protocol violation:

- occurrence during followup of concomitant disease that would interfere with a clear classification of the treatment outcome;
- detection of a mono-infection with another malaria species during followup;
- Miss-classification of a patient due to a laboratory error (parasitaemia).

Data of withdrawn patients were censored during analysis and reasons for discontinuation or protocol violation were recorded on the case record form.

4.9.3. Assessment of adverse events

An adverse event is defined as signs, symptoms or abnormal laboratory finding not presented at enrolment, but occurring during followup, or presented at D0 and increased in intensity during followup despite clearance of parasitaemia; serious AE was defined, as any adverse experience that resulted in death, life threatening experience, participant hospitalization, persistent or significant disability or incapacity, or specific medical or surgical intervention to prevent serious outcome (WHO 2009).

Evaluations of AE were determined through clinical and physical examination, and questioning with standard list of malaria associated and AL related adverse events. The caregiver was asked to inform any unusual phenomenon occurred after the administration of the drug (side effect of the drug, the child’s tolerability to the treatment). All demographic and clinical information were recorded on a standard record form at all time points.

4.10. Study endpoints

Study endpoints were defined according to the revised WHO (2009) guide line and it include treatment failure (early treatment failure, late clinical failure, and late parasitological failure), completion of followup without treatment failure (adequate clinical and parasitological response), lost to followup, withdrawal from the study and protocol violation.

Results were classified in to primary and secondary; the primary outcome was the D28 overall efficacy of Art-L expressed as PCR uncorrected cumulative success rate (or the cumulative failure rate) and the proportion of adequate clinical and parasitological response (or proportion of early treatment failure, late clinical failure or late parasitological failure). The primary outcome was analysed using the Kaplan-Meier survival estimator and Per-protocol analysis methods.

The secondary outcomes were:

Parasite clearance rate: proportion of patients with negative blood smears on D1, D2 and D3.

Fever clearance rate: proportion of patients with out fever (body temperature $<37.5^{0}C$) on D1, D2 and D3.

Gametocyte carriage rate: proportion of patients with gametocytes during the 28 day followup period.

Hematological recovery: change in mean blood Hb concentration from baseline to D14 and D28.

4.11. Classification of treatment outcomes

Classification of response to treatment was based on the revised WHO guideline classification system (Annex 4):

4.11.1. Early treatment failure (ETF)

Treatment outcome was classified as ETF if the following conditions were met (WHO, 2009):

- Occurrence of danger signs of severe malaria on D1, D2 or D3 in the presence of parasitaemia;
- Parasitaemia on D2 higher than on D0, irrespective of axillary temperature;
- Parasitaemia on D3 with axillary temperature ≥ 37.5 ºC;
- Parasitaemia on D3 $\geq 25\%$ of the count on D0.
- Severe AE requiring change in treatment on D0 and D2.

4.11.2. Late treatment failure (LTF)

LTF has two categories:

Late clinical failure (LCF)

The treatment outcome was classified as LCF if the following conditions were met:

- Danger signs of severe malaria in the presence of parasitaemia on any day between D4 and D28 in patients who did not previously meet any of the criteria of ETF;

- Presence of parasitaemia on any day between D4 and D28 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)

Treatment outcome was classified as LPF if parasitaemia occured on any day between D4 and D28 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.

4.11.3. Adequate parasitological and clinical response (APCR)

This was the target treatment outcome which shows the overall efficacy of the study drug and it was defined as absence of parasitaemia on D28, 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.

4.12. Data management and monitoring

Enrollment and case record forms were completed for each study subject. Their initials and study code on the case record form were used to identify the participants. The principal investigator was responsible to complete case record forms at each visit. All corrections were made on the case record form by striking through the incorrect entry with a single line and entering the correct information adjacent to it.

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

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

4.13. Statistical analysis

Every data from recruited patients were imported into the WHO Excel sheet (double entry) which is designed for analysis of therapeutic efficacy study data. Data were also entered in to IBM SPSS (version-20) software to calculate descriptive statistics (mean, median, standard deviations, range). Independent sample t-test was used to compare baseline temperature and parasitemia between children and adults; mean blood Hb level at D0, D14 and D28 between patients with parasitemia ≥ 10,000 and < 10,000/µl blood. Non-parametric (median) test for independent sample was used to compare D1 parasitemia between children and adults and paired sample t-test was used to compare mean Hb level between D0 and D14, D0 and D28, D14 and D28. All comparisons were performed at 95% CI and significance level of 0.05.

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

5. ETHICAL CONSIDERATIONS

All participants were consented to be part of the study. Written informed consents were obtained from all study participants (parents or caregivers for children under the age of twelve) after thorough information on the study were provided in the local language. Name of study participants were not mentioned and incorporated in to the final database. Medical services were provided for volunteer study participants free of charge during the followup period. At all times, patient well-being was given priority over his/her continuation in the study. In addition, round trip transport fare (30.00 Ethiopia Birr) was provided for every patient every scheduled visit.

The study protocol was approved by College of Natural Sciences Institutional Ethics Review Board, Addis Ababa University.

6. RESULTS

6.1. The study population

A total of 1878 malaria suspected symptomatic patients visiting SHHC were screened for malaria during the study period. Of these, 1258 (67%) were malaria slide positive; 986 (78%) *P. falciparum*, 253 (20%) *P.vivax* and 19 (2%) mixed (*P. falciparum/P. vivax*) infections. Most of those patients were mobile agricultural labor workers from different parts of the country and hence were not potential candidates for inclusion in the study. Only 120 patients were potential candidates to be included in the study residing within the targeted distance from the health center (5-10 Km). However, 18 patients refused to give their consent, 4 were with parasitaemia below 1000/µL, 4 lactating women, 2 pregnant, and hence were excluded before enrollment.

Thus, only 92 patients finally satisfied the inclusion criteria and enrolled. At baseline, 57 (61.9%) patients were males yielding 1.63 male to female ratio (Table 1). Three (3.3%) were under-five (U5) children, 33(35.9%) were 5-14 years old, and the remaining 56 (61.8%) were over 14. Mean body temperature was 38.5±1.25°C. While 61 patients (66.3%) were febrile; the rest had self-reported history of fever within the previous 24 hours. The mean body temperatures of children and adults were 38.3±1.20 and 38.8±1.27 respectively with no significant difference (p=0.211).The mean body weight was 39.6±14.13.

Baseline mean Hb level was 13.2±1.83 and 26 patients (28.3%), 17 children and 9 adults, were anemic (6 moderately anemic 21 mild and 6 moderate cases).Overall baseline mean and median parasitaemia were 27798 and 12970 respectively. There was no significant variation (p=0.615) between mean parasitaemia for children (30259±41518) and those over 14 years of age (26214±34642). There were 6 patients with parasitaemia>100,000/µl. Overall, 16 of the 26 anemic patients were males. Gametocyte carriage rate was 7.6% with gametocyte number ranging from 48-2224/µl of blood. 82.6% and 95% of the study participants/parents replied "yes" to "previous malaria history" and "availability of atleast one bed net at home" respectively.

Among the 92 participants 12 exclusions were recorded on different follow-up days for various reasons (Figure 5). Two were excluded on D1 (1 repeated vomiting of the evening dose, 1 withdrawal of consent); 1 on D3, 2 D7, 4 on D14 and 1 on D28 all due to LFU; 1 on D23 and 1 on D25 both had *P. vivax* infection; and 1 on D21 because of intake of non-study antimalarial drug.

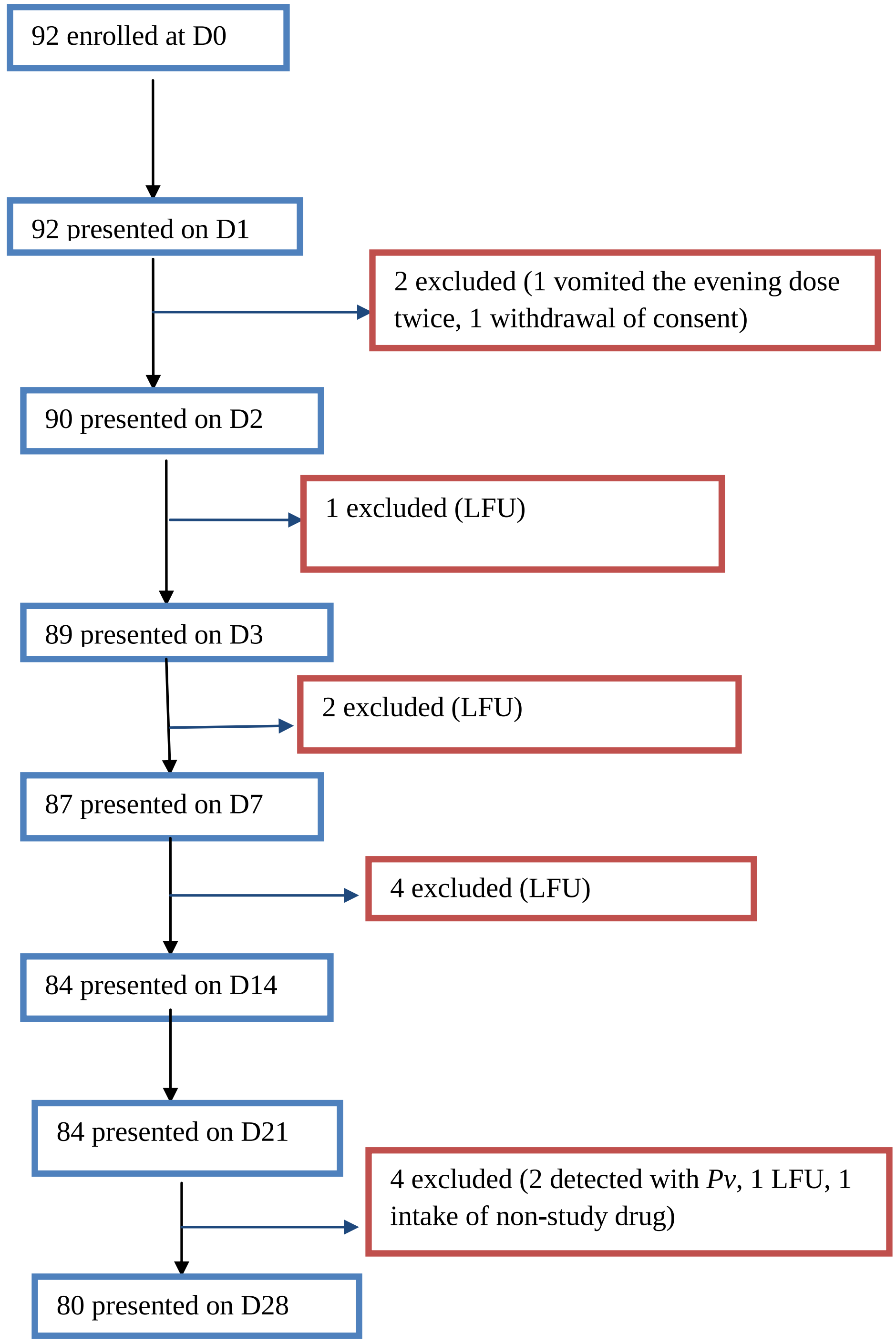

Figure 5: Diagram presenting total enrolled and excluded study participants

Table 1: Base line characteristics of the study population

Age category		U5	5-14	≥15	Total
No. of participants		N=3	N=33	N= 56	N=92
Mean age (range) in Yrs		3 (2-4)	9.73 (5-14)	18.96 (15-28)	15.13 (15-28)
Sex (n) (%)	Male	3 (3.3)	22 (23.9)	32(34.8)	57 (61.9)
	Female	0 (0)	11 (12%)	24 (26.1)	35 (38.1)
Mean body temperature (°C) (M±SD)		39.3±1.37	38.7±1.20	38.4±1.27	38.5± 1.25
Average body weight (Kg) (M±SD)		11.3 ±2.30	26.4±8.67	48.9±7.10	39.6±14.13
Hemoglobin (g/dL) level (M±SD)		11.4±1.05	12.3±1.52	13.8±1.74	13.2±1.82
Mean paras. (per µl)		28846.67	30389	26215	27798
Median paras. (per µl)		20202	10320	12970	129970.00
Gametocyte carriage (%)		1(1.1%)	3(3.3%)	3(3.3)	7(7.6%)
Self- reported bed net availability at home					98.95% (91/92)
Self-reported previous malaria attack					82.6%(76/92)

M±SD: Mean±Standard deviation, N= initial number, Kg: in Kilo gram, Paras. : Parasitemia

6.2. Primary outcome

The cure rate was 98.7% based on PP analysis method with one LCF (parasitaemia 9200/µl on D28) and no ETF or LPF (Table 2). PCR-uncorrected cure rate was 98.8% (95% CI: 0.915–0.998) as the K-M survival estimate analysis showed (Table 3).

Table 2: Summary of treatment outcomes based on PP analysis stratified by age category

Age cat.	U5 N=3	5-14 N=33	≥15 N=56	Total N= 92
	n (%)	n (%)	n(%)	n (%)
ETF	0 (0)	0 (0)	0 (0)	0 (0)
LCF	0 (0)	1(3.1)	0 (0)	1 (1.3)
LPF	0 (0)	0 (0)	0 (0)	0 (0)
TTF	0 (0)	1(3.1)	0 (0)	1(1.3)
ACPR	3 (100)	32 (96.9)	45 (100)	78 (98.7)
LFU	0 (0)	0 (0)	8 (14.3%)	8 (8.7)
Withd.	0 (0)	2 (6.1%)	3 (6.5%)	5 (5.4)

ACPR: Adequate clinical and parasitological response, Age cat.: Age category, ETF: Early treatment failure, LCF: Late clinical failure, LFU: Lost follow up, LPF: Late arasitological failure, N: Baseline number, n: Final number, TTF: Total treatment failure. Withd: Withdrawn

Table 3: Summary of PCR-uncorrected cure rate based on K-M survival analysis

Follow-up day	n	Failures	Censored	Success cumulative incidence	Failure cumulative incidence
0	92	0	0	1.000	0.000
1	92	0	1	1.000	0.000
2	91	0	1	1.000	0.000
3	89	0	1	1.000	0.000
7	89	0	2	1.000	0.000
14	87	0	4	1.000	0.000
21	83	0	1	1.000	0.000
23	82	0	1	1.000	0.000
25	81	0	1	1.000	0.000
28	80	1	1	0.988	0.013
Total	78	1	13	95% CI: 0.915-0.998	95% CI: 0.002-0.085

6.3. Secondary outcomes

Parasite clearance rate was high that 33% of the patients cleared parasitaemia on D1, 84.4% on D2 and 100% on D3. The mean parasitaemia declined from 27798 on D0 (baseline) to 2864 on D1, 84 on D2 and to 0 on D3 (Figure 6). Taking D2 median parasitaemia as a comparing variable, parasite clearance rate was compared between children and adults and there was no significant difference (p=0.334).

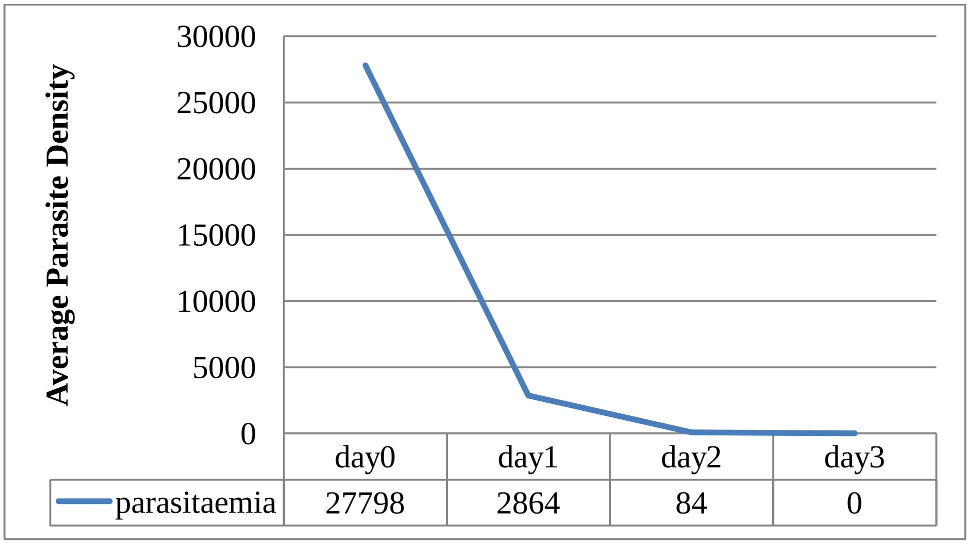

Figure 6: Mean parasite density on the first three follow-up days

At baseline all study participants were considered as febrile; 61 (66.3%) of them had actual fever, while the remaining had self-reported fever within 24 hours before enrollment. The mean body temperature declined from 38.5 ^{0}C on D0 to 37.4 on D1, 36.9 ^{0}C on D2 and to 36.8^{0}C on D3 (Figure 7) with 80% of the participants clearing fever on D1, 97.8% on D2 and 100% on D3.

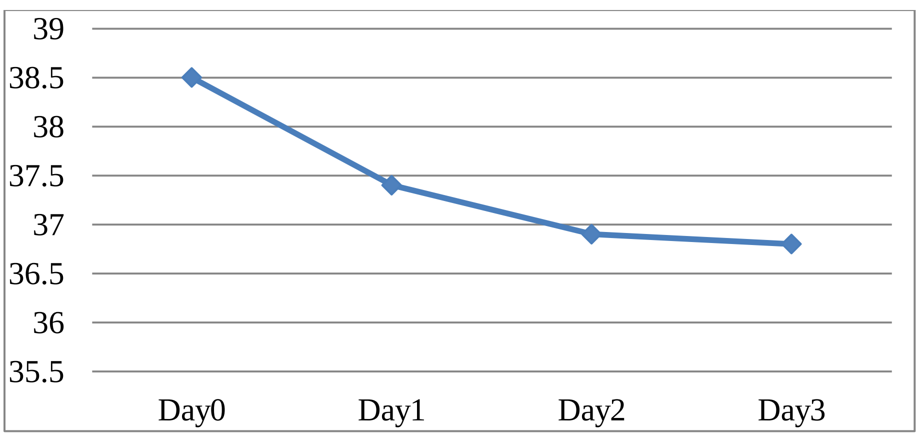

Figure 7: Mean body temperature on the first three follow-up days

The number of gametocyte carriers had declined from 7 on D0 to 5, 4 and 2 on D1, D2 and D3 respectively; only one patient remained gametocyte carrier on D7 and D14 and no gametocytes were detected beyond D14 (Figure 8).

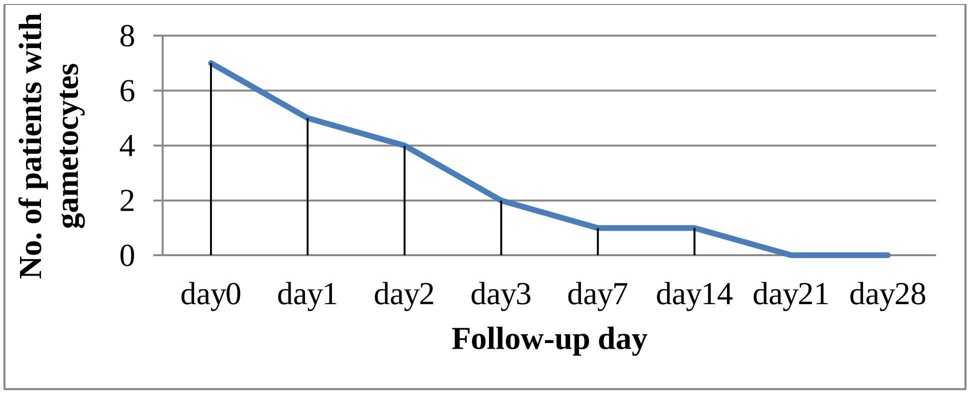

Figure 8: Number of participants with gametocytes on each follow-up day

The number of anemic patients that was 26 at baseline had risen to 42 (20 adults, 22 children) and 30 (18 adults, 12 children) on D14 and D28 respectively. Accordingly, blood mean Hb level showed slight decline from D0 (13.2±1.82) to D14 (12.2±1.64) (Table 4). Comparison of D0, D14 and D28 mean Hb level between patients with parasitaemia ≥10,000 and <10,000/µl showed no significant difference (13.0±1.88, 13.6±1.71, p=0.136; 12.0±1.66, 12.36±1.67, p=0.403; 12.8±1.50, 12.8±1.32, p=0.845 for ≥ 10,000 and < 10,000/µl; and for D0, D14 and D28 respectively.

Table 4: Mean hemoglobin concentration (g/dl) of study population by age and follow-up days

Age cat.	U5 children	5-14	Adults	Average total
Follow up day	Mean Hb ±SD (g/dl)	MeanHb±SD (g/dl)	Mean Hb±SD (g/dl)	Mean Hb±SD (g/dl)
D0	11.4±1.05	12.3±1.52	13.8±1.75	13.2±1.82
D14	11.4±0.95	11.3±1.45	12.7±1.55	12.2±1.64
D28	11.3±1.23	12.3±0.86	13.2±1.56	12.8±1.42

At baseline, a number of signs and symptoms were either self-reported or witnessed. Headache was the most frequent being reported by 68 patients followed by anorexia (41), diarrhea (13), vomiting (8), cough (23), abdominal pain (13), dizziness (10), nausea (16), weakness/fatigue (8), and sleep disorder (2). During follow-up some of these events persisted among some participants and new ones also emerged in totally 54 (53%) participants (Table 5). Most of these probable AEs disappeared with the clearance of parasitaemia except cough and peri-oral ulcer. Whereas cough persisted for sometimes beyond parasite clearance, peri-oral ulcer was noted after clinical manifestations subsided.

Table 5: **Probable drug-related AEs observed during the course of the study**

Probable adverse event	**Number of patients**	**Percent**
Headache	8	8.6
Perioral ulcer	7	7.6
Anorexia	6	6.5
Cough	6	6.5
Sleep disorder	5	5.4
Abdominal disorder	5	5.4
Dizziness	4	4.3
Weakness/fatigue	4	4.3
Diarrhea	3	3.4
Vomiting	2	2.2
Total	54	53

7. DISCUSSION

The 67% malaria prevalence indicates the heavy burden of malaria in the study area calling for scaling-up of control intervention and identifying possible risk factors. Malaria outbreak was confirmed in Setit Humera town in 2014 (Gidena 2015). According to that study, there were many risk factors associated with high prevalence and elevated risks of outbreak in Humera town. The first and most important factor was the amount and duration of rainfall. Rainfall was heavy and extended up to the end of October. The second factor was the availability of many natural and man made mosquito breeding sites; interrupted rivers crossing the town, poor drainage system, huge number of infrastructural and housing construction, uncontrolled irrigation areas covered with thick grasses all provides ideal breeding site for mosquitoes. Further more, although ITNs coverage is high, proper use of it and extensive out door activities at night with out any malaria protective measure undermines its preventive values.

The 98.9% PCR-uncorrected cure rate shows high therapeutic efficacy of AL nine years after its introduction for the treatment of uncomplicated falciparum malaria in the study site. Although this result is slightly lower compared to the drug's baseline efficacy (100%) in the area (Jima *et al.* 2005) it is relatively higher than PCR-corrected AL cure rates (96.3-97.8%) documented in other parts of Ethiopia (Assefa *et al*. 2010; Kinfu *et al*. 2012; Kebede *et al*. 2015). Factors such as host nutritional and immune status, initial parasitaemia level, pharmacokinetics and pharmacodynamics of the drug may influence therapeutic efficacy of a drug apart from inherent parasite susceptibility (WHO 2009). These and other related factors may result in treatment failure showing low efficacy of otherwise an efficient drug. At the same time, resistant parasites may be cleared with the help of the immune system which may result exaggerated efficacy of less efficient antimalarial drugs. As a result, the WHO recommends the integration of pharmacological studies as standard protocol of therapeutic efficacy studies to increase the determining capacity of therapeutic efficacy studies (WHO 2009).

The absence of ETF confirms the absence of "suspected artemisinin-resistance" in the current study area. Detecting parasitaemia on D3 is key indicator of "suspected artemisinin resistance" (WHO 2014). The absence of ETF and low recurrent malaria (only 1 LCF) in the present study could imply high therapeutic efficacy of both components of Art-L in the study site. One study in

Ethiopia (Getnet *et al*. 2015) and Nigeria (Anthony and Nnanna 2013) reported two and three ETF cases of Art-L treatment respectively.

In this study, the baseline mean and median parasitaemia were 27798 and 12970 respectively (range: 1128-199346). Parasitaemia is linked to the degree of malaria severity and hence is an important parameter to help in the decision of the type of treatment to be initiated. It has also epidemiological implication as it indicates the level of transmission intensity in a specific area. Although the level of malaria endemicity in the area is not thoroughly determined, Mengesha *et al*. (2000) characterized it as mesoendemic. According to local health office data, malaria is unstable and seasonal with varying duration and intensity mainly depending on the amount and length of rainfall. Accordingly, the level of transmission was high during the study period as rain fall amount was high with extended duration.

Clearance of parasitaemia was achieved by all patients on D3 indicating high parasite clearance rate. This is in line with the established quality of Art-L, rapid clearance of parasitaemia. This could be due to the inherent capacity of artemether to rapidly metabolize to its active ingredient, dihydroartemisinin, and rapidly absorbed to the blood stream which results in rapid clearance of parasite bio-mass (WHO 2014). The rate of elimination of artemether is also fast with half-life time ranging from 2-3 hours (Djimde and Lefevre 2009). The partner drug, lumefantrine, is slowly acting drug with long half-life time ranging from 4-7 days (Muller and Hyde 2010). This leads to successive accumulation of the drug after the completion of the full dose sufficient enough for the elimination of residual parasites and possibly prevention of new infection. The high parasite clearance rate of Art-L is also indicated in other efficacy studies conducted in Ethiopia (Kinfu *et al*. 2012; Getnet *et al*. 2015). Slower parasite clearance rate (detection parasites after D3) was associated with Art-L resistance in the Great Mekong region (Noedl *et al*. 2010; Ashley *et al*. 2014).

Fever is the main clinical manifestation of malaria causing much discomfort. In this study, the baseline mean body temperature was 38.5±1.25 ^{0}C and all study participants were considered as febrile. There was no significant difference in baseline mean body temperature between children and adults. This may be explained by the mesoendemic endemicity level, seasonal and unstable nature of transmission in the study site. It is well known that partial immunity is not acquired in areas with unstable and seasonal transmission pattern. The fever clearance was rapid notably less than three days. The fast fever resolving capacity of Art-L is also observed in other efficacy studies conducted in Ethiopia (Assefa *et al*. 2010; Kebede *et al*. 2015) as well as elsewhere in sub-Saharan Africa (Hwang *et al*. 2011; Ngasala *et al*. 2011; Shayo *et al*. 2014) as opposed to findings from Southeast Asian countries (Noedl *et al.* 2010; Ashley *et al*. 2014). Like delay in parasite, fever clearance rate is considered the main phenotypic presentation of artemisinin resistance in Southeast Asia.

The high parasite and fever clearance rate could be explained by the fast act of artemether to clear parasite biomass leading to rapid resolution of clinical manifestations (WHO 2015). There are many factors that determine parasite clearance rate; the initial parasitaemia, immune and nutritional status of the host, concomitant infection with geohelminths (WHO 2009).

Baseline mean Hb level (13.2±1.82) was significantly higher than that on D14 (12.2±1.64) and D28 (12.8±1.42). This is contrary to what was expected. Hb level is expected to increase or at least remain unchanged following parasite clearance. Results of other studies conducted in Ethiopia and other African countries showed slight to significant increase in mean Hb level following treatment with Art-L (Hwang *et al*. 2011; Shayo et al. 2014; Getnet et al. 2015).

Assuming the presumed higher safety of AL, Hb level was expected to increase (or atleast remain at baseline level) following parasite clearance. Results of studies conducted in Ethiopia and other African countries showed slight to significant increase in mean Hb level following treatment with Art-L (Hwang *et al*. 2011; Shayo *et al*. 2014; Getnet *et al.* 2015). However, Gray and colleagues reported anemia as severe AE of AL treatment (Gray *et al*. 2015). The authors reported that one patient had developed haemolytic anaemia and was rehospitalized, and a second one had received an outpatient blood transfusion due to ongoing haemolysis. Another report from the US found a total of 20 cases of what is described as post-artemisinin delayed hemolysis (PADH) (Paczkowski *et al*. 2014). These authors discussed that PADH which is characterized by a decline in Hb level could occur 1–3 weeks post initiation of treatment with ACT.

According to the PADH syndrome theory, Asn-containing drugs in general, and parenteral artesunate in particular, are known for their rapid killing of ring stage parasites and most of the killed ring stage parasites are cleared rapidly by the spleen by ‘pitting’ of erythrocytes whereby the dead parasite is removed from within the erythrocytes (Arguin 2014). These ‘once infected’ erythrocytes are returned to the circulation but they have a reduced lifespan of about 7-15 days. The delayed destruction of ‘once infected’ erythrocytes corresponds with the time course of post-treatment delayed anaemia. As reviewed in Rehman *et.al.* (2014), there are evidences of delayed anaemia occurring in patients from malaria endemic regions of Southeast Asia and Africa. Haemolysis following treatment of severe malaria with Asn drugs is a common clinical phenomenon typically occurring in the second week after initiation of antimalarial therapy. This review also indicated that late hemolysis leading to significant decrease of haemoglobin was evident associated with all artemisinin derivatives and all routes of administration (intravenous, intra-muscular, intra-rectal and even oral administration).

However, post-treatment haemolysis is commonly associated with the treatment of severe malaria and hyperparasitaemic patients and not all patients with hyperparasitaemia may develop haemolysis following treatment with artemisinins. Furthermore, this study was conducted in patients with uncomplicated falciparum malaria and comparison of D14 and D28 mean blood Hb level between patients with parasitemia >10,000/µl and <10,000/µl showed no significant difference. So, the PADH theory seems to be poor explanation for the current result calling for for further study.

Although individual difference in reporting of possible drug adverse events, difference in tolerance and other factors pose difficulty in comparing frequency and magnitude of occurrence of possible drug AE in different studies, the probable AEs observed in this study was almost similar with observation of other studies conducted in Ethiopia (Hwang *et al.* 2011; Kinfu *et al*. 2012; Lemma *et al*. 2012; Ebstie *et al* 2015). Most of the possible AE reported from study participants and/or observed during physical and clinical examination were similar to the symptoms of malaria itself. There were about 11 probable drug AEs; most of them were typical of malaria symptoms (headache, anorexia, diarrhea, nausea, vomiting, abdominal pain, and cough).

Although cough is common symptom of malaria, its longer persistence (up to D14) after subsidence of other malaria symptoms observed in this study makes it suspected drug related AE. Further study is required to exactly determine whether it is drug related or due to other concomitant upper respiratory tract infections that cause chronic cough. Similarly, peri-oral ulcer, (not commonly known malaria symptom), had observed in 7 patients; it was occurred mostly after resolution of other malaria related clinical manifestations). It was also reported in other efficacy studies (Jima *et al*. 2005; Asefa *et al*. 2010; Kassa 2010). It is therefore, very unlikely to consider the occurrence to be purely due to chance although further study is required. The PADH syndrome theory may partly explain Hb reductions on D14 and D28 compared to the baseline (D0) in this study. But further studies are warranted understand whether observation was drug-related or due to other causes.

The result of this study showed strong gametocidal effect of AL; it reduced the gametocyte carriage from 7 (7.6%) at D0 to 1 (1.1%) at D7 and D14, and complete clearance was achieved at D21. This result is consistent with other studies conducted in Ethiopia and elsewhere (Jima *et al*. 2005; Assefa *et al*. 2010; Hamde 2011; Hwang *et al*. 2011; Ngasala *et al*. 2011; Lemma 2012; Ebstie *et al*. 2015). Two separate studies conducted in Ethiopia showed higher gametocyte clearance rate (all cleared at D7) (Kassa 2010; Getnet *et al*. 2015). Another study conducted in Southern Ethiopia showed persistence of gametocytes up to D28 (Kebede *et al.* 2015). This calls for further studies to be conducted in all malaria endemic areas of the country to determine the local efficacy of AL against gametocytes. One comparative efficacy study indicated that Art-L decreasesed gametocytes by 6-8 folds compared with SP and CQ (Mårtensson *et al*. 2005). In other study, Art-L was reported to reduce gametocyte infectivity down to 40% (Mulenga *et al*. 2006). The inherent gametocidal effect of Art-L has important implication for the current malaria elimination agenda where transmission blocking is vital. Currently, sexual stages of *P. falciparum* and hypnozoites of *P. vivax* are targeted by primaquine and tefenoquine; but these drugs have a potential of inducing hemolytic anemia in glucose-6-phosphate dehadrogenase deficient patients (Biamont *et al*. 2013).

8. CONCLUSION

The result of this study has implied that the therapeutic efficacy of Art-L is excellent nine years after its introduction against uncomplicated falciparum malaria with high parasite clearance rate and rapid resolution of fever. The study has also implied that Art-L is effective against the transmissible sexual phase of the parasite. This gametocidal effect makes the drug very important for blocking transmission; thereby reducing the transmission intensity and leading to malaria elimination. Although blood Hb level was expected to increase following parasite clearance, the result of this study showed a significant decrease from baseline to D14 and D28; suggesting the potential occurerence of Art-L induced delayed haemolytic anaemia. Apart from this, there was no serious drug adverse event observed implying excellent safety of the drug.

9. RECOMMENDATIONS

1. The potential occurrence of post-treatment delayed hemolytic anemia in patients treated with Art-L needs to be investigated.
2. Malaria control interventions should be extended and reached to the migrant workers who bear the highest burden of malaria in the study site. Furthermore, much has to done on awareness rising regarding the benefit of bed nets.
3. Further studies using other malaria drug resistance detecting methods (prevalence of resistance molecular markers, in-vitro susceptibility tests) are required to complement therapeutic efficacy studies in this study site.

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ANNEXES

Annex 1: Patient screening form

1	Patient aged 6 months and over	Yes:□	No:□
2	Patient has severe malnutrition	Yes:□	No:□
3.	Patient has mono infection with Pf	Yes:□	No:□
4.	Body weight 5 kg or more	Yes:□	No: □
5.	Patient with fever or history of fever in the previous 24 hours	Yes: □	No:□
6.	Non-pregnant or breast-feeding female	Yes:□	No:□
7.	Residents living within 5-10 km radius of the health center	Yes:□	No:□
8.	12. Evidence of concomitant febrile illness If "YES", indicate illness. If "NO", leave blank. Pneumonia/RTI □ Measles□ Otitis Media □ UTI □ Gastroenteritis □ Other: □	Yes:□	No: □
9.	. Evidence of severe malaria / danger signs If "YES" indicate 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	Yes:□	No:□

	□Unable to drink or breast feed □Vomiting everything □Unable to stand/sit due to weakness □Severe anaemia (Hb < 5.0 gm/dL) □Respiratory distress (labored breathing at rest) □Jaundice (yellow coloring of eyes)		

Annex 2: Enrollment form

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

4. Patients Full name:
5. Family head:
6. Mother's/Wife's (if married) name:
7. Caregiver's name and relationship:
8.Kebele/Street:
9. Home parish:
10. Locality/village:
11. Home address and localizing features/Owners name/Direction:
Phone number (s) and the owner(s):
12. Previous antimalarial intake: Yes ______No ______ If yes, CQ/SP/AL? 13. Hold Bed net: Yes ___No If yes, Bed net use Yes_______ No _______

Annex 3: Case record form

PIN……………………… No. of Tablets ………………

Name…………………………………………………

	day 0	day 1	day 2	day 3	day 7	day 14	day 21	day 28	Extra
Date									
Success of treatment at home*									
Axillary T^o C									
Parasite asexual									
Gametocyte count									
Haemoglobin									
Adverse events**									
Concomitant treatment									
Reasons for withdrawal									
Remarks									

*1. Observed by the health extension worker and successfully took medication 2. Not observed by the health extension worker but successfully took medication 3. Vomited the drug, came to clinic and were successfully re-administered 4. Vomited medication and did not come to clinic 5. Forgot to take medication or lost the tablets 6. Did not take the medication

**1) Headache 2) Anorexia 3) Nausea 4) Vomiting 5) Abdominal pain 6) Diarrhea 7) Cough 8) Behavioral Change 9) Dizziness 11) Mouth ulcer 12) other, specify__________

Annex 4: Classification of response to treatment

Early treatment failure

Danger signs or severe malaria on D1, 2 or 3 in the presence of parasitaemia; parasitaemia on D2 higher than on D0 irrespective of axillary temperature; parasitaemia on D3 with axillary temperature ≥ 37.5 ºC; parasitaemia on D3 ≥ 25% of the count on D0; severe AE requiring change in treatment on D1 and D2

Late clinical failure (LCF)

Danger signs or severe malaria in the presence of parasitaemia on any day between D4 and D28 in patients who did not previously meet any of the criteria of ETF; presence of parasitaemia on any day between D4 and D28 with axillary temperature ≥ 37.5 ºC (or history of fever) in patients who did not previously meet any of the criteria of ETF.

Late parasitological failure (LPF)

Is presence of parasitaemia on any day between D7 and D28 and axillary temperature < 37.5 ºC in patients who did not previously meet any of the criteria of ETF or LCF.

Annex 5: Weight-based administration of Art-L

Weight (kg)	Coartem® (20mg /120 mg tablet)					
	Day 0		Day 1		Day 2	
	am	pm	am	pm	am	pm
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

Annex 6: Definition of severe falciparum malaria

Clinical manifestations:

Altered consciousness (e.g. sleepiness, confusion, drowsiness, coma); prostration, i.e. generalized weakness so that the patient is unable to walk or sit up without assistance; unable to eat or drink; repeated vomiting; resulting in inability to retain oral medication, inability to eat or drink; severe dehydration; convulsion or recent history of convulsions; difficult breathing; jaundice (yellowish discoloration of the eyes); anemia (paleness of palms is most reliable symptom in children); hemoglobinuria (cola colored urine); abnormal spontaneous bleeding; no urine output in the last 24 hours.

Laboratory findings

Severe anaemia (haemoglobin < 5 gm/dL, haematocrit < 15%); Hypoglycaemia (blood glucose < 2.2 mmol/l or 40 mg/dL); Acidosis (plasma bicarbonate < 15 mmol/l),hyperlactataemia (venous lactic acid > 5 mmol/l) ;Hyperparasitaemia (> 4% in non-immune patients); renal impairment (serum creatinine above normal range for age).

Annex 7: Adult consent form in English

Title of the study: EFICACY OF AL AGAINST UNCOMPLICATED FALCIPARUM MALARIA IN SETIT HUMERA HEALTH CENTER, NORTHWEST ETHIOPIA

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Addis Ababa university, Addis Ababa, Ethiopia

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Your health care provider has determined that you have malaria infection caused by Pf that needs treatment with a medication called Art-L (Coartem®). Art-L is currently the recommended 1st line drug by the FMoH for the treatment of uncomplicated Plasmodium falciparum malaria in Ethiopia. Regardless of whether you decide to participate in this study, this health care facility will provide you with Art-L medication for your malaria infection at no cost to you.

We would appreciate your help if you are volunter for this research study that will help us to carefully follow and document the course of your malaria infection after treatment with Art-L. The Ministry regularly conducts clinical studies to make sure that the recommended malaria treatments are still working well. In this study we intend to assess how well Art-L works to cure malaria at six locations in Ethiopia, and Setit Humera is one of the sites selected. The information from this study should help national malaria control program managers to determine whether there is evidence of Art-L drug resistance and whether we may need to find other medications to substitute for Art-L.

We are inviting all malaria patients aged 6 months and over living in this area to take part in this study.

If you agree, you will be treated with six doses of Art-L given twice daily for 3 days. (This is the same treatment that you would receive if you decide not to volunteer for this study.) The first dose will be given at the clinic supervised by study nurse and the 2nd dose will be given to you to be taken at home. The study will take place over 28 days. During that time, you will be asked to come to the health facility on scheduled days (D1, 2, 3, 7, 14, 21 and 28). You will also be asked to come to the clinic at any other time if you become sicker, develop new symptoms, or if you fail to get better. Ttransportation fees will be provided to you during each scheduled study follow-up visit.During each follow-up visit, we would like to obtain a finger prick blood samples from you by a qualified technician that would be used only for malaria diagnosis, to detect the presence of markers for malaria drug resistance, and to see the outcome of treatment. There is no serious risk in participating, but you may experience a small pain during finger pricking. The pain should disappear within one day.

The Art-L medicine can have some unwanted side-effects or some effects that we are not currently aware of; however, we will follow you closely and ensure proper medical treatment. If you take Art-L as directed, the course of your illness and possible side effects from Art-L should not be any different whether you volunteer for this study or not.

The Art-L medicine may have some unexpected effects; however, we will follow you closely and keep track of these effects, if they arise. Patients showing deterioration in their clinical status will be immediately admitted to the clinic free of charge for appropriate treatment according to the national policy till they recover. A physician will be responsible for every trial related medical decision of the patient throughout the study period.

Your participation in this study is completely voluntary and you can refuse to participate or are free to withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or all the services you receive at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services you receive at the clinic will continue.

If you decide to participate in this study, the information in your records is strictly confidential and your name will not be used in any report and any illnesses related to malaria or to the malaria treatment will be treated at no charge to you.

Do you understand what has been said to you? If you have any questions you have the right to get proper explanation.

Certificate of consent

I have been invited to participate in Art-L efficacy study. I have read the information in this consent form or have been readout to me in my own language. I clearly understand the content. I am also aware of my right to opt out of the study at any time during the course of the study without having to give reasons for doing so. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction and I voluntarily consent to participate in the study.

Name of participant___________________Signature__________ Date__________

Witness' signature:

I confirm that the participant has given consent freely.

Name of witness______________________Signature__________ Date________

Investigator's signature:

I confirm that the participant has given consent freely.

Name of investigator:____________________Signature__________ Date__________

Annex 8: Children Consent Form in English

Title of the study: EFFICACY OF AL UNCOMPLICATED FALCIPARUM MALARIA IN SETIT HUMERA HEALTH CENTER, NORTH WEST ETHIOPIA

Name of principal investigator: MICHAEL TEKLEMARIAM

Department of Microbial, cellular and molecular biology (Biomedical stream)

Addis Ababa university, Addis Ababa, Ethiopia

ID. No:GSR/1312/06; E-mail address:-miketek81@gmail.com

Your health care provider has determined that your child has malaria infection caused by *P. falciparum* that needs treatment with a medication called Art-L (Coartem®).

Artemether/Lumefantrine is currently the recommended 1st line drug by the FMoH for the treatment of uncomplicated Plasmodium falciparum malaria in Ethiopia. Regardless of whether you decide to allow your child to participate in this study, this health care facility will provide your child with AL medication for this malaria infection at no cost to you. We would appreciate your help if you decide to allow your child to volunteer for this research study that will help us to carefully follow and document the course of this malaria infection after treatment with AL. The Ministry regularly conducts clinical studies to make sure that the recommended malaria treatments are still working well. In this study we intend to assess how well AL works to cure malaria at six locations in Ethiopia, and to determine whether it is still working as well as in our earlier studies. The information from this study should help national malaria control program managers to determine whether there is evidence of Art-L drug resistance and whether we may need to find other medications to substitute for Art-L.

We are inviting all malaria patients aged 6 months and over living in this area to take part in this study. Thus, you are being asked to consent to have your child participate in this study. If you agree, your child will be treated with six doses of Art-L given twice daily for 3 days. (This is the same treatment that your child would receive if you decide not to volunteer your child for this study).

The first dose will be given at the clinic supervised by study nurse and the 2nd dose will be given to you to be taken at home. The study will take place over 28 days. During that time, you will be asked to come to the health facility on scheduled days (D1, 2, 3, 7, 14, 21 and 28). You will also be asked to bring your child back to the clinic at any other time if your child becomes sicker, develops new symptoms, or if your child fails to get better transportation fees will be provided to you during each scheduled study follow-up visit. During each follow-up visit, we would like to obtain a finger prick blood samples from your child by a qualified technician that would be used only for malaria diagnoses, to detect the presence of markers for drug resistance, and to see the outcome of treatment. There is no serious risk in participating but your child may experience a small pain during finger pricking. The pain should disappear within 1 day. The Art-L medicine can have some unwanted side-effects or some effects that we are not currently aware of; however, but we will follow your child closely and ensure proper medical treatment. If your child takes the Art-L as directed, the course of your child's illness and possible side effects from Art-L should not be any different whether you volunteer your child for this study or not. Children showing deterioration in their clinical status will be immediately admitted to the clinic free of charge for appropriate treatment according to the national policy till they recover. A physician will be responsible for every trial related medical decision of the patient throughout the study period.

Your decision to have your child participate in this study is entirely voluntary and you can refuse your child to participate or are free to withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or all the services your child receives at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services your child receives at the clinic will continue.

If you decide that your child will participate in this study, the information in your child records is strictly confidential and your child name will not be used in any report and any illnesses related to malaria or to the malaria treatment will be treated at no charge to you.

Do you understand what has been said to you? If you have any questions you have the right to get proper explanation.

Certificate of consent

I have been invited to have my child participate in Art-L efficacy study and I have read the information in this consent form or have been readout to me in my own language. I clearly understand the content. I am also aware of my right to opt out of the study at any time during the course of the study without having to give reasons for doing so. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction and I voluntarily consent to my child's participation in this study.

Name of participant _______________ Name of caregiver_______________

Signature of caregiver _______________Date_______________

Witness' signature:

I confirm that the participant has given consent freely.

Name of witness:____________________Signature_______________Date___________

Investigator's signature:

I confirm that the participant has given consent freely.

Name of investigator:_____________________ Signature ____________ date ____________

Annex 9: Adult consent form in Amharic

ለአዋቂ የተሰናዳ የስምምነት ቅጽ

ሀ/ ጥናቱን የተመለከቱ መረጃዎች

1. **የጥናቱ ርዕስ**

ለፕላዝሞዲየም ፋልሲፓረም ህክምና ጥቅም የሚውለውን የኮአርተም መድሐኒት ፍቱንነት ጥናት በሰቲት ሑመራ ጤና ጣቢያ

ጥናቱን የሚያጠናው ተመራማሪ ስም፡ – ሚኪኤል ተክለሚርያም

አድራሻ፡ – አዲስ አበባ ዩኒቨርሲቲ

2. **የጥናቱ ዓላማ**

ኮአርተም የወባ መድሐኒት ለፕላዝሞዲየም ፋልሲፓረም ወባ ህክምና የሚሰጥ የመጀመሪያ ደረጃ መድሐኒት ነው፡፡ የፌደራል ጤና ጥበቃ ሚኒስቴር የወባ መድሐኒቶችን ፍቱንነት በየጊዜው በሚደረጉ ጥናቶች ይፈትሻል፡፡ ይህ ጥናትየ የዚህ አይነቱ ፍተሻ አካል ሲሆን በኢትዮጵያ ሕብረተሰብ ጤና ኢንስቲትዩት የሚካሄድ የኮአርተም መድሐኒት ፍቱነን ፍተሻ ጥናት ነው፡፡

ይህ ጥናት በአገር አቀፍ ደረጃ በ6 የተመረጡ የጥናት ጣቢያዎች ወባን በሚወስ ረገድ ኮአርተም ምን ያህል እየሰ እንደሆነ በማምገም መድሐኒቱ አሁንም ድረስ ፍቱን ሆኖ መዘለቁንና አለመዘለቁን ለማረጋገጥ የሚደረግ ሲሆን የሰቲ ሑመራ ጥናት ጣቢያ ከነዚህ ጣቢያዎች አንዱ ነው፡፡ ከጥናቱ የማገኘው መረጃ ወባው መድሐኒቱን ማላመዱን አለማላመዱን ለማየት ብሎም ለመድሐኒቱ ምትክ ማስፈለጉንና አለማስፈለጉን ለመወሰን ጥቅም ላይ ይውላል፡፡ የጥና

ዓብይ ዓላማም ይሄው ነው፡፡

3. በጥናቱ የሚካተቱ ታካሚዎች

ይህን ጥናት ለማጥናት እድሜያቸው 6 ወር እና ከዛ በላይ የሆናቸው የወባ ታማሚዎች በበጎ ፈቃደኝነት ተሳታፊ ይሆናሉ፡፡ ከዚህ አኳያ እርሰዎ በኮአርተም መድሐኒት በሚታከም ወባ ሳቢያ መታመምዎ በምርመራ ስለተረጋገጠ በጥናቱ እንዲካተቱና ለጥናቱ እገዛ እንዲያደርጉ የእርሰዎ ፈቃድ ያስፈልጋል፡፡

4. እርሰዎ ፈቃድዎን ገልጸው በጥናቱ ከታቀፉ በጥናቱ የሚከተሉት እንዴት ነው?

ጥናቱ የሚካሄደው ለ28 ቀናት ሲሆን ከነዚህም ወስጥ በጥናቱ ሞርህ ግብር ማስረት ለ7 ቀናት ይሄውም ወባው ከተገኘብዎ በኋላ ባሉት የመጀመሪያ፣ ሁለተኛ፣ ሶስተኛ፣ ሰባተኛ፣ አስራ አራተኛ፣ ሃያ አንደኛ እና ሃያ ስምንተኛ ቀናቶች ወደ ጤና ጣቢያው እየመጡ በጥናቱ በማተፍ ህክምናወን ይከታተላሉ፡፡ በእያንዳንዱ የክትትል ቀናት ከጣታቸው ከ2-3 የደም ጠብታ ለወባ ምርመራ ይወሰዳል፡፡ ህክምናው በቀን ሁለት ጊዜ ለ3 ቀናት የሚሰጥ ሲሆን እርሰዎ በቀጣዩ ቀናት ጧት ጧት ወደ ጤና ጣቢያው እየመጡ መድሐኒቱን ይወስዱና ማታ የሚወጠትን ደግሞ ተቀብለው ወደቤትዎ ይመለሳሉ፡፡ መድሐኒቱን ወስደው ሳይሻለዎት ቢቀርና አዲስ የወባ በሽታ ምልክት ቢያሳዩ ከሞርህ ግብሩ በተጨማሪ በማናቸውም ወቅት ወደ ጤና ጣቢያው እየመጡ ክትትል እየተደረገለዎት ይታከማሉ፡፡

5. በጥናቱ ወቅት ለእርሰዎ የሚደረጉት የወባ ምርመራዎች

በጥናቱ ወስጥ ህክምናወን በሚከታተሉበት ወቅት ከጣት ላይ ደም እየተወሰደ የወባ ምርመራ ይደረግለዎታል፡፡ ከጣት ደም ሲወሰድ ጣት ስለሚወጋ ጥቂት ለአጭር ጊዜ ብቻ የሚቆይ የህመም ስሜት ይኖረዋል፡፡ በተወሰደው ደም የሚደረገው ምርመራ እየተሻለዎ ማምጣቱን እንዲሁም ወባው ለመድሐኒቱ ያለወን ብግርነት ያሳያል፡፡

6. ለእርሰዎ የሚሰጠው የኮአርተም መድሐኒት

የኮአርተም መድሐኒት በወል ተለይቶ ያልታወቀ የጎንዮሽ ጉዳት ሊኖረው እንደሚችል ይገመታል፡፡ሆኖም ይህ ከጥናቱ ጋር የሚገናኘው አንዳች ነገር የለም፡፡ የመድሐኒቱን ተጽእኖ በማጥናት ሂደት ሳይሻለው እየባሰበት የሚሄድ ታካሚ ከተገኘ በወባ የህክምና መመሪያ ማስረት በተሻለ መድሐኒት እንዲታከም ይደረጋል፡፡

7. በጥናቱ በማተፍዎ ያለዎት መብትና ጥቅም

በዚህ ጥናት ወስጥ መካተት የሚቻለው በፈቃደኝነት ብቻ ስለሆነ በጥናቱ ያለመሳተፍ ሙሉ መብት አለዎት፡፡ በጥናቱ ወስጥ አልሳተፍም በማለትዎ የሚያጡት እንክብካቤም ሆነ ህክምና አይኖርም፡፡ በጥናቱ ወስጥ ማተፍ ከጀመሩም በኋላም ከጥናቱ መውጣት ቢፈልጉ ሙሉ መብት ያለዎት በመሆኑ የሚያስፈልገወን ህክምና በሙሉ አንደም ሳይጎድልብዎት ከጤና ጣቢያው ያገኛሉ፡፡

በሌላ በኩል በዚህ ጥናት ወስጥ የሚካተፍ ማንኛወም ታካሚ በተሰናዳው ሞርሃ ግብር ማስረት ወደ ጤና ተቋሙ ሲመላለስ ለእያንዳንዱ ቀን የመሄጃና የመመጫ የመጓጓዣ አበል ይሰጠዋል፡፡ በተጨማሪም ጥናቱ ከታካሚው የሚያገኘወን ሚጃ ፍጹም ምስጢራዊነት የሚጠበቅ ሲሆን ከጥናቱ ጋር ተያይዞ በሚወጣ ማንኛወም ዓይነት ሪፖርት ወስጥ የታካሚው ስም አይጠቀስም፡፡ ከላይ የተዘረዘሩትን ጥናቱን የተመለከቱ ሚጃዎች በሚባ ሚዳትዎን ያረጋግጡ፡ ያልገባዎት አረፍተ ነገር ወይም ግልጽ ያልሆነ ሃሳብ ካለ ማንኛወንም ማብራሪያ ማጠየቅ ይችላሉ፡፡ ሁሉም ሚጃዎች በሚባ ግልጽ ከሆኑልዎትና በጥናቱ እንዲታቀፉ የሚፈቅዱ ከሆነ ከስር የተቀመጠወን የስምምነት ሰነድ ተረድተው ይፈርሙ፡

ለ/ የስምምነት ሰነድ

ጥናቱን የተመለከተወን ሚጃ አንብቤ /በቋንቋዬ ተነቦልኝ ይዘቱን በወል ተገንዝቤአለሁ፡፡ አስፈላጊ ሆኖ ካገኘሁት በፈለኩት በማኛወም ጊዜጥ ናቱን የማቋረጥ መብት እንዳለኝ በሚባ አወቄአለሁ፡ ሚጃወን ካነበብኩ/ከተነበበልኝ በኋላ ያልገባኝን ነገር ጠይቄና ተረድቼ በጥናቱ እንድታቀፍ በፈቃደኝነት ተስማምቻለሁ፡

የጥናቱ ተሳታፊ ስም____

ፊርማ____________________________ቀን______________________________

የምስክር ስም____

ፊርማ_________________________________ ቀን _________________________________

የጥናት አድራጊው ስም_____________________________ፊርማ_____________________________ቀን______
____

Annex 10: Children consent form in Amharic

ለህፃናት የተሰናዳ የስምምነት ቅጽ

ሀ/ ጥናቱን የተመለከቱ መረጃዎች

1. <u>የጥናቱ ርዕስ</u>

ለፕላዝሞዲየም ፋልሲፓረም ህክምና ጥቅም ላይ የሚውለውን የኮአርትም መድሐኒት ፍቱንነት ጥናት በሰቲት ሁመራ ጤና ጣቢያ

ጥናቱን የሚያጠናው ተመራማሪ ስም፡ <u>ሚኪኤል ተክለሚካኤል</u>

አድራሻ፡ - <u>አዲስ አበባ ዩኒቨርሲቲ</u>

2. <u>የጥናቱ ዓላማ</u>

ኮአርተም የወባ መድሐኒት ለፕላዝሞዲየም ፋልሲፓረም ወባ ህክምና የሚሰጥ የመጀመሪያ ደረጃ መድሐኒት ነው፡፡ የፌደራል ጤና ጥበቃ ሚኒስቴር የወባ መድሐኒቶችን ፍቱንነት በየጊዜው በሚደረጉ ጥናቶች ይፈትሻል፡፡

ይህ ጥናትም የዚህ አይነቱ ፍተሻ አካል ሲሆን በኢትዮጵያ ሕብረተሰብ ጤና ኢንስቲትዩት የሚካሄድ የኮአርተም መድሐኒት ፍቱነንት ፍተሻ ጥናት ነው፡፡ይህ ጥናት በአገር አቀፍ ደረጃ በ6 የተመረጡ የጥናት ጣቢያዎች ወባን በመዉሰስ ረገድ ኮአርተም ምን ያህል እየሰራ እንደሆነ በማግግም መድሐኒቱ አሁንም ድረስ ፍቱን ሆኖ ማዘለቁንና አለማዘለቁን ለሚጋገጥ የሚደረግ ሲሆን የሰቲት ሁመራ ጥናት ጣቢያ ከነዚህ ጣቢያዎች አንዱ ነው፡ ከጥናቱ የሚገኘው መረጃ ወባው መድሐኒቱን መላመዱንና አለመላመዱን ለማየት ብሎም ለመድሐኒቱ ምትክ ማስፈለጉንና አለማስፈለጉን ለመወሰን ጥቅም ላይ ይወላል፡፡የጥናቱ ዓብይ ዓላማም ይሄው ነው፡፡

3. <u>በጥናቱ የሚሳተፉ ታካሚዎች</u>

ይህን ጥናት ለማጥናት እድሜያቸው 6 ወር እና ከዛ በላይ የሆናቸው የወባ ታማሚዎች በበጎ ፈቃደኝነት ተሳታፊ ይሆናሉ፡፡ ከዚህ አኳያ ልጅዎ በኮአርተም መድሐኒት በሚታከም ወባ ሳቢያ መታመሙ በምርመራ ስለተረጋገጠ በጥናቱ እንዲካተትና ለጥናቱ እገዛ እንዲያደርግ የእርሰዎ ፈቃድ ያስፈልጋል፡፡

4. <u>እርሰዎ ፈቃድዎን ገልጸው ልጅዎ በጥናቱ ከታቀፈ በጥናቱ የሚሳተፈው እንዴት ነው</u>?

ጥናቱ የሚካሄደው ለ28 ቀናት ሲሆን ከነዚህም ወስጥ በጥናቱ መርሀ ግብር ማስረት ለ7 ቀናት ይሄወም ልጅዎ ወባው ከተገኘበት በኋላ ባሉት የመጀመሪያ፣ ሁለተኛ፣ ሶስተኛ፣ ሰባተኛ፣ አስራ አራተኛ፣ ሃያአንደኛ እና ሃያ ስምንተኛ ቀናቶች ወደ ጤና ጣቢያው እየመጣ ጥናቱ በሚሰተፍ ህክምናወን ይከታተላል፡፡ በእያንዳንዱ የክትትል ቀናት ከጣታቸወከ2-3 የደም ጠብታ ለወባ ምርመራ ይወሰዳል፡፡ ህክምናው በቀን ሁለት ጊዜ ለ3 ቀናት የሚሰጥ ሲሆን ልጅዎ በቀጠሮ ቀናት ጧት ጧት ወደ ጤና ጣቢያው እየመጣ መድሐኒቱን ይወስዱና ማታ የሚወጠወን ደግሞ ተቀብሎ ወደ ቤቱ ይመላሳል፡፡ ልጅዎ መድሐኒቱን ወስዶ ሳይሻለው ቢቀርና አዲስ የወባ በሽታ ምልክት ቢያሳይ ከመርሀ ግብሩ በተጨማሪ በሚፈቸወም ወቅት ወደ ጤና ጣቢያው እንዲመጣ ተደርጎ ክትትል እየተደረገለት ይታከማል፡፡

5. <u>በጥናቱ ወቅት ለልጅዎ የሚደረጉት የወባ ምርመራዎች</u>

ልጁ በጥናቱ ወስጥ ህክምናወን በሚከታተልበት ወቅት ከጣት ላይ ደም እየተወሰደ የወባ ምርመራ ይደረግለታል፡፡ ከጣት ደም ሲወሰድ ጣት ስለ ሚወጋ ጥቂት ለአጭር ጊዜ ብቻ የሚቆይ የህመም ስሜት ይኖረዋል፡፡ በተወሰደው ደም የሚደረገው ምርመራ ልጅዎ እየተሻለው መምጣቱን እንዲሁም ወባው ለመድሐኒቱ ያለወን ብግርነት ያሳያል፡፡

6. **<u>ለልጅዎ የሚሰጠው የኮአርተም መድሐኒት</u>**

የኮአርተም መድሐኒት በወልተለይቶ ያልታወቀ የጎንዮሽ ጉዳት ሊኖረው እንደሚችል ይገመታል፡፡ሆኖም ይህ ከጥናቱ ጋርየሚገናኘው አንዳችነገርየለም፡የመድሐኒቱን ተጽእኖ በማጥናት ሂደት ሳይሻለው እየባሰበት የሚሄድ ታካሚ ከተገኘ በወባ የህክምና መመሪያ ማስረት በተሻለ መድሐኒት እንዲታከም ይደረጋል፡፡

7. **<u>በጥናቱ በመሳተፉ ልጅዎ ያለው መብትና ጥቅም</u>**

በዚህ ጥናት ወስጥ መካተት የሚቻለው በፈቃደኝነት ብቻ ስለ ሆነ ልጅዎ በጥናቱ ያለመሳተፍ ሙሉ መብት አለው፡፡ ልጅዎ በጥናቱ ወስጥ አልሳተፍም በማለቱ የሚያጣው እንክብካቤም ሆነ ህክምና አይኖርም፡፡ በጥናቱ ወስጥ መሳተፍ ከጀመረ በኋላም ከጥናቱ መውጣት በፈልግ ሙሉ መብት ያለው በመሆኑ የሚያስፈልገወን ህክምና በሙሉ አንድም ሳይጎልበት ከጤናጣቢያው ያገኛል፡፡

በሌላበኩል በዚህ ጥናት ወስጥ የሚታቀፍ ማንኛወም ታካሚ በተሰናዳው መርሃ ግብር ማስረት ወደ ጤና ተቋሙ ሲመላለስ ለእያንዳንዱ ቀን የሜጃና የመጫጫ የመጓጓዣ አበል ይሰጠዋል፡፡ በተጨማሪም ጥናቱ ከታካሚው የሚያገኘወን መረጃ ፍጹም ምስጢራዊነት የሚጠበቅ ሲሆን ከጥናቱ ጋር ተያይዞ በሚወጣ ማንኛወም ዓይነት ሪፖርት ወስጥ የታካሚው ስም አይጠቀስም፡፡ ከላይ የተዘረዘሩትን ጥናቱን የተመለከቱ መረጃዎች በሚገባ ሚዳትዎን ያረጋግጡ፡፡ ያልገባዎት አረፍተ ነገር ወይም ግልጽ ያልሆነ ሃሳብ ካለም ማንኛወንም ማብራሪያ ማጠየቅ ይችላሉ፡፡ ሁሉም መረጃዎች በሚገባ ግልጽ ከሆኑልዎትና በጥናቱ ልጅዎ እንዲታቀፍ የሚፈቅዱ ከሆነ ከስር የተቀመጠወን የስምምነት ሰነድ ተረድተው ይፈርሙ፡፡

ለ/ <u>የስምምነት ሰነድ</u>

ጥናቱን የተመለከተወን መረጃ አንብቤ/በቋንቋ ተነቦልኝ ይዘቱን በወል ተገንዝቤአለሁ፡፡ አስፈላጊ ሆኖ ካገኘሁት በፈለኩት በማኛወም ጊዜ ልጄ ጥናቱን የማቋረጥ መብትእንዳለወም በሚገባ አወቄአለሁ፡መረጃወን ካነበብኩ/ከተነበበልኝ በኋላ ያልገባኝን ነገር ጠይቄና ተረድቼ ልጄ በጥናቱ እንዲታቀፍ በፈቃደኝነት ተስማምቻለሁ፡፡

የጥናቱ ተሳታፊ ስም________________________ፊርማ____________________ቀን ____________________

የምስክር ስም________________________ፊርማ________________________ቀን ____________________________

የጥናት አድራጊው ስም________________________ፊርማ__________________ቀን __________________

Annex 11: Medications with antimalarial activity

- Chloroquine, amodiaquine, Quinine, Quinidine, Mefloquine, halofantrine, lumefantrine
- Artemisinin and its derivatives (artemether, arteether, artesunate, dihydroartemisinin)
- Proguanil, chloroproguanil, pyrimethamine, Sulfadoxine, sulfalene, sulfamethoxazole, Primaquine, Atovaquone, Pentamidine, Dapsone
- Antibiotics- tetracycline, doxycycline, erythromycin, azythromycin, clindamycin, rifampcin, trimethoprim

Annex 12: Declaration

I hereby declare that this thesis is my original work and has not been submitted for award of a degree in any institution of learning to the best of my knowledge.

Name…………………………………………………Signature……………………