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Monitoring the efficacy of Coartem® for uncomplicated *falciparum* malaria in Selekleka town, Tigray Region, Northern Ethiopia

By

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Abbreviations

%: Percent

µl: Microlitre

µm: Micrometer

ACPR: Adequate clinical and parasitological response

ACT: Artemisinin-based combination therapy (Artesunate + Amodiaquine + Artemether + Lumefantrine)

AL: Artemether-lumefantrine

ANOVA: Analysis of variance

CDC: Center for disease control and prevention

CQ: Chloroquine

D: Day

DHS: Demographic and Health Surveys

EHNRI: Ethiopian health nutrition and research institution

ETF: Early treatment failure

FMOH: Federal Ministry of Health

GDP: Gross domestic Product

Hb: Hemoglobin

IPTp: Intermittent preventive treatment during pregnancy

IRS: Indoor residual spray

ITNs: Insecticide treated bed nets

LCF: Late clinical failure

LLINs: Long lasting insecticide treated bed nets

LPF: Late parasitological failure

No.: Number

OPD: Outpatient department

RBM: Roll back malaria

RDTs: Rapid diagnostic tests

SP: Sulphadoxine pyrimethamine

SPSS: Statistical package for the social science

TNF: Tumor necrosis factor

URTI: Upper respiratory tract infections

US$: USA dollar

WBC: White blood cells

WHO: World health organization

Abstract

Drug resistance is the most serious problem in achieving control of malaria. The spread of *Plasmodium falciparum* resistance to almost all available affordable mono-therapy, in many malaria endemic regions, is a serious impediment on malaria control. The current WHO recommendation for treatment of uncomplicated *P. falciparum* malaria is the use of Coartem®, an artemisinin based combination therapy (ACT). Coartem® (20mg artemether and 120mg lumefantrine) is an artemisinin based tablet that provides effective antimalarial treatment against uncomplicated *falciparum* malaria in many parts of the world, including sub-saharan Africa. The present study was conducted to monitor the efficacy of this drug in patients ≥ 6 months with uncomplicated *falciparum* malaria in Selekleka town, Northwestern Tigray, Ethiopia. A total of 98 study participants, microscopically confirmed for *P. falciparum* mono-infection, were included in the study. Majority of the study participants were adults above 15 years (87.8%). At enrollment, 65 (66%) patients were febrile (T ≥ 37.5ºC) and the overall parasite mean density was 22,679. Six doses of Coartem were given over 3 days, two doses each day, on D0, D1, and D2; with a follow up on D3, D7, D14, D21 and D28. The clinical and parasitological conditions of the patients were assessed at each visit. The level of hemoglobin in the study participants was determined by using hemocue reader. 89 (90.8%) patients completed the 28-day follow-up while 9 (9.2%) patients were excluded from the study because of loss-to-follow-up and withdrawal of consent. The ACT treatment rapidly cleared parasitaemia and fever by D2 and complete gametocyte clearance was obtained on D21. Significant (p= 0.05) hemoglobin recovery was observed among patients with adequate clinical and parasitological response. No severe adverse side-effects, clinical failures or parasitological failures were observed among these patients. Overall, the 28-day clinical and parasitological cure rate was 100%. Coartem, therefore, was efficacious for the treatment of uncomplicated *falciparum* malaria in Selekleka town. However, the reported increasing trend in *P. falciparum* prevalence since 2007/8 in Selekleka, despite free availability of Coartem and ITN coverage, needs further investigation on the efficacy of the malaria measures in use.

Key words: Malaria, *P. falciparum*, Coartem, Selekleka town, Cure rate, Hemoglobin recovery, Adverse effects

1. INTRODUCTION

Malaria is the most important tropical disease, widespread throughout the tropics and subtropics, but also occurring in many temperate regions (Guerra *et al*., 2008; Mwine, *et al*., 2010). It exacts a heavy toll of illness and death - especially amongst children and pregnant women as a result of immature and weakened immunity, respectively. It also poses a risk to travelers and immigrants that may not have developed protective immunity as a result of lack of previous exposure to malaria. Imported cases are also increasing in non-endemic areas as a result of population movements facilitated by international transports (Brooker *et al.,* 2009).

Malaria is transmitted by mosquitoes of the genus *Anopheles,* which are most abundant in tropical/subtropical regions, although they are also found in limited numbers in temperate climates. Transmission is associated with changes in temperature, rainfall, humidity as well as level of immunity (Ayanlade *et al.,* 2010).

About 20 different *Anopheles* species are important around the world. All of the important vector species bite at night. They breed in shallow collections of freshwater like puddles, rice fields, and hoof prints. Transmission is more intense in places where the mosquito is relatively long-lived (so that the parasite has time to complete its development inside the mosquito) and where it prefers to bite humans rather than other animals. For example, the long lifespan and strong human-biting habit of the African vector species mainly *Anopheles gambiae* is the underlying reason why the highest disease burden, with more than 85% of the world's malaria deaths are in Africa (WHO, 2011).

Malaria typically is found in warmer regions of the world - in tropical and subtropical countries. Higher temperatures allow the *Anopheles* mosquito to thrive. Malaria parasites, which grow and develop inside the mosquito, need warmth (21-32ºC) to complete their growth before they are mature enough to be transmitted to humans. In humans, the parasite must function at 37ºC or higher, since the infection induce a significant rise in core temperature (Despommier and Hotz, 2005; Ermert*, et al*., 2011). At temperatures below 20°C, *Plasmodium falciparum* (which causes severe malaria) cannot complete its developmental cycle in the *Anopheles* mosquito, and thus cannot be transmitted (HuangShuisen *et al*., 2011).

Yet malaria does not occur in all arid climates. For example, malaria has been eliminated in some countries with warm climates, while a few other countries have no malaria because *Anopheles* mosquitoes are not found there (Sainz-Elipe, *et al*., 2010)

According to CDC (2008) even in many malaria-endemic countries, malaria transmission does not occur in all parts of the country. This includes very high altitudes (≥2,500m) and colder seasons.

The highest transmission is found in Africa South of the Sahara and in parts of Oceania such as Papua New Guinea. In cooler regions, transmission will be less intense and more seasonal. There, *P. vivax* might be more prevalent because it is more tolerant of lower ambient temperatures (CDC, 2010).

In many temperate areas, such as Western Europe and the United States, public health measures and economic development have succeeded in eliminating malaria. However, most of these areas have *Anopheles* mosquitoes that can transmit malaria, and reintroduction of the disease is a constant risk (Greenwood *et al*., 2008).

1.1. Global malaria situation

At present, about 100 countries or territories in the world are considered malarious, and more than two billion of the world's total population is still exposed to the risk of malaria. Large areas of Africa and South Asia and parts of Central and South America, the Caribbean, Southeast Asia, the Middle East, and Oceania are considered areas where malaria transmission occurs. It is estimated that approximately 225 million people worldwide are affected by malaria and about 781,000 people die from it every year. (WHO, 2009; Kant, 2011).

1.2. The Malaria Parasites and epidemiology

Malaria parasites are micro-organisms that belong to the genus *Plasmodium*. There are more than 100 species of genus *Plasmodium*, which can infect many animal species such as reptiles, birds, and various mammals. Four species of *Plasmodium* have long been recognized to infect humans in nature (McKenzie *et al.*, 2006; Pongsumpun and Tang, 2008; Cox-Singh, 2008). In addition

there is one species (*P. knowlesi*) that naturally infects macaques which has recently been recognized to be a cause of zoonotic malaria in humans (Baird, 2009).

The species infecting humans are:

P. falciparum, which is found worldwide in tropical and subtropical areas. *P. falciparum* is the most common species in Africa and it accounts for 95 - 98% of all malaria infections (Usher, 2010). *P. falciparum* can cause severe malaria because it multiples rapidly in the blood, including in the young reticulocytes and can thus cause severe hemoglobin degredation and red blood cell destruction (hypoxia, jaundice, anemia, etc.). In addition, the infected parasites can clog small blood vessels. When these occur in the brain, cerebral malaria results, a complication that can be fatal (Scarlata *et al*., 2002; Mzilahowa *et al*., 2007; CDC, 2011).

P. vivax is found mostly in Asia, Latin America, and in some parts of Africa. Because of the population densities especially in Asia it is probably the most prevalent human malaria parasite (RBM, 2008; Hulden and Hulden, 2011).

P. ovale is seldom seen except in sub-Saharan Africa (especially West Africa) and the islands of the western Pacific. It is biologically and morphologically very similar to *P. vivax*. However, differently from *P. vivax*, it can infect individuals who are negative for the Duffy blood group (a receptor of *P. vivax* on red blood cells), which is the case for many residents of sub-Saharan Africa. This explains the greater prevalence of *P. ovale* (rather than *P. vivax*) in most of Africa (Collins and Jeffery, 2005).

P. malariae, found worldwide, is the only human malaria parasite species that has a quartan cycle (four-day cycle)-(the three other species have a tertian, three-day cycle.) If untreated, *P. malariae* causes a long-lasting, chronic infection that in some cases can last a lifetime (CDC, 2010).

P. knowlesi - a non-human anthropoid malaria, is recognized as the fifth species of *Plasmodium* infecting humans. It is found throughout Southeast Asia as a natural pathogen of long-tailed and pig-tailed macaques. It has recently been shown to be a significant cause of zoonotic malaria in that region, particularly in Malaysia (Lee *et al.,* 2011).

The life cycle of the four species of human malaria parasites consists of two phases: the sexual and asexual phases (schizogony). Schizogony begins when an infective female anopheline mosquito injects sporozoites into the human host while taking a blood meal. The sporozoite stage of the parasite disappears from circulation within thirty minutes. Those avoiding the host immune system invade the liver and undergo development and multiplication to form schizonts. Over the next five to fifteen days, the schizonts mature, rupture the liver cell, and invade the circulation as merozoites. These merozoites bind to the red blood cell membrane; then penetrate the red blood cell, where they develop as ring forms and grow into trophozoites. Further division inside red blood cell forms mature schizonts, which consists of merozoites. The blood cell swells and ruptures, releasing merozoites that go on to invade other red blood cells. Clinical symptoms result when the blood cell ruptures and releases cellular debris and toxic substances from infected cells into the bloodstream. The host response to these toxins produces the classic paroxysms of fever and chills, which are closely timed with the cycles of red blood cell schizogony. The timing of the blood cell phase differs depending on the species of the parasite. *P. vivax* and *P. ovale* classically have cycles of forty-eight hours, *P. malariae* seventy-two hours, and *P. falciparum* forty-eight hours, although this may vary. After a period of time, some of the merozoites develop into male and female sexual forms called gametocytes. The gametocytes are ingested by the female anopheline mosquitoes during a blood meal. Inside the mosquito stomach, the male and female gametocytes fuse to form a zygote, which quickly becomes a mobile ookinete. The ookinete penetrates the stomach wall where sporogony takes place to form an oocyst. The oocyst then bursts, releasing sporozoites that migrate to the salivary glands, ready to be injected into a human host, thus completing the cycle. The parasite generally develops within the mosquito in nine to twelve days, with time variations according to parasite species and external temperature (Baer *et al.,* 2007).

P. vivax and *P. ovale* differ from the other two species in that some hepatic trophozoites, called hypnozoites, may remain dormant and persist in the liver for months to up to four years. Periodic release of merozoites formed from these hypnozoites can produce recurrent parasitemia and clinical symptoms. Recurrent parasitemia can also occur with *P. falciparum* and *P. malariae*, although these species do not form hypnozoites. Infection with these parasites may remain in the blood at subclinical levels because of either the host immune system or use of antimalarial drugs

that do not completely clear the blood-stage parasites. The level of parasitemia can increase weeks to month's later, giving rise to another clinical attack. While *P. falciparum* rarely recrudeses more than several months after the initial infection, *P. malariae* may become active again up to forty years after the infection (Richter *et al.,* 2010).

1.3. Clinical features of malaria

Malaria is a febrile illness characterized by fever, chills, sweats, headaches, nausea, vomiting and related symptoms. All the clinical features of malaria are caused by the erythrocytic schizogony in the blood. The growing parasite progressively consumes and degrades intracellular proteins, principally hemoglobin, resulting in formation of the malarial pigment and hemolysis of the infected red cell. This also alters the transport properties of the red cell membrane, and the red cell becomes more spherical and less deformable. The rupture of red cells by merozoites releases certain factors and toxins (such as red cell membrane lipid, glycosyl phosphatidyl inositol anchor of parasite membrane protein), which could directly induce the release of cytokines such as TNF and interleukin-1 from macrophages, resulting in chills and high grade fever. This occurs once in 48 hours, corresponding to the erythrocytic cycle. In the initial stages of the illness, this classical pattern may not be seen because there could be multiple groups (broods) of the parasite developing at different times, and as the disease progresses, these broods synchronise and the classical pattern of alternate day fever is established. It has been observed that in primary attack of malaria, the symptoms may appear with lesser degree of parasitemia or even with submicroscopic parasitemia. However, in subsequent attacks and relapses, a much higher degree of parasitemia is needed for onset of symptoms. Further, there may be great individual variation with regard to the degree of parasitemia required to induce the symptoms (Kakkilaya, 2009).

1.4. Anti-malaria Interventions

Malaria control strategy is a concerted effort meant to bring about changes in the way malaria problem is addressed. As a result, this strategy stresses the selective use of preventive and therapeutic measures wherever they can lead to sustainable results. Several interventions have been recommended to curb the rising burden of the disease in endemic regions. These interventions form the pillar of the global campaign for effective malaria intervention,

particularly in sub-Saharan Africa. Key interventions to control malaria include: prompt diagnosis and effective treatment and vector control (Enato and Okhamafe, 2005).

1.4.1. Prompt and effective treatment of malaria

Prompt access to effective malaria treatment is central to the success of malaria control worldwide. However, most African countries are far below these targets, with only a minority of fevers being treated promptly and effectively (Chuma *et al.,* 2010).

However, it is generally accepted that most malaria deaths can be prevented when clinical cases are promptly diagnosed and effectively treated. In young children, malaria can progress from a mild to severe case within 24 hours after the onset of symptoms. Prompt diagnosis and timely malaria treatment within 24 hours after onset of first symptoms can reduce illness progression to severe stages and, therefore, decrease mortality (Getahun *et al*., 2010).

1.4.2. Vector control

There are various methods of vector control and they are not necessarily mutually exclusive. Because the *Anopheles* mosquito normally bites in the early evening and through the night, not during the day, many of these methods focus on protecting dwellings and their inhabitants (Mboera *et al*., 2007). The major vector control strategies include:

- Environmental management (source reduction)
- Larvicidal strategies (chemical and biological control)
- Adult mosquito control – mainly, IRS and ITNs based

1.5. Malaria in Sub-Saharan Africa

The vast majority of malaria deaths occur in Africa, south of the Sahara, where malaria also presents major obstacles to social and economic development. Kibret *et al.,* (2009) indicated that malaria causes great economic loss in many African countries and is considered a major barrier to the socioeconomic development of the continent. Malaria has been estimated to cost Africa more than US$ 12 billion every year in lost gross domestic product (GDP), even though it could be controlled for a fraction of that sum. Ninety per cent of deaths due to malaria occur in Africa

south of the Sahara mostly among young children. Malaria kills an African child every 30 seconds (Barnes, *et al.,* 2009). Many children who survive an episode of severe malaria may suffer from learning impairments or brain damage. Malaria is Africa's leading cause of under-five mortality (20%) and constitutes 10% of the continent's overall disease burden (Kulkarni *et al*., 2010). It accounts for 40% of public health expenditure, 30-50% of inpatient admissions, and up to 50% of outpatient visits in areas with high malaria transmission (RBM, 2010).

Africa is the most affected due to a combination of factors (Plowe *et al.,* 2007; Salam *et al.,* 2009; Mourou *et al*., 2010; Chrispinus *et al*., 2011):

- A very efficient mosquito (*Anopheles gambiae*) is responsible for high transmission.
- The predominant parasite species is *Plasmodium falciparum*, which is the species that is most likely to cause severe malaria.
- Local weather conditions often allow transmission to occur year round.
- Scarce resources and socio-economic instability have hindered efficient malaria control activities.
- Malaria parasites are increasingly resistant to antimalarial drugs, presenting one more barrier to malaria control on the continent.

Since 2005, malaria control scale-up has progressed in many African countries. Controlled studies of insecticide-treated mosquito nets (ITNs), indoor residual spraying (IRS), intermittent preventive treatment during pregnancy (IPTp) and malaria case management suggested that when incorporated into national programmes a dramatic health impact, likely more than a 20% decrease in all-cause childhood mortality, was possible (Steketee and Campbell, 2010).

1.6. Malaria in Ethiopia

The African region south of the Sahara is heavily affected by malaria. Ethiopia is among the 30 high burden countries in malaria infection and contributes about 6% of the malaria cases in Africa. Due to climatic changes and geographic factors in Ethiopia, malaria occurs everywhere except the central high lands. Approximately 75% of Ethiopia's landmass is malarious (Baume *et al.,* 2009; Amare *et al*., 2010).

According to Getahun et al., (2010) malaria is a major public health problem in Ethiopia; it contributes up to 20% of under-five deaths. Tragically, in epidemic years, mortality rates of nearly 100,000 children are not uncommon. In the last major malaria epidemic in 2003, there were up to 16 million cases of malaria. Out of an estimated 9 million malaria cases annually, only 4-5 million will be treated in a health facility. The remainder will often have no medical support. It is estimated that only 20% of children under five years of age that contract malaria are treated in a facility.

Malaria transmission in Ethiopia is seasonal, depending mostly on altitude and rainfall. The two main seasons for transmission of malaria in Ethiopia are September to November, sometimes extended to December after heavy summer rains, and March to May, after the light rains. Malaria epidemics involving highland or highland fringe areas are relatively frequent in areas 1,000–2,000 meters above sea level, in which the population lacks immunity to malaria (Endeshaw *et al*., 2008).

Demographic and Health Surveys (DHS) were carried out in Ethiopia in 2000 and 2005, and included a malaria module. Recognizing the low coverage and use of malaria interventions in the country, in 2005 the Government of Ethiopia's Federal Ministry of Health (FMOH) developed a 5-year National Malaria Prevention and Control Strategy. According to the strategy, areas <2,000 m were considered 'malarious' and targeted to receive key malaria control interventions, including insecticide-treated nets (ITNs), indoor residual spraying of households with insecticide (IRS), and rapid diagnostic tests (RDTs) for malaria coupled with prompt and effective case management with artemisinin-based combination therapy (ACT). The strategy outlined an ambitious national goal of 100% household ITN coverage in malarious areas with a mean of two LLINs per household through distribution of about 20 million LLINs by the end of 2007. Moreover, the strategy stated that IRS should be scaled-up to cover 30% of households targeted for IRS and also included the rapid scale-up of provision of RDTs and ACT to newly established community health posts (Jima *et al*., 2010).

1.7. Malaria in Tigray

1.7.1. General background of Tigray

Tigray is the northern most region of Ethiopia, extending from 12º 15' to 14º 50' North latitude and from 36º 27' to 39º 59' East longitude. It is bounded with the state of Eritrea in the North, the state of Afar in the East, the state of Amhara in the South, and the Republic of Sudan in the West. The mean annual temperature of the region is estimated to be 18ºC. In summer the Eastern low lands have a mean temperature in excess of 27.4ºC (Ghebreyesus *et al.,* 2000).

1.7.2. The malaria situation in Tigray

Almost 75% of Tigray region is malarious and about 56% of population lives in malarious areas. Malaria transmission is seasonal and depends on both altitude and rainfall. As in the rest of Ethiopia, malaria is unstable in Tigray. The unstable nature of malaria makes the region prone to out breaks and malaria is a major public health problem. In Western Tigray, a malaria epidemic in 1987 was responsible for 142,317 cases and 349 deaths. In 1991 an outbreak in Southern Tigray affected 198 localities with population of 172,139 and 523 deaths were recorded (Ghebreyesus *et al.,* 2000).

This indicates that in Tigray malaria is a high priority public health problem. Malaria is also a serious problem in Medebay Zana Woreda which is one of the Northwestern Woreda of the region. This disease occurs in the Woreda at the end of kiremt from September- December and sometimes April and May in the form of epidemic. *P. falciparum* is the most prevalent or dominant species among the other species of malaria in the Woreda (Fig. 2). It is also among the top ten diseases of the Woreda (Table 1). Due to this fact regional health bureau department of malaria and other vector borne disease provides the Woreda with spraying chemical and distribution of ITNs (Source: Medebay Zana Woreda Department of Malaria and Other Vector Borne Diseases).

1.8. History of malaria parasite resistance

Drug resistance is the most serious problem in achieving control of malaria. The spread of *Plasmodium falciparum* resistance to available cheap drugs, the increased cost of insecticides, the vector's resistance to insecticides and lack of an effective vaccine, together with a socio-economic instability in many malaria-endemic regions, had a negative impact on malaria control. Therefore, surveillance and prevention of drug resistance and also effective curative chemotherapy have become more important to be considered as the primary approach to malaria control (Zakeri *et al*., 2007).

The emergence and spread of antimalarial drug resistance is one of the most important factors undermining malaria control programmes in most of the malaria endemic world (Price *et al.,* 2007). Antimalarial drug resistance is the ability of a parasite strain to survive and multiply despite the administration and absorption of a drug given in doses equal or higher than dose usually recommended, but within the limits of tolerance of the subject. One of the mechanisms by which resistance to antimalarial drugs arises is as a result of spontaneously-occurring mutations that affect the structure and activity at the molecular level of drug target in the malaria parasite that affect the access of the drug to that target (WHO, 2011).

In the late 1950s and early 1960s, the eradication of malaria seemed possible because the parasite does not have an animal reservoir and effective agents to interrupt transmission or to obtain a radical cure existed. In 1955, WHO declared a global war in malaria (CDC, 2011).

On the basis of such observations, the World Health Organization spearheaded projects for malaria eradication by using indoor residual spraying and large mass drug administration program using chloroquine (CQ) and pyrimethamine. However, many of the national programs lacked adequate epidemiological skills and knowledge and administrative organization. These deficiencies were overlooked because of the humanitarian appeal of the program, the sense of urgency, and the feeling that peer pressure could eventually shake the chronic apathy of the health services (Talisuna *et al*., 2004).

As time progressed, evidence started to accumulate indicating that although it was possible to reduce or even interrupt malaria transmission by insecticide spraying in large areas; it was very

difficult to establish effective surveillance in the absence of solid health infrastructure. Some of the factors responsible for the lower than expected impact of the eradication programme includes the following (Mills *et al*., 2008; Song *et al.,* 2010):

- DDT resistance in vector mosquitoes;
- High levels of refusals by house owners to have their house sprayed and replastering of walls following spraying;
- Selection of exophilic mosquitoes which do not rest long enough indoors to pick up a lethal dose.

Furthermore, it was realized that in the great majority of countries, eradication was not a realistic goal and that there was a need to change from highly prescriptive, centralized control program to flexible, cost-effective, and sustainable programs adapted to local conditions and responding to local needs (Najera *et al*., 2011).

The geographic distribution of *P. falciparum* resistance to CQ corresponds almost exactly to that of the parasite, and the prevalence of the resistance is high in many countries (Ghanchi *et al.,* 2011). Despite reports of Chloroquine failure suspected to be a result of drug resistance in the 1980s, CQ has remained the first-line drug for treatment of uncomplicated *Plasmodium falciparum* malaria. The combination of sulphadoxine pyrimethamine (SP) has been an effective and affordable alternative single dose treatment for CQ-resistant parasites since its first introduction to Africa in 1960s. Use of SP increased rapidly in countries with CQ resistance over the next 30 years. As a result, resistance to SP is now globally widespread, leading most endemic countries in Africa to abandon SP alone as first-line treatment (Gatton, *et al.,* 2004; Dlamini *et al.,* 2010).

Parasite resistance to SP has developed very quickly in Southeast Asia. Ever since the discovery of the first case of chloroquine resistance along the Thai-Combodian border in the late 1950s, Southeast Asia has played an important role as a focus for the development of drug resistance in *Plasmodium falciparum.* Although the first case of quinine resistance had been reported much earlier from South America, the onset of chloroquine resistance marked the beginning of a new chapter in the history of malaria in Southeast Asia and by 1973 chloroquine finally had to be replaced by the combination of sulphadoxine and pyrimethamine (SP) as first line drug for the

treatment of uncomplicated malaria in Thailand (Farooq and Mahajan, 2004; Escalante *et al.,* 2009).

In Africa, following increased chloroquine resistance, the use of SP for first-line purposes are an interim measure while different anti-malarial combinations are being evaluated for long-term use. However, the fact that *Plasmodium falciparum* rapidly develops resistance to SP following wide use of the drug poses a serious threat to malarial control efforts. High levels of SP resistance have been recorded in highly endemic African countries including Kenya, Burundi, Rwanda, Tanzania and Malawi where pyrimethamine and sulfadoxine were used at different periods between 1950 and 1994 for prophylactic and therapeutic trials, respectively. Therefore, several African countries have switched to SP combination therapies following the fail of SP to clear parasitemia (Mugittu *et al*., 2004).

Drug resistance is a major impediment to treatment of *P. falciparum* malaria. In the majority of the regions where *P. falciparum* predominates, the parasites are resistant to one or multiple of the common antimalarial drugs such as Chloroquine and sulphadoxinepyrimethamine (SP), and resistance to more recent treatment options such as mefloquine and atovaquone-proguanil is emerging (Boggild *et al.,* 2007; WHO, 2008). Therefore, a new treatment option for malaria is necessary.

1.9. Current WHO Recommendations for Treatment of uncomplicated *falciparum* Malaria

Because of the rising threat of resistance to available antimalarial drugs, the WHO recommends use of an artemisinin based combination therapy for treatment of uncomplicated *P. falciparum* malaria (Travassos and Laufer, 2010).

Artemisinin-based combination therapies have been widely adopted worldwide. The combination of an artemisinin derivative with another effective antimalarial drug that has a complementary mechanism of action and pharmacologic profile can overcome the emergence of drug resistance. Artemisinin derivatives have the most potent and rapid onset of anti-parasitic activity of any antimalarial drug available today and are active against all *Plasmodium* species that infect humans. They allow more parasite clearance than any other antimalarial drug. When

combined with antimalarial drugs with slower elimination rates (eg, lumefantrine), shorter courses of treatment (3 days) are effective. Combinations of antimalarial drugs are now recommended by the WHO, because combination therapy is usually more effective than monotherapy and minimizes the risk of treatment failure due to the development of drug resistance during treatment. If a parasite resistant to one component of a combination emerges during treatment, it should be killed by the other component (WHO, 2006).

Coartem® is the first-dose ACT prequalified by the WHO and is widely available internationally. Artemether and lumefantrine have both been included on the WHO model list of Essential Medicines since 2002 and on the first WHO model list of Essential Medicines for children since 2007 (WHO, 2007).

Coartem is a combination of two antimalarial active ingredients, artemether (an artemisinin derivative) and lumefantrine (a racemic mixture of a synthetic racemic fluorine derivative formerly known as benflumetol) with a fixed ratio of components in a single tablet (20mg artemether and 120mg lumefantrine). Both components are blood schizontocides, with complementary pharmacokinetics and dissimilar modes of action, thus providing synergistic antimalarial activity. The mechanisims of action of artemisinins and lumefantrine is uncertain. The antimalarial activity of artemisinin may result from the production of free radicals that follows the iron-catalyzed cleavage of the artemisinin endoperoxide bridge in the parasite food vacuole or from inhibition of a parasite calcium ATPase. The antimalarial mechanism of action of lumefantrine is not well defined. However, available data suggests lumefantrine inhibits the formation of β-hematin by forming a complex with hemin, a byproduct of hemoglobin digestion, which is present in the parasite in high amounts (Bhisutthibhan *et al.,* 2011).

Artemether is a white, crystalline powder that is freely soluble in acetone, soluble in methanol and ethanol. Lumefantrine or benflumetol is a yellow, crystalline powder that is freely soluble in N,N-dimethylformamide, chloroform, and ethyl acetate; soluble in dichloromethane; slightly soluble in ethanol and methanol. Lumefantrine is only used in combination with artemether (Toovey *et al.,* 2003).

The artemisinin component of these combinations produces a characteristic rapid reduction in parasite biomass immediately after treatment, but these compounds are metabolized in hours, and thus combination with a partner drug (lumefantrine) is required to provide complete parasite clearance with short treatment regimens, and to minimize the opportunity for evolution of parasites resistant to either component drug. This allows better compliance to treatment than free combination of loose tablets (Beshir *et al*., 2010).

Following the original approval of coartem in 1998, resistance to older antimalarial drugs continued to increase. By 2006 median clinical failure rates of chloroquine as high as 70– 80% are found in several countries in Asia, Africa, and the Americas which were greater than threshhold (25%) for a change in antimalarial policy (Vestergaard and Ringwald, 2007).

Following the rapid development of significant drug resistance of *Plasmodium falciparum* to chloroquine and then sulphadoxine-pyrimethamine (the first line therapy in Ethiopia 1998-2004), artemether- lumefantrine (Coartem® or AL) was adopted as first line therapy in Ethiopia in 2004. According to the current national malaria diagnosis and treatment guidelines, first-line treatment for uncomplicated *falciparum* infection is artemether-lumefantrine (CDC, 2010). In this study the therapeutic efficacy of artemether-lumefantrine (Coartem®) for the treatment of uncomplicated *P. falciparum* infection in Selekleka town, North-western Tigray, Ethiopia, has been assessed.

2. Objectives

2.1. General objective

- To generate reliable data on the current level of coartem efficacy for *P. falciparum* in Selekleka town and provide credible information to control managers for appropriate decision making.

2.2. Specific objectives

- To assess the current clinical and parasitological cure rate of coartem in patients with uncomplicated (mild) *P. falciparum* malaria;
- To measure the effect of treatment with coartem on parasite clearance and fever clearance time;
- To measure gametocyte carrier rates in patients Coartem treated for uncomplicated *P. falciparum* malaria;
- To assess hemoglobin (Hb) changes from baseline to day 14 and 28 after *P. falciparum* treatment with coartem;
- To see the adverse drug reactions and tolerability of coartem during *P. falciparum* treatment.

3. Materials and methods

3.1. Study site

The study was carried out between October and January, 2010/11 at a Health Center situated in Medebay Zana Woreda, Selekleka town (Fig. 1). Medebay Zana is situated in North Western Tigray and it is bounded by:

- Tahtay Maychew and Naeder Adet Woreda in the East;
- Tahtay Koraro and Asigede Tsimbela Woreda in the West;
- La'ilay Adiabo Woreda and Mereb Lake in the North and
- Naeder Adet and Tselemti Woreda in the South.

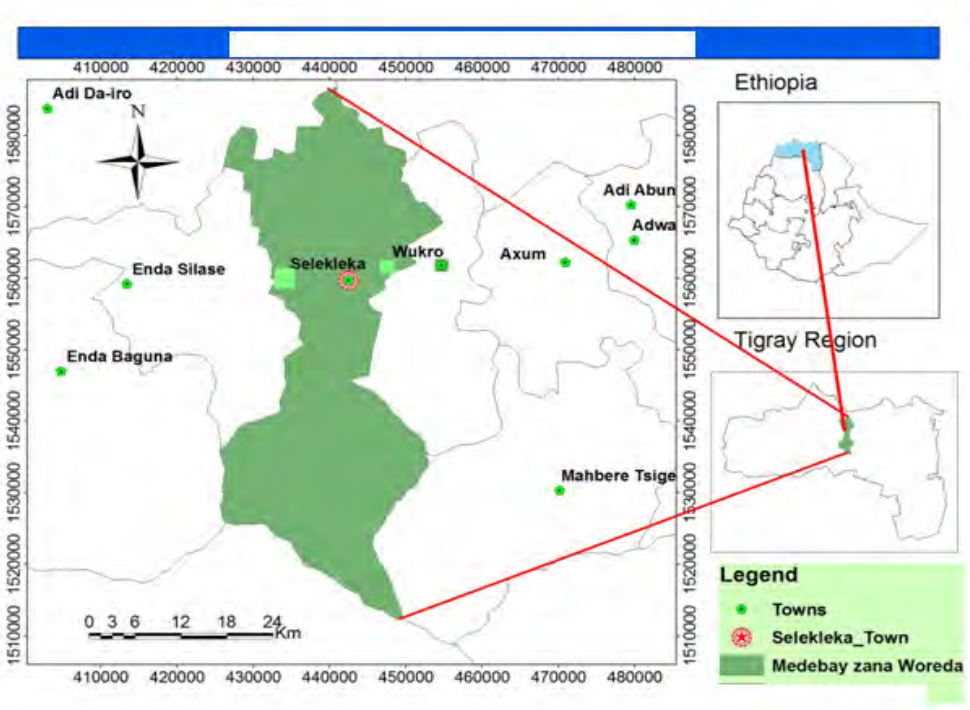

Figure 1. Selekleka Town in Medebay Zana Woreda (Source: modified from Ethio-GIS)

Selekleka town is situated at a distance of 1060km from Addis Ababa and 306km from Mekele. The location of Selekleka town is 30km west of the ancient historical town Axum and 30km East of Endaslasie (Shire) with absolute location of 14º07'N to 14º117'N and 38º29'E to

38º483'E. The Woreda is categorized under sub tropical climate zone and found at an elevation ranging from 1500-2107 meter above sea level with a mean annual temperature of 17.5ºC and annual rainfall of 500-900mm. The total area of the Woreda has been estimated to be about 16,880,600 square meters. The total population of the Woreda is 139,083. The annual growth rate of the population is 2.5% and total number of household is 31,609. On average, 5 family members live in the same shelter. Malaria disease occurs in the Woreda at the end of kiremt from September-December and during the small rains during April and May, in the form of epidemic. Subsistence farming is the main economic activity in this area and the main crops grown include: teff, corn/maize, sorghum and Degussa/finger millet (Source: Medebay Zana Woreda Department of Malaria and Other Vector Borne Diseases).

3.2. Sample size determination

The WHO (2003) protocol was used to calculate the required sample size. It assumes a 95% Adequate Clinical and Parasitological Response rate with Coartem® on day 28, considering the very low proportion of clinical failure of Coartem resistance in Ethiopia. Thus, with desired precision of 5% and 95% confidence interval a minimum of 73 patients should be enrolled.

$N = (z/d)^2 P (1-P)$

$= (1.96/0.05)^2 0.05 (1-0.05)$

$= 73$

Where, N = number of samples

P = the expected population proportion of clinical failure (5%)

z = confidence interval (95%)

d = precision (5%)

Assuming an additional 20% loss to follow-up rate and withdrawal of consent (15 patients) during the study, at least 88 (73 + 15) patients should be recruited to bring about a representative sample.

N = (1 + 0.2)73 = 88

3.3. Patients

This study followed the World Health Organization (2003) recommendation, for the *in-vivo* investigation of antimalarial drug efficacy, in terms of treatment, follow up and data analysis. The study was conducted in patients with fever aged ≥ 6 months, who were attending Selekleka Health Center, using the WHO 28 day *in-vivo* protocol; patients were enrolled in the study, if they satisfied the following inclusion criteria:

- Both sexes ≥ 6 months of age,
- Microscopically confirmed *P. falciparum* mono-infection, with asexual parasitemia between 500 and 100,000 parasites/µl, or higher with no complications.
- Body weight > 5kg,
- Fever (axillary temperature ≥ 37.5ºC) or history of fever in the previous 24 hours,
- Non-pregnant or breast-feeding women (since very limited data are available),
- Patients living within facility catchment area (i.e. 5-10km radius of the health center),
- Informed consent by each patients (Appendix I) or by caregivers for children (Appendix II) and patient's agreement to return for all scheduled visits.

Key exclusion criteria were:

- Mixed or mono-infection with another *Plasmodium* species (besides *P. falciparum*),
- Hemoglobin < 5g/dl,
- Coartem intake in the 2 weeks prior to study enrollment,
- Unable to take oral medication or repeated vomiting,
- Known hypersensitivity to Coartem,
- Severe malnutrition,
- Evidence of severe malaria or other danger signs according to WHO: not able to drink or breast-feed, vomiting (i.e. more than twice in past 24 hours), recent history of convulsions (i.e. more than once in the past 24 hours), unconscious state, unable to sit or stand.

3.4. Drug administration

Enrolled patients were treated with the standard six-dose regimen of Coartem given twice daily for three consecutive days. On days 0, 1, and 2, the first dose of the medication administered in the study Health Center under direct supervision, while the second dose of day 0 medication administered, 8 hours later and of day 1 and day 2, 12 hours later at home by the patients or caregivers.

Dosing was administered according to weight-based guidelines (Guthmann *et al.,* 2006) (Appendix III). Study medications given to young children were crushed, mixed with water, sugar and administered as suspension. Study medications administered to older children and adults were given as tablets or fractions of tablets to be taken orally with a glass of water. Patients were provided with some biscuits after each dose. At each supervised drug administration the patient was observed for 30 minutes. If vomiting occurs before 30 minutes, the dose was repeated and the patient was observed for an additional 30 minutes. Patients vomited more than once were withdrawn from the study and referred immediately to the OPD (outpatient department) for rescue treatment with intramuscular or intravenous quinine (Mangen, *et al.,* 2005).

Patients were taken the evening dose that would be taken at home each day. These were given to patient or caregiver with proper and clear verbal instruction on when and how to take the medication. They were advised to administer the drugs with some fatty food such as a glass of milk, some peanuts, etc., since this improves absorption of artemether and lumefantrine (WHO, 2003).

3.5. Microscopic diagnosis

At screening prior to enrollment, thick and thin blood films were stained with 10% Giemsa for ten minutes. Thick films were examined at 1000× magnification using an oil immersion objective lens to quantify the parasitemia, while thin smears were used for species identification. Parasitemia was measured by counting the number of asexual parasites against a number of white blood cells in the thick blood film based on putative count of 8000 WBC per microlitre of blood. The number of asexual parasites was counted against 200 WBC using a

hand tally counter. The number of parasites per microlitre of blood was calculated by using the formula (Opera *et al*., 2010):

$$\text{No. parasites/µl} = \frac{\text{parasite count}}{200\ \text{WBC}} \times 8000$$

Moreover, gametocytes were counted per 1000 WBC, based on mean WBC count of 8000/ µl.

$$\text{No. gametocytes/µl} = \frac{\text{gamete count}}{1000\ \text{WBC}} \times 8000$$

One experienced laboratory technician working in Selekleka Health Center examined each blood smear. All *P. falciparum* positive slides, at day of enrollment were re-examined by the investigator. Finally the slides were examined by senior technician and staff of Ethiopian Health and Nutrition Research Institute (EHNRI) for confirmation.

3.6. Hemoglobin measurement

A drop of finger prick blood was taken for heamoglobin measurement on D0, D14 and D28. The finger tips were cleaned with alcohol soaked cotton and pricked with a sterile blood lancet, and the first drop wiped away with dry cotton. Then, the next drop of blood was used to fill the microcuvette by touching the middle of the blood drop with its tip until the microcuvette was completely filled. Finally the filled cuvette was pushed into hemocue instrument [hemoglobinomter (HemoCue, Anglom, Sweden)] and the displayed value was recorded in g/dl. Anemia is defined as a reduction of the hemoglobin (Hb) concentration, below normal levels. Mild anemia is defined as an Hb concentration of <11 g/dl for both sexes under five, a Hb concentration of <12 g/dl for both sexes between 5 and 14 years (10-11.9g/dl - mild, 7-9.9g/dl - moderate and <7 - severe) and a Hb concentration of <13g/dl (10-12.9g/dl - mild, 7-9.9g/dl moderate and <7g/dl - severe) for male and (10-11.9g/dl - mild, 7-9.9g/dl - moderate and <7 - severe) and female ≥15 years respectively. (WHO, 2001).

3.7.Treatment patient follow-up protocol

On day 0 in which patients are enrolled in the study, enrollment form (Appendix IV) was used to record the general information and all baseline demographic information and a case record form (Appendix V) was used to record all clinical and laboratory information. Each patient who was successfully treated with the first dose was provided with appointment card (Appendix VI) and the evening dose to be taken at home. Patients were then asked to come back for treatment the following 3 days (D1, D2 and D3) and for follow-up on days, 7, 14, 21 and 28 and on any unscheduled day if they did not feel well. On day 0 and at each follow up visit, clinical and laboratory assessment were performed (Annex VII) and results for each patient was entered on the patient record sheet (VIII).

3.8. Conditions for patient withdrawal after enrollment

Patients were withdrawn from the study in case of: Protocol violation, withdrawals and loss to follow up

Patients were excluded from the study on day 1 if:

- Day 0 repeat parasite count <500/µl;
- Mixed *Plasmodium* species infection detected;
- Missing the previous evening dose.

In addition, patients were excluded from the study on any day if the following occurs after treatment:

- Vomiting any study dose twice;
- Missing any treatment dose;
- Experiences serious adverse event;
- Intake of any drug with antimalarial properties;
- Detection of other malaria species during the follow-up;
- Development of febrile illness (e.g. pneumonia, dysentery, measles) that interferes with outcome classification;

- Withdrawal of consent;
- Loss to follow-up (Patients who missed follow-up visits and unable to be located within 24 hours on days 1-3 or within 48 hours on days 4-28).

3.9. Study outcome

Outcome of the treatment was defined according to the standard WHO classification (WHO, 2003) as follows:

Early Treatment Failure (ETF) was defined as

- ❖ Danger signs or severe malaria on days 1, 2 or 3 in the presence of parasitemia;
- ❖ Parasite density at day 2 greater than at day 0;
- ❖ Parasitemia on day 3 with axillary temperature ≥ 37.5ºC and
- ❖ Parasite density at day 3 ≥ 25% of count on day 0.

Late Clinical Failure (LCF) was defined as

- Danger signs or severe malaria in the presence of parasitemia on any day between day 4 and day 28 in patients who did not previously meet any of the criteria of early treatment failure;
- Presence of parasitemia on any day between day 4 and day 28 with axillary temperature ≥ 37.5ºC in patients who did not previously meet any of the criteria of ETF.

Late Parasitological Failure (LPF) was defined as

- o Reappearance of parasitemia on any day between day 7 and day 28 and axillary temperature < 37.5ºC in patients who did not previously meet any of the criteria of ETF or LCF.

Adequate Clinical and Parasitological Response (ACPR) was defined as

- Absence of parasitemia on day 28, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of ETF, LCF or LPF. The overall rate of treatment failure (Total Treatment Failure) was computed as if the patient had an ETF, LCF or LPF. In LPF Only parasitemia confirmed by PCR as recrudescence is considered as treatment failure.

All adverse events were recorded on the Case Record Form. An adverse event was defined as "any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the drug administered (WHO, 2003).

3.10. Ethical clearance

This study was approved by the ethical committee of Addis Ababa University, Biology Department and ethical committee of Ethiopian Health and Nutrition Research Institution (EHNRI). Informed consent was obtained from adult patients while informed consent for the minors was obtained from their parents and guardians. All work was performed according to the World Health Organization guidelines for human experimentation in clinical research.

The study participants' transportation expenses were covered as the patients return for the scheduled follow-ups.

3.11. Statistical analysis

All the data from recruited patients were imported into an Excel spreadsheet and the WHO designed Excel data analysis program was used for analysis. Data was also analyzed with the aid of statistical package for social science, SPSS (version 16.0) using analysis of variance (ANOVA) - one-Way-ANOVA. Patients lost to follow up and withdrew consent were excluded from the analysis. Correlation was also used for the relationships between variables.

4. Results

4.1. Malaria prevalence

Malaria transmission in Medebay Zana Woreda peaks from September to December and sometimes from April to May coinciding with major harvesting season with serious consequences for the subsistence economy of the Woreda. As indicated in the five consecutive years data (2005-2010) of malaria documented by different Health Centers of Medebay Zana Woreda, *P. falciparum* was more prevalent than *P. vivax* and few numbers of mixed infections was recorded. The figure below (Fig. 2) shows a decline in the prevalence of *P. falciparum* upto 2007/8 followed by increasing trends thereafter, while *P. vivax* and mixed infection shows the same trends.

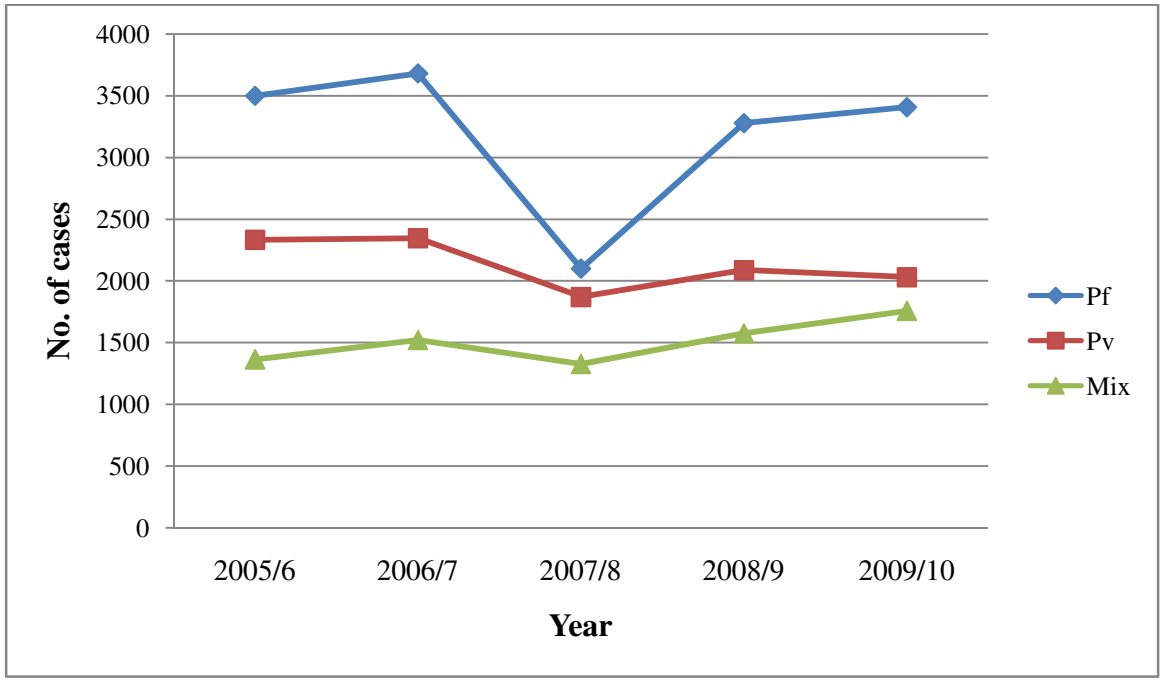

Figure 2. Prevalence of malaria infection in Medebay Zana Woreda, Northwestern Tigray Zone from 2005 to 2010 (Source: Medebay Zana Woreda Department of Malaria and Other Vector Borne Diseases); (Pf = *P. falciparum*; Pv = *P. vivax*; Mix = Mixed infection).

Malaria (unspecified) is the major health problem of the Woreda (29.3%), followed by URTI (16.2%) and *P. falciparum* mono-infection (13.6%) (Table 1).

Table 1. The top ten diseases reported from Medebay Zana Woreda (2009/10)

Types of disease	Total no. of cases	%
Malaria unspecified	**7344**	**29.3**
URTI	**4068**	**16.2**
Malaria (Pf)	**3407**	**13.6**
Gastric deodonitis	**1646**	**6.6**
Pneumonia	**1606**	**6.4**
Dysentery unspecified	**1514**	**6.0**
Skin infection	**1373**	**5.5**
Helminths	**1178**	**4.7**
Eye disease	**814**	**3.2**
Other diseases	**2130**	**8.5**
Total	**25080**	**100**

(Adapted from Medebay Zana Woreda, Department of Malaria and Other Vector Borne Diseases).

4.2. Malaria Control measures in Medebay Zana Woreda

Malaria is the first among the top ten diseases and therefore, the Woreda uses different intervention methods to control it. Malaria control programs of the Woreda depends on the control of vector mosquitoes, and early diagnosis and treatment at the community health worker levels using rapid diagnostic test and at Health Center with the use of microscopic diagnosis. Vector control strategies in the Woreda involve environmental sanitation (once per week) during transmission season; indoor residual spray every six months and free distribution of insecticide treated bed nets (two insecticide treated bed nets per household) that were obtained from health bureau. However, the information collected from 98 enrolled patients in the study showed lack of full coverage (81.6% coverage) and inappropriate use of nets with 60 (61.2%)

patients using the bed nets sometimes; only 6 (6.1%) using it always and 14 (14.3%) never using their insecticide treated nets.

Regarding the indoor residual spray, the Woreda had sprayed 99.6% of the households in 2009/10 which did not seem to have protected the population from *falciparum* malaria (Fig. 2).

4.3. Study participants screening and treatment follow-up

Between October, 2010 and January, 2011, 286 patients were positive for malaria (121-*P. falciparum* mono-infection, 140-*P. vivax* infection and 25-mixed infection) from 896 total examined cases. Among 121 *P. falciparum* mono-infection, 17 patients were excluded as they did not fulfil the inclusion criteria. That is, 14 were excluded on day 0 since they were out of the catchment area; 1 because of pregnancy; 1 because of prior antimalarial intake and 1 because of refusal of consent. From 104 patients enrolled in the study, 4 were excluded for having mixed malaria infection and 2 for low parasite density by day 0 afternoon parasite counts. Finally, 98 patients meeting the inclusion criteria participated in the study. Out of which 9 (9. 2%) who had qualified for the study were excluded during follow-up; 8 (8.2%) for lost to follow up; and 1 (1.0%) for withdrawal of consent. Thus, the study participants that successfully completed the 28 day follow-up Coartem efficacy monitoring study were 89 (Fig. 3). Demographic characteristics of 98 patients at the time of enrollment are given in table 2. The age distribution showed 5 (5.1%) under five, 7 (7.1%) between 5 and 14 and 86 (87.8%) were adults of 15 years and above. At enrollment the mean temperature of the patients was 38°C and paracetamol was given to all patients with axillary temperature ≥37.5°C. Mean hemoglobin concentration was increased with age and parasite load was high in children under five. Gametocyte carriage was observed only in the adult patients.

Table 2. Characteristics of the study participants included on the 28 day follow-up *in vivo* Coartem efficacy study at the time of enrollment in Selekleka town, Tigray Region, October, 2010 to January, 2011.

	Age in years			
Characteristics	**< 5**	**5-14**	**≥ 15**	**Total**
	n = 5	**n = 7**	**n = 86**	**n = 98**
Mean age (range)	2.94 (2-4)	10.23 (6-14)	25.44 (15-58)	23.2 (2-58)
Male	1	4	84	89
Female	4	3	2	9
Average weight (kg)	11.2	24.57	50.07	46.27
Average Temp (ºC)(range)	38.6 (38-40)	37.6 (35.6-40)	38.2 (36-39.8)	38.0 (35.6-40)
Mean hemoglobin (g/dl)(range)	9.38 (5.4-12.4)	11.27 (6.7-13.1)	13.02 (7.2-17.4)	12.71 (5.4-17.4)
Mean parasite density/µl (range)	58,208 (11,280-177,120)	20,351 (3,080-65,420)	20,478 (1920-144,400)	22, 679 (1,920-177,120)
Gametocyte carriage (n)	0	0	16	16

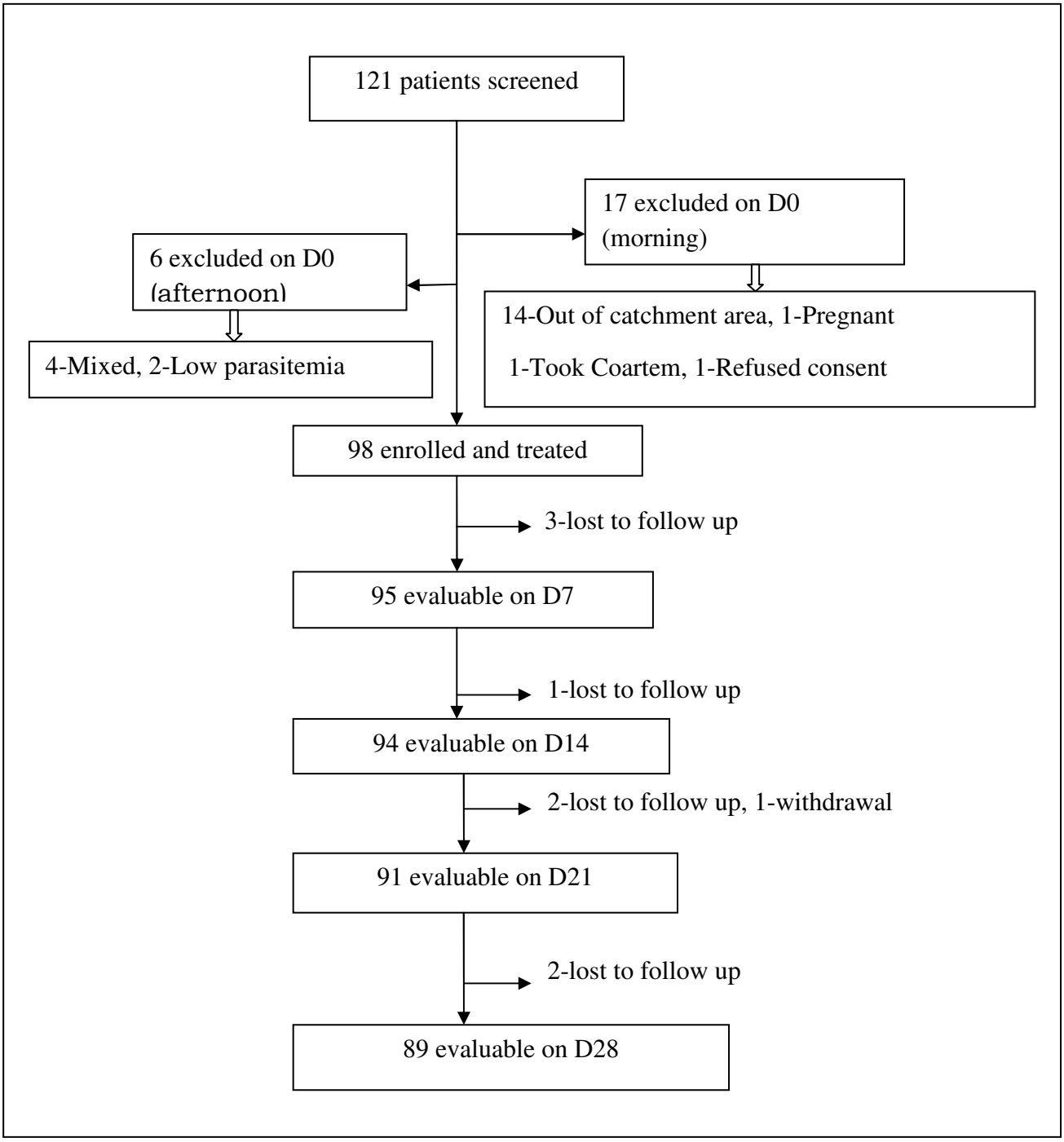

Figure 3. The follow-up of patients through the trial at Selekleka Health Center, October, 2010 to January, 2011.

The adequate clinical and parasitological response after 28 days follow up was 100%. There were no early treatment failures, late clinical failures and late parasitological failures (Table 3).

Table 3. Cure rate of Coartem treated *falciparum* malaria infected study participants on day 28 in Selekleka town, October, 2010 to January, 2011.

	Age in years			
Treatment status	**Under 5**	**5-14**	**≥ 15**	**Total**
	n = 5	n = 7	n = 77	n = 89
	(%)	(%)	(%)	(%)
Early treatment failure	0.0	0.0	0.0	0.0
Late clinical failure	0.0	0.0	0.0	0.0
Late parasitological failure	0.0	0.0	0.0	0.0
Adequate clinical and parasitological response	100.0	100.0	100.0	100.0
Total analyzed	**5.6%**	**7.9%**	**86.5%**	**100**

4.4. Fever clearance

Sixty-five febrile (T ≥ 37.5ºC) patients and the remaining twenty-four patients with history of fever participated in the study. Fever had cleared in 48/65 (73.8%) patients on D1 and no febrile cases were found on D2, that is, overall fever clearance was fast following initiation of treatment as shown in Figure 4. That is, there was a negative correlation between fever and parasitemia (r= -0.8).

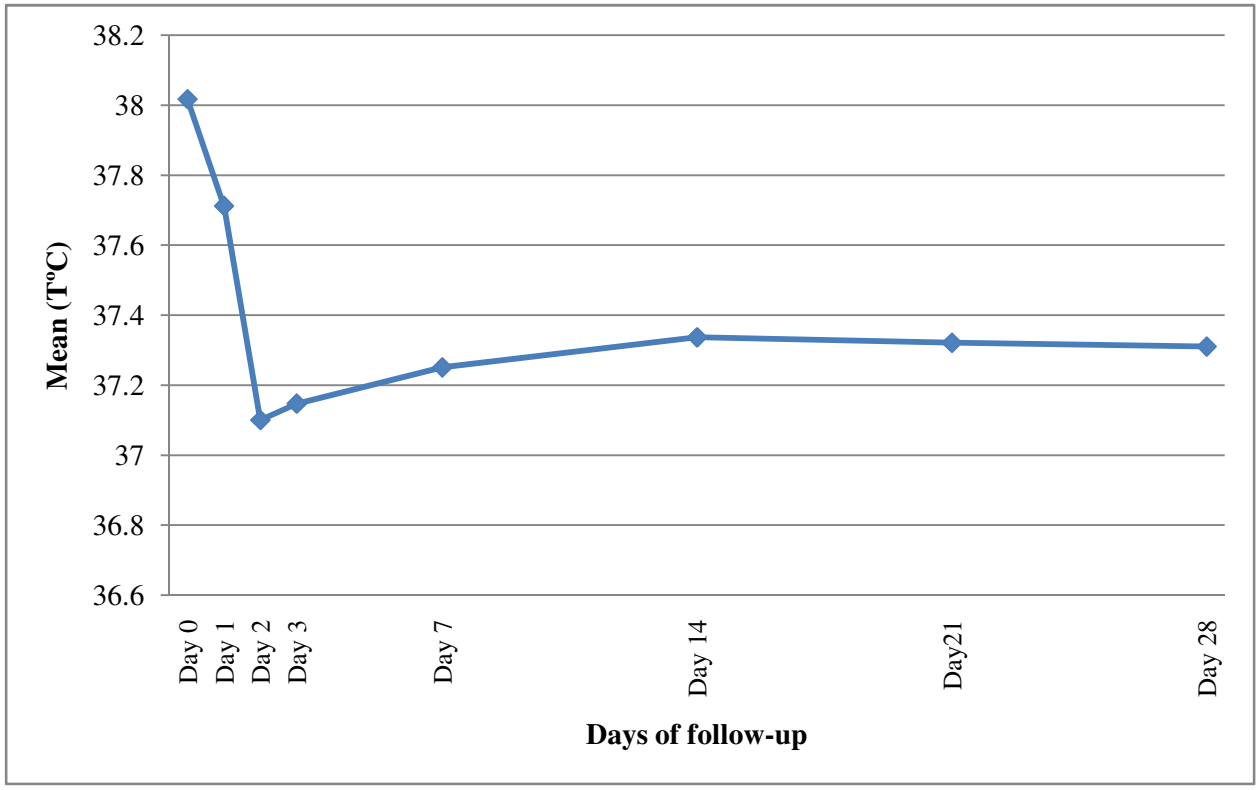

Figure 4. Fever clearance in Coartem-treated *falciparum* malaria patients during the follow up period at Selekleka town, October, 2010 to January, 2011.

4.5. Parasite clearance

Parasite clearance was also rapid as shown in Figure 5. Two patients with hyperparasitemia were included into the study. They were not excluded from the analysis, because there were no disease complications. Parasitemia was cleared from 73 (82%) of the patient on D1 and all patients had cleared their parasitaemias by D2 (Fig. 5). The same was also true for the two patients with hyperparasitemia, which were included in the analysis because of the absence of malaria complications.

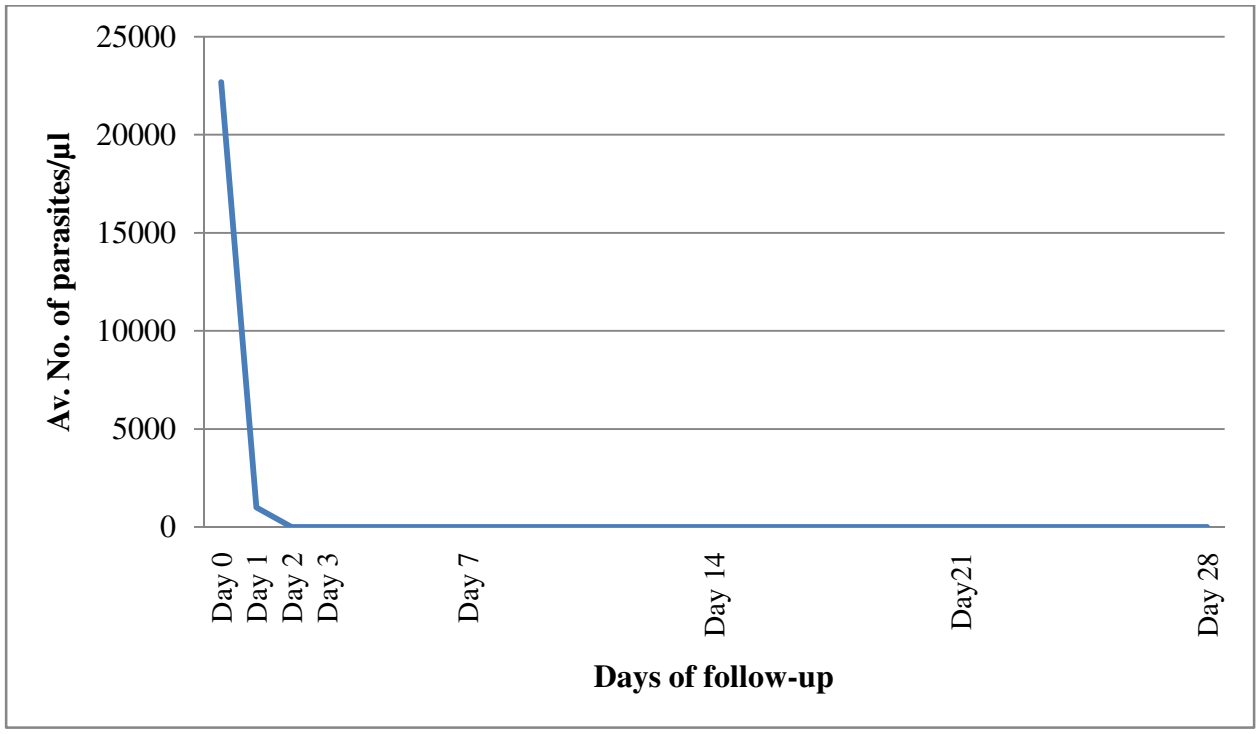

Figure 5. Parasite clearance following Coartem-treatment of *falciparum* malaria infected patients in Selekleka town, October, 2010 to January, 2011.

4.6. Gamete clearance

Twenty-three out of 89 patients (25.8%) had gametocytemia and of this 16/23 (69.6%) were detected at enrollment (D0) while the rest were found on later days. Gametocyte density in the patients ranged between 16 and 2208/µl of blood. 18%, 19%, 11.2%, 10%, 3.4% and 1.1% of the patients were gametocyte carriers on days 0, 1, 2, 3, 7 and 14, respectively and Complete gametocyte clearance was obtained on D21 (Fig. 6).

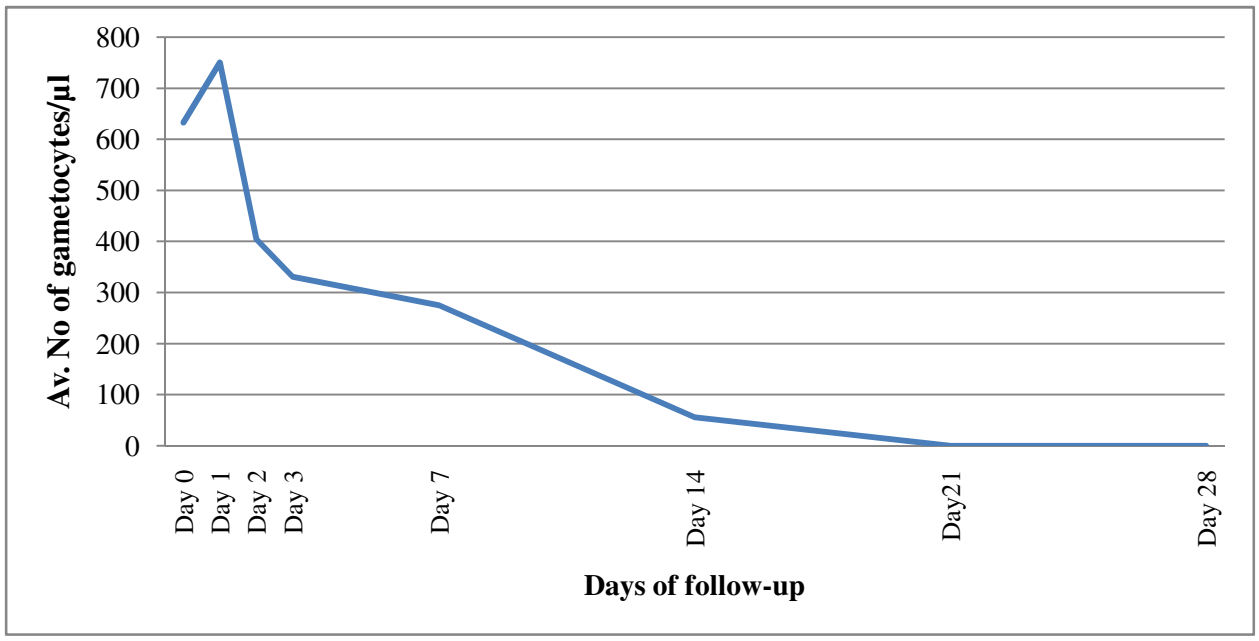

Figure 6. Gametocyte clearance following Coartem-treatment of *falciparum* malaria infected study participants in Selekleka town, October, 2010 to January, 2011.

4.7. Hemoglobin recovery

At enrollment 19 (21.3%) patients were found to have mild anemia; 9 (10.1%) patients were found to have moderate anemia and 3 (3.4%) patients were found to have severe anemia. But they were improved with the resolution of malaria which resulted only in 10 (11.2%) mild anemia on D28 (Table 4). Low level decreases in hemoglobin concentration from baseline to day 28 were detected in some patients. However, significant increase in the mean hemoglobin level from baseline to day 14 and day 28 among the study participants with adequate clinical and parasitological response was noted in all patients.

Mean hemoglobin was 12.7 g/dl (ranging from 5.4 g/dl to 17.1 g/dl) and 13.4g/dl (ranging from 8.3g/dl to 17.1) at enrollment and on day 14 respectively. After 28 days of follow up, the level of mean hemoglobin improved to 14. 3 g/dl (ranging from 10.9-17.4) (p= 0.05). These observed improvements in hemoglobin concentration are consistent with effective parasite clearance.

Table 4. Resolution of anemia observed in *falciparum* malaria infected study participants following Coartem-treatment in Selekleka town, October, 2010 to January, 2011.

	Follow-up days n(%)					
Anemia	Day 0		Day 14		Day 28	
	Male	Female	Male	Female	Male	Female
Mild	18(20.2)	1(1)	15(16.9)	1(1)	10(11.2)	-
11.4 (10-12.9)*						
Moderate	9(10.1)	-	6(6.7)	-	-	-
9.1 (7.0-9.9)						
Severe	2(2.2)	1(1)	-	-	-	-
6.8 (<7)						

* Mean Hb (range) in g/dl

4.8. Adverse events following Coartem treatment

Minor and mild clinical signs and symptoms following Coartem treatment of *falciparum* malaria were reported by study participants during the study. Most of these were reported within the first week of treatment. The signs and symptoms included - headache, fever, joint pain, weakness, anorexia, cough, dizziness, abdominal pain, and diarrhea (Table 5). However, none of them were severe and all resolved spontaneously.

Table 5. Characteristic clinical signs and symptoms reported by patients following Coartem-treatment of *falciparum* malaria in Selekleka town, October, 2010 to January, 2011.

	Number of patients (%) on days of follow-up							
Clinical symptoms	D0	D1	D2	D3	D7	D14	D21	D28
Headache	78	47	38	27	10	5	3	1
	(87.6)	(52.8)	(42.7)	(30.3)	(11.2)	(5.6)	(3.4)	(1)
Fever	70	24	14	6	1	0	0	0
	(78.7)	(27)	(15.7)	(6.7)	(1)	(0)	(0)	(0)
Joint pain	54	20	14	7	5	3	4	2
	(60.7)	(22.5)	(15.7)	(7.9)	(5.6)	(3.4)	(4.5)	(2.2)
Weakness	36	23	15	18	6	4	0	0
	(40.4)	(25.8)	(16.9)	(20.2)	(6.7)	(4.5)	(0)	(0)
Anorexia	34	7	2	1	1	0	0	0
	(38.2)	(7.9)	(2.2)	(1)	(1)	(0)	(0)	(0)
Dizziness	17	3	2	1	1	0	0	0
	(19)	(3.4)	(2.2)	(1)	(1)	(0)	(0)	(0)
Abdominal pain	18	13	11	9	9	4	2	1
	(20.2)	(14.6)	(12.4)	(10)	(10)	(4.5)	(2.2)	(1)
Cough	15	13	20	16	11	10	9	6
	(16.9)	(14.6)	(22.5)	(18)	(12.4)	(11.2)	(10)	(6.7)
Diarrhea	7	4	1	0	2	0	0	0
	(7.9)	(4.5)	(1)	(0)	(2.2)	(0)	(0)	(0)

5. Discussion

Since drug resistant *falciparum* malaria is a major public health problem, rapid and effective case management is key to its control. This requires proper diagnosis of cases and administration of effective antimalarial drugs (White *et al.,* 2009). The adequate clinical and parasitological response on the 28 days *in vivo* drug efficacy test (WHO, 2003) undertaken in the present study has demonstrated the effectiveness of Coartem for the treatment of uncomplicated *falciparum* malaria in Selekleka town. This was shown by the finding that all patients who received Coartem treatment were cured with no treatment failure with 100% adequate clinical and parasitological response. These results are comparable to a previously published report from Ethiopia (Kefyalew *et al.,* 2009), but is at variance with other reports that have shown low levels of treatment failures - Wondo Genet (3.2%) (Kassa *et al*., 2009), Serbo (2.5%) (Assefa *et al.,* 2010); and Alemata, Humera, Assendabo and Nazareth (0.9%) (Jima *et al.,* 2005). These variations in Coartem sensitivity between different malaria endemic locations need further investigation.

The reason why more male adult malaria patients were recruited into the present study appears to be the result of more malaria cases in the adult male population in the area. This may have resulted from the limited access of the adults to the ITNs. That is, each household, which on the average has 5 family members in the Woreda, was provided with only two insecticide treated bed nets and priority to its use was given to children under 5 years of age and to pregnant women. This may be a plausible explanation because more than 87% of the study participants that could fulfil the recruitment criteria, at Selekleka Health Center were adult men, who may have been exposed to malaria as a result of lack of insecticide treated bed nets. In addition, the information provided by the study participants indicated that even those who had access to the insecticide treated bed nets were not using them regularly and properly and this poor knowledge atitude practice may partly explain the inadequate reduction in malaria prevalence inspite of the on-going control program in Selekleka town and the Tigray Region in general.

Another source of adult male malaria infection is their frequent travel to the Tekeze valley to engage in gold mining and to work as farm labourers in Humera area, both of which are highly malarious. Thus imported malaria from these highly a malarious endemic region, among the male population is to be expected. On the other hand, since children and women do not normally move far into such areas with intense malaria transmission, the numbers of children and females who came to the Selekleka Health Center seeking treatment for malaria will be expected to be significantly lower compared to adult males that were recruited into the study. And the information obtained from the study participants on their history of mobility suggests a high proportion of malaria cases to have been imported into Selekleka town.

Treatment with Coartem resulted in significant clearance of fever within the first two days. It came down to normal body temperature during the later follow up days (D3, D7, D14, D21 and D28). This is the major advantage of the artemisinins and their combinations, which is their ability to rapidly reduce the parasite biomass with resultant rapid fever resolution (Okafor *et al.,* 2010). This finding confirms the previous studies carried out in Southwestern Nigeria that showed rapid fever clearance (Sowunmi *et al.,* 2007).

The antigametocyte effect of the six dose Coartem regimen was compatible with decreased risk of developing drug resistance. Prompt treatment of *falciparum* malaria infections with effective drugs is often associated with low gametocyte carriage and may invariably reduce transmission of gametocytes to mosquitoes (Robert *et al.,* 2000). So, it is preferable to employ drugs with schizontocidal action to reduce the development of gametocytes. In the present study gametocyte clearance was obtained on (95.7%) of patients on day 14 and complete gametocyte clearance obtained on day 21. Therefore, Coartem would mitigate not only the problem of resistant malaria but also reduce the chances that an infected person might pass the infections to mosquitoes and thus, to other members of the community (Kiszewski, 2010). However, transmission is not completely blocked by treatment with Coartem only, which can pose a problem in areas with high vectorial capacity since gametocytes survived up to D21. This finding is comparable to the report by (Opera *et al.,* 2010), but different from another study in which gametocytes survived up to D42 (Okafor *et al.,* 2010) on Coartem treatment. Therefore, gametocytocidal properties of

Coartem has been shown to be effectively complemented by adding a single dose of primaquine (45mg) to the end of a course of treatment, because primaquine has proven to reduce gametocyte clearance time to 2 to 3 days (Lederman *et al.,* 2006). However, its use may result in hematological reactions /especially in patients with glucose-6-phosphate-dehydrogenase/ (Reddy, 2007).

Gametocytes are refractory to many of the early front-line therapies; including chloroquine and sulphadoxine-pyrimethamine since neither of the two drugs has any significant gamotocytocidal activity (Sutanto *et al.,* 2004). As a result, gametocytes may continue to circulate for weeks to months after all asexual parasites have been eliminated from the bloodstream after treatment. Therefore, Coartem is advantageous over these (CQ and SP) drugs, since it results in a relatively rapid clearance of gametocytes.

Differential diagnosis of mild anemia is difficult because malnutrition and other common disorders contribute to it (Murphy and Breman, 2001). Therefore, the mild anemic patients observed in this study, may also get it as a result of malnutrition, because Tigray region as a whole, and Medebay Zana Woreda particularly are known to be affected by malnutrition according to the rural nutritional survey in Ethiopia, carried out in 2000 (Belachew *et al.,* 2001). In addition, helminth infections may also be contributing to the mild anemia observed in the patients, as helminth infections are among the top ten diseases of the Woreda. However, the hemolytic property of Coartem has not been studied in this study. Thus, it was difficult to incriminate Coartem as a partial cause of anemia.

However, anemia due to *falciparum* malaria is usually moderate to severe and the effect of infection usually non-ambiguous. Therefore, treatment of *falciparum* malaria with the appropriate drug will be expected to improve the patients' hemoglobin levels with time (Murphy and Breman, 2001). Thus, the significant increase in the mean haemoglobin level from day 0 to day 14 and then day 28 post-treatment after adequate clinical and parasitological response were consistent with successful treatment and resolution of malaria. That is, there was a negative correlation between hemoglobin concentration and parasitemia, which is consistent with the fact

that hemoglobin increases with decreasing parasite density. Similar findings were also observed in other studies in Ethiopia (Kefyalew *et al.,* 2009).

Since all the post-Coartem clinical signs and symptoms were minor and mild, and all of them disappeared spontaneously following the resolution of malaria (within the first week), it can be concluded that Coartem is safe and adequately tolerated by all participants. Similar findings were also reported from study in many different populations around the world (Makanga and Krudsood, 2009). However, cough was the only clinical manifestation that did not resolved rapidly.

A similar result was observed in other study in Serbo, Southwestern Ethiopia (Assefa *et al.,* 2010). Most of these clinical signs and symptoms were induced by malaria when the blood cell ruptures and releases toxic cellular debris from infected cells into the bloodstream (Kakkilaya, 2009).

6. Conclusion

This study showed the efficacy of six-dose regimen of Coartem in the treatment of uncomplicated malaria. Treatment was well tolerated by patients; and resulted in:

- Rapid clearance of parasitemia;
- Rapid clearance of fever;
- Gradual elimination of gametocytes;
- The recovery of hemoglobin was evident from baseline (D0) to D14 and above.
- The six-dose Coartem regimen, therefore, fulfils the requirements of the WHO for an effective and safe therapy of *falciparum* malaria in patients in Selekleka town.

7. Recommendation

- Purchasing Coartem from private pharmacy is the main problem. Since the cost of the drug is expensive (35.00 birr), usually the patients are obliged to buy half or lower dose. It is purchased without blood film confirmation to all febrile condition. It is a problem the Woreda health office and other concerned bodies should find solution for.

- Most of the people do not use insecticide treated bed nets regularly and properly. Especially adults traveling to more malarious area for gold mining and other economic activity. So Health education and access to ITNs to the mobile adult male population that travels to Tekeze valley and Humera is important.

- The possibility of Coartem's drug reaction as a cause for anemia could not be ruled out and needs to be studied in the future.

- To interpret the results uniformly, to follow trends over time and to compare levels of resistance between different regions, studies must be carried out with the same standardized protocol, in different sentinel sites, if possible, and, at the same time of year in a given site.

8. References

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Appendix I. Adult consent form

Title of the project: Monitoring the efficacy of Coartem for uncomplicated *falciparum* malaria

Name of investigator: _____

Address: _____

Patient code _____ Date: _____

Your health care provider has determined that you have malaria infection caused by *Plasmodium falciparum* that needs treatment with a medication called Coartem. Coartem is currently the recommended first line drug by the Federal Ministry of Health for the treatment of uncomplicated *Plasmodium falciparum* malaria in Ethiopia. Regardless of whether you decide to participate in this study, this health care facility will provide you with Coartem medication for your malaria infection at no cost to you. We would appreciate your help if you decide to volunteer for this research study that will help us to carefully follow and document the course of your malaria infection after treatment with coartem. The Ministry regularly conducts the clinical studies to make sure that the recommended malaria treatments are still working well. In this study we intend to assess how well Coartem® works to cure malaria at six locations in Ethiopia, and to determine whether it is still working as well in our earlier studies. The information from this study should help national malaria control program managers to determine whether there is evidence of Coartem drug resistance and whether we may need to find other medications to substitute for Coartem.

We are inviting all malaria patients aged 6 months and over living in this area to take part in this study. If you agree, you will be treated with six doses of Coartem given twice daily for 3 days. (This is the same treatment that you would receive if you decide not to volunteer for this study). The first dose will be given at the clinical supervised by study nurse and the second dose will be given to you to be taken at home. The study will take place over 28 days. During that time, you will be asked to come to the health facility on scheduled days 1, 2, 3, 7, 14, 21 and 28. You will also be asked to come to the clinic at any other time if you become sicker, develop new symptom, or if you fail to get better. Transportation fees will be provided to you during each scheduled study follow-up visit. During each follow-up visit, we would like to obtain fingr prick blood samples from you by a qualified technician that would be used only for malaria diagnosis, to detect the presence of markers for malaria drug resistance, and to see the outcome of treatment. There is no serious risk in participating, but you may experience a small pain during finger pricking. The pain should disappear within 1 day.

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

The Coartem medicine may have some unexpected effects; however, we will follow you closely and keep track of these effects, if they arise. Patients showing deterioration in their clinical status will be immediately admitted to the clinic free of charge for appropriate treatment according to

the national policy till they recover. A physician will be responsible for every trial related medical decision of the patient throught the study period.

Your participation in this study is completely volunterly and you can refuse to participate or are free to withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or all the services you receive at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services you receive at the clinic will continue. If you decide to participate in this study, the information in your records is strictly confidential and your name will not be used in any report and any illness related to malaria or to the malaria treatment will be treated at no charge to you. Do you under stand what has been said to you? If you have any questions you have the right to get proper explanation.

Certificate of consent

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

Name of participant: _____

Signature of participant: _____

Date: _____

(dd/mmm/yyyy)

Witness' signature:

I confirm that participant has given consent freely.

Name of witness: _____

Signature of witness: _____

Date: _____

(dd/mmm/yyyy)

Investigator's signature:

I confirm that participant has given consent freely.

Name of investigator: _____

Signature of investigator: _____

Date: _____

(dd/mmm/yyyy)

Appendix II. Children consent form

Monitoring the efficacy of Coartem for uncomplicated *falciparum* malaria

Name of investigator: _____

Address: _____

Patient code _____ Date: _____

Your health care provider has determined that the child have malaria infection caused by *Plasmodium falciparum* that needs treatment with a medication called Coartem. Coartem is currently the recommended first line drug by the Federal Ministry of Health for the treatment of uncomplicated *Plasmodium falciparum* malaria in Ethiopia. Regardless of whether you decide to participate in this study, this health care facility will provide you with Coartem medication for your child malaria infection at no cost. We would appreciate your help if you decide to volunteer for this research study that will help us to carefully follow and document the course of your malaria infection after treatment with coartem. The Ministry regularly conducts the clinical studies to make sure that the recommended malaria treatments are still working well. In this study we intend to assess how well Coartem® works to cure malaria at six locations in Ethiopia, and to determine whether it is still working as well in our earlier studies. The information from this study should help national malaria control program managers to determine whether there is evidence of Coartem drug resistance and whether we may need to find other medications to substitute for Coartem.

We are inviting all malaria patients aged 6 months and over living in this area to take part in this study. If you agree, your child will be treated with six doses of Coartem given twice daily for 3 days. (This is the same treatment that you would receive for the child if you decide not to volunteer for this study). The first dose will be given at the clinical supervised by study nurse and the second dose will be given to you to administer at home. The study will take place over 28 days. During that time, you will be asked to bring the your child to the health facility on scheduled days 1, 2, 3, 7, 14, 21 and 28. You will also be asked to bring the child to the clinic at any other time if he/she become sicker, develop new symptom, or if he/she fail to get better. Transportation fees will be provided to you during each scheduled study follow-up visit. During each follow-up visit, we would like to obtain fingr prick blood samples from the child by a qualified technician that would be used only for malaria diagnosis, to detect the presence of markers for malaria drug resistance, and to see the outcome of treatment. There is no serious risk in participating, but the child may experience a small pain during finger pricking. The pain should disappear within 1 day.

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

The Coartem medicine may have some unexpected effects; however, we will follow the child closely and keep track of these effects, if they arise. Patients showing deterioration in their clinical status will be immediately admitted to the clinic free of charge for appropriate treatment

according to the national policy till they recover. A physician will be responsible for every trial related medical decision of the patient throught the study period.

Participation in this study is completely volunterly and you can refuse the participation of your child or withdrawal from the study at any time is possible. Refusal to participate will not result in loss of medical care provided or all the services you receive to your child at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services you receive for your child at the clinic will continue. If you decide the participation of your child in this study, the information in your records is strictly confidential and the name of your child will not be used in any report and any illness related to malaria or to the malaria treatment will be treated at no charge to the child. Do you under stand what has been said to you? If you have any questions you have the right to get proper explanation.

Certificate of consent

The study was explained and I have had the opportunity to ask questions about it and any questions that I have asked have been answeredto my satisfaction. I consent volunterly participate my child as a participant in this study.

Name of parent/guardian: _____ Name of child: _____

Signature _____ Signature: _____

Date: _____

(dd/mmm/yyyy)

Witness' signature:

I confirm that participant has given consent freely.

Name of witness: _____

Signature of witness: _____

Date: _____

(dd/mmm/yyyy)

Investigator's signature:

I confirm that participant has given consent freely.

Name of investigator: _____

Signature of investigator: _____

Date: _____

(dd/mmm/yyyy)

Appendix III. Weight-based administration of Coartem®

Weight (Age)	20mg of Artemether and 120 of Lumefantrine (Coartem®)					
	0 hours	8 hours	24 hours	36 hours	48 hours	60 hours
5-14kg	1 (tablet)	1	1	1	1	1
15-24kg	2	2	2	2	2	2
25-34kg	3	3	3	3	3	3
>34kg	4	4	4	4	4	4

Appendix IV. Enrollment form

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

4. Study Number 5. Number of tablets 6. Start Date:(dd/mm/yy)

7. Patients Full name:

8. Family head:

9. Mother's/Wife's (if married) name:

10. Caregiver's name and relationship:

11. Town:

12. Kebele:

13. Village:

14. History of travel to other malarious area: ……. When ……..

15. Home address and localizing features/Owners name/Direction:

16. Phone number (s) and the owner (s):

17. Previous malaria attack: Yes….. No……

18. Previous antimalarial intake: Yes…… No…… If yes, CQ…… SP……

19. Hold Bed net: Yes…… No…… If yes, Bed net use Yes…… No……

Appendex V. Case Record Form

Pin No._____ No. of Tablets_____ Name _____

	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28	Extra Day
1. Date									
2. success of treatment at home*									
3. Axillary TºC									
4. Parasite asexual									
5. Gametocyte count									
6. Haemoglobin									
7.DBS/PCR									
8. Adverse events**									
9. Concomitant treatment									
10. Reasons for withdrawal									
11. Remarks									

* 1. Successfully took the drug 2. Vomite the drug 3. Miss the evening dose

** 1. Headache 2. Anorexia 3. Nausea 4. Vomiting 5. Weakness 6. Joint pain 7. Abdominal pain

8. Diarrhea 9. Cough 10. Behavioural change 11. Dizziness 12. Mouth ulcer 13. Skin rash 14. Others _____

Appendix VI Patient Follow-up Card

Appointment card

PIN. No.:……

Name…….Dose # ……

Appointment day

Day	D1	D2	D3	D7	D14	D21	D28
Appoitment day							

Appendix VII. Follow up activity/procedure

Days	0	1	2	3	7	14	21	28	Extra day
Study medication	x	x	x						
Success of drug intake at home		x	x	x					
Clinical examination	x	x	x	x	x	x	x	x	x
Temperature measurement	x	x	x	x	x	x	x	x	x
Adverse drug event		x	x	x	x	x	x	x	x
Parasitology/blood smears	x	x	x	x	x	x	x	x	x
Filter paper sample					x?	x?	x?	x?	x?
Haemoglobin	x					x		x	

Appendix VIII. Patient Record Sheet

Efficacy Study Patient Record Sheet

Date: _____

Name: _____ PIN. No. _____

Follow up date: _____

Lab. Results

Parasite: _____ Gamete: _____

Hb: _____

DBS: _____

Declaration

I, the undersigned, declare that this thesis is my own original work and has not been presented for a degree in any other University, and all sources of materials used for the thesis have been duly acknowledged

Student: Feyissa Hamde

Signature: _____

This thesis has been submitted for examination with my approval as University advisor

Advisor: Professor Beyene Petros

Signature: _____