ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

THERAPEUTIC EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *P. FALCIPARUM* MALARIA IN ALAMATA DISTRICT, TIGRAY, NORTHERN ETHIOPIA; FIVE YEARS AFTER DEPLOYMENT

By

Gebremedhin Kinfu Gebrewahid, B.Sc.
Department of Microbiology, Immunology and Parasitology
Faculty of Medicine, Addis Ababa University

April, 2010
Addis Ababa

Therapeutic Efficacy of Artemether-Lumefantrine for the Treatment of Uncomplicated *P. falciparum* Malaria in Alamata District, Tigray, Northern Ethiopia; Five Years after Deployment

Name of M.Sc Candidate: Gebremedhin Kinfu Gebrewahid

Advisors:

Dr. Solomon Gebre-Selassie (MD, M.Sc, Department of Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University)

Mr. Nigus Fikrie (M.Sc, Department of Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University)

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Masters of Science in Medical Parasitology

April, 2010

Addis Ababa

Acknowledgements

I deeply thank my advisors Dr. Solomon GebreSelassie and Mr. Nigus Fikrie for their extensive support and assistance throughout the course of this research. I also deeply thank to my co-advisors Mr. Hailemariam Lema and Mr. Alem Desta for their constructive comments, and support in data entry and analysis.

I am grateful to Tigray Regional health Bureau and Alamata District Health office staff, who were co-operative during training and from day of screening through to the follow up days of the study, particularly Mr. Goitom Mehari, Mr. Berhanu Amare, Mr. Areki Hagezom and Mr. Abreham G/Tsadik.

My special thanks also go to Addis-Ababa University Faculty of Medicine and Tigray Region Health Bureau for their financial and technical support for the operation of the research. I also wish to thank the Department of Microbiology, Immunology and Parasitology. Finally I would like to express my best gratitude to my family, particularly my wife Kiros G/Hiwot for her patience and moral support through out the research project including data collection, data entry, analysis and writing of this thesis.

Contents **Page No**

List of Tables **Page No**

List of Figures **Page No**

Abbreviations

ACPR	Adequate Clinical and Parasitological Response
ACT	Artemisinin based combination therapy
AL	Artemether Lumefantrine
AQ	Amodiaquine
AS	Artesunate
BP	Blood pressure
CM	Cerebral Malaria
Cytb	Cytochrome b
CD36	Cluster of differentiation 36
CQ	Chloroquine
DHA	Dihydroartemisinin
DNA	Deoxyribonucleic acid
DHPS	Dihydropteroate synthase
DHFR	Dihydrofolate reductase
ETF	Early treatment failure
ELISA	Enzyme linked immunosorbent assay
FMOH	Federal ministry of health
HPF	High power fields
HA	Haemagglutination
Hb	Haemoglobin
HMIS	Health management and information system
HIV	Human Immunodeficiency Virus
HO	Health officer
ICAM1	Intercellular adhesion molecule 1
IFA	Immuno fluorescence
ITNs	Insecticide treated nets
ITT	Intent to treat
LCF	Late clinical failure
LLINs	Long-lasting insecticide nets

LPF	Late parasitological failure
LTF	Late treatment failure
MOH	Ministry of Health
PABA	Para-amino benzoic acid
PCT	Parasite clearance time
PP	Perprotocol
Pfcrt	*Plasmodium falciparum* chloroquine resistance transporter gene
Pf	*Plasmodium falciparum*
Pfmdr1	*Plasmodium falciparum* multidrug resistance protein1
Pgh-1	P-glycoprotein homolog1
PfHRP2	*Plasmodium falciparum* Histidine rich protein 2
PCR	Polymerase chain reaction
P.v	*Plasmodium vivax*
RDTs	Rapid diagnostic tests
RBC	Red blood cells
SERCA	Sarco-endoplasmic reticulum ca-ATPase
SP	Sulphadoxine –Pyrimethamine
TTF	Total treatment failure
TB	Tuberculosis
TNF	Tumour necrosis factor
TRHB	Tigray Regional Health bureau
WBC	White blood cells
WHO	World Health Organization

Abstract

Background: Malaria is one of the most important infectious diseases, causing hundreds of millions of illness and more than one million deaths worldwide annually. The emergence and spread of *Plasmodium falciparum* resistance to commonly used anti-malarials such as chloroquine and sulfadoxine pyrimethamine has posed major challenges to malaria control in sub Saharan Africa where the problem is most chronic. In response to the anti-malarial drug resistance situations, artemisinin-based combination therapy is the treatment of choice for uncomplicated *P. falciparum* malaria. In Ethiopia, malaria is a major health problem where climatic factors and altitude play roles to the malaria epidemics and drug resistance aggravates the problem.

Objective: The aim of the study was to determine the therapeutic efficacy of Artemether-Lumefantrine against *P. falciparum* malaria in Alamata District of Tigray Region, North Ethiopia.

Methods: During August to November, 2009 a prospective study was conducted on 73 subjects who were above six months of age and fulfilled the inclusion criteria set by WHO. Thick and thin blood smears was employed to determine the presence of parasitemia and parasite density. The standard six-dose regimen of Artemether-lumefantrine was administered over three days and was followed-up with clinical and parasitological evaluations over 28 days. Finally, WHO excel data analysis sheet and SPSS version 15 were used for data entry and analysis.

Results: Out of the 73 patients enrolled in the study, 71 were successfully followed and 2 cases were excluded during the 28 days follow up for different reasons. The cure rate was found to be high (97.2 %) in this study. The mean parasite and fever clearance time was 26.5 and 26 respectively.

Conclusions and Recommendations: This 97.2% cure rate and /or 2.8% failure rate result is encouraging that the drug could still continue as first-line drug for the treatment of uncomplicated *P. falciparum* malaria in the study area. However the efficacy of Artemether-lumefantrine needs to be carefully monitored periodically in sentinel sites representing different areas of the region and the country.

Key words: Malaria, *Plasmodium falciparum*, Artemether-lumefantrine, Alamata,

1. Introduction

1.1. Background

Malaria is one of the most important infectious diseases in the world, causing hundreds of millions of illness and more than one million deaths each year. It is the main public health problem world- wide and occurs in more than 90 countries, inhabited by a total of about 2.4 billion people representing 40% of the global population (Hastings, 2003; Rosenthal, 2008). The greatest burden of malaria in Africa occurs in endemic areas, where the parasite is continuously present in the community. Where control measures are inadequate, the distribution of the disease is closely linked to seasonal patterns of climate and the local environment. Those most at risk from endemic malaria are very young who have not acquired immunity and pregnant women whose immunity is reduced during pregnancy. In contrast, epidemic malaria occurs where the exposure of the population is infrequent. Since immunity is low all age groups are vulnerable and fatality rates could be high (Gebreyesus *et al*., 2009).

There is a steady increase of drug-resistant *Plasmodium falciparum* malaria across many tropical areas (Makanga *et al.*, 2006). The emergence and spread of *Plasmodium falciparum* resistance to commonly used anti-malarials such as chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) has posed major challenges to malaria control programe in sub-Saharan Africa (Kabanywanyi *et al*., 2007). In response to the anti-malarial drug resistance situations, the World Health Organization (WHO) recommends that treatment policies for *falciparum* malaria in all countries experiencing resistance to mono-therapies should be combination therapies, preferably those containing an artemisinin derivative. It has been suggested that the wide spread use of artemisinin-based combination therapies (ACTs) could have a major impact on the treatment of malaria (Kabanywanyi *et al.*, 2007).

In Ethiopia, malaria is a major health problem. Climatic factors and altitude make the country prone to epidemics (WHO, 1999). It has been consistently reported as one of the three leading causes of morbidity and mortality in the past years. The magnitude of the problem in 2002/2003 has even worsened and mortality accounting for 15.5% outpatient consultations, 20.4% admissions and 27% inpatient deaths (FMOH, 2004). According to the Federal Ministry

of Health, about 68% of the population in Ethiopia is at risk of malaria, representing approximately 52 million people in 2007. Malaria is seasonal in most parts of Ethiopia, with unstable malaria transmission, rendering the country prone to epidemics. The transmission patterns and intensity vary greatly due to the large diversity in altitude, rainfall, and population movement; areas below 2000 meters are considered to be malarious or potentially malarious (FMOH, 2008).

In non-epidemic year, 5-6 million clinical malaria cases and over 600,000 confirmed cases were reported from health facilities (FMOH, 2004). However, as the potential health service coverage is accessible to about 61% of the population and due to the low service utilization rate, the number of malaria cases reported by health facilities is only a portion of the actual magnitude. *P. falciparum* and *P. vivax* are the two dominant parasite species with relative frequency of 60% and 40% respectively. This proportion varies from place to place and from season to season. In malaria epidemic situations, *P. falciparum* is the dominant parasite species that causes severe manifestations and almost all malaria death happen due to infection by this parasite (FMOH, 2004).

The biological diversity of *P. falciparum* and its ability to develop resistance to a number of anti-malarial drugs has been a major challenge in malaria chemotherapy. Treatment failure of chloroquine was indicated in an *in vivo* study conducted at Humrera in 1993 (Wezam, 1993). The high treatment failure rates of chloroquine for the treatment of uncomplicated *falciparum* malaria as documented through a nationwide study conducted in 1997/98, led to a treatment policy change that recommends the use of SP as first line drug for the treatment of uncomplicated *falciparum* malaria and CQ for the treatment of v*ivax* malaria (FMOH, 2004).
At the time of introduction of SP as first line drug, the level of treatment failure observed was about 5% probably due to the earlier and informal distribution of the drug in private pharmacy. A nationwide study on the therapeutic efficacy of SP for the treatment of uncomplicated *falciparum* malaria was conducted in 11 sentinel sites from October- December 2003. Results obtained from the study showed a mean treatment failure rate of 35.9 % (range; 26.7-53.4%) on the 14 days follow up and 71.8 % (range; 53.8-85.7) on the 28 days follow up. *In vivo* therapeutic efficacy and safety baseline study on Artemether-lumefantrine was also conducted

in 4 sites by enrolling 213 subjects and after a follow up period of 14 days; no treatment failure cases and drug side effects were reported (FMOH, 2004). Large scale-up of malaria control interventions, especially distribution of long-lasting insecticide nets (LLINS) and nationwide deployment of Artemether-Lumefantrine are being implemented since 2005 (FMOH, 2004).

Tigray is the northern most region of Ethiopia with 80,000 km^2 that is inhabited by 81% rural (Barnes *et al*., 2009) and 4.5 million populations (WHO, 2009). About 75 % of the Region is malarious and an estimated 56% of the total population live in malarious areas. The transmission pattern of malaria in Tigray is unstable and the community at large has no immunity to infection. Intensive construction of microdams and wells aggravates the transmission of malaria and has the potential for adverse effects on health (Alemayehu *et al.,* 1998). The average temperature ranges from about 22^0C in areas below 2400m above sea level to less than 16^0C at higher altitudes, where the average temperature in most of the low land areas is about 27^0C (WHO, 2009). As in the rest of the country, *Plasmodium falciparum* is the dominant parasite and *Anopheles arabiensis* is the major malaria vector in the region (WHO, 1999).

As a result of this, the disease is one of the leading causes of illness, hospital admissions and death in Tigray Region. The 1999, 2000 and 2001 E.C. regional report indicates that of the total admissions about 17.2%, 13.36%, and 14.96 % were malaria cases respectively. Of the total death 16.67%, 9.2%, and 7.45% respectively were malaria cases. To avert the impact of the disease on socio-economic development endeavours, organised malaria control activities has been implemented in the region (WHO, 1999). The primary objective of malaria control in the region is to reduce morbidity and prevent malaria related mortality. The major components of the malaria control strategy are provision of early diagnosis and prompt treatment services and implementing selective and sustainable preventive activities.

Emerging biological phenomenon related to parasite drug resistance is challenging the success of anti-malarial treatments. Parasite drug resistance, mainly resistance to anti-malarials by *Plasmodium falciparum* has been increasing ever since. *P falciparum* already resistant to CQ and SP. The first line drug since 2005 is Artemether Lumefantrine (FMOH, 2004). Although the efficacy of Artemether-lumefantrine was 100% as indicated in the *in vivo* baseline study

conducted in 2003, assessing its efficacy status after 4-5 years of deployment in the area will be helpful for monitoring of the drug efficacy. Therefore the objective of this study was to assess the clinical and parasitological therapeutic efficacy of Artemether-lumefantrine (Coartem) against *P. falciparum* malaria in Alamata District, Tigray Region, North Ethiopia.

1.2. Literature review

Malaria is caused by obligate intra-erythrocytic protozoa of the genus *Plasmodium* (Trampuz *et al.*, 2003) and among the four *Plasmodium* species, that cause malaria in humans, *Plasmodium falciparum* is the most virulent (Greenwood *et al*., 2008). The genus *Plasmodium* is characterized by the type of the asexual multiplication (shizogony) in the vertebrate host and sexual multiplication (sporogony) in the mosquito host ((Bruce-Chwatt, 1991). The female anopheles mosquito is responsible for the spread of all malaria parasites (Clark *et al.*, 2004).

1.2.1. Plasmodium life cycle

Female *Anopheles* mosquitoes first ingest the malaria parasite by feeding on an infected human carrier and the infected mosquito carry plasmodium sporozoites in their salivary glands. Once ingested the parasite gametocytes taken up in the blood will further differentiate in to male or female gametes and then fuse in the mosquito gut (Bruce-Chwatt, 1991). After maturation the gametocytes form gametes and the fusion of the male and female gametes form the zygote (Trampuz *et al.*, 2003). With in 12-24 hours the resulting zygote develops into an ookinete which penetrate and encysts in the mosquito's gut wall (Greenwood *et al*., 2008; Rosenthal, 2008). The ookinete is a motile invasive stage. After reaching the extracellular space between the epithelial cells and the basal lamina, the ookinete develops into an oocyst. The oocyst undergoes an asexual replication, called sporogony, which culminates in the production of several thousands of sporozoites. This generally takes 10-28 days depending on species and temperature (Greenwood *et al*., 2008).

When the mosquito feeds on blood after piercing the human skin, the sporozoites are injected with the saliva and human infection is initiated during mosquito feeding (Bruce-Chwatt, 1991). The sporozoites enter the circulatory system carried via the blood stream to the liver and with in 30-60 minutes will invade a liver cell and begin a period of asexual reproduction (Trampuz

et al., 2003). This replicative stage is often called exoerythrocytic (pre-erythrocytic) schizogony. By budding or segmentation a progeny called merozoites, are released in to the circulatory system following rupture of the host hepatocyte (Greenwood *et al*., 2008). In *P. vivax and P. ovale* some of the sporozoites do not immediately undergo asexual replication, but enter a dormant phase known as the hypnozoite. This hypnozoite can reactivate and undergo schizogony at later time resulting in relapse (Greenwood *et al.*, 2008).

Merozoites released from the infected liver cells invade erythrocytes. The merozoites recognize specific proteins on the surface of the erythrocyte and actively invade the cell. The merozoite enters the cell by five stages: (a) Initial recognition and attachment, (b) formation of a junction, (c) creation of a vacuole membrane continuous with the red cell membrane, (d) entry into the vacuole through the moving junction, and (e) sealing of the erythrocyte after entry (Bruce-Chwatt, 1991). After entering the erythrocyte the parasite undergoes a trophic period followed by an asexual replication. The young trophozoite is often called a ring form due to its morphology in stained smear. As the parasite increases in size this 'ring' morphology disappears and it is called a trophozoite. During the trophic period the parasite ingests the host cell cytoplasm and breaks down the hemoglobin into amino acids. A by product of the hemoglobin digestion is the malaria pigment, or hemozoin (Greenwood *et al.*, 2008).

Nuclear division marks the end of the trophozoite stage and the beginning of the schizont stage. Erythrocytic schizogony consists of 3-5 rounds (depending on species) of nuclear replication followed by a budding process. The host erythrocyte ruptures and releases the merozoites. Symptoms of malaria typically occur at the time of schizont rupture, when parasite toxins act on host cells to release cytokines, such as tumour necrosis factor (TNF) (Greenwood et al., 2008; Trampuz *et al.*, 2003). These merozoites invade new erythrocytes and initiate another round of schizogony. As an alternative to schizogony some of the parasites will undergo a sexual cycle and terminally differentiate into either micro- or macrogametocytes. Gametogenesis, or the formation of micro and macro gametes, is induced when the gametocytes are ingested by a mosquito (Bruce-Chwatt, 1991).

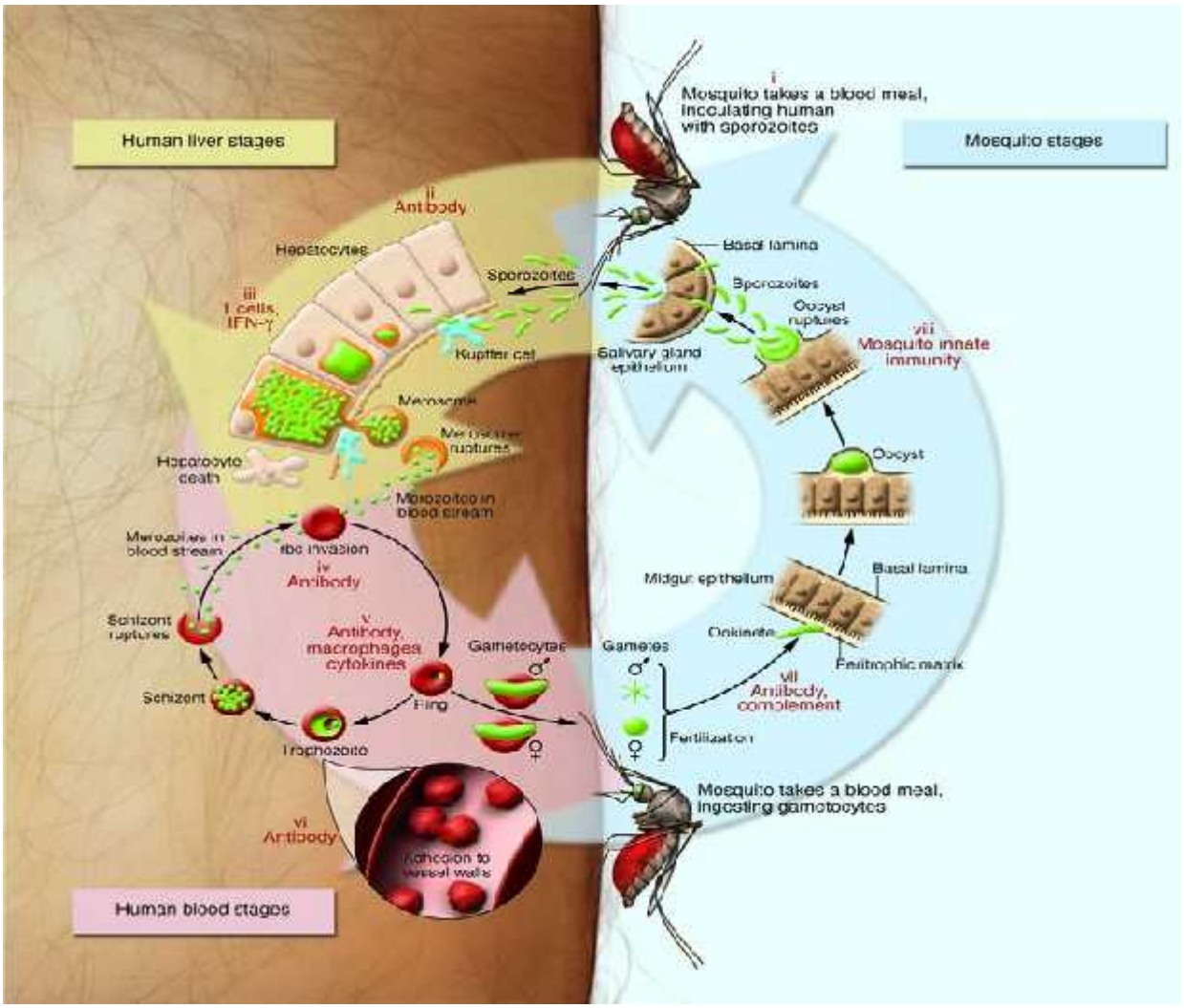


Figure 1. The life cycle of plasmodium species (Adapted from Greenwood *et al*., 2008)

1.2.2. Pathophysiology

The invasion of red blood cells which follows the pre-erythrocytic phase of the life cycle is the basic pathological process in malaria infection. Pathology associated with all malaria species is related to the rupture of infected erythrocytes and the release of parasite material and metabolites, hemozoin (malaria pigment). Several severe complications can be associated with *falciparum* malaria, with cerebral malaria being the most notable and a frequent cause of death (Bruce-Chwatt, 1991). The neurological manifestations such as impaired consciousness, severe headache, convulsions etc., are believed to be due to the sequestration of the infected erythrocytes in the cerebral microvasculature. This sequestration provides several advantages for the parasite. The major advantage is the avoidance of the spleen and the subsequent elimination of infected erythrocytes. The slower blood flow and low oxygen tension provides a favorable environment for further parasite development (Greenwood *et al.,* 2008; Trampuz *et al*., 2003).

Adherence of trophozoite and schizont-infected erythrocytes in target organs appears to be a major feature of the pathophysiology of *P. falciparum* malaria (Rosenthal, 2008). Knobs are expressed during the trophozoite and schizont stage and are formed as a result of parasite proteins exported to the erythrocyte membrane. Among human P*lasmodium* species, knobs are restricted to *P. falciparum* and thus suggest that the knobs play a role in cytoadherence (Greenwood *et al*., 2008). The parasite also alters the RBC membrane by changing its transport properties, exposing cryptic surface antigens, and inserting new parasite derived proteins. The RBC becomes more irregular in shape, more antigenic, and less deformable (Trampuz *et al.*, 2003).

The molecular mechanism of cytoadherence involves receptor-ligand interactions. In other words, proteins expressed on the surface of the infected erythrocyte (ligand) will bind to proteins expressed on the surface of the endothelial cells (receptor). PfEMP-1 (erythrocyte membrane protein) is a parasite protein, which has been implicated as the cytoadherence ligand. In contrast to the usually highly conserved nature of receptor-ligand interactions, pfEMP-1 is a member of a highly variable (var) gene family with different genes (Baruch *et*

al., 1996). The expression of different pfMP-1 gene is correlated with different receptor-binding phenotypes. This antigenic variation associated with the surface exposed PfMP-1 allows the parasite to evade the immune system. However, the cytoadherence function is preserved through its ability to recognize multiple receptors. This antigenic variation may also account for different disease outcomes (Smith *et al*., 2000). Several vascular receptors have been identified, of which intercellular adhesion molecule1 is probably the most important in the brain, chondroitin sulfate B in the placenta, and CD36 in most other organs (Greenwood *et al.*, 2008).

Initially it was assumed that the cytoadherence would lead to a mechanical blockage (i.e., cerebral ischemia) and subsequently hypoxia. In addition, the parasite could also cause localized metabolic effects such as hypoglycemia and/or lactic acidosis. The hypoxia and metabolic effects would then cause the coma and subsequent death (Baruch *et al*., 1996). Other suggestion is that the coma is mediated by short-lived molecules (cytokines, nitric oxide) that affect cerebral function. In this cytokine theory, malarial antigens would stimulate TNF-alpha which could then induce nitric oxide or have other pathological effects. Nitric oxide is known to affect neuronal function and it could also lead to intracranial hypertension through its vasodilator activity (Allan *et al.*, 1995).

The sequestration hypothesis and cytokine theory for the pathophysiology of cerebral malaria are not mutually exclusive, and both phenomenon are likely to be involved. For example, parasite exo-antigenes, which are released at erythrocyte rupture, are known to stimulate macrophages to secret TNF-alpha. This cytokine is known to up regulate the expression of adhesion molecules such as ICAM-1 on the surface of brain endothelial cells (Clark *et al.*, 2004). This would lead to increase binding of infected erythrocytes and amplify the effects whether they are due to vascular blockage, soluble mediators, metabolic effects, or a combination.

At the cellular level, it is probable that at least two primary events underlie the pathology of cerebral malaria: the sequestration of shcizonts in small cerebral blood vessels and shcizont rupture. Among the debris released by rupturing shcizonts are toxins that stimulate monocytes

and macrophages to produce tumor necrosis factor (TNF) and related cytokines. Circulating TNF levels are correlated with disease severity in childhood cerebral malaria (CM) (Allan *et al.,* 1995). Some studies indicate that TNF may contribute to pathogenesis of CM in several ways: TNF produced in response to shcizont rupture may exacerbate parasite sequestration by stimulating the endothelium to express parasite binding receptors such as ICAM-1. It has also been proposed that TNF generated with in the cerebral vasculature may lead to coma by stimulating the endothelium to release high levels of nitric oxide, which diffuses into the surrounding brain and disturbs neurotransmission (Clark *et al.,* 2004).

1.2.3. Epidemiology

Malaria is primarily a disease of the tropics and subtropics & is wide spread in hot humid regions of Africa, Asia and South & Central America (Greenwood *et al.,* 2008). According to world health organization estimates, between 300 million and 500 million people are infected with malaria every year. More than 90% of all malaria cases are in sub-Saharan Africa. Mortality due to malaria is in the range of 1.5 million to 2.7 million deaths annually. Death occurs mostly among young children in Africa, especially in remote rural areas with poor access to health services. Nearly 85% of malaria is caused by *P. falciparum* and is also responsible for about 90% of the deaths from malaria (Lou *et al.,* 2001).

Malaria is caused by protozoan parasites that belong to the genus *Plasmodium*. Under this genus, there are several species that cause the infection in a multitude of vertebrates. But in man, only 4 species of *Plasmodium* are responsible for malaria: *P. falciparum, P. vivax, P. ovalae, and P. malariae.* The disease is complex in epidemiological pattern and distribution including the vector involved. This is due to the fact that the human Plasmodium species also differ in geographical distribution, in the type of clinical manifestation they cause, level of virulence, potential to drug resistance development, genetic variation, and other factors (Greenwood *et al.,* 2008).

Man is the only source of *falciparum* malaria. Infected man suffering from acute and chronic malaria and carriers harboring gametocytes are the reservoirs of malaria (Bruce-Chwatt, 1991). The parasite's definitive hosts and transmission vectors are female mosquitoes of the anopheles

genus (Rosenthal, 2008). Malaria is transmitted by the bite of female anopheles mosquitoes during their blood meal at night. Anopheles mosquitoes comprise several species depending on the geographical settings. Based on the type of vector involved in the transmission, the potential of the vector in transmission of the disease differ between regions and within a region where malaria is endemic (Barnes, White, 2005).

The epidemiology of malaria can be viewed in terms of being stable (endemic) or unstable (epidemic). Stable malaria refers to a situation in which there is a measurable incidence of natural transmission over several years. This would also include areas, which experience seasonal transmission. Different areas can experience different levels of incidence rates and this is often denoted by hypo endemic, meso-endemic, hyper endemic and holo-endemic (Greenwood *et al.,* 2008). In areas where transmission is low full protective immunity is not acquired, and symptomatic disease may occur at all ages. This situation usually exists in hypo endemic areas and is termed unstable transmission). Persons living in highly endemic areas usually exhibit a high level immunity and tolerate the infection well (Trampuz *et al*., 2003).

The intricate interactions between host, parasite and vector are the major factors for epidemiological complexity. Malaria infections are characterized by an initial acute phase followed by a longer relatively asymptomatic chronic phase. This is due in part to the ability of the parasite to avoid complete clearance by the immune system. For example *Plasmodium falciparum* exhibits an antigenic variation that allows it to stay one step a head of the immune system. In addition, *P. vivax and P. ovale* exhibit the hypnozoite stage and are capable of relapses. This allows the parasite to maintain the infection with in the human host even after the blood stage of the infection has been cleared (Greenwood *et al.,* 2008).

In regards to the host, the immune status of the individual and their prior experience with malaria will influence the course of the infection. Pregnant women, especially during the first pregnancy, are more susceptible to falciparum malaria as illustrated by a higher prevalence of infection and higher parasitemmias (Bruce-Chwatt, 1991).

The potential of the mosquito to serve as a vector depends on the ability to support sporogony, mosquito abundance, and contact with humans, which are all influenced by climatic and ecological factors. The ability to support sporogony is largely dependent upon species in that not all species of *Anopheles* are susceptible to *Plasmodium* infection. Temperature and mosquito longevity are other key factors affect in the parasites interaction with the vector. In general, at temperatures less than 16-18^{o}C, sporogony is not completed and transmission does not occur (Greenwood et al., 2008; Trampuz et al., 2003). Development of *Plasmodium falciparum* requires a minimum temperature of 20^{o}C; where as the minimum temperature for the other species is 16^{o}C.

Temperature also affects the time of development in that the duration of sporogony is substantially shorter at higher temperatures. Mosquito density and feeding habits also influence the transmission of malaria. Mosquito density is affected by temperature, altitude, rainfall and the availability of breeding places, whereas human-mosquito contact will be influenced by the mosquito behavior (Greenwood *et al*., 2008).

1.2.4. Diagnosis

Diagnosis of malaria requires a high degree of clinical suspicion (Bruce-chwatt, 1991). Malaria must be recognized promptly in order to teat the patient in time and to prevent further spread of the disease. Delay in diagnosis and treatment is the cause of death in malaria patients (Clark *et al.,* 2004).

1.2.4.1. Clinical diagnosis: is based on the patient's symptoms and on physical findings at examination. The first symptoms of malaria (most often fever, chills, sweats, headaches, muscle pains nausea and vomiting) are not specific and are also found in other diseases such as the "flu" and common viral infections. Likewise, the physical findings are often not specific (elevated temperature, perspiration, tiredness) (Bruce-chwatt, 1991).

1.2.4.2. Microscopic diagnosis: The diagnosis of malaria is usually made by the examination of Geimsa-stained thick and thin blood smears for interaerythrocytic ring stage parasite using an oil immersion lens. This technique remains the golden standard for laboratory confirmation of malaria (Trampuz *et al.,* 2003)

1.2.4.3. Antigen detection: Rapid diagnostic tests (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available. Rapid diagnostic antigen tests using monoclonal antibodies to the *P. falciparum* histidine rich protein-2 have been shown to be highly sensitive & reliable (Trampuz *et al.,* 2003). Some of these tests carry a second antibody, which allows *falciparum* malaria to be distinguished from the less dangerous malarias. PfHRP2 based tests may remain positive for several weeks after acute infections. This feature is a disadvantage in high transmission areas where infections are frequent but is of value in the diagnosis of severe malaria in patients who have taken antimalarial drugs and cleared peripheral parasitemia (Greenwood et al., 2008).

1.2.4.4. Molecular diagnosis: Parasite nucleic acids are detected using polymerase chain reaction (PCR). This technique is more accurate than microscopy. However, it is expensive and requires a specialized laboratory (Bruce-Chwatt, 1991). PCR techniques could have application with low parasitemia, possible mixed infections or uncertain parasite speciation, as well as for reference studies and micro-epidemiology or as research tools for detection of low parasitemia in sophisticated laboratories. Serology detects antibodies against malaria parasite, using immunofluorescence (IFA), indirect haemagglutination (HA) and enzyme linked immunosorbent assay (ELISA). These are inappropriate for use in the diagnosis of acute malaria, as they reflect exposure rather than acute infections (Trampuz *et al.,* 2003).

1.2.5. Chemotherapy and anti-malarial drugs

Several anti-malarial drugs are available. Many factors are involved in deciding the best treatment for malaria. These factors include the parasite species, the severity of disease, the patient's age and immune status, the parasite's susceptibility to the drugs and the cost and availability of drugs (Greenwood *et al.*, 2008). Anti-malarial drugs may be generally divided into causal prophylactic drugs which prevent the establishment of the parasite in the liver and schizontocidal drugs which attack the parasite in the red blood cell, preventing or terminating the clinical attack (Bruce-Chwatt, 1991).

Fast acting blood schizonocides, such as quinine, chloroquine, artemisinin derivatives etc., which act up on the blood stage of the parasite, are used to treat acute infections and to quickly relieve the clinical symptoms (Trampuz *et al.,* 2003). Tissue-stage schizonticides for example artemisinin derivatives and premaquine kill the asexual stages developing in the liver, including liver schizonts (all species) and quiescent hypnozoites (*P. vivax* and *P. ovale*), thus preventing primary or secondary attacks (relapses) of clinical malaria. Blood stage schizonticides interrupt asexual schizogony (mitotic division) in red cells, preventing or terminating clinical attacks of malaria. Gametocytocides such as premaquine and artemisinin derivatives kill or sterilize sexual stages in the blood, thus preventing infection of mosquitoes and transmission of the disease (Baird, 2005). Chloroquine is generally the recommended treatment for patients with *P. vivax, P. ovale, P. malariae*, and uncomplicated chloroquine sensitive *P.falciparum* infections (Greenwood *et al.,* 2008). Chloroquine resistant strain may be sensitive to sulfadoxine-pyrimethamine in some areas. Where there is resistance to SP, quinine, mafloquine, Artemether lumefantrine are alternative drugs (Rosenthal, 2008).

1.2.5.1 Artemisinin-based combination therapy

Multidrug resistance has rendered monotherapy for malaria useless in most parts of the world, and has also compromised the usefulness of many of the available combination chemotherapies (Kremsner and Krishna, 2004). Due to wide spread resistance of *P. falciparum* to drugs such as chloroquine and sulfadoxine-pyrimethamine, artemisinin combination therapy (ACTs) is currently advocated in Africa as a means of improving treatment efficacy and slowing the spread of resistance (Dokomajilar *et al.,* 2006) Currently, the WHO recommended the use of combination therapy to provide effective treatment against malaria and to slowdown the spread of drug resistance. The recommendation was particularly in favor of an Artemisinin based combination therapy (Barnes and White, 2005).

Under low transmission, ACTs have a public health benefit of reducing malaria transmission due to their rapid parasite clearance time; treating early cases of uncomplicated malaria with ACTs may prevent its progression to severe disease with consequent reduction in severe case and malaria mortality rate (Mutabingwa, 2005; Dondrop *et al.,* 2009). The effect of

combination therapy is enhancing by the inclusion of an artemisinin derivative. Artemisinin antimalarials decrease parasite density more rapidly than other anti-malarial drug when used alone, the short half-life of the artemisisnin derivatives minimizes the period of parasite exposure to sub-therapuetic blood levels (Boland *et al.,* 2000).

The selection and spread of resistant parasites can be limited by the widespread use of artemisinin-based combination therapy that delays the spread of antimalarial resistance. Wide-scale use of artesunate plus mefloquine decreased mefloquine resistance in northwest Thailand. This is explained both by the advantage of using two drugs with different mechanisms of action, preventing further selection of resistance and resulting in higher cure rates, and by the effect of artesunate in reducing gametocyte carriage, even in mefloquine resistant infections (Barnes and White, 2005).

Artemether-lumefantrine is a fixed dose combination of Artemether (20mg) and Lumefantrine (120mg). Artemether will reduce parasitaemia, giving symptomatic relief, and Lumefantrine will eliminate residual parasites. As the parasites are never exposed to Artemether alone (because of its rapid elimination), this is thought to minimize the development of resistance (Shanks, 2006). This combination combines the benefits of a rapid schizonticidal effect of artemether with a slow but longer acting schizonticidal effect of lumefantrine.

Artemisinin-based combination therapies tested in Sudan were found to be highly efficacious in the treatment of uncomplicated *P. falciparum* malaria. The ACTs tested also had an effect on gametocytes. In general, the gametocidal action of Artisunate (AS) appears to work through preventing the development of new gametocytes rather than clearance of existing ones (Vandenbroek *et al*., 2005). Decreased gametocyte carriage following artemether-lumefantrine treatment has been shown to limit post-treatment transmission of *P. falciparum* to *Anopheles* mosquitoes (Barnes and White J, 2005; Mkulama *et al.,* 2008).

1.3 Anti-malarial drugs resistance

The emergence and spread of *Plasmodium falciparum* resistance to commonly used anti-malarials, such as chloroquine and sulphadoxine- pyrimethamine poses major challenges to

malaria control in malaria endemic setting across the world especially in sub-Saharan Africa (Barnes and White, 2005).

The term" parasite resistance" has been used with different meanings. It has been defined as "the ability of a parasite strain to survive and /or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but with in the limits of tolerance of the subject. This definition was subsequently modified to specify that" the drug must gain access to the parasite or the infected red blood cells for the duration of the time necessary for the normal action of the drug (WHO, 2005).

1.3.1 Factors contributing to development and spread of drug resistance

Observed treatment failure may result from either actual drug resistance or sub-therapeutic levels. If treatment failure is not a direct result of resistance, then exposure of organisms to suboptimal levels of drug will certainly contribute to developing resistance. Systemic drug levels are influenced by metabolism, absorbance, compliance or drug quality. For example, age, genetics, drug interactions, diet and disease state affect metabolism and absorption rates, while high drug costs, inconvenient dosing regimen and side effects have an adverse influence on compliance (Green, 2006 and Baird, 2005). In self treatment, individuals may only take the drug until symptoms clear or will take lower doses to save money. In poor compliance patients may not complete the full course of treatment because of drug side effects. The widespread (mass administration) use of a drug in an area of intense transmission increases drug pressure by exposing a larger parasite population to the drug. Drugs that are slowly eliminated (long drug half-life) will lead to a longer exposure of the parasite to sub therapeutic drug concentrations. In high levels of malaria transmission there could be re-infection while drugs are at sub-therapeutic levels (Greenwood *et al.,* 2008).

Generally, there are factors contributing to drug resistance. A combination of drug pressure and drug misuse can trigger the development of drug resistance. Vector control can, by reducing transmission, have an impact on each of these if it reduces the size of the circulating gene pool. A smaller gene pool will result in lower drug pressure and less drug misuse, because fewer disease episodes will occur. Conditions particularly conducive to the development of

drug resistance include: a limited range of drug treatment options, a history of high drug pressure, high levels of population transience/migration, substantial under dosing, availability of fake or substandard anti-malarial, inappropriate drug-prescribing practices and wide spread failure to complete the prescribed treatment course (Molyneux *et al.*, 1999).

1.3.2. Genetic markers of resistance

Resistance emerges de-novo through spontaneous mutations or gene duplications, which are thought to be independent of drug selection pressure, but these mutants, are then selected for and spread as a result of the drug pressure which provides a selective advantage to resistant parasites.

There are some genes that appear to play a role in the regulation of resistance to the principal chemical families of anti-malarials (WHO, 2005). These are: *pf*crt (*P. falciparum* Chloroquine transporter protein), *pf*mdr1 (*P. falciparum* multidrug resistance), *dhfr* (dihydrofolate reductase), *dhps* (dihydropetraote synthatase), and Cytb (cytochrome b). Briefly, Cytb is a molecular marker of resistance to Atovaquine, an anti-malarial drug. The mode of action of the drug is by interfering with the electron transport controlled by Cytb gene. Hence, a mutation in Cytb gene leads to Atovaquine resistance (WHO, 2005). The pfmdr1 and pfATPase6 are thought to be associated with the resistance of Artemisinin derivatives.

A). Plasmodium falciparum multidrug resistance gene1 (pfmdr1)

The cheap and widely available first line drug chloroquine has become largely ineffective to treat *falciparum* malaria. A homolog of the major multi-drug transporter in mammalian cells was identified, *Plasmodium falciparum* multi-drug resistance protein1 (pfmdr1), also known as the p-glycoprotein homolog1 (pgh-1) (Duraisingh and Cowman, 2005). Multidrug resistance of *Plasmodium falciparum* is spreading throughout Asia and is impeding efforts to control malaria (Kabanywanyi *et al.*, 2007).

Level of resistance is also modulated by another transporter encoded by the pfmdr1 gene on chromosome 5 of the parasite. An allele encoding tyrosine at codon 86 of the pfmdr1 was associated with treatment failure (Conway, 2007). A strong association has been observed

between possession of the wild type form of pfmdr1, amplification of pfmdr1 and resistance to hydrophobic drugs such as the arylaminalcohol mefloquine and the endoperoxide Artemisinin derivatives in field isolates. The aryl aminalcohol and endo peroxide drugs are structurally unrelated drugs and this resistance resembles true multi-drug resistance (Duraisingh and Cowman, 2005; Kabanywanyi *et al.,* 2007).

In addition, point mutations in *Plasmodium falciparum* multidrug resistance gene1 (pfmdr1) such as N86Y, Y183F, S1034C, N1042D, and D1246Y have been shown to modulate CQ resistance and possibly Lumefantrine resistance (Talisuna *et al.,* 2004).

B) Targets for Artemisinins

Combinations of chemotherapeutic agents are generally used to accelerate therapeutic response, improve cure rates, and protect the component drugs against resistance. Artemether-lumefantrine has been given priority as a first line artemisinin-based combination therapy (ACT) recommended by the WHO for the treatment of uncomplicated *P. falciparum* malaria (Kabanywanyi *et al.,* 2007). However, artemisinin resistance has been reported and efficacy of such therapies has declined on the Thailand-Cambodian border, historically a site of emerging antimalarial-drug resitance (Dondrop *et al.,* 2009).

The pfmdr1 alleles 86N, 184F, and 1246d significantly increased in prevalence after treatment with Artemether-lumefantrine (AL). Treatment with AL selects for polymorphisms that may alter antimalarial drug response (Dokomajilar *et al.,* 2006). Several individual molecules have been proposed as targets for artemisinin. Some suggest that artemisinin inhibit the parasite encoded sarco-endoplasmic reticulum Ca2+-ATPase (SERCA). PfATPase6 has gained support from recent observations that a polymorphism in the gene encoding PfATPase6 is associated with invitro resistance to Artemether infield isolates of *P. falciparum* (Krishna *et al.,* 2006). DNA sequencing in a subsample of 60 isolates lends support to SERCA-pfATPase6 as the target for artemisinins (Jambou *et al.,* 2005). More over, the long-term usefulness of ACTs in high transmission areas remains unclear and it has been suggested that documentation of the

S769N PfATPase6 mutations may be used as an emergence of artemisinin resistance of *Plasmodium falciparum* in field isolates (Jambou R *et al.,* 2005).

1.3.3 Therapeutic Efficacy and Treatment Failures of Anti-malarial Drugs

Malaria related morbidity and mortality has been increasing in Sub-Saharan Africa, primarily as a result of increased resistance to the commonly used first line treatments, chloroquine and sulphadoxine-pyrimethamine (Muheki *et al.,* 2004). Anti-malarial drug resistance is a severe and growing problem in Africa (Zongo *et al*., 2007). In response to the anti-malarial drug resistance situations, the WHO recommends that treatment policies for *falciparum malaria* in all countries experiencing resistance to monotherapies should deploy combination therapy; preferably those containing an artemisinin derivative. It has been suggested that the wide spread use of artemisinin-based combination therapies (ACT) could have a major impact on malaria (Makanga *et al*., 2006). Artemisinin enhances efficacy and has the potential to lower the rate at which resistances emerges and spreads. Artemether-lumefantrine, a tablet formulation of the anti-malarial compounds artemether and lumefantrine has been approved in a large number of countries. It is a well tolerated, fast-acting, and effective blood schizontocidal drug used primarily in the treatment of uncomplicated *falciparum* malaria that is resistant to other anti-malarial drugs (Mizuno *et al.,* 2009).

Artemether- lumefantrine (CoArtem) is a new oral anti-malarial drug that consists of a fixed-dose combination of artemether & lumefantrine given in a six-dose regimen over a three-day period. Treatment with four dose AL has demonstrated 28 day cure rates of more than 95% in patients from China, Africa and India with known immunity to malaria or with non-drug resistant infections, but gave inferior cure rates (approximately 85%) in Thailand compared with treatment of mefloquine plus artesunate, with Adequate Clinical and parasitological Response (ACPR) 97.7% (Lefevre *et al.,* 2001), although at the beginning it was indicated more than 97% (Vugt *et al.,* 2000).

In 2005 a study conducted in Eastern Sudan to investigate the efficacy of AL suspension for the treatment of uncomplicated *P. falciparum* malaria in children, aged 6-59 months. Here treatment with AL rapidly cleared parasitaemia and fever. The overall 28 days cure rate was 100% and no clinical or parasitological failures were observed (Salah et al., 2006). A 42 follow

up study was undertaken to assess the efficacy of CoArtem in South Africa. The study did not prove treatment failure with the use of CoArtem for *Plasmodium falciparum* malaria (Vanghan-williams, *et al*., 2004).

In Ethiopia, effective treatment is essential for malaria control. However, drug resistant malaria has become a challenge in malaria control programs in recent years (Woldearegai *et al*., 2005). A study conducted in 1991 in Humera, Northern Ethiopia on the susceptibility of *P. falciparum* to CQ and SP had shown no treatment failure with in 7 days of follow-up (Wezam, 1993). However, another study in 1998, intense resistance of *P. falciparum* to chloroquine necessitated a change to sulphadoxine-pyrimethamine (SP) as first line antimalarial drug in Ethiopia (Schunk *et al.,* 2006). Therapeutic efficacy study conducted in Jimma town in 2003 had indicated a treatment failure of SP in 14 days follow-up in children less than 15 year. Only 54.7% children had responded successfully both clinical and parasitological to SP treatment (Worku *et al*., 2005). Recent data had shown a high mean SP treatment failure rate (72%) in some areas. Consequently, a change to artemether -lumefantrine was suggested in 2004 (Schunk *et al*., 2006).

2. Statement of the Problem

Widespread resistance of malaria parasites to commonly available anti-malarial drugs has necessitated countries to review and deploy new anti-malarial drug policies to ensure effective case management (Kabanywanyi *et al*., 2007).

Malaria is one of the leading health problems in Ethiopia. In 2002/2003, the disease has been reported as the first cause of outpatient consultations, admission and inpatient deaths. Because of the unstable nature of malaria transmission, protective immunity of the population in general is low or absent, leaving the country at large prone to malaria epidemics (FMOH, 2004).

The effort to achieve radical cure has been challenged mainly by the ever-increasing failure of the therapeutic efficacy of various anti-malarial drugs in use. The previously effective drugs Chloroquine and Sulphadoxine-Pyrimethamine are no more effective to treat uncomplicated *P.*

falciparum. The new drug Artemether-lumefantrine is the recently deployed first line anti-malarial drug since 2005 (FMOH, 2004).

In Tigray, as in most parts of Ethiopia, Malaria is one of the leading causes of morbidity and mortality. Malaria transmission is seasonal and depends largely on rainfall and altitude. In the region and study area malaria transmission is unstable and due to this unstable nature of malaria transmission, the large majority of the population has no protective immunity against the disease. Likewise failure of the therapeutic efficacy of various anti-malarial drugs so far used was a challenge in the region (WHO, 1999).

The new drug seems effective to treat uncomplicated *P. falciparum* malaria. However, since 2004, no therapeutic efficacy assessment was undertaken. In addition, this costly drug is used to treat patients clinically based on sign and symptoms and using RDTs by health extension workers (FMOH, 2004).

Treatment of malaria based on sign and symptoms (especially during shortage of RDTs) may increase drug pressure in the population, which in turn aggravates drug resistance. On the other hand Artemether-lumefantrine is an efficacious drug to different parasites that specially shows anti-schistosomal properties (EI-Lakkany *et al.,* 2004). In the area there is more likely to find co-infected individuals, as the prevalence of *S. masoni* in the area in two surveys is 27% and 36.4% respectively (Tadesse *et al*., 2009). Although it is advantageous to treat many diseases using single drug, there could be development of drug resistance because of lowering of the drug concentration, which is acting on multiple parasites (EI-Lakkany *et al.,* 2004). Therefore, this study will give a clue about the status and may be helpful as base line data for further studies during monitoring the efficacy of the drug.

3. Significance of the study

The study is intended to describe the therapeutic efficacy of the anti-malarial drug Artemether-lumefantrine (AL) to *P. falciparum* in the study area, Alamata District, Tigray Region, North Ethiopia. Since the introduction of Coartem in the area, no efficacy study was conducted at

national level and the result obtained from this study will be used as basic information or baseline data on therapeutic efficacy status of the anti-malarial drug, AL to uncomplicated *P. falciparum* malaria for further monitoring of the drug.

4. Objectives and hypothesis

4.1 General objective

To assess the clinical and parasitological therapeutic efficacy of Artemether-lumefantrine (CoArtem) against *P. falciparum* malaria in Alamata District, Tigray Region, North Ethiopia

4.2 Specific objectives

- To determine the level of efficacy of Artemethe-lumefantrine for the treatment of uncomplicated *P. falciparum* malaria in Alamata.

- To assess the early and late therapeutic failure of Artemether-lumefantrine for the treatment of uncomplicated *P. falciparum* malaria in Alamata.

4.3. Hypothesis

The current Adequate Clinical and Parasitological Response of Artemether-lumefantrine is efficacious as when it was deployed in 2004 for 28 days follow up therapeutic *in vivo* study in Alamata District, Tigray.

5. Methods and materials

5.1. Study design:

A cross sectional, one arm and prospective study was implemented to evaluate the therapeutic efficacy of Artemether-lumefantrine (CoArtem) for treatment of uncomplicated *P. falciparum* malaria based on WHO protocol (WHO, 2003).

5.2 Study Area:

The study was conducted in Alamata district, Tigray National Regional State Northern Ethiopia between August and November 2009. It was conducted in Tumuga Health center, about 12 kilometers South of the Town, Alamata. The area (Figure 2) is located in the southern zone of the region and inhabited by an estimated population of 89,377 (WHO, 2009) in 12 sub-districts (locally referred to as “Tabias”) and in 64 villages (referred to as “kushets”). The altitude of the District ranges between 1,438 and 2,571 meters above sea level. Majority of the population (81%) live in altitudes less than 2,000 meters above sea level. The study area has two malaria transmission seasons, September to December (main transmission season) and may to June (minor transmission season). The 2000, and 2001 E.C. report of the District indicated that there were about 25.8%, & 29.8%, malaria cases respectively.

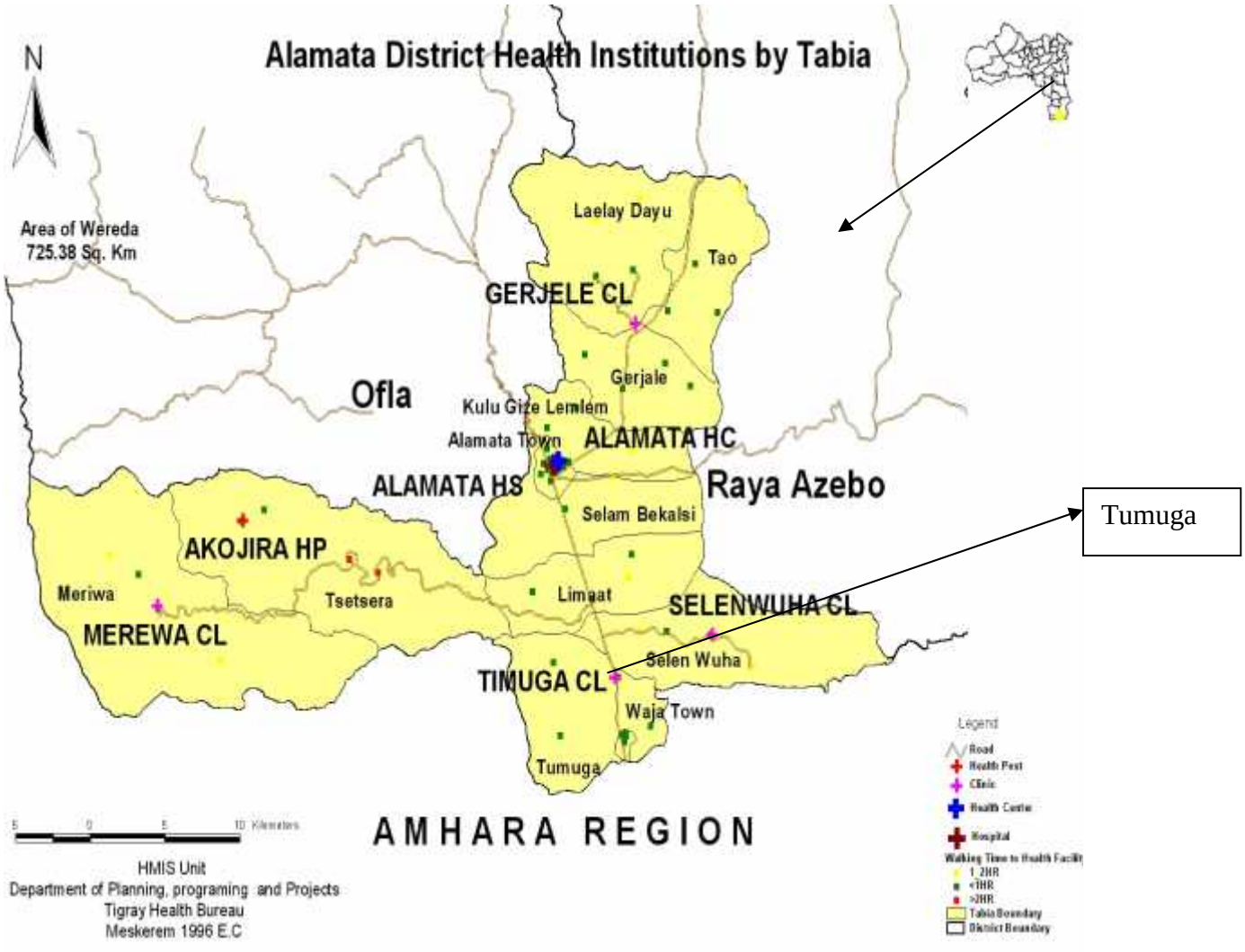


Figure 2 Map of the study area, Tumuga, Alamata District November 2009 (Adapted from TRHB, HMIS unit)

5.3. Source and Study Population: All the population in the study area was considered as source population for the study and those individuals age greater than 6 months were considered as target groups of the study population.

5.4. Sample Size and Sample Size Determination:

Based on WHO, 2003 standard protocol a classical statistics of sample size determination was used to obtain the proper sample size. Assuming the anticipated population proportion of clinical failure is 20% (because below 20% doesn't, required representative minimum sample size and 50% exaggerates the proportion of clinical failure and complicates management of follow up) with 95% confidence interval, and 10% precision 61 subjects were to be included in the study area. But, the sample size was adjusted and raised to 73, by 20% as a contingency for a loss of study subject during follows up.

5.5. Sampling Technique

The sampling technique was conducted using a convenient method as outlined in standard protocol procedure, on the assessment of therapeutic efficacy test for anti- malaria drugs, (WHO, 2003).

Patients were selected on the basis of certain criteria; in the *in vivo* efficacy study subjects were included with patients age above 6 months, those with a slide confirmed infection with *Plasmodium falciparum* only (i.e., no mixed infections), with the range of initial parasite density between 1,000 and 100,000 asexual parasites/micro liter, with absence of severe malnutrition and with absence of general severe signs. Other criteria were also used to select study subjects, such as those with auxiliary temperature greater or equal to 37.5^0C or history of fever during the previous 24 hours, ability to attend stipulated follow-up visits and easy access to the health facility, informed consent provided by patient /guardian, and absence of history of hypersensitivity reactions to the drug being evaluated.

Generally presence of one or more of the general danger signs or any sign of severe or complicated malaria causes exclusion from the study. In addition presence of severe malnutrition, presence of one or more of the mixed infection, pregnancy, significant concomitant febrile illness which would interfere with follow-up, chronic infectious diseases

other than malaria (e.g. Tuberculosis), history of allergy and/or intolerance to drug(s) being tested, and age below 6 months were other criteria for exclusion.

5.6. *In vivo* test Enrolment and Procedures

A rapid screening procedure was employed in an outpatient setting to identify patients who may meet enrollment criteria. This was done by identifying all patients with age above 6 months coming to health facilities. Measuring their auxiliary temperatures, and recording basic demographic information using enrolled patient forms (Annex-2) was employed in the study site. When measured temperature was >37.5^{o}C, blood was collected using blood film slip form (Annex-3) for malaria smear examination. Patients who did not meet these basic enrollment criteria were treated by staff of the health facility in accordance with routine practice (WHO, 2005).

For initial clinical evaluation/enrollment evaluation, all patients meeting the basic enrolment criteria during the screening procedure were evaluated in depth by clinical staff (health officer). Special care was taken to detect the presence of early signs of febrile diseases other than malaria, as these were probably necessitating exclusion of the patient from the evaluation. On enrolment day (day 0), a brief history of each patient was obtained. Each patient was then sent for laboratory examination to confirm malaria case and to identify if parasitaemia was adequate for his/her enrolment.

A case record form (Annex-4) was used to record the general information (Name, Age, Sex, Address, etc) and clinical observation for each patient passed from screening into the study. Particular care was taken to record detailed instructions on how to find the patient's home using location form (Annex-5) to ensure that follow-up at home was possible if the patient fails to return to the health facility for the scheduled visit.

Regarding informed consent, formal informed consent (Annex-6) was obtained from all patients meeting the enrollment criteria, following briefing on the objective and purpose of the study in verbal or/ and written form (Annex-6), to participate. The procedure for obtaining

formal consent was conforming to national and regional/ local guidelines for human research subjects.

Concerning treatment, *Plasmodium falciparum* positive cases were treated with 6 doses of CoArtem scaled by age/weight as outlined in the monogram of the producer. CoArtem was given as a tablet containing 20mg of Artemether and 120mg of Lumefantrine (Novartis, Geneva, Switzerland), Batch No F1309. All study participants were followed up for 28 days. Patients who fail to respond to the first line anti-malarial drug (CoArtem) were treated with Quinine. Moreover, treated patients were observed for at least 30 minutes to ensure that they don't vomit the medicine. When vomiting occurs within 30 minutes of treatment, the full treatment dose was repeated. The first dose was given on observation and the rest five doses were given to the patients to be taken at home under the supervision of trained community health workers. Moreover, recruited patients were advised to use ITNS regularly in order to minimize re-infection.

Duration of follow-up and schedule of the study subjects were based on the WHO 2003, *in vivo* study protocol. All study subjects were followed for the recommended minimum length of follow-up 28 days. Those who agreed to participate in the study had informed to come or bring participant (participant children) back during the scheduled visit for follow up on day 1, 2, 3, 7, 14, 21, 28. Participants who came on these scheduled visiting days were followed up for both clinical and parasitological assessment using follow up card (Annex-7). Moreover, they were informed to come back whenever there is a danger sign of severe illness in between scheduled visits. When the patient shows any danger sign of malaria (Annex-8), referral form (Annex-9) was used to send the patient to the health facility and the patient was obtaining further assessment and appropriate management. A tracer (nurse) had visited patients who did not turn up for scheduled visit at home on the same day.

5.7. Sample Blood collection and processing for microscopic analysis

Microscopy: Following the procedures outlined in Malaria Microscopy, Part 1 (WHO, 1991), finger prick blood sample collection, preparation, and staining of blood slides, using Giemsa staining at pH 7.2 were conducted for each patient. The blood films were prepared by a

laboratory technician before treatment on day 0 and on days 1, 2, 3, 7, 14, 21, and 28 including any other day that the patient was brought to the Health Center before the next scheduled visit.

Duplicate slides were prepared with both thin and thick film preparation, using conventional slides that can be labeled with a permanent ink or diamond pencil. One of the slides was used for rapid staining with 10% Giemsa stain for 10-15 minutes and reading while the patient was in attendance. The second slide was used for subsequent standard staining with 3% Giemsa stain for 30-45 minutes. This slide was used to determine parasite density.

The first blood smear for screening was examined for the presence of parasitaemia by counting the number of asexual parasites and the number of white blood cells (WBC) in limited microscopic fields. Adequate parasitaemia for enrollment requires at least 1 parasite per 6-8 WBCs, which corresponds to approximately 1000 asexual parasites/µl.
The second blood smear was examined to determine parasite density (one hundred high power fields (HPF) was examined and the number of asexual parasites per HPF was recorded), according to the method described in the WHO protocol. Parasitaemia was measured by counting the number of asexual parasites against a number of leucocytes in the thick blood film, based on a putative mean count of 8000 leucocytes per µl. The Number of asexual parasites was counted against 200+ leucocytes using hand tally-counter. Thus, the parasitaemia (per µl) was calculated by using the formula): *Parasiteamia/ µl = Number of Parasites x 8000/Number of leucocytes* (WHO, 2003).

When 500+ parasites were identified before counting 200 leucocytes, counting was stopped and the parasitaemia was calculated according to the formula above. A blood slide was declared negative when the examination of 100 HPF did not show the presence of asexual forms of *P. falciparum*. When gametocytes were detected, this was noted irrespective of the presence or absence of asexual forms, but was not figured during evaluation of the test.

5.8. Classification of therapeutic response (outcomes)

Based on the WHO, 2003 modified protocol, generally the following categories of therapeutic responses, namely Early Treatment Failure (ETF), Late Treatment Failure (LTF) which in turn divides in to Late Clinical Failure (LCF) and Late Parasitological Failure (LPF), and Adequate Clinical and Parasitological Response (ACPR) were used.

The definition and description of the therapeutic response (outcomes) is based on WHO guideline (WHO, 2003):

Early treatment failure (ETF); is the development of danger signs for severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitemia; parasitemia on Day 2 higher than Day 0 count irrespective of auxiliary temperature; parasitemia on Day 3 with auxiliary temperature ≥ 37.5 °C; parasitemia on Day 3 ≥ 25 % of count on Day 0.

Late Clinical Failure (LCF); is the development of danger signs for severe malaria after Day 3 in the presence of parasitemia, without previously meeting any of the criteria of ETF; Presence of parasitemia and auxiliary temperature ≥37.5 °C (or history of fever) on any day from Day 4 to Day 28, without previously meeting any of the criteria of ETF.

Late Parasitological Failure (LPF); is the presence of parasitemia on any day from Day 7 to Day 28 and auxiliary temperature < 37.5 °C, without previously meeting any of the criteria of early treatment failure or late clinical failure.

Adequate Clinical and Parasitological Response (ACPR);

Is the absence of parasitemia on Day 28 irrespective of axillary temperature without previously meeting any of the criteria of ETF, LTF or LPF.

The Secondary outcomes were fever clearance rate; proportion of patients who have fever cleared at day 1, 2, and 3. Parasite clearance rate: proportion of patients with negative thick

blood film smears on days 1, 2, 3. Gametocyte carriage: proportion of patients with gametocytes during the course of the study.

Fever clearance time (FCT) was defined as the time from drug administration until the body temperature decreased to < 37.5°C and remained so for 48 hours. Parasite clearance time (PCT) was defined as the time from drug administration until the first in a series of negative blood smears.

5.9 Ethical Considerations

The therapeutic efficacy test was conducted under direct supervision of qualified medical Personnel's (Health Officer) at all times for the safety and welfare of the individual patient. Ethical clearances to conduct this study were obtained from Tigray Regional Health Bureau as well as from Department Research and Ethical Committee of the Department of Medical Microbiology, Immunology and Parasitology, and Institutional Review Board of the Medical Faculty, Addis Ababa University.

Regional and District health institutions were formally informed about this study and support letter was obtained from Tigray Regional Health Bureau. Formal written consent was obtained from all eligible participants/ parents or guardian for study participants of under age of 18 years old. Moreover, community and religious leaders were informed about the purpose of the study and its benefit for the district, Region and the country.

5.10. Data management and analysis

The case enrolment form was used for the patient's case history and it also contained clinical and parasitological data for day 0. The case record form was used to record the patients' study number, directions, study drug, and all the clinical and parasitological data for day 0 to day 28, and the final classification of therapeutic response. The data forms were thoroughly checked on daily basis, not only for completeness but also to ensure that they were being filled out clearly, that the information collected makes sense, and, most importantly, that study patients were classified correctly .

The data was entered in to computers using WHO Excel data analysis sheets and SPSS version 15 soft wares at the Regional Health Bureau Heath management information system unit. Result from in vivo test was summarized using summary sheet (Annex-10).

Descriptive statistics are presented as counts, percentages, mean, median, standard deviation, and range, as appropriate. The efficacy assessment was done by modified intention to treat (ITT) and per protocol (PP) analysis. The ITT analysis included all patients enrolled into the study. The PP employs Kaplan Meir analysis and excludes all lost to follow up and was used for analysis of primary outcomes, i.e. the ETF, LCF, LPF and ACPR. The ITT population was used for analysis of background variables, primary and secondary efficacy end points. In addition bivaraite analysis to compare means and statistically significance of p-value <0.05 and 95% confidence intervals were used. Analyzed data was demonstrated using tables, graphs and figures.

5.11. Quality control

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

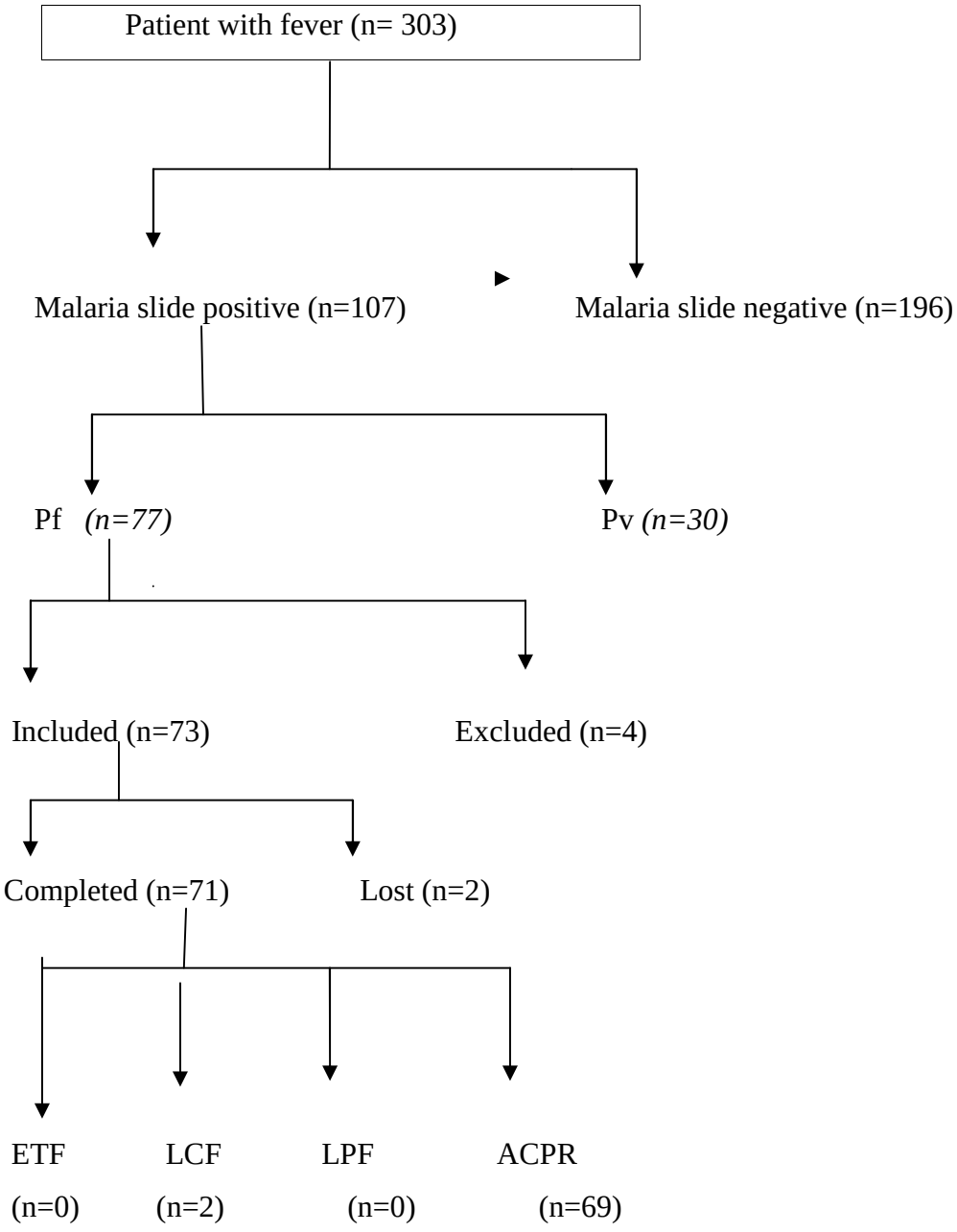
Microscopic results were assessed following the procedure that emphasizes reproducibility of final outcome classification over reproducibility of exact parasite counts by crosschecking 10% of the total slides. To avoid errors in recording data, the study supervisors on a regular basis were reviewing all case report forms during the assessment for completeness and accuracy. Data had computerized using double entry using WHO data analysis excel sheets and SPSS version 15 and a random sample of 10% of computerized records were selected and compared to hard-copy case report forms for confirmation of consistency.

6. Results

6.1. Characteristics of the study population (Baseline information)

During the study period (August to November 2009) a total of 303 patients with fever were screened for malaria and out of these, 107 (35.3%) had a peripheral blood slides with plasmodium species of which 77 (72%) were with *Plasmodium falciparum* and 30 (28%*)* with *Plasmodium vivax.* Among the 77 patients with *Plasmodium falciparum*, 73 (94.8%) patients were enrolled into the study that fulfilled the inclusion criteria set by WHO (WHO, 2003) and 4 were excluded due to different reasons. Out of the enrolled subjects, only 71 patients were successfully followed up.

Of the total 73 cases included in the study, 2 cases were lost during the 28 days follow up for different reasons: one on day 1 and the other one on day 7. Through out the course of study, there was no withdrawal from the study. The flow diagram of recruitment and follow up is shown below (Figure 3).



ETF: Early treatment failure, LCF: Late treatment failure, LPF: Late parasitological failure, ACPR: Adequate clinical and parasitological response

Figure 3. The flow diagram of recruitment and follow up of the study, Tumuga health center, Alamata, November, 2009

Most patients were in the age range between 5 and 15 years (n= 30, 41.1%) followed by the group above 15 years of age (n=29, 39.7%) (Table 1). The smallest age group was under 5 years (n= 14, 19.2%). The median age of the study population was 14 years (2 - 70 years), and the majority (61.6%, n= 45) of the study cases were males. The body weight range was 65 kg (9-74 kg), with mean of 36.2 (SD ±17.3) kg. On recruitment, 87.7% (64/73) of the patients receiving Artemether-lumefantrine had temperatures greater than 37.6^{o}C. The mean temperature and the parasite count were 38.2^{o}C and 20672 rings/µL, respectively.

Table 1. General Baseline characteristics of the study population Tumuga health center, Alamata, November 2009

Details	values	Age distribution		
		Age category	No	%
No screened	303	<5	14	19.2
No enrolled	73	5-15	30	41.1
Parasite rate (%)	35.3	>15	29	39.7
Mean Parasite density	20672	Total	73	100
		Mean	15.2	
Mean weight (kg)	36.2	Median	14	
Female (%)	38.4	SD	11.8	
Mean temp. D0	38.2	Range	68(2-70)	

D0 = Day zero; SD = Standard deviation

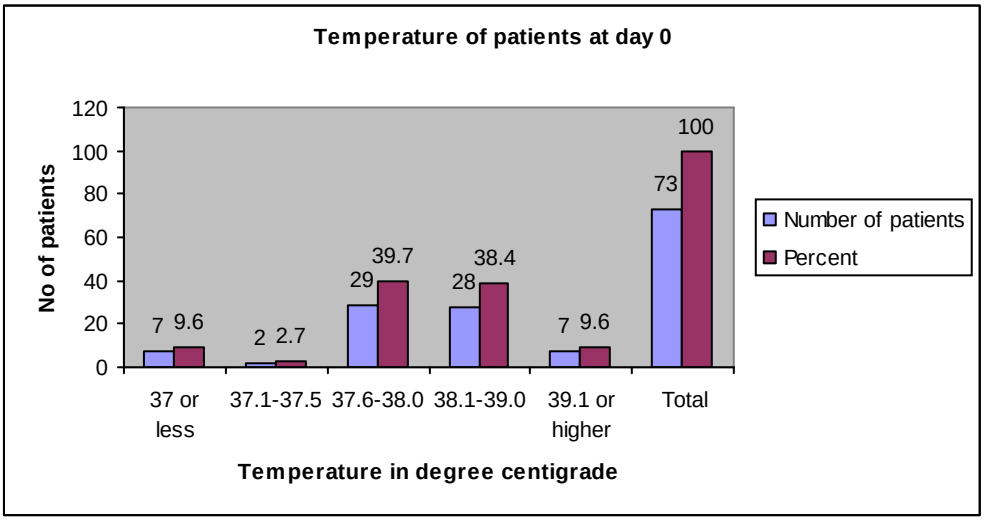


Figure 4. Temperature of patients at recruitment, Tumuga Heath center, Alamata, November 2009.

6.2. Association between Temperature at recruitment, initial parasitaemia, and clearance time

Using bivariate analysis, no statistical significant association was found between Temperature of patients at recruitment and parasite clearance time (p-value = 0.392) and fever clearance time (p-value =0.178) (Table 2). Association between initial parasite density at recruitment and length of parasite clearance time was statistically significance at the 0.05 confidence level (*p*-value = 0.030). This indicates that high parasite counts density on recruitment day results in the parasite clearance time to be prolonged. Concerning the fever clearance time and parasite density at day 0, no significant association was found (p-value = 0.067) (Table 3).

Table 2 Temperature at recruitment versus mean parasite and fever clearance time, Alamata, Tumuga Health center, November 2009.

Temperature Day 0	Mean PCT	P-value	Mean FCT	P-value
0-37	25.43		25.57	
37.1-37.5	29.00		29.50	
37.6-38	26.52		26.00	
38.1-39	26.61		26.32	
>=39.1	26.00		25.00	
Total	26.47	0.392	26.08	0.178

Table 3. Parasite densities at day 0 versus Mean parasite and fever clearance time, Alamata, Tumuga Health center, November 2009.

Parasite dens. Day 0	Mean PCT	P- value	Mean FCT	P-value
1000-11000	25.53		25.20	
11001-21000	26.93		26.47	
21001-31000	26.90		26.80	
31001-41000	27.38		26.50	
41001-51000	29.00		28.40	
51001-61000	26.00		27.00	
>61000	25.67		25.00	
Total	26.47	0.030	26.08	0.067

PCT; Parasite clearance time, FCT; Fever clearance time

6.3 Treatment Responses

Clinical failure (LCF) is 2.8% and is observed on day 21 and day 28, 1.4% LCF on each days. The cure rate (ACPR) is 97.2% on the study site and the parasite clearance is rapid and complete; clearance of parasitaemia with in 32 hours was observed in all (100.0%) patients. The mean parasite clearance time (PCT) was 26.5 (±2.3 hrs). Proportion of patients with gametocytes at enrolment was 1.4% by day 7. From day 14 onwards no patient had gametocytes. Fever and parasite cleared rapidly over 32 hours after starting treatment (Table 5). There fore, all (100%) patients had cleared fever after 32 hours. In terms of parasitemia, the rate of parasite clearance was also 100 % after 32 hours of starting treatment and patients were no longer parasitemia by day 2. No major adverse events were observed through out the 28 days follow up.

Table 4. General characteristics of out comes and cure rates of Artemether-lumefantrine by follow up days, Alamata, Tumuga Health center, November 2009.

Details	Values (%)
Parasitological failure, day14	0
Clinical failure, day14	0
ACPR, day 14 (n=71))	100
Gametocyte carriage, day 14	0
Parasitological failure, day 21	0
Clinical failure, day 21 (n=1)	1.4
ACPR, day 21 (70)	98.6
Gametocyte carriage, day 21	0
Parasitological failure, day 28	0
Clinical failure, day 28 (n=2)	2.8
ACPR, day 28 (n=69)	97.2
Gametocyte carriage, day 28	0

ACPR; Adequate clinical and parasitological response

Table 5. Patient parasite counts at recruitment, Parasite clearance time & fever clearance time Tumuga Health center, Alamata, November, 2009.

	Parasite count per micro liter blood	Parasite clearance time(hors)	Fever clearance time(hours)
Mean	20672	26.5	26
Median	14800	26	25
SD	47295	2.3	2.4
Range	72480 (1440-73920)	9 (23-32)	8 (24-32)

SD: Standard deviation

The Kaplan Meier survival analysis of the data shows estimates of success 1.00 from day 0 to day 20, 0.986 from day 21 to day 27 and 0.972 on day 28. The estimate of cumulative incidence of treatment failure is indicated as 0.00 from day 0 to day 20, 0.014 from day 21 to day 27 and 0.028 on day 28. Here the proportion of success and failure of patients at each point in time is not significant as the CI is (0.933-1.010) and (-0.010-0.067) respectively (Annex 11). Thus, the estimate of success (cure rate) is 97.2% and estimate of failure is 2.8% during the 28 days follow up of study.

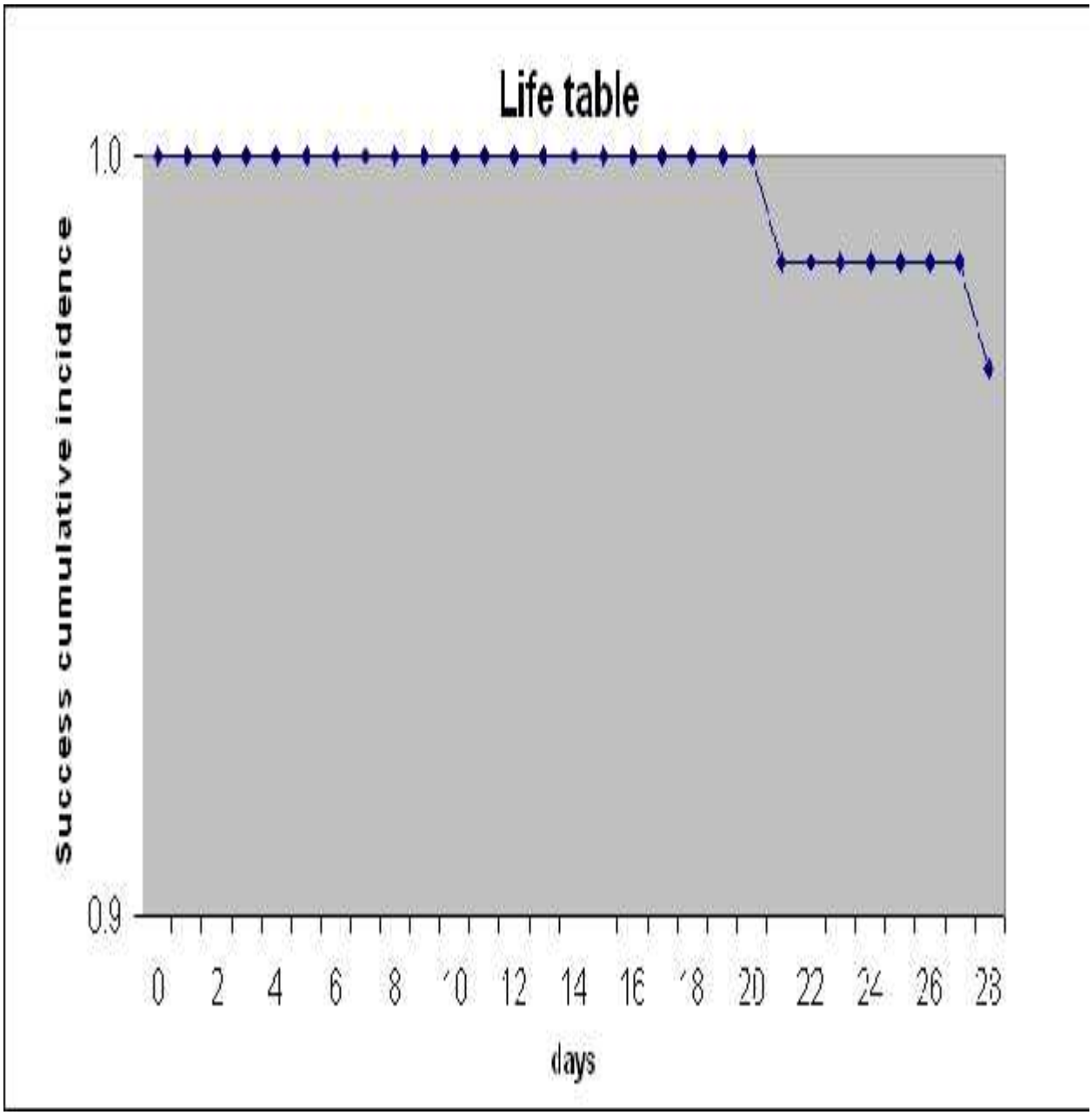


Figure 5. Kaplan Meier survival curve of the study outcomes (success cumulative incidence) by follow up days, Tumuga, health center, Alamata, Nov., 2009.

In the intent to treat analysis the lost to follow up were included in the denominator of the study analysis, but were excluded in the per-protocol analysis approach (Table 6).

Table 6. Primary treatment outcomes on day 28 in patients with uncomplicated *P. falciparum* malaria, in intent to treat analysis and per-protocol analysis, Tumuga Health center, Alamata, 2009.

Outcome of treatment	Intent to treat analysis*	Per-protocol analysis
	Values [n/N (%)]	Values [n/N (%)]
ETF	0/73(0)	0/71(0)
LCF	2/73(2.7)	2/71(2.8)
LPF	0/73(0)	0/71(0)
TTF	2/73(2.7)	2/71(2.8)
ACPR	71/73(97.3)	69/71(97.2)
Lost	2/73(2.7)	2/73(2.7)

*Lost to follow up included

ETF; Early treatment failure, LCF; Late clinical failure, LPF; late Parasitological failure; TTF; total treatment failure; ACPR; Adequate clinical and parasitological response.

The study outcomes by follow up days shows 0% on day 7 and day14, but 1.4% LCF on day 21 and 2.8% LCF by day 28. The ACPR was 100% on day 7 and day14 but 98.6% on day 21 and 97.2% on day 28 (Table 8)

Table 7. Classification outcomes by follow-up days in percent, Tumuga Health center, Alamata, November 2009

Outcome	Day 7	Day14	Day 21	Day 28
ETF	0%	0%	0%	0%
LCF	0%	0%	1.4%	2.8%
LPF	0%	0%	0%	0%
ACPR	100%	100%	98.6%	97.2%

ETF; Early treatment failure, LCF; Late clinical failure, LPF; late Parasitological failure, ACPR; Adequate clinical and parasitological response.

Table 8. Summary of Classification outcome, Tumuga Health center, Alamata, November 2009

Outcome	Value	%	Remark
ETF	0	0	
LCF	2	2.8	Both female and >5
LPF	0	0	
TTF	2	2.8	
ACPR	69	97.2	
LOST	2	2.8	

ETF; Early treatment failure, LCF; Late clinical failure, LPF; late Parasitological failure; TTF; total treatment failure; ACPR; Adequate clinical and parasitological response.

7. Discussion

When Ethiopia deployed SP as first line drug to treat uncomplicated malaria at national level, a treatment failure rate of SP was 5%. After 3-5 years of its deployment, study findings showed that, SP treatment failure was 10-30% (Jima *et al.,* 2005). Based on a nationwide study, SP treatment failure was shown to be 35.9% and 71.8% (PCR unadjusted) for 14 and 28 days follow up, respectively. The country, thus deploy coArtem, a combination of artemether-lumefantrine in 2004. Treatment failure for CoArtem in 2004 was zero (14 days follow up) and 0.09% (28 days follow up) when deployed as first line anti-malarial drug to treat uncomplicated *P. falciparum* malaria at national level (FMOH, 2004).

The therapeutic efficacy study on artemether-lumefantrine (AL) to uncomplicated *P. falciparum* malaria was conducted from August to November, 2009 in Alamata District, Tumuga Health Center. The study was conducted to assess the clinical and parasitological efficacy of the six-dose regimen of artemether-lumefantrine (CoArtem) for treating uncomplicated *P. falciparum* malaria five years after its deployment into the area.

This study had shown that a standard dose of Artemether-Lumefantrine is highly effective against uncomplicated *P. falciparum* malaria after 5 years of use as first line treatment in the area. Treatment with six-dose artemether-lumefantrine cleared fever and parasitaemia rapidly and proved highly effective, resulting in 28 days cure rates (PCR uncorrected) of 97.2% using the per-protocol analysis. By employing Kaplan Meier survival analysis in the data obtained was 0.972 with 95% CI, (0.933-1.010) the success cumulative incidence and the failure cumulative incidence was 0.028; 95% CI, -0.010-0.067 (Fig 5). This Kaplan Meier estimates of success 97.2% with 95% CI (0.933-1.010) shows that the drug is effective for the treatment of uncomplicated *falciparum* malaria.

The AL combination offers the advantage of avoiding rapid emergence of resistance to the new chemical entity lumefantrine, as a result of the rapid parasite reduction obtained by the administered artemether. Furthermore, no parasites are exposed to artemether alone, and these two drugs are mutually protective. As with the other artemisinin-containing combinations,

Artemether-Lumefantrine can also reduce transmission of *falciparum* malaria, because of its excellent efficacy and associated low transmission potential (Vugt *et al.,* 2000).

The finding with 97.2% adequate clinical and parasitological response (ACPR) is consistent with the therapeutic efficacy study done in the country during deployment of the drug in 2004 which enables as to accept our hypothesis. The aggregated mean of clinical and parasitological treatment response to AL was 100% and 99.01% (PCR unadjusted) for 14 and 28 days follow up respectively (FMOH, 2004). In addition the study results also shows consistency with other therapeutic efficacy study results conducted three years after its introduction in Ethiopia, in which treatment of AL resulted in 100% adequate clinical and parasitological response (Kefyalew *et al*., 2009). Another study conducted in 2007/2008 on therapeutic efficacy of CoArtem for the treatment of uncomplicated *Plasmodium falciparum* malaria in two rural endemic area of Ethiopia, Wondogenet and Sebro showed PCR unadjusted ACPR of 95.8% and 96.2%, respectively and the PCR corrected ACPR in the two sites was 96.8% and 97.4%, respectively (Ashenafi *et al.,* 2010).

A study was conducted to assess the efficacy of AL administered to African children weighing 5-25 kg, with acute, uncomplicated *falciparum* malaria. Treatment with six-dose AL rapidly cleared parasitaemia and fever. The over all 28 day cure rate was 86.5% (PCR uncorrected) and 93.9% corrected by PCR for re-infection (Falade *et al.,* 2005).
Our study result had shown 97.2% ACPR without PCR correction. This is much higher than the study done in African children, mentioned above, where they found 86.5% ACPR without PCR correction. The higher efficacy of coArtem in our study may be due the drug is being used for relatively shorter period, i.e. 4-5 years or may be due to differences in immunity in the different populations.

Another study conducted in Zambia, which assessed therapeutic efficacy of a pediatrics formulation of AL for the treatment of uncomplicated malaria in children less than 10 kg in 2005 found similar ACPR after 28 days follow up (100% PCR uncorrected) but 96.0% PCR corrected (Chanda *et al.,* 2006). The study conducted in India in 2006 also shown a cure rate

100% (PCR corrected) in the treatment of uncomplicated *P. falciparum* malaria with six-dose AL regimen (Valecha *et al.*, 2009).

Results of our study (97.2 cure rate) was in line with these studies and nearly found similar results. Our findings confirmed, the results from the health center showed that the AL six-dose treatment for the patients with uncomplicated *P. falciparum* malaria is highly efficacious. The combination of artemether lumefantrine (CoArtem) showed excellent efficacy (cure rates 97.2%) and safety when given as six dose regimen in the treatment of *falciparum* malaria in the area.

The study also showed fast parasite clearance time (mean time 26.5 hours, 95% CI, 23 - 32 hours). The fast parasite and fever clearance may be due to the fast reduction in parasite biomass and prompt symptomatic improvement. The lumefantrine that persist in the blood after the three-day treatment course eliminate any remaining parasites to prevent recrudescence (Premiji, 2009). AL is thus safe and effective drug for the treatment of acute uncomplicated *falciparum* malaria in the study area.

The proportion of female patients was found low (38.4 %) in the study group compared with male patients (61.6%). The proportion of female patients was found 30% in a study done in Thailand (Lefevre *et al.*, 2001), and 49% and 54.6% in two study sites in Zambia (Chanda *et al.*, 2006). In a study conducted among children less than 5 years in Eastern Sudan the proportion of female patients was 31.8% (Salah *et al*., 2006). The low proportion of female in our study may be due to different socioeconomic condition or may be related to difference in health seeking behavior rather than difference in disease prevalence. The mean temperature of the patients on day 0 was 38.2^0C ($\pm$ 0.86). Similar mean ($\pm$SD) temperature was found in a study done in Eastern Sudan (38.5^0C) ($\pm$0.6) (Salah *et al.*, 2006) and in Thailand 37.5^0C (Lefevre *et al.*, 2001) and 37.7 and 38.7^0C in two site studies in Zambia (Chanda *et al*., 2006).

In our study the mean fever clearance time was found 26 hours, and mean parasite clearance time 26.5 hours. In a study conducted in 2003 for the efficacy of Artemether lumefantrine for uncomplicated malaria, fever clearance time was found 47 hours, parasite clearance time 36

hours (Omari *et al.*, 2004). The median fever clearance time and parasite clearance time was found 29 hours, and 24, hours respectively in a study conducted between September 1998 and June 1999 in Thailand (Lefevre *et al.*, 2001). In other study the median fever clearance time was found 24 hours and the median parasite clearance time was 48 hours (Makanga *et al.*, 2006). In our study the median fever clearance time and parasite clearance time was found to be 25 hours and 26 hours, respectively. The variation of fever and clearance time in different areas may be partly due to differences in parasite density at day 0 or due to differences in times of the drug deployments in the different regions. Generally the fever clearance time is fast because the drug has anti-pyretic property and the drug is fast acting on different stages of the parasite which makes the parasite clearance time shorter (Premiji, 2009).

Analysis using bivariate showed that the only base line characteristics that affected parasite clearance time were parasite density at day 0. There was an association between the mean parasite clearance time and parasite density (p= 0.030). The higher the parasite density at recruitment, the lower is the estimated probability to reach parasite clearance. This is because of the higher density of the parasite at day 0 which prolongs the parasite clearance time (Lefevre *et al.*, 2001). Regarding temperature at recruitment and clearance time, no association was found (p-values= 0.392 and 0.178) and this may be due to anti-pyretic property of the drug and some of the patients were taking paracetamol, which count down fever. Similarly there was no association between parasite density at day 0 and fever clearance time (p= 0.067).

Therefore it can be concluded that, Artemether-lumefantrine remains to be safe and effective drug for the treatment of uncomplicated *Plasmodium falciparum* malaria at least in the study area. The efficacy of this ACT needs to be carefully monitored periodically since the treatment failures can occur due to resistance as well as sub-therapeutic levels due to inadequate absorption or low adherence to the drug (Mutabingwa, 2005), as the adherence study in the area in patients treated in heath institution is 53% (probably adherent) (WHO, 2009).

8. Limitations of the study

The study conducted had not used polymerase chain reaction (PCR) technique to correct the cure rate or treatment failure whether it is due to recrudescence or re-infection at day 21 and day 28; because of budget, equipment and time constraints.

In this study the course of drug administration was not highly supervised by health professionals, although the first dose was administered in front of the clinician and instructions were given to patients/guardians how to administer the drug and there was also an attempt to visit by community health workers & health extension worker.

9. Conclusion

The study suggest that Artemether-lumefantrine seems to be very effective drug for the treatment of uncomplicated *falciparum* malaria as observed in the study area, Alamata district, Tigray North Ethiopia, 5 years after the deployment of the drug in to the area. This highly potent drug is with rapid fever and parasite clearance (26 and 26.5 respectively) and the efficacy (97.2%) is consistent to met the World Health Organization (WHO) criteria for efficacy (>95%) in malaria endemic regions.

10. Recommendations

The study results show that Artemether-lumefantrine is safe and an effective drug for the treatment of uncomplicated *Plasmodium falciparum* malaria in the study area, Alamata, Tigray, North Ethiopia and this result is encouraging to continue the drug as first line untimalarial for the treatment of uncomplicated *Plasmodium falciparum* malaria in the area.

However, the efficacy of Artemether-lumefantrine needs to be carefully monitored periodically, since the treatment failures can occur due to resistance as well as sub-therapeutic levels because of poor adherence (WHO, 2009). Moreover, therapeutic efficacy study should be conducted in sentinel sites representing different areas of the region and the country.

11. References

Alemayehu T, Ye-ebiyo Y, Gebreyesus TA, Witten KH (1998). Malaria, schistosomiasis, and intestinal helminthes in relation to micro-dams in Tigray, Northern Ethiopia. *Parasitologia* **40**: 259-267.

Allan RJ, Beattie P, Bate C, Vanhensbroek MB, Morris-jones S, Greenwood BM, Kwiatkowski D (1995). Strain variation in Tumor Necrosis factor induction by parasites from children with acute *Falciparum* malaria. *Infect Immune* **63**(4): 1173-1175.

Ashenafi A, Moges K, Gemechu T, Hassen M, Abebe A, Tesfaye M (2010). Therapeutic efficacy of Artemether-Lumefantrine(Coartem) against Plasmodium falciparum in Kersa, South west Ethiopia. *Parasite and Vectors* 3: 1.

Baird JK (2005). Effectiveness of antimalarial drugs. *New Engl J Med* **352**: 1565-1577.

Barnes KI, Chanda P, Barnabas G (2009). Impact of the large-scale deployment of artemether/lumefantrine on the malaria disease burden in Africa: case studies of South Africa, Zambia and Ethiopia. *Malar J* 8.

Baruch DI, Gormley JA, Ma C, Howard RJ, Pasloske BL (1996). *Plasmodium falciparum* erythrocyte membrane protein1 is a parasitized erythrocyte receptor for adherence to CD36, thrombospondin, and intercellular adhesion molecule1. *Proc. Natl. Acad. Sci. USA* **93**:3497-3502.

Bloland PB, Ettling M, Meek S (2000). Combination therapy for malaria in Africa: hype or hope? *Bull World Hlth Org* **78**: 285-294.

Bruce-Chwatt LJ (1991). Essential malariology. Second edition. Geneva, Switzerland.

Clark IA, Alleva LM, Mills AC, Cowden WB (2004). Pathogenesis of malaria and clinically similar conditions. *Clin Microbiol Rev* **17**(3): 509-539.

Chanda P, Hawela M, Kango M, Sipilanyambe N (2006*).* Assessment of the therapeutic efficacy of a paediatric formulation of artemether-lumefantrine(coartisiane) for the treatment of uncomplicated *Plasmodium falciparum* in children in Zambia. *Malar J* 5:75.

Conway DJ (2007). Molecular epidemiology of malaria. *Clin Microbiol Rev* **20**(1): 188-204.

Cooper RA, Ferdig MT, Su XZ, UrsosL MB, Mu J, Nomura T, Fujioka H, Fidock DA, Roepe PD, Wellems TE (2002). Alternative mutations at position 76 of the vacolar transmebrane protein PfCRT are associated with chloroquine resistance and unique stereospecific quinine and quinidine responses in Plasmodium falciparum. *Molecular Pharmacology* **61**(1): 35-42.

Dokomajilar C, Nsobya SL, Greenhouse B, Rosenthal PJ, Dorsey G (2006). Selection of *Plasmodium falciparum* in an area of Uganda where malaria is highly endemic, *Antimic Agents Chemother* **50**(5): 1893-1895.

Dondrop AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotvanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon, Nicholas PJ, Lindegardh N, Socheat D, White NJ (2009). Artemisinin resistance in *Plasmodium falciparum* malaria. *New Engl J Med* **361**(5): 455-467.

Druaisingh MT, Cowman AF(2005*)*. Contribution of the pfmdr1 gene to antimalarial drug-resistance. *Acta Tropica* **94**:181-190.

EI-Lakkany N.N., Seifeldin S.H., Badawy A.A., Ebeid F.A (2004). Effect of artemether alone and incombination with grapefruit juice on hepatic drug-metabolising enzymes and biochemical aspects in experimental *Schistosoma mansoni. Inern J Parasitol* **34**(12):1405-14 12.

Esterre P, Fandeur T, Mercereau-puijalon O (2005). Resistance of *Plasmodium falciparum* field isolates to in-vitro arthemether and point mutations of the SERCA-type pfATPase6. *Lancet* **366**: 1960-1963.

Façade C, Makanga M, Ortmannd C, Stockmeyerd M, Depalaciosd PI (2005*)*. Efficacy and safety of artemether-lumefantrine Coartem) tablets (six-dose regimen) in African infants and children with acute, uncomplicated falciparum malaria. *Trop Med Hygiene* **99**(6): 459-467.

Federal Ministry of Health (2004). Malaria diagnosis and treatment guide lines for health workers in Ethiopia. Second Ed. FMOH, Addis Ababa.

Gebreyesus TA, Tadesse Z, Jima D, Bekele E, Mihretie A, Yihdego YY, Dinku T, Connor SJ, Rogers DP (2009*)*. Using climate information in the health sector. *Field Actions Science Report*; **2**:63-67.

Green MD (*2006)*. Antimalarial drug resistance and their importance of drug quality monitoring. *J Post Grad Med* **52**(4): 288-290.

Greenwood BM, Fidock DA, Kyle DE, Kappe Stefan HI, Alonso PL, Collins FH, Duffy PE (2008). Malaria: Progress, perils, and prospects for eradication. *JClin Investigation* 118(4): 1266-1276.

Hastings IM (2003*)*. Malaria control & the evolution of drug resistance: an intriguing link. *Trends parasitol* **19**(2):70-73.

Jambou R, Legrand E, Niang M, Khim N, Lim P, Volney B, Ekala MT, Bouchier C, Smith JD, Craig AG, Kriek N, Hudson-Taylor D, Kyes S, Fagen T, Pinches R, Barunch DI, Newbold CI, Miller LH (2000). Identification of a plasmodium falciparum intercellular

adhesion molecule1 binding domain: a parasite adhesion trait implicated in cerebral malaria. *PNAS* 97(4):1766-1771.

Jima D, Tesfaye G, Medhin A, Kebede A, Argaw D, Babaniyi O (2005). Efficacy of sulfadoxine pyrimethamine for the treatment of uncomplicated *falciparum* malaria in Ethiopia. *East Afr Med J* **82**(8): 391-395.

Kabanywanyi AM, Mwita A, Sumari D, Mandike R, Mugittu K, Abdulla S (2007). Efficacy and safety of artemisinin-based antimalarial in the treatment of uncomplicated malaria in children in southern Tanzania. *Malar J* **6**:146.

Ketema T, Bacha K, Birhanu T, Petros B (2009). Chloroquine-resistant Plasmodium vivax malaria in Sebro Town, Jima zone, South-west Ethiopia. *Malar J* 8(177).

Kefyalew T, Animut A, Tamene T, Jima D,Hailemariam A, Legesse M (2009*).* Efficacy of six dose regimen of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria, three years after its introduction in to Ethiopia. *Parasite* **16**(2): 129-134.

Kremsner PG, Krishna S(2004). Antimalarial combinations. *Lancet* **364**: 285-294.

Krishna S, Woodrow CJ, Staines HM, Haynes RK, Mercereau-puijalon O (2006). Re-evaluation of how artemisinins work in light of emerging evidence of in vitro resistance. *Trends in Molecular Med* **12**(5): 200-205.

Lefevere G, Looareesuwan S, Treeprasertsuk S, Krudsood S, Silachamroom U, Gathmann I, Muu R, Bakshi R (*2001).* A clinical and pharmacokinetic trial of six doses of Artemether-lumefantrine for multidrug resistant Plasmodium falciparum malaria in Thailand. *Am J Trop Med Hygiene* **64**(5): 247-256.

Lou J, Lucas R, Grau GE (*2001).* Pathogenesis of cerebral malaria: Recent experimental data & possible applications for humans. *Clin Microbiol Rev* **14(**4): 810-820.

Makanga M, Permji Z, Falade C, Karbwang J, Mueller EA, Andriano K, Hunt P, Depalacios PI(2006). Efficacy and safety of the six dose regimen of artemether lumefantrine in pediatrics with uncomplicated Plasmodium falciparum malaria. A pooled analysis of individual patient data. *Am J Trop MedMygiene* **74**(6): 991-998.

Ministry of Health, Ethiopian national malaria indicator survey (2008). *FMOH*, 2008.

Mizuno Y, Kato Y, Kudo K, Kanos S (2009). First case of treatment failure of Artemether lumefantrine in a Japanese traveler with imported *Falciparum* malaria. *Japanese J Infect Dis* **62:**139-141.

Molyneux OH, Floyd K, Barnish G, Fevre EM (*1999).* Transmission control and drug resistance in malaria: A crucial interaction. *Parasitol Today* **15**(6): 238-240.

Muheki C, Mclntyre D, Barnes KI (2004). Artemisinin-based combination therapy reduces expenditure on malaria treatment in Kwazulu Natal, South Africa. *Trop Med Intern Health* **9**(9): 959-966.

Mutabingwa TK (2005). Artemisinin-based combination therapies (ACTs): Best hope for malaria treatment but in accessible to the needy. *Acta Tropica* **95**: 305-315.

Omari AA, Gamble C, Garner P (*2004)*. Artemethr-lumefantrine for uncomplicated malaria: a systematic review. *Trop Med Intern Health*; **9(**2): 192-199.

Plowe CV (*2003)*. Monitoring antimalarial drug resistance: making the most of the tools at hand. *J Experimental Biol* **206**: 3745-3752.

Prmji ZG (2009). Coartem: the journey to the clinic. *Malaria J* 8.

Salah MT, Faroung M, Magzoub MM, Adam I (*2006)*. Efficacy of Artemether-lumefantrine (Coartem) suspension in the treatment of uncomplicated Plasmodium falciparum malaria among children under 5 years in eastern Sudan. *TropJ Pharmaceutical Research* **5**(1):551-555.

Rosenthal P (2008). Artesunate for the treatment of severe falciparum malaria. *New Engl J Med* **358**(17):1829-1836.

Schunk M, Kumma WP, Miranda IB, Osman ME, Roewer S, Alano A, Loscher T, Bienzle U, Alano AP (2006). High prevalence of drug-resistance mutations in Plasmodium falciparum & Plasmodium vivax in southern Ethiopia. *Malaria J* **5**(54).

Tadesse D, Tsehaye A, Mekonen T (2009). Intestinal Helminthes Infection and Re-infections with Special Emphasis on Schistosomiasis by Mansoni in Waja, North Ethiopia. *MEJS* **1**(2): 4-16.

Talisuna AO, Bloland P, Alessandro UD (2004). History, dynamics, and public health importance of malaria parasite resistance. *Clin Microbial Rev* **17**(1):235-254.

Trampuz A, Jereb M, Muzlovic I, Prabhu RM (2003) Clinical review: Severe malaria. *Clinical care* **7**:315-323.

Valecha N, Srivastiva P, Mohanty S, Mittra P, Sharma SK, Tyagi PK, Pradhan K, Dev V, Singh R, Dash AP, Sharma YD (2009). Therapeutic efficacy of artemether-lumefantrine in uncomplicated *falciparum* malaria in India. *Malar J* **8**(107).

Vandenbroek I, Amsalu R, Balasegaram M, Hepple P, Alemu E, Hussein E, Al-faith M, Montgomery J, Checchi F(*2005)*. Efficacy of two artemisisnin combination therapies for uncomplicated falciparum malaria in children under 5 years, Malakal, upper Nile, Sudan. *Malar J* 4:14.

Vanghan Williams CH, Lacock C, Bredenkamp BLF(*2004)*. Audit of efficacy of coartem to clear *Plasmodium falciparum* malaria parasitemia at single forty-two day follow up. *SAFAM Prac*; **46**(6): 21-24.

Vugt MV, Looareesuwan S, Wilairatana P, Megready R, Villigas L, Gathmann I, Mull R (*2000)*. Artemether-lumefantrine for the treatment of multidrug-resistant falciparum malaria. *Transac Roy Soc Trop Med Hygiene* **94**:545-548.

Wezam A (*1993*. *Plasmodium falciparum* sensitivity to antimalarials at Humera, North western Ethiopia. *Ethiop Med J* **31**: 271-275.

White NJ, Breman JG (2004). Malaria and babesiosis: Diseases caused by red blood cell parasites, 16th Ed. Harisons principles of internal medicine.

Woldearegai TG, Hailu A, Grobusch MP, J.Kun JF (2005). Molecular surveillance of mutations in dihydrofolate reductase & dihydropteroate synthase genes of Plasmodium falciparum in Ethiopia. *Am J Trop Med Hygiene* 73(6): 1131-1134.

Worku S, Girma T, Shiferaw Y (*2005)*. Therapeutic efficacy of sulfadoxine/ pyrimethamine in the treatment of uncomplicated malaria in children. *Ethiop J Health Dev* **19**(1): 11-15.

World Health Organization (2003). Assessment and monitoring of anti-malarial drug efficacy for the treatment of uncomplicated f*alciparum* malaria. *Geneva, WHO*, 2003 (WHO/HTM/2003.50).

World Health Organization (2009). Deployment at community level of artemether-lumefantrine and rapid diagnostic tests, Raya valley, Tigray, Ethiopia.

World Health organization (2005). Susceptibility of Plasmodium falciparum to antimalarial drugs. Report on global monitoring 1996-2004. *World Health Organization*, Switzer land Geneva. WHO/HTM/mal/2005.11.3.

World Health Organization (1999). The community-based malaria control program in Tigray, Northern Ethiopia. A review of program set-up activities, outcomes and impact. *WHO, 1999*. WHO/mal/99.1090.

Zhong D, Afrane Y, Githeko A, Cui L, Menge DM, Yan G (*2008)*. Molecular epidemiology of drug resistant malaria in western Kenya highlands. *Biomedical central* 8:105.

Zongo I, Dorsey G, Rouamba N, Tinto H, Dokomajilar C, Guiguemde RT, Rosenthal P, Ouedraogo JB (2007). Artemether lumefantrine versus amodiaquine plus sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Burkinafaso: a randomized non-inferiority trial. *Lancet* 369: 491-498.

12. Annexes

Annex-1 Information sheet

Annex 1.1 English versions

1. Aim of the study

This study is designed to determine the therapeutic efficacy of Artemether-lumefantrine to un complicated *P. falciparum* malaria in Alamata district, northern Ethiopia.

2. Role of participants in the study

Study participants are expected to cooperate during the sample collection, and the scheduled follow up visit. During sample collection, sterile cotton and lancet will be used.

3. Risk associated with sample collection

The risks associated with the sample collection are minimal since the collection of these would follow routine procedures for laboratory investigation.

4. Confidentiality

All the information contained within the questionnaire is to be kept confidential.

5. Right

The study participants have a right to:

Keep hold information

Decline to cooperate in the study

Withdraw from the study..

To refuse provision of specimens

6. Whom to contact

The study participant can ask any questions that are not clear. The participants/ parents/guardians have full right to ask information about the research before they decide to participate. You can contact the principal investigator or co-investigator for any doubts you want to clear.

7. Approval

This research project has got ethical clearance from TRHB ethical review committee; it has also got approval from Addis Ababa University Medical Faculty Post Graduate Program, Department of Medical Microbiology, Immunology and parasitology.

8. Sponsors: AAU and the rest are to look from Tigray Regional health Bureau.

ANNEX-2 *IN VIVO* THERAPEUTIC EFFICACY STUDY ENROLLED PATIENTS

Study Site _____ Microscopist _____ Date from ___/____/____ to ___/____/____

DATE	PATIENT NAME	PATIENT CASE NUMBER	PATIENT STUDY DAY	SLIDE LABEL	SLIDE READING			
					Number of WBC	Number of PF asexual forms	PF gametocyte present (check if present)	Pf asexual parasite density = Number of Pf asexual forms X 8000 / Number of WBC

Annex-3: Blood film request and result slip

Study Site_____ Date _____

Patient Name _____

Age _____ Sex _____

Case Number _____

Kebele _____

Parasite's species type_____

WBC Count _____

Parasite Count _____

Fg (Yes/No) _____

Lab. Tech. Sign. _____

ANNEX-4: Daily case record form

Region…… Zone……Wereda……Health Institution name…….

year……

Name of investigator (s)…… Page 1

Record (Slide) Number		Study site		Full Name		Guardian's Name	
Contact (home) address				Length of residence		Occupation	
Age Years/Months	Weight (kg)	Hb/Ht/D0	Hb/Ht/D28	Sex	Drug name		Total drug dose (mg base)
Previous antimalrials (Y/N/Unknown)	Dosage				Urine Test (drug)		(Concentration)

Day		Day-0	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7	Day-8	Day-9	Day-10	Day-11	Day-12	Day-13	Day-14
Date																
Clinical Assessment	Danger signs															
(Yes/No)	History of fever (last 24 hrs)															
Axillary temperature																
Asexual parasite count/200 WBC																
Gametocyte count																
Treatment (no. of tabs) - Co-Artem - Alternative treatment																
Concomitant treatment																
Possible drug side-effects																
Reasons for withdrawals or loss to follow-up																
Overall Assessment	*ETF* *LTF* *ACPR* *Withdrawals* *Loss to Follow-up*															

DAILY CASE RECORD FORM

Record (Slide) Number		Study site		Full Name						Guardian's Name			
Day	Day15	Day16	Day17	Day18	Day19	Day20	Day21	Day22	Day23	Day24	Day25	Day26	Day27	Day28
Date														
Clinical assessment (Yes/No) — Danger signs														
Clinical assessment (Yes/No) — History of fever (Last 24 hrs)														
Axillary temperature														
Asexual parasite count/ 200WBC														
Gametocyte count														
Treatment (no. of tabs) - CoArtem - CQ - Alternative treatment														
Concomitant treatment														
Possible drug side-effects														
Reasons for withdrawals or loss to follow-up														
Overall Assessment	*ETF*		*LTF*		*ACPR*		*Withdrawals*				*Loss to Follow-up*			

Annex-5: Patients location form
Case Record Number _____

Name: _____Age _____Sex _____

Kebele _ _____ House number _____

Father's name _____

Mother's name _____

Name of household head where living now _____

Name of nearest neighbour _____

If student, name of school _____ Class _____

If employed, place of work _____

Annex-6: Consent form

6.1 English version

Code number: _____

Name of study Subject: _____

Parents/ Guardians Name: _____

Relationship:

I have been informed about the study, which plans to assess the therapeutic efficacy of anti-malarial drugs. The objective and the application of the study were explained to me. I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also well informed of my right to keep hold of information, decline to cooperate and dropout from the study if I want and that none of my actions will have any bearing at all on my overall health care and access to health facilities.

It is therefore with full understanding of the situations that I agreed to give the informed consent voluntarily to the researcher to use the finger-pricked blood samples from fingertip, for the investigation and to be visited for 7 days. I also agreed that the parasites isolated might be stored and investigated further on similar grounds. Moreover I have had the opportunity to ask questions about the project and I have received clarifications to my satisfaction.

I was also told that results would be reported timely to the requesting physicians for appropriate treatment and management if there is any danger sign of complicated malaria. I agree that I am contributing to the improved treatment of the community at risk of malaria infection and infected by participating in this project.

I _____ hereby give my consent for giving of the requested information and blood samples for the purpose of determining therapeutic efficacy and choice of treatments.

Annex-7: Patient follow-up cards

Case Record Number _____
Patient's name _____

Age _____Sex _____

Day	Date	Signature
0		
1		
2		
3		
7		
14		
21		
28		
Any Other Day		
Day	Date	Signature

Patient follow-up cards

CaseRecordNo----
Patient's name _____

Age _____ Sex __

Day	Date	Signature
0		
1		
2		
3		
7		
14		
21		
28		
Any Other Day		
Day	Date	Signature

Annex-8: Definition of severe Malaria and complications

One or more of the following criteria in the presence of asexual parasitaemia define severe falciparum malaria:

Defining criteria of severe disease

- Cerebral malaria (unarousable coma i.e,. After generalized convulsion, coma should persist for at least 30 minutes to make the distinction from transient post-ictal coma)
- Severe normocytic anaemia (Hb < 5 g/dl)
- Renal failure (serum creatinine > 3.0 mg/dl)
- Pulmonary oedema
- Hypoglycemia (< 40 mg/dl)
- Circulatory collapse/shock (systolic BP ~70 mm Hg in adults; or ~50 mm Hg in children < 5 years)
- Spontaneous bleeding/disseminated intravascular coagulopathy
- Repeated generalized convulsion(s)
- Acidaemia/acidosis
- Macroscopic haemoglobinuria

Other manifestations

- Impaired consciousness but arousal
- Prostration, extreme weakness (inability to stand or sit)
- Hyperparasitaemia (> 5% RBC infected)
- Jaundice (total serum bilirubin > 3 mg/dl)
- Hyperpyrexia (Axillary temp > 39.5^{o}C)

Annex-9: Referral forms

Name: _____ Case Number _____

Age: _____Sex: _____

Date: ___/___/___ Study site: _____

This child is enrolled in a national study of anti-malarial drug sensitivity. Blood film at the time of enrolment was positive for *P. falciparum*. An anti-malarial drug was administered under supervision, with dose calculated according to weight as follows:

Date: _____ drug and dose _____

Date: _____ drug and dose _____

Date: _____ drug and dose _____

Additional treatment has included:

Date: _____ drug and dose _____

Date: _____ drug and dose _____

The child is being referred for the following reason:

Please return this form with the results of your evaluation.
Date: ___/___/___

Health institution _____

Evaluation and treatment given:
Name _____ Signature _____

Annex-10: Summary Sheet for In-vivo Drug Sensitivity Study

Study Site _____*Clinician*_____ *Technicians* _____*Date from* _____*to*_____

Patient Name	Patient Case Number	Age	Sex	Address	DAY0 (Date)		DAY 1 (Date)		DAY2 (Date)		DAY3 (Date)		DAY7 (Date)		DAY14 (Date)		DAY21 (Date)		DAY28 (Date)		Remarks Outcomes
					Temp	PS/200W	Temp	PS/200W	Temp	PS/200W	Temp	PS/200W BC	Temp	PS/200W	Temp	PS/200W	Temp	PS/200W	Temp	PS/200W	

Annex 11 . The Kaplan Meier survival analysis of success cumulative incidence and Failure cumulative incidence in each point in time, Tumuga health center, Alamata, November 2009.

Day	Number of patients	Failures	Loss + With	Success cumulative incidence	Failure cumulative incidence
0	73	0	0	1.000	0.000
1	73	0	1	1.000	0.000
2	72	0	0	1.000	0.000
3	72	0	0	1.000	0.000
4	72	0	0	1.000	0.000
5	72	0	0	1.000	0.000
6	72	0	0	1.000	0.000
7	72	0	1	1.000	0.000
8	71	0	0	1.000	0.000
9	71	0	0	1.000	0.000
10	71	0	0	1.000	0.000
11	71	0	0	1.000	0.000
12	71	0	0	1.000	0.000
13	71	0	0	1.000	0.000
14	71	0	0	1.000	0.000
15	71	0	0	1.000	0.000
16	71	0	0	1.000	0.000
17	71	0	0	1.000	0.000
18	71	0	0	1.000	0.000
19	71	0	0	1.000	0.000
20	71	0	0	1.000	0.000
21	71	1	0	0.986	0.014
22	70	0	0	0.986	0.014
23	70	0	0	0.986	0.014
24	70	0	0	0.986	0.014
25	70	0	0	0.986	0.014
26	70	0	0	0.986	0.014
27	70	0	0	0.986	0.014
28	70	1	0	0.972	0.028
Total		2	2	95 % CI	95 % CI
				0.933	0.067
				1.010	-0.010

DECLARATION

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate: Gebremedhin Kinfu

Signature _____

Place _____

Date ____/_____/_____

Name of advisor: Dr. Solomon Gebre-Selassie

Signature _____

Place _____

Date _____

Name of advisor: Nigus Fikrie

Signature _____

Place _____

Date _____