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ASSESSMENT OF THERAPEUTIC EFFICACY OF COARTEM® IN PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN HALABA SPECIAL WOREDA, SOUTHERN ETHIOPIA

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A Thesis Submitted to the School of Graduate Studies of the Addis Ababa University in Partial Fulfilment of the Requirements for the Degree of Master of Science in Biology (Biomedical Science)

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Assessment of therapeutic Efficacy of Coartem in Patients with Uncomplicated *Plasmodium falciparum* Malaria in Halaba Special Woreda, SNNPR

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in (Biomedical Sciences Stream)

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LIST OF ABBREVIATIONS

ACT	Artemisinin Combined Therapies
AL	Artemether– Lumfantrine
CDC	Center for Disease Control and Prevention
DNA	Deoxy Ribonucleic Acid
EHNRI	Ethiopian Health and Nutrition Research Institute
ETF	Early Treatment Failure
FMOH	Federal Ministry of Health
IRS	Indoor Residual Spray
ITNs	Insecticide Treated Nets
LCF	Late Clinical Failure
LPF	Late Parasitological Failure
MOVBDCU	Malaria and Vector-Born Diseases Control Unit
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
RBM	Roll Back Malaria Cabinet Project
SNNPR	Southern Nations Nationalities and Peoples' Region
SP	Sulfadoxine - Pyrimethamine
WHO	World Health Organisation

ABSTRACT

Emergence of malaria parasite drug resistance is a serious problem in malaria control. Artemether-lumefantrine (Coartem®) is used as a first line treatment for uncomplicated *falciparum* malaria since 2004 in Ethiopia. The objective of the study was to assess the efficacy of Coartem® against *falciparum* malaria in Halaba Special Woreda, Southern Ethiopia. 5922 individuals that were clinically suspected of malaria were screened for infection. Giemsa stained thin smears were used for identification of *Plasmodium* species, and thick smears for detection and quantification of the parasites. Among the screened, 1826 (30.8%) were malaria positive, of which 273 (14.9%) were due to *Plasmodium flaciparum* and 1553 (85.1%), due to *Plasmodium vivax*. Among *Plasmodium flaciparum* positive patients, 89 (32.6%) fulfilled the inclusion criteria and were enrolled in the study. Haemoglobin concentration of the study participants was measured on days 0, 14 and 28 using portable spectrophotometer. Study outcomes were classified as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) and adequate clinical and parasitological response (ACPR). Out of the 89 study participants, 6 (6.7%) were lost to follow up and 3 (3.4%) withdrew from the study. Following drug administration, parasitemia cleared from all the study participants on day 3, and almost all fever cleared on day 2. Gametocytes were detected in 9% of the study participants on the day of inclusion into the study, and none could be detected after day 7. During follow up, one patient became positive for *Plasmodium flaciparum,* amounting to a 1.3% LCF, as a result of which the cure rate of Coartem® in the study area was 98.7% (95% CI=93.2-100%). However, as a confirmation of emergence of Coartem® resistance in the study area, it will be necessary to do a PCR correction to determine whether the 1.3% LCF was a new infection or a recrudescence. The mean haemoglobin level of the study participants was 10.8g/dl (Range: 9.2g/dl-13.7g/dl) on day 0, of which 57.3% were anemic. On day 28 of follow up, mean haemoglobin level significantly increased (P=0.002), compared to day 0, and the proportion of anemic individuals declined. The overall findings of the study have shown high efficacy and safety of Coartem® for treatment of uncomplicated *falciparum* malaria, in the study area. Also, the high prevalence of *P. falciparum* and *P. vivax* malaria, detected in the study area, indicated the ineffectiveness of the control measures in practice. Therefore, the malaria situation in Halaba Special Woreda requires initiation of more effective integrated control measures and periodic monitoring of anti-malarial drug efficacy.

Key words: Coartem®, *falciparum* malaria, fever clearance, gametocyte clearance, Halaba Special District,Parasiteclearance,Haemoglobin

1. INTRODUCTION

Malaria is among the killer infectious diseases, particularly in tropical countries. Malaria is a mosquito borne disease that is caused by protozoan parasites of the genus *Plasmodium* (WHO, 2012). Until very recently, the etiologic agents of human malaria were believed to belong to four species: *Plasmodium falciparum, P.vivax, P.malariae* and *P.ovale.* However, recent studies have revealed that a fifth malaria parasite of non-human primates, *P. knowlesi,* can also naturally infect humans (Cox- Singh and Singh, 2008; Collins and Barnwell, 2009). Among the five species, *P. falciparum* causes the most deadly type of infection.

The life cycle of *Plasmodium* parasites begins when a female anopheline mosquito acquires blood meal from humans (Fig. 1). During such a meal, a mosquito with prior infection with *Plasmodium* parasites injects sporozoites (Garcia *et al*., 2006). Sporozoites are carried by the circulatory system to the liver and invade hepatocytes within minutes. The parasites in hepatic cells undergo an asexual replication through an exoerythrocytic schizogony. Exoerythrocytic schizogony culminates when the schizonts rupture and release 20, 000 -30, 000 merozoites per original sporozoite into the hepatic venous circulation, from where they disseminate systemically (Sinden, 2010). A proportion of the liver-stage parasites from *P. vivax* and *P. ovale* go through a dormant period instead of immediately undergoing asexual replication. These hypnozoites reactivate several weeks to months or years after the primary infection and are responsible for relapse (White, 2011). Each merozoite that is not picked up by phagocytic cell and destroyed, invades an erythrocyte and starts a 48-h cycle of replication, in the case of the three species except *P. malariae,* which exhibits a 72-h cycle of replication. Replication is followed by schizont rupture and invasion of new red blood cells. Some merozoites differentiate into

gametocytes in a process that takes 7-15 days in the case of *P.falciparum* and 1-3 days in the case of other human malaria species (Talman *et al*., 2004).

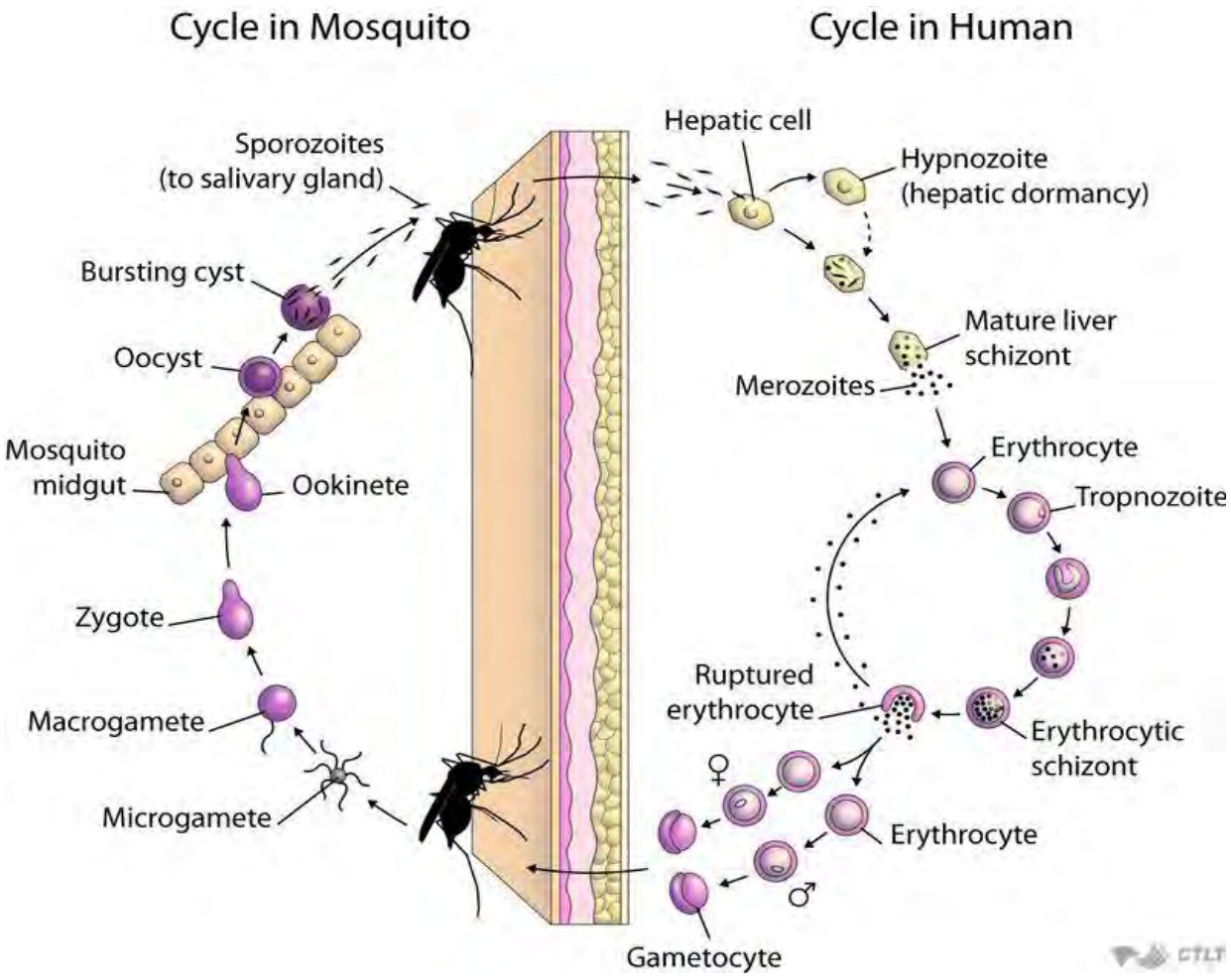

Figure 1. Generalized life cycle of *Plasmodium* parasite (Source: http://ocw.jhsph.edu/- Accessed on August 25, 2011)

The cyclical infection of human red blood cells by malaria parasites causes symptomatic malarial illness, which manifests as recurring episodes of chills, fever, and sweating. Other symptoms such as headache, malaise, fatigue, loss of appetite and body ache can last for weeks (van der Heyde *et al*., 2006). This is due to the release of parasite products that stimulate lymphocytic

host cells to release cytokines while the erythrocytes burst to release merozoites (Eling and Kremsner, 2005).

The growing malaria parasites progressively consume and degrade intracellular proteins, principally haemoglobin, causing the red blood cell to become more irregular in shape, and leading to its eventual destruction (Francis *et al.,* 1997). Malaria parasite inserts parasite derived proteins, which mediate attachment of the red blood cells to the walls of blood vessels, an event termed cytoadherence (Kirchgatter and del Portillo, 2002; Rathore *et al*., 2003). This cytoadherence causes accumulation of infected red blood cells inside the small blood vessels.

At the same stage, the malaria infected red blood cells may also adhere to uninfected red blood cells to form rosette, which causes accumulation of the blood cells inside the blood vessels (Carlson *et al.,* 1994). The processes of cytoadherence and rosetting are central to the pathogenesis of *P. falciparum* malaria as they result in the accumulation of the parasite infected red blood cells in vital organs, particularly the brain, heart and lungs, where they interfere with organ function by affecting blood circulation (van der Heyde *et al*., 2006).

1.1 The Global Burden of Malaria

Malaria is the leading cause of disease and death in Africa (CDC, 2010). Asia, Latin America, the Middle East and parts of Europe are also affected. According to WHO (2009), half of the world's population live in areas at risk of malaria transmission. In 2009, there were an estimated 225 million malaria cases worldwide, and 781, 000 deaths due to malaria were reported, with the majority of deaths occurring in sub- Saharan Africa, in children under the age of five (WHO, 2010**)**.

Malaria and poverty are also strongly related. According to Gallup and Sachs (2001), countries with intensive malarial attack had only 33% income level of that of countries without malaria in 1995, and significant economic growth was detected within 5 years in countries that are found in subtropics and islands after eliminating malaria. In Africa, the direct economic burden of the disease is calculated to be $12 billion per year (Sinden, 2010).

1.2. The Global Malaria Control Strategies

The World Health Organisation (WHO) identified malaria as a priority health issue in the world and initiated the Roll Back Malaria Cabinet Project (RBM) in 1998 (Binka, 2000). In this initiative the WHO, United Nations Children's Fund (UNICEF), United Nations Development Programme (UNDP), World Bank (WB) and various other collaborators joined forces to fight malaria.

RBM aimed to achieve a 50% reduction of the malaria burden by 2010. The initiative had identified six elements to achieve the objective; early detection of malaria illness, rapid treatment of those who are ill, multiple means for prevention of infection, strengthening of health sector and intersectorial activities, a powerful sustained social involvement and movement and focused research for new tools and better implementation (Nabarro, 1999). These main elements were an elaboration of the basic elements of the global malaria control strategy devised in 1993 (WHO, 1993b).

In September 2008, the RBM partnership added three additional targets as part of the Global Malaria Action Plan. The first was to reduce the total number of malaria deaths worldwide to near-zero preventable deaths by 2015. The second was to eliminate malaria in 8–10 countries by 2015, and afterwards in all countries that were in the pre-elimination phase in 2008. The third

goal was to eradicate malaria worldwide by reducing the global incidence to zero through progressive elimination in countries in the long term (WHO, 2008a).

Generally, malaria control strategies fall into two major areas; prevention and case management. Taken together, these strategies work against both the transmission of the malaria parasite and the development of illness and severe disease in humans, respectively (WHO, 2010).

Vector control aims to protect people against infective mosquito's bite by reducing vector longevity, vector density and vector contact with human host. For malaria vector control, insecticide treated nets (ITNs) and indoor residual spray (IRS) are the most widely applied interventions. In addition to ITNs and IRS, environmental management could be an additional measure. In order to have effective malaria vector control with ITNs, IRS or environmental management, the measures must have high coverage and need to be sustainable. This will require specialized personnel with practical experience at national, regional and district levels (WHO, 2006b).

1.3. Malaria in Ethiopia

Malaria was reported to be endemic in Ethiopia, first and foremost by the Italian and British scientists from the mid 1930s to the late 1950s (Tulu, 1993). According to WHO (2010) report, malaria is present everywhere in Ethiopia, except in the central highlands, and 56 million people are at risk.

The predominant malaria parasite species in Ethiopia is *P. falciparum* and it accounts for 60% of all malaria infection, and *P. vivax* follows with 39% (WHO, 2007a). *P. malariae* accounts for

less than 1% and *P. ovale* is rarely reported (Tulu, 1993). *Anopheles arabiensis , An. pharoensis, An. funestus a*nd *An. nili* are the major malaria vectors in the country (WHO 2010).

In most parts of the country, malaria transmission peaks bi-annually, from September to December and April to May, coinciding with the major harvesting and planting seasons, respectively, with serious consequences for the subsistence economy of the country. Contrary to this, some parts of the country, for example, low-lying western regions, experience perennial malaria transmission, because of the situations permitting continual breeding of vectors in permanent breeding sites (Gebre-Mariam *et al*., 1988; MOVBDCU, 1999; WHO, 2005).

The incidence of the disease in most parts of the country is unstable, mainly due to the country's topographical and climatic variabilities (Abose *et al.,* 2003). Rainfall and temperature are the most important determinants of malaria transmission (Senay and Virdin, 2005). Although areas below 2,000 meters are considered malarious (Tulu, 1993), malaria epidemics have been recorded up to 2400 meters (Negash *et al*., 2004), during periods when increased temperature and adequate precipitation are conducive for both vector survival and parasite development within the vector.

The disease is one of the country's leading health problems in terms of morbidity, mortality and impediment to socioeconomic development and top ranking in the list of common communicable diseases, consistently ranking in the top 10 causes of outpatient visits, admissions, and deaths at health centers and hospitals (FMOH, 2004). Malaria causes 70,000 deaths, and accounts for 17% of outpatient consultations, 15% of admissions and 29% of in-patient deaths in the country

(Adugna, 2010). Since a significant proportion of the population is out of reach of the health service coverage, these figures could under estimate the actual burden of malaria in Ethiopia (PMI, 2008).

According to WHO, the number of reported malaria cases decreased by 41% from an annual average of 3.2 million between 2000 and 2005 to 1.75 million in 2009. During the same period, 33% decline in malaria admission, and 60% reduction in the death of under five children was also reported (WHO,2010).

1.4. An Overview of Malaria Control Actions in Ethiopia

Ethiopia's fight against malaria started more than half a century ago, which initially began as pilot control projects in the 1950's and then launched a national eradication campaign in the 1960's followed by a control strategy in the 1970's (Gebere-Mariam, 1984). However, efforts to achieve malaria eradication in the country were not successful, partly due to some technical and financial constraints in countries and institutions that were supporting the eradication efforts (FMOH, 2000).

In recent times, early diagnosis and effective treatment, easy and universal accessibility to ITNs, residual spray of dwellings and environmental management are the main components of prevention and control strategies (Sheleme, 2007). Giving training to increase malaria control manpower, educating the society on various aspects of control, including the use of ITNs and setting effective and permanent program to monitor and evaluate the control actions are decisive measures to control malaria. Based on WHO (2009) report, around 75% households in the country owned ITNs in year 2008. In addition, IRS further expanded and protected 50% of

people at risk. Also in 2008 and 2009, about 8 million ACT treatment courses were delivered, which were sufficient to treat all reported malaria cases (WHO, 2010).

1.5. Malaria Case Management

1.5.1. Malaria Diagnosis

Prompt and accurate diagnosis is one of the main malaria intervention measures which is the key to effective disease management (WHO, 1993a). Malaria can be diagnosed by clinical signs (i.e. presumptive diagnosis), detection of parasites by microscopy or parasite antigens by rapid diagnostic tests (RDTs) or DNA by polymerase chain reaction (PCR) (CDC, 2007).

Clinical diagnosis has been the only feasible diagnosis in rural areas where laboratory support to the presumptive diagnosis doesn't exist (WHO, 1999). The drawback of clinical diagnosis is its lack of specificity, thus it should be confirmed by laboratory tests when it is possible (Redd *et al.,* 1996). However microscopic diagnosis is time consuming and labour intensive, the careful examination by an expert microscopist of a well prepared and well stained blood film remains currently the gold standard for detecting and identifying malaria parasites (WHO, 1999).

RDTs are based on the detection of antigens derived from malaria parasites in lysed blood, using immunochromatographic methods (WHO, 1999). RDTs are simpler to perform and easy to interpret. However they give false positive results and only capable of detecting *P.falciparum* (Bell *et al*., 2005*)*. Furthermore, microscopy using fluorochromes, PCR and antibody detection by serology are also available for malaria diagnosis, but they are not suitable for field application unlike microscopy and RDTs (Voller and Draper, 1982; Wilson, 1993).

1.5.2. Malaria Treatment

For centuries there was no specific treatment, and it was not until the seventeenth century that Spanish colonisers brought back from Peru cinchona tree bark from which quinine was later extracted (CDC, 2010). In the twentieth century, synthetic alternatives to quinine were developed. Of these, chloroquine was the most successful, but by the 1970s widespread resistance developed and the world was left without an effective treatment for malaria (Mehlotra *et al.,* 2008). During the same decade, Chinese scientists extracted from the Chinese herb qinghao (Artemisia annua), the drug artemisinin, which is proved to be very effective against chloroquine resistant malarial parasites (Nasongelya, 2006; Butler *et al.,* 2010). Currently, use of ACT has made malaria treatment possible.

In Ethiopia chloroquine was the most common antimalarial drug used to treat *falciparum* malaria for decades. Chloroquine resistance to *Plasmodium falciparium* was first documented in 1986 from areas bordering Somalia, Kenya and Sudan, where population movements are intense in both directions (Teklehaimanot, 1986), followed by gradual spread of the resistant strain throughout the country (Desta and Gebrat, 1991; Gebreyesus and Alamerew, 1998; Gebreyesus, 1999; Kebede *et al.,* 1999). In 1998, intense resistance of the parasite to chloroquine necessitated a change to sulphadoxine pyramethamine (SP) (Schunk *et al.,* 2006). Faster drop in therapeutic efficacy of SP for the treatment of uncomplicated *falciparum* malaria enforced the adoption of artemether-lumefanthrine (Coartem®) as a first line treatment in 2004 (FMOH, 2004). Since 2004, the drug Coartem® serves as a first line treatment against uncomplicated *Plasmodium falciparum* malaria in Ethiopia.

1.5.3. Mechanism of Anti-malarial Drugs Action

1.5.3.1. Antifolates

Prokaryotic and eukaryotic cells require reduced folate cofactors for the biosynthesis of many cellular components. In plants and most microorganisms folate is synthesized through the folate biosynthesis pathway. However, higher eukaryotic cells including mammal cannot synthesize folate and are totally dependent on exogenous folate as the only source for tetrahydrofolate (THF) production by dihydrofolate reductase (DHFR). The differences in folate biosynthesis capacity between mammals and microorganisms makes the pathway an attractive antimicrobial target (Bermingham and Derrick 2002; Djapa *et al*., 2006).

In normal physiological state the parasite's dihydropteroate synthase (DHPS) catalyses the condensation of p-aminobenzoic acid (p-ABA) with 2-amino-4-hydroxy-6-hydroxymethyl-7, 8 dihydropteridine pyrophosphate (DHPPP) to form dihydropteroate(DHP). Subsequently the dihydrofolate synthase (DHFS) adds a glutamate to DHP to form dihydrofolate (DHF) which is finally reduced by DHFR to form THF. THF and its derivatives are used as cofactors in biosynthesis of amino acids like serine, methionine, glycine and histidine and purines and thymidylate for normal cell growth and function (Patel *et al*., 2004).

Sulfa drugs and p-ABA show high degree of structural similarities, thus competitively bind to DHPS (Nzila, 2006). Therefore, by binding to DHPS sulfa drugs competitively inhibits the activity of this enzyme. Whereas, Pyrimethamine selectively binds with several folds higher affinity to DHFR of the parasite than the human host (Djapa *et al*., 2006). Thus, sulfadoxine and pyrimethamine exert their parasitocidal effect by synergistically inhibiting the parasite's folic acid biosynthesis pathway.

Resistance to the dihydrofolate inhibitors (pyrimethamine and biguanides) and dihydropteroate inhibitors (sulphonamides) is mediated by single or double mutations in genes encoding DHFR and DHPS, respectively (Gama *et al.,* 2011). These mutations are considered as molecular markers for surveillance of antifolate resistance.

1.5.3.2. Aminoquinolines

The class includes chloroquine and amodiaquine. Many studies have been done to elucidate the parasitocidal activity of these quinolines. However, to date their modes of action remain largely unclear. Nonetheless, a large body of knowledge accumulated for many years shows that the drugs act primarily in the parasite digestive vacuole by interfering with detoxification of heme, a by-product of hemoglobin digestion (Fitch, 2004). Parasites resistant to chloroquine have been found to accumulate much less chloroquine in their digestive vacuoles than their sensitive counterparts (Martin and Kirk, 2004). Chloroquine resistance have been clearly associated with multiple mutations in genes encoding for membrane transporters located in the digestive vacuole, namely the chloroquine resistance transporter *PfCRT* and the multi-drug resistance transporter 1 *Pfmdr*1 (Djimde *et al.,* 2001; Mehlotra *et al*., 2008).

1.5.3.3. Quinoline-4-methanols

Quinine, mefloquine, lumefantrine and halofantrine are belong to this group of drug. However, the precise mode of these quinolines is not known. Mefloquine, quinine and other quinoline-4-methanol subclass bind with high affinity to phospholipid targets in malaria parasites (Mu *et al*., 2003). Mefloquine and quinine antagonize chloroquine induced abnormalities in malaria parasites, primarily by inhibition of hemoglobin ingestion, and secondarily they inhibit membrane recycling that lead to the parasite death (Famin and Ginsburg, 2002).

The inverse effects of 4-aminoquinolines and quinoline-4-methanol on parasite morphological abnormalities are consistent with observations made on the role of the two membrane transport genes on resistance to these antimalarial drugs (Mu *et al*., 2003; Bray *et al.,* 2005). Resistance to mefloquine and halofantrine is mainly limited to South East Asia and appears to be related in part to both mutations and amplification of the *pfmdr1* gene like chloroquine (Price *et al.,* 1999; Reed *et al*., 2000).

1.5.3.4. Artemisinins

The artemisinins including artesunate and artemether, derived from the Chinese herb qinghao (*Artemisia annua*), have been introduced recently as malaria treatment (Butler *et al.,* 2010). However, the mechanism of action of artemisinin is not clear. Artemisinins interferes with parasite transport proteins, disrupts parasite mitochondrial function, modulates host immune function and inhibits angiogenesis (Golenser *et al.,* 2006). Artemisinin eliminates almost all asexual forms of the parasite in its intraerythrocytic stage, and prevents its maturation to more pathological forms (Moles *et al*., 2011). Furthermore, artemisinin also eliminates some sexual stages, the gametocytes, avoiding the disease transmission to new hosts.

PfATP6, a transporter found on membranous structures within the parasite cytoplasm, plays a key role in artemisinins sensitivity. Artemisinins interact and selectively inhibit PfATPase6, the only sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) type Ca^{2+} ATPase in the *P. falciparum* genome (Uhlemann *et al.,* 2005). Therefore, Mutations in the PfATPase6 and Pfmdr1 are the only currently available markers that may be used as warning signals for emergence of *in vivo* resistance to artemisinins (Duraisingh *et al.,* 2000; Mugittu *et al.,* 2006). Uncontrolled use of

artemisinins monotherapies or in combination with ineffective partners might lead to faster selection of resistance (Duffy and Sibley, 2005).

1.5.4. Anti- malarial Drug Resistance

Drug resistance is defined as the ability of a parasite to survive and multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within the limits of tolerance of the subject (WHO, 2001).The successful treatment of malaria requires the administration of an effective anti-malarial regimen that results in the clearance of all the parasites from the blood, resolution of symptoms of acute disease and prevention of recrudescent infections (Price and Nosten, 2001).

P. falciparum which is the most deadly *Plasmodium* species in Africa has developed resistance to the cheap and safe anti-malarials such as chloroquine and sulfadoxine-pyrimethamine (SP) (Witkowski *et al*., 2009). Due to spread of resistance to SP and chloroquine monotherapies, the use of artemisinin based combination antimalarial therapies was recommended by WHO, (2006a).The recommended ACTs include artemether– lumfantrine (AL), artesunate-amodiaquin(AS-AQ) and artesunate-mefloquine (AS+MQ). For an effective combination therapy, both partner drugs must be reasonably efficacious (Krishna *et al.,* 2004).

Artemisinins are highly potent anti-malarial drugs and are also active against early stage gametocytes (Ashely *et al*., 2007). To date clinical resistance has been reported only at Thai-cambodian border since they were first introduced in 1972 (WHO, 2011a). The drugs have short half-lives and act very fast. They clear over 90% of the parasitic load within the first 6 hours of administration and the rest of the load is slowly eliminated by the partner drug that usually has longer half-life and acts slowly. Hence fewer parasites are exposed to sub-therapeutic level,

which is a potential factor for the selection and spread of resistance (Makanga and Krudsood, 2009).

1.5.4.1. Factors Responsible for the Generation of Drug Resistance

1.5.4.1.1. Natural selection

The process of selection will depend upon a variety of factors, including the size of the infecting biomass, the immunity of the host, the pharmacokinetic profile of the drug and the drug susceptibility and fitness of the mutants (White, 1999). Selection can occur either when a primary infection consists, in part, of resistant parasites capable of surviving treatment or when a subset of parasites with spontaneous mutations encounter residual concentrations of a slowly eliminated antimalarial drug. The number of parasites exposed to selective pressure will be far greater in the first case than the second and thus will provide the greater opportunity for resistant mutants to arise and spread (Price and Nosten, 2001).

1.5.4.1.2. Substandard drugs

Widespread use of subtherapeutic antimalarial regimens is also likely to play a major role in facilitating the emergence of drug resistance. Substandard drugs have been widely available in the private sector like, pharmacies, clinics, drug shops and market stall. Fake drugs with inadequate amounts of active ingredient may kill off some susceptible parasites, but leave those more likely to develop tolerance to multiply (WHO, 2011a).

1.5.4.1.3. Monotherapies

Monotherapies are perceived as having fewer side effects and often cheaper than the ACTs. However, it is easier for a parasite to develop resistance to a single drug treatment as it only needs to adapt to the characteristics of one drug (Krishna *et al.,* 2004). If a treatment involving

two or more drugs is used, it is likely to kill the parasite even if it has developed resistance, to one of the drugs. It is far more complicated for a parasite to develop resistance to effective ingredients in both drugs (Elbashir and Adam, 2008).

1.5.4.1.4. Lack of compliance

Patients often stop therapy as soon as their acute symptoms have resolved. This habit of poor compliance may arise because of the occurrence of adverse side effects, the cost of medication or because therapies are prolonged and complicated (White, 1997). Failure to take the full course of the drug means that while some susceptible parasites are killed, resilient ones live on, leading to resistance, to the drugs to which they were initially exposed (WHO, 2011a). Most of the times, this occurs because of the luck of proper information to the patient, regarding the use of the drug and the consequences of misusing it.

1.6. Coartem® (Artemether-Lumfantrine)

Coartem® is a fixed dose, oral combination of artemether (20 mg) and lumefantrine (120 mg), that is specifically indicated for the treatment of acute, uncomplicated *falciparum* malaria in patients that have 5 kg and above bodyweight (Nosten and White, 2007; Novartis, 2009). Artemether and lumefantrine are white and yellow crystalline powders, respectively, that are practically insoluble in water (Krishana *et al.,* 2001).

Both components are blood schizontocides. Artemether interferes with parasite transport proteins, disrupts parasite mitochondrial function, inhibits angiogenesis and modulates host immune function (Golenser *et al.,* 2006), whereas the exact mechanism by which lumefantrine exerts its anti-malarial effect is not well defined. However, available data suggests that lumefantrine

prevents detoxification of heme, such that toxic heme and free radicals induce parasite death (Kokwaro *et al.,* 2007).

Artemether is absorbed with peak plasma concentration that reaches in about 2 hours after dosing, with an elimination half- life of 2-3 hours. On the other hand, absorption of lumefantrine starts after a lag-time of up to 2 hours, with peak plasma concentrations about 6 to 8 hours after administration, with an elimination half life of 3 to 6 days (Ashley *et al.,* 2007). Food enhances absorption of both artemether and lumefantrine, although this effect is more apparent for lumefantrine (Kibwika *et al.,* 2010). Administration of AL with high fat meal increased bioavailability of both artemether and lumefantrine by 2 fold and 16 fold, respectively (White *et al.,* 1999; Ezzet *et al.,* 2000).

Artemether is metabolized rapidly to dihydroartemisinin (DHA) which in turn is converted to inactive metabolites (Aweeka and German, 2008). Both artemether and DHA offer potent antimalarial properties causing significant reduction in asexual parasite mass, with prompt resolution of symptoms. Lumefantrine is metabolized to desbutyllumefantrine that has 5 to 8 fold higher anti-parasitic effect (Novartis, 2010).

The majority of the reported adverse events of Coartem® are mild or moderate in severity and tend to affect the gastrointestinal and the nervous systems (Falade and Manyando, 2009). These adverse events, which are common in both adults and children, are also typical symptoms of malaria. In addition, repeated administration is not associated with an increased risk of adverse drug reactions including neurological adverse events (Toovey and Jamieson, 2004). This is especially relevant for children from regions with high malaria transmission rates who often receive many courses of anti-malarial medications during their lifetime.

1.7. Hypothesis

Arthemether – Lumfantrine (Coartem®) that serves as the first line anti-malarial drug in Halaba Special Woreda, SNNPR, may have high cure rate, with prevalence of treatment failure less than 5 %.

2. OBJECTIVES OF THE STUDY

2.1. General Objective

To assess the present therapeutic efficacy of Coartem® against uncomplicated *P.falciparum* malaria after seven years use.

2.2. Specific Objectives

- To evaluate the efficacy of Coartem® treatment on parasite clearance, fever clearance and gametocyte clearance on patients with uncomplicated *faciparum* malaria.

- To detect possible incidence of adverse drug reaction and tolerability of Coartem®.

- To determine the effect of Coartem® on anemia in uncomplicated *falciparum* malaria.

3. MATERIALS AND METHODS

3.1. The Study Area

The study was conducted in Halaba Special Woreda , Southern Ethiopia, which is located 313 km south of Addis Ababa. Halaba's projected population, as of July 1998 E.C was 251,385 with an estimated rate of natural increase (RNI) of 3% per annum and the percentage of males and females was 49% & 51%, respectively. 87.8% of the population dwell in rural parts of the Woreda and about 12.2% reside in urban. The land feature includes 61.3% flat, 21.3% rolling and 17.4% hilly. The agro-climate of the area consists of 86% weinadega and 14% kola. The annual rainfall varies from 857 to 1085 mm, while the annual mean temperature is between 17 ^{0} C to 20 ^{0}C with mean value of 18^{0}C. The altitude of the Woreda ranges from 1554 to 2149 meters above sea level, but most of the population in the Woreda is found at about 1800 meters above sea level. Agriculture is the backbone of the Woreda. Crops such as sorghum, maize, teff, wheat, millet, barely, Pepper, beans and potato are the main products. Particularly, pepper, potato, maize, teff, wheat, and bean are the main cash crops. In addition, farmers rear livestock.

The Woreda is known in the regional state as well as in federal level with its largest and most serious attack of malaria. Malaria is distributed in all kebeles and always ranks high in terms of outpatient morbidity, admission and deaths. This is followed by pneumonia, acute upper respiratory infections, helminthiasis, infection of skin, urinary tract infection. Malaria transmission in Halaba is unstable, seasonal and depends on altitude and rainfall. There are two main seasons for transmission; September to December, after the heavy summer rains, and March to May, after the light rains (Source: Halaba Special Woreda Health Office).

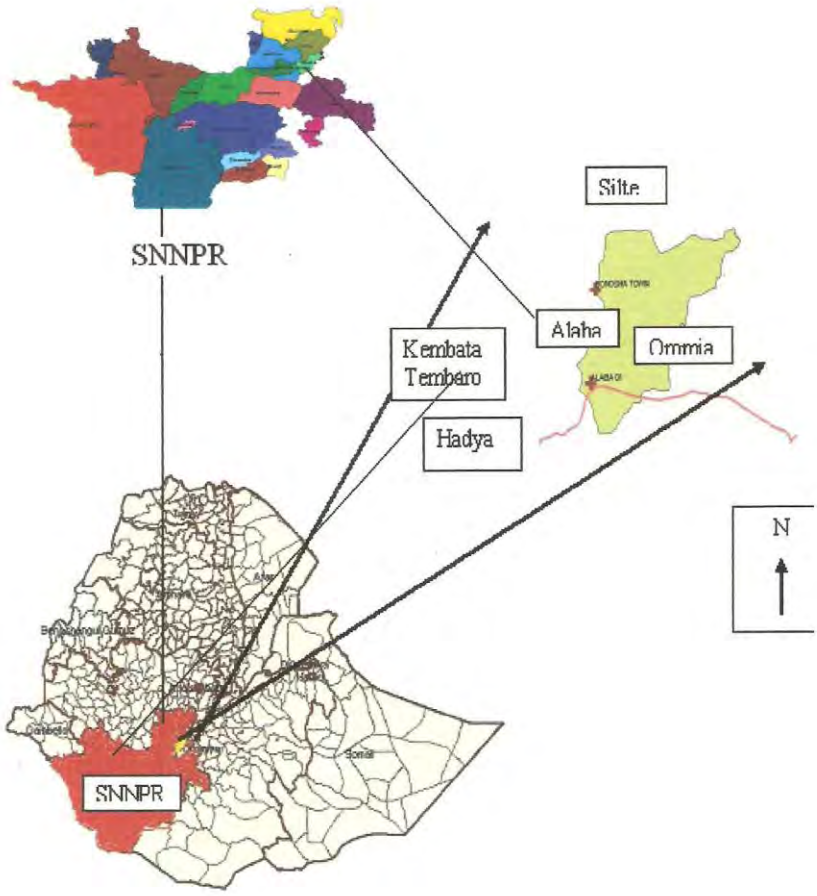

Figure 2. Map of the study area (Halaba Special Woreda, Southern Ethiopia)

3.2. Study Subjects

The study commenced in late October 2010 and ended in January 2011. The study subjects were recruited from malaria suspected individuals that were febrile or with history of fever visiting Halaba Health Center , Qulito town. All age groups beginning from six months regardless of their sex were screened for the study. In addition to age, additional factor that qualified the representative samples was the weight of the patients. Those patients whose age is above six months and weigh above 5kg, and those fulfilled the inclusion criteria according to WHO (2003)

guideline for the assessment and monitoring of anti-malarial drug efficacy for the treatment of uncomplicated *P. falciparum* malaria were recruited after providing the informed consent.

3.2.1. Inclusion Criteria

Patients that enrolled in the study were selected on the basis of the following criteria; aged between 6 months and above, absence of severe malnutrition and infection with *P. falciparum* only (i.e. no mixed infection). The range of initial parasitemia appropriate for inclusion was between 500 and 100,000 parasites/μl. Absence of sign of severe malaria was among the major criteria that were used to select representative samples according to WHO 28 day *in vivo* protocol (WHO, 2003).

3.2.2. Exclusion Criteria

Patients that did not fulfil WHO (2003) recommendation for inclusion were rejected from the study. The exclusion criteria includes; mixed infection of *P. falciparum* with other *Plasmodium* species, clinical and laboratory manifestation of severe sign of malaria, history of allergic reaction to the study drug (Coartem®), concomitant presence of other febrile conditions (e.g. acute respiratory infection, measles, severe diarrhea and others), severe malnutrition and persistent vomiting (i.e. necessitating more than a single repeat dose after 30 minute observation). Pregnant and lactating women were excluded from the study.

3.3. Sample Size Determination

According to WHO (2003) protocol, sample size was calculated using 5% precision, 95% confidence interval and by considering low prevalence of clinical failure of Coartem® from previous studies in Ethiopia. Thus, a minimum sample size for the study was 73. In addition, 20%

were added as contingency for lost to follow up and withdrawal of the study because the study has 28 days of follow up, and the final sample size became 88.

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

$= (1.96/0.05)^2 0.05 (1-0.05) = 73$

$n= (1+0.2) 73= 88$

Where, N = number of samples

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

z = confidence interval

d = precision (5%)

3.4. Specimen Collection and Microscopic Diagnosis

3.4.1 Malaria Parasite Identification and Determination of Parasitemia

Thick and methanol fixed thin smears were prepared from capillary blood by finger prick using disposable sterile lancet on clean slides. Giemsa stain whose P^H value was maintained between 6.8 - 7.2 using buffer solution of NaH_2PO_4 and K_2HPO_4 was used to stain thick and thin blood films during screening and the study period. Thick and thin blood films were rapidly stained using 10% Giemsa for 10 minutes and regularly stained using 3% Giemsa for 30 minutes for best result, during screening and at each day of the study period, respectively. The stained slides were examined under a light microscope using 100x oil immersion objective by an experienced laboratory technician from the health center. In addition, the slides were examined for the 2^{nd}

time by experienced laboratory technologist from Ethiopian health and nutrition research institute (EHNRI) who is blinded for the first reading. The kappa value between the two readers was 0.875. Finally the slides were examined by the principal investigator for confirmation. Thick blood films were used to determine parasite density, whereas, Methanol fixed thin smears were used to identify *Plasmodium* species. A blood slide was considered negative after examining 100 fields on a thick film. Parasite count was based on the number of parasites observed against 200 WBCs by assuming 8000WBCs/ µl of blood using the following formula (Cheesbrough, 1998). In addition, gametocytes were counted against 1000WBCs.

$$\text{No of parasites/ µl} = \frac{\text{8000WBCs/ µl x parasite count}}{\text{200WBCs}}$$

$$\text{No of gametocytes/ µl} = \frac{\text{8000WBCs/ µl x gametocyte count}}{\text{1000WBCs}}$$

The level of parasitemia was determined according to Garcia, (2001). Accordingly, 100-10,000 parasites/µl was categorized as medium parasitemia and 10,000-100,000 parasites/µl as high parasitemia.

3.5. Haemoglobin Measurement

Haemoglobin was measured at day 0, 14 and 28 by taking finger prick capillary blood using disposable sterile lancet, and portable spectrophotometer (Hemocue HB 201, Anglom, Sweden) was used for the reading. Anemia was defined according to WHO (2011b) categorization as presented below in table 1.

Table 1. WHO's hemoglobin threshold used to define anemia in different age and gender groups

Age or gender group	Non anemia (g/dl)	Anemia (g/dl)		
		Mild	Moderate	Sever
Children (0.5–5.0 yrs)	≥11.0	10-10.9	7-9.9	<7
Children (5.0–12.0 yrs)	≥11.5	11-11.4	8-10.9	<8
Children (12.0–15.0 yrs)	≥12.0	11-11.9	8-10.9	<8
Women, non-pregnant (>15yrs)	≥12.0	11-11.9	8-10.9	<8
Women, pregnant (>15 yrs)	≥11.0	10-10.9	7-9.9	<7
Men (>15yrs)	≥13.0	11-12.9	8-10.9	<8

3.6. Treatment and Follow up Schedule

All *P. falciparum* malaria patients that fulfilled the inclusion criteria were treated using Coartem® with batch number f1655 and expiry date, August, 2011 for three consecutive days (day 0 to day 2) twice a day (Annex III). The drug dose was determined by the weight of the patients (Annex III). The morning dose had given under supervision, and patients/parents or guardians took the second evening dose for administration at home with clear verbal instruction on how and when to take the drug. Patients / parents or guardians were told to return back to the clinic on study days (Day 1, 2, 3, 7, 14, 21 & 28) and on any other day when they feel sick. Patients who failed to come to the clinic were traced by a member of the study team by going to their homes assisted by the registered addresses, and provided the scheduled drug dose and obtained the necessary sample that is required for the study. The drug was crushed and mixed with water and sugar for the small children who couldn't swallow. After drug administration 30 minutes follow up was made to repeat the administration of the drug if vomiting occurs.

3.7. Conditions for Patient Withdrawal after Enrolment

Patients withdrew from the study after enrolment in case of:

- Withdrawal of consent
- Lost to follow-up
- Detection of *P. vivax (* those patients were treated with chloroquine)

3.8. Classification of *in vivo* Drug Response

Patients were classified as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) or adequate clinical and parasitological response (ACPR) according to the following definitions;

3.8.1. Early Treatment Failure (ETF)

Development of sign of severe malaria on day 1, 2 or 3, in the presence of parasitemia; parasitemia on day 2 higher than day 0 irrespective of axillary temperature; parasitemia on day 3 with axillary temperature ≥ 37.5°C; parasitemia on day 3 ≥ 25% of day 0.

3.8.2. Late Clinical Failure (LCF)

Development of sign of severe malaria after day 3 in the presence of parasitemia; presence of parasitemia and axillary 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.

3.8.3. Late Parasitological Failure (LPF)

Presence of parasitemia on any day from day 7 to day 28 and axillary temperature < 37.5°C, without previously meeting any of the criteria of early treatment failure or late clinical failure.

3.8.4. Adequate Clinical and Parasitolgical Response (ACPR)

Absence of parasitemia on day 28, irrespective of axillary temperature, without previously meeting any of the criteria of ETF, LTF or LPF.

3.9. Ethical Consideration

Ethical clearance for the study was obtained from the Ethical Clearance Committee of the Department of Biology, Addis Ababa University and from Ethiopian Health and Nutrition Research Institute (EHNRI) before its initiation. In addition, Permission to conduct the study in the area was obtained from Halaba Special Woreda Health Director's office. Informed consents were obtained from parents or guardians for children and the study participants themselves for adults (Annex II). The communities of the study participants were also informed of the risk and benefits of the study through meetings.

3.10. Screening and Follow up of the Study Participants

Between October 2010 and December 2010, 3305 (55.8%) female and 2617 (44.2%) male patients that were clinically suspected for having malaria were screened in Halaba Health Center, Qulito town. Of the 5922 screened patients, 52.9% were adults followed by 28.5% pre-school children and 18.6% children aged above 5 years.

Among the screened patients, 1826 (30.8%) were positive for malaria. A total of 273 (14.9%) of these patients were microscopically positive for *Plasmodium falciparum* mono infection, and 1553 (85.1%) were positive for *Plasmodium vivax.* Out of 273 positive patients for *Plasmodium falciparum,* only 89 (32.6%) patients fulfilled the recruitment criteria and included in the study. Others were excluded from the study for failure to fulfil the inclusion criteria.

Of the 89 eligible patients, 9 were excluded from the study during follow up: 6 (6.7%) were lost to follow up, and 3 (3.4%) withdrew from the study. In addition, one treatment failure was detected and classified as a late clinical failure (LCF) (Fig. 3).

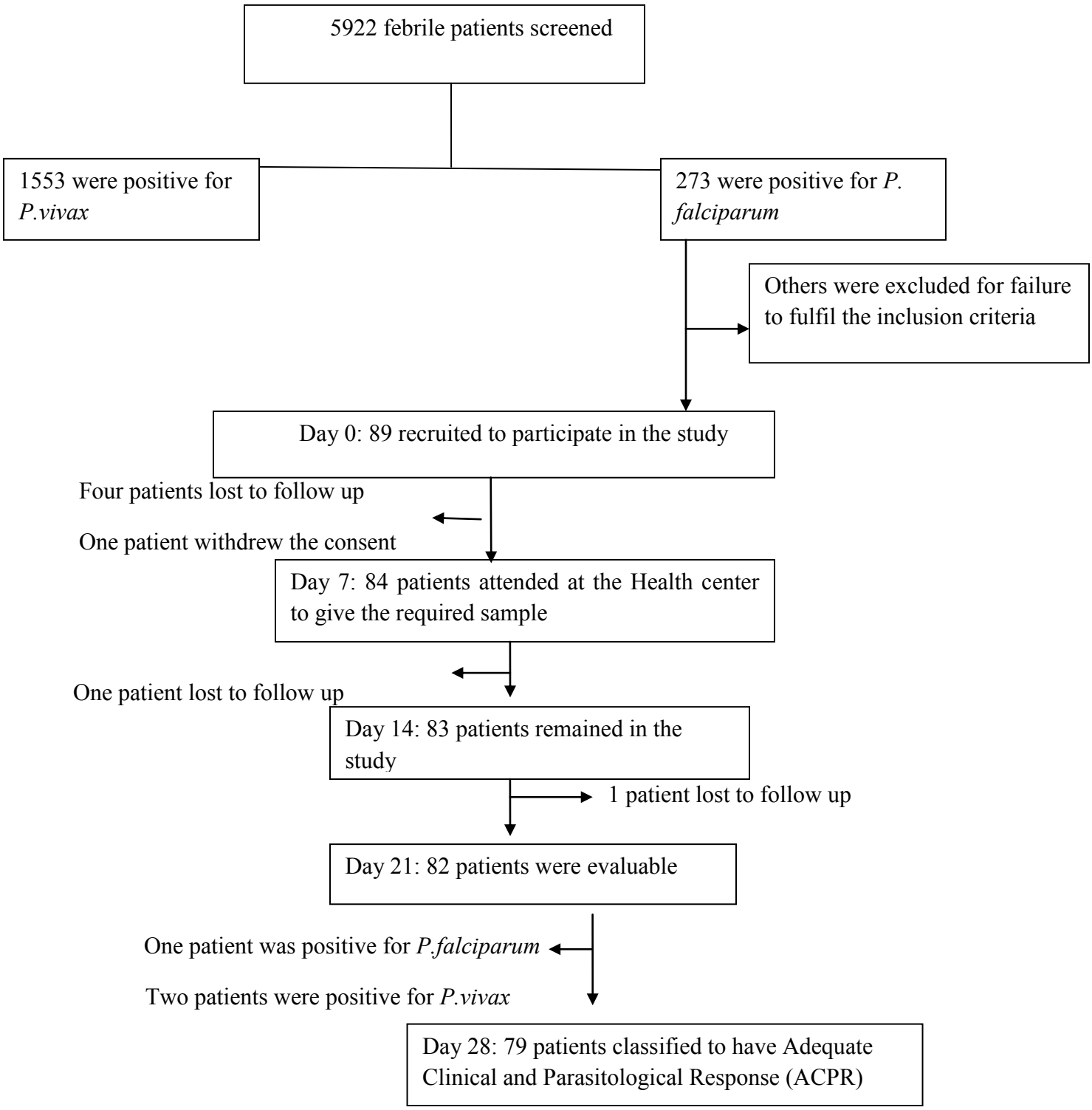

Figure 3. Details of screening and follow up process of the study participants, Halaba Special Woreda, Southern Ethiopia, 2010/11

3.11. Statistical Analysis

All the data from recruited patients were imported into the WHO designed Excel data analysis sheet and Excel 2007. Independent t-test in SPSS version 17.0 was used to check the presence or absence of statistically significant differences between the haemoglobin means of different age groups on the day of inclusion. Change in mean haemoglobin level between day 0 and 28 was compared using paired t- test. P value less than 0.05 was considered statistically significant.

4. RESULT

4.1. Retrospective Malaria Prevalence Data of Halaba Special Woreda

According to six yearly malaria reports that were available in Halaba Special Woreda Health Office, high prevalence of malaria characterizes the Woreda. Based on the reports, fluctuation in yearly malaria prevalence was evident. In the Woreda, mixed infection showed smaller proportion compared to single infections by one malaria parasite species (Table 2).

Table 2. Retrospective data on prevalence of malaria in Halaba Special Woreda, Southern Ethiopia, between 2005/06 and 2010/11

year	2005/06	2006/07	2007/08	2008/09	2009/2010	2010/11
P.falciparum	10482 (53%)	15171 (53.3%)	8054 (41.4%)	8568 (42.1%)	11772 (35.7%)	18270 (45. 5%)
P. vivax	8421 (42.4%)	12114 (42.5%)	10679 (54.9%)	11621 (57.1%)	17043 (51.7%)	20590 (51.2%)
Mixed infection	917 (4.6%)	1207 (4.2%)	721 (3.7%)	154 (0.8%)	4162 (12.6%)	1329 (3.3%)
Total positive	19820 (74.2%)	28492 (75.5%)	19454 (65.3%)	20343 (69.5%)	32977 (67.5%)	40189 (64%)
Total examined	26693	37739	29781	29284	48892	62762

4.1.1 Malaria Control in the Woreda

In Halaba Special Woreda, distribution of insecticide treated nets (ITNs), application of indoor residual spray (IRS) using the chemical deltamethrin, once in a year; environmental management of breeding sites of the vector, and early case detection and free of charge treatment using anti-

malarial drug, Coartem® are the malaria intervention actions that have been taken by the Woreda Health Office. 1 to 4 ITNs were provided for each household based on the size of the family with priority given to under five children and pregnant women.

Among the recruited 89 study participants, males were higher in proportion compared to females, and the median age was 5 years as shown in table 3. On the first day of the study period, relatively higher average parasitemia and lower mean haemoglobin concentration were observed in pre- school children as compared with the other age groups. Gametocytes were detected in the study participants under the age of 15 yrs, during the time inclusion.

Table 3. Baseline characteristics of the study participants with uncomplicated *falciparum* malaria, Halaba Special Woreda, Southern Ethiopia, October 2010 to December 2010

Characteristics	Age group			Total n=89
	7mon-4yr (n=37)	5-14yr (n=25)	15-45yr (n=27)	
Median age (yr)	2	6	32	5
Male n(%)	24 (64.9 %)	12 (48%)	15 (55.6%)	51 (57.3%)
Female n(%)	13 (35.1 %)	13 (52%)	12 (44.4%)	38 (42.7%)
Average temperature (range), (°C)	38.7(37.6-40.2)	39(37.2-41)	38.2 (36.5-40.4)	38.6(36.5-41)
Average weight(kg)	10.6	19.3	55.2	26.6
Mean Haemoglobin (range), (g/dl)	9.2 (5.3-13.6)	10.3(5.2-15.3)	13.7(8.8-16)	10.8(5.2-16)
Average parasitemia (parasites/µl)	6416	5184	3066	5053
Gametocyte carriage n (%)	4 (10.8%)	4 (16 %)	0 (0%)	8 (9%)

91% of the recruited patients experienced malaria attack in the past, and 27% of them took anti-malarial drug other than Coartem®, within two weeks prior to the study. In addition, 67 (75.3%) study participants had ITNs, of whom only 38 (56.7%) reported proper use. The remaining 17 (25.3%) patients used the ITNs sometimes, and 12 (18%) did not use them at all.

4.3. Cure Rate of Coartem®

PCR uncorrected cure rate of Coartem® was 98.7% (95% CI=93.2-100%) in the study area. During the 28-day follow up, a reappearance of *P. falciparum* was found and classified as a *LCF*. The cure rate of Coartem® was 100% for children above five years and adults as shown in table 4.

Table 4: Cure rate of Coartem® treated uncomplicated *falciparum* malaria infected study participants on day 28, Halaba Special Woreda, Southern Ethiopia, 2010/11

	Age in years n(%)			
Parameters	Under 5	5-15	15+	Total
ETF	0 (0%)	0 (0%)	0 (0%)	0 (0%)
LCF	1 (3.3%)	0 (0%)	0 (0%)	1 (1.3%)
LPF	0 (0%)	0 (0%)	0 (0%)	0 (0%)
ACPR	29 (96.7%)	23 (100%)	27 (100%)	79 (98.7%)
Total analysis	30 (81.1%)	23 (92%)	27(100%)	80 (89.9%)
Withdrawal	2 (5.4%)	1 (4%)	0 (0%)	3 (3.4%)
Lost to follow up	5 (13.5%)	1 (4%)	0 (0%)	6 (6.7%)
Total	37	25	27	89

4.4. Fever Clearance

Average temperature of the study participants at the time of inclusion was 38.6°C ± 0.09, and 94.3% of the study participants were with fever. Almost all fever cleared from the study participants on day 2 (Fig. 4). After clearance of parasitemia, body temperature ≥37.5°C was detected in small proportion of the study participants.

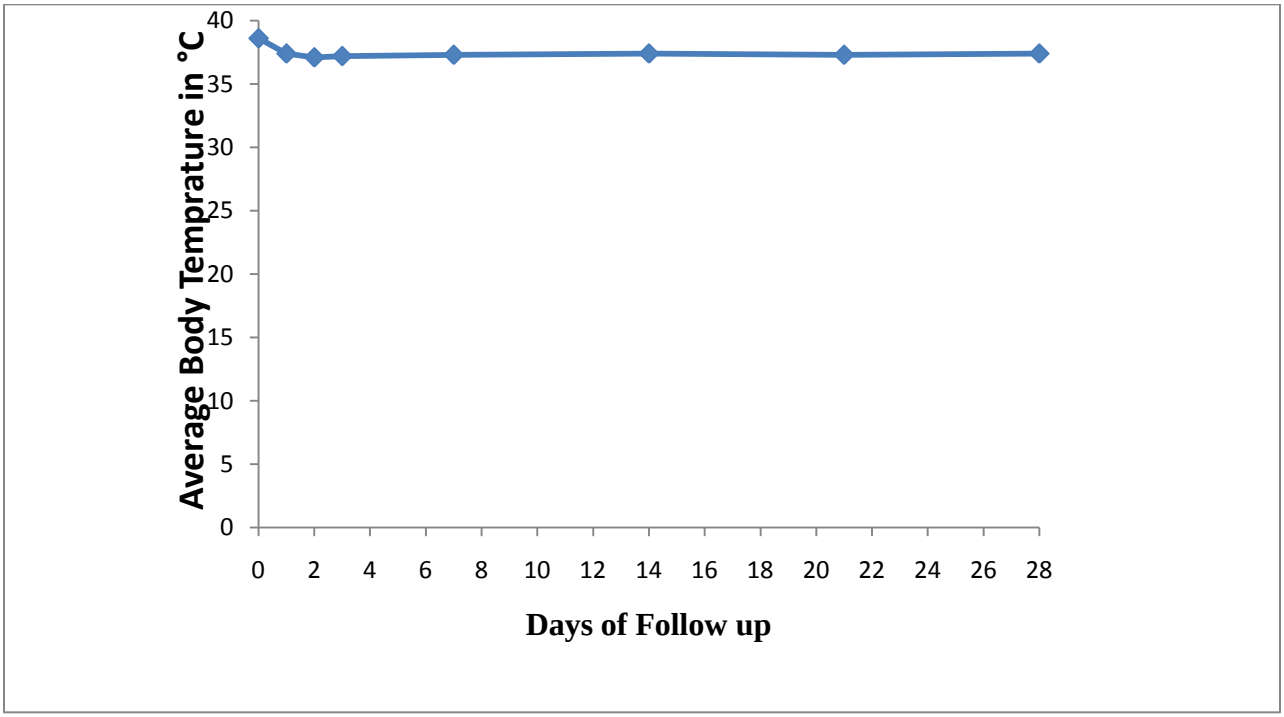

Figure 4. Average temperature of the study participants at day 0 and after Coartem® treatment of uncomplicated *falciparum* malaria, Halaba Special Woreda, Southern Ethiopia, 2010/11

4.5. Parasite Clearance

At day 0, before drug administration, average parasitemia of the study participants was 5053 para/µl, and 15.7% of them had high parasitemia. The average parasitemia reduced to 483 para/µl and 18 para/µl on day 1 and 2, respectively. On the 2nd day of the study period, almost 90% of parasitemia cleared, and the number of positive patients declined in 40.5% as shown in figure 5. All parasitemia cleared from the study participants on day 3.

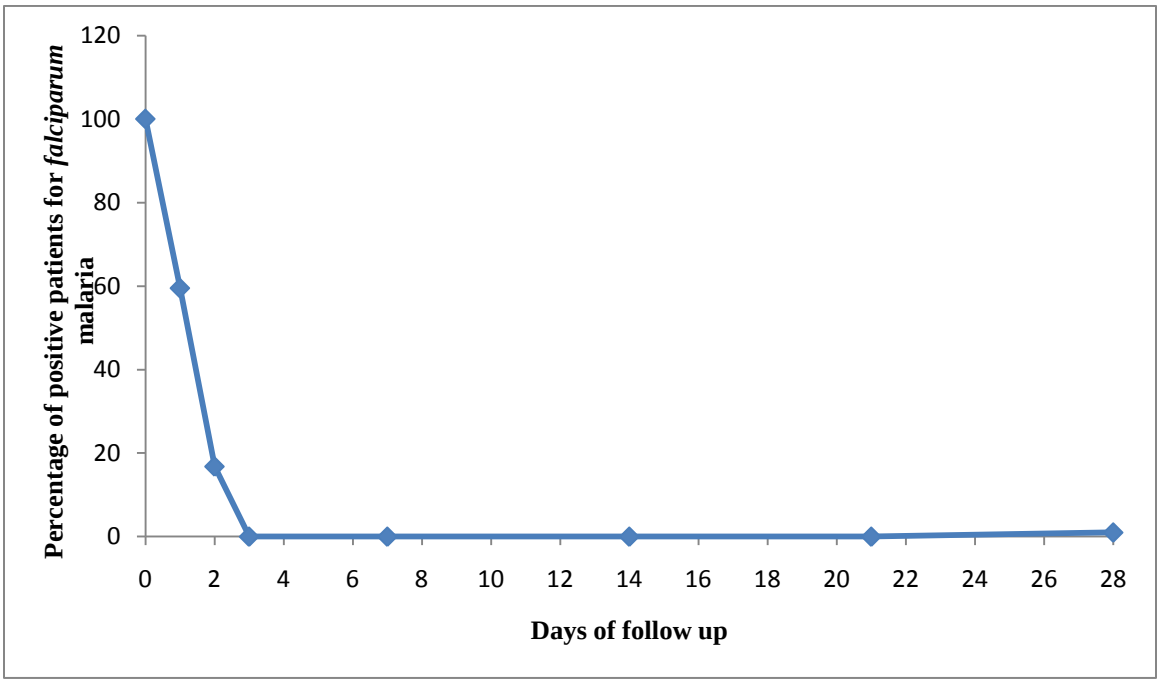

Figure 5. Percentage of *falciparum* malaria positive patients during follow up days, Halaba Special Woreda, Southern Ethiopia, 2010/11

4.6. Gametocyte Clearance

Gametocytes were observed on 9% of the study participants, on the day of inclusion, no new patient with gametocyte was detected after initiation of treatment. At day 0, average gametocytemia was 27 gametocytes/ µl (Range: 40-928 gametocyte/µl) as shown in figure 6. The percentage of gametocyte positive individuals declined to 6.7% (day 1), 3.3% (day 2) and 1.1% (day 3 and 7), and completely disappeared at day 14. No gametocytemia observed among adults during the study period.

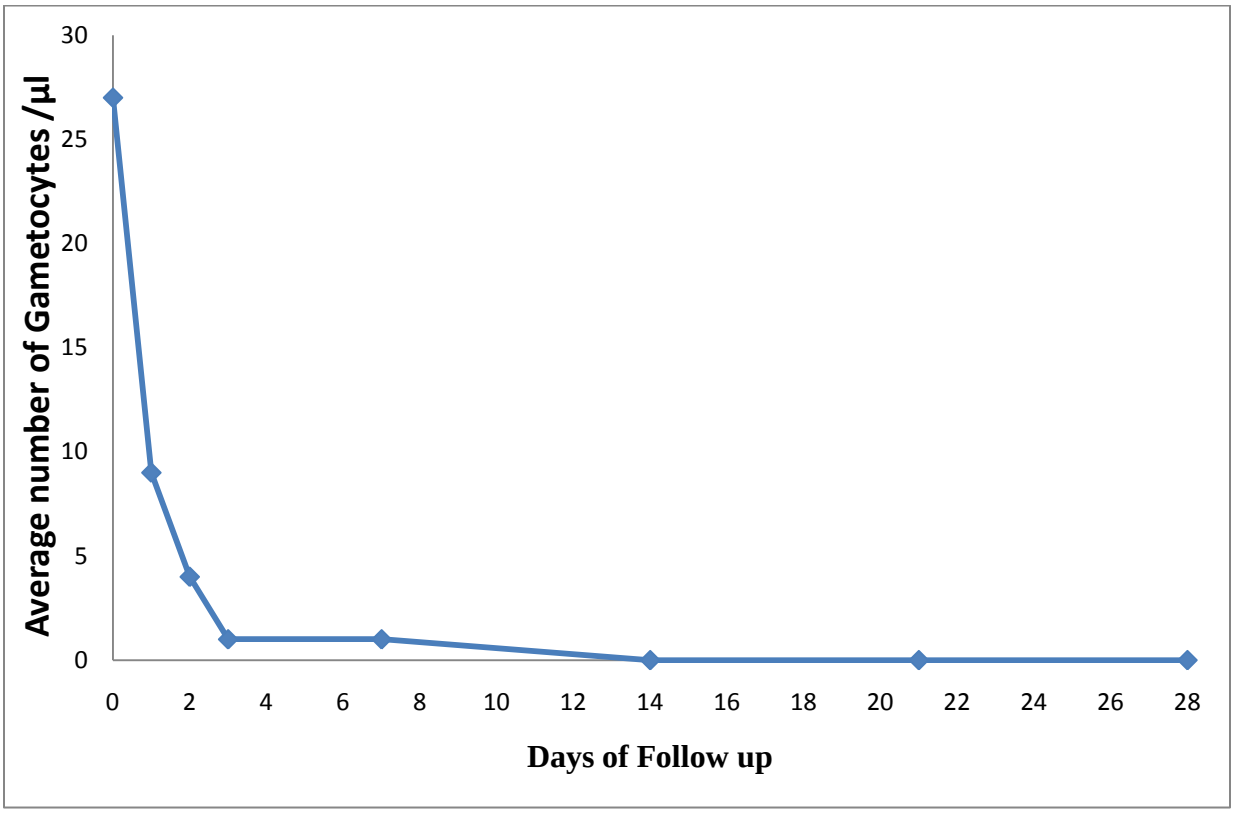

Figure 6. Average number of gametocytes of the study participants with uncomplicated *falciparum* malaria, before and after treatment with Coartem®, Halaba Special Woreda, Southern Ethiopia, 2010/11

4.7. Assessment of Haemoglobin Level and Anemia

At day 0, the mean haemoglobin level of the study participants was 10.8g/dl, and the mean haemoglobin level in children was significantly lower than in adults (P=0.00). Over all, the mean haemoglobin level showed improvement to 11.3g/dl (range: 10.2g/dl -13.4g/dl) and 11.8g/dl (range: 10.5g/dl -13.8g/dl) at day 14 and 28, respectively. In addition, by day 28, mean haemoglobin level had significantly increased (P = 0.002), compared to day 0.

Furthermore, 78.4% of under five children, 64% of children above 5 yrs old and 22.2% of adults were anemic, at the time of inclusion. The proportion of anemic individuals declined from 57.3% on day 0 to 54.2% and 44.3% on day 14 and 28, respectively.

Table 5. The percentage of anemic individuals, before and after Coartem® treatment of uncomplicated f*alciparum* malaria, Halaba Special Woreda, Southern Ethiopia, 2010/11

Day of follow up	Percentage of anemic individuals		
	Mild (10-12.9 g/dl)	Moderate (7-10.9 g/dl)	Severe (<7g/dl)
Day 0 N=89	10 (11.2%)	32 (35.9%)	9(10.1%)
Day 14 N=83	9 (10.8%)	29 (34.9%)	7(8.4%)
Day 28 N=79	8(10.1%)	22(27.8%)	5(6.3%)

4.8. Adverse Events

Novartis listed out a number of adverse events of Coartem®, nine of these adverse events were observed in the present study after clearance of the malaria parasite. Most of the adverse events of Coartem® observed were similar with common symptoms of malaria. Almost all symptoms declined with parasite clearance as shown in table 6. After the completion of the study dose, anorexia, cough, headache and weakness were reported by higher proportion of the study participants, compared to others. There was no serious drug adverse event observed in the present study.

Table 6. Adverse events reported by the study participants after treatment of uncomplicated *falciparum* malaria by Coartem®, Halaba Special Woreda, Southern Ethiopia, 2010/11

Adverse events	**The percentage of the study participants with an adverse event**				
	Day 0	Day 3	Day 7	Day 14	Day 28
Headache	57.3	12.6	20.2	9.6	6.3
Anorexia	69.6	12.6	8.3	5.4	6.3
Nausea	32.5	1.1	-	-	-
Vomiting	53.9	1.1	-	-	1.2
Weakness	94.3	35.6	21.4	9.6	7.5
Joint pain	29.2	3.4	5.9	3.6	1.2
Abdominal pain	62.9	9.1	2.3	6	5
Diarrhoea	33.7	3.4	7.1	8.4	5
Cough	60.7	29.8	23.8	7.2	5

5. DISCUSSION

As the present study demonstrated a high prevalence of *P.falciparum* and *P.vivax* malaria in Halaba Special Woreda, Southern Ethiopia, the malaria control interventions that are mainly based on ITNs distribution, IRS application and Coartem® treatment, did not seem to be effective. This might be due to the improper use of ITNs and weakness in other malaria intervention measures.

The abundance of *P. vivax* infected patients detected during screening compared to those infected with *P. falciparum* may be partly explained by the relapsing charachterstic of *P. vivax.* Moreover, the free distribution of Coartem® at the health posts might have reduced the number of patients with *P. falciparum* malaria in the health center (Personal communication, Mr Adem Seman, Disease Prevention Officer in Halaba Special Woreda Health Office).

The low level of treatment failure detected in the present study is comparable with other reports from different parts of Ethiopia - Kersa (3.7%) (Assefa *et al*., 2010) and Alemata, Humera, Assendabo and Nazareth (0.9%) (Jima *et al.,* 2005), and from India (1.4%) (Valecha *et al*., 2009). On the other hand, a higher treatment failure (6.4%) reported from Lao people's Democratic Republic (Stohrer *et al*., 2004) shows that *P.falciparum* to Coartem® resistance of a higher magnitude can develop. In line with this, the present finding of a 1.3% LCF in the same location (Halaba Special Woreda) where 100% efficacy (Kefyalew *et al*., 2009) was reported two years ago may be a sign of emerging resistance to Coartem®.

In the treatment of malaria, background immunity to malaria from repeated infections usually acts synergistically with anti malarial drugs to achieve better cure rate than in non-immune individuals, such as young children (Ezzet and Karbwang, 1998). This may be the reason for the

only LCF observed in the present study in under five children, in which acquired immunity to malaria is poorly developed (Mannan *et al*., 2003). Therefore, a close monitoring of children during assessment of therapeutic efficacy of anti-malarial drug is required.

The strong association of fever clearance with malaria parasite clearance can be explained by the fact that malaria toxins and various metabolic end products are associated with fever induction and some of these with shorter half lives, disappear with parasite elimination (Kwiatkowski *et al.,* 1997). The fast rate of fever clearance, following Coartem® treatment detected in the present study, is comparable with earlier reports (Fanello, *et al.*, 2007; Kefyalew *et al*., 2009; Assefa *et al*., 2010; Faye *et al.,* 2010; Hamde, 2011). However, the presence of low grade fever in small proportion of the study participants, after clearance of parasitemia, may be the result of circulating toxins and molecules of malaria parasite that have longer half life or other parasites (Olaleye *et al.,* 1998).

The finding that parasitemia cleared from the study participants very rapidly following treatment with Coartem® is similar to the reports from other studies from Ethiopia (Kefyalew *et al*., 2009; Assefa *et al.,* 2010; Hamde, 2011) and other countries - Lao People's Democratic Republic (Stohrer *et al.,* 2004); Rwanda (Fanello *et al*., 2007); India (Valecha *et al*., 2009) and Senegal (Faye *et al.,* 2010). This shows the potent anti-malarial property of Coartem® targeting the blood stage parasites.

In agreement with the present study, studies from different parts of Ethiopia (Jima *et al*., 2005; Kefyalew *et al*., 2009; Assefa *et al*., 2010, Hamde, 2011) and other countries (Fanello *et al*., 2007; Valecha *et al*., 2009; Faye *et al.,* 2010) had demonstrated the gametocidal activity of

Coartem®. Earlier studies had also shown that Coartem® prevents gametocyte maturity and kills the early developmental stages and hence prevents malaria transmission (Ashley *et al.,* 2007).

As a combination therapy, the three day regimen of Coartem® targets the asexual life cycle phase with artemether that has a shorter half-life, and exposure to lumefantrine prevents the late appearing recrudescence (Makanga and Krudsood, 2009). Therefore, the 1.3% LCF detected in the present study could be an indication of a genuine drug resistance. However, PCR verification of the LCF parasite isolates, for possible variation from the day 0 parasite population, will be necessary to draw a firm conclusion.

The prevalence of anemia in malaria infected pre-school children recorded at enrolment (78.4%) was higher than the 75.2% that was reported by WHO (2008b) for under five children in Ethiopia, indicating that anemia is a serious public health problem in this age group in Halaba Special Woreda. The higher prevalence of anemia in under 5 children may be an indication of their poor nutritional state. Poor nutrition, together with the lack of partial protective immunity in children, which is acquired over a period of years after repeated exposure to malaria parasites (Marsh and Kinyanjui, 2006), may have contributed to the high prevalence of anemia. The possible contribution of poor nutrition to the high anemia prevalence was indicated by the large proportion of anemic individuals on day 14 and 28, despite the clearance of malaria parasite following Coartem® treatment.

The fact that the population of Halaba Special Woreda is not teff (an iron-rich grain) consumer could be one factor that may account for the high prevalence of anemia in all age groups. In addition, according to Halaba Special Woreda Health Office report, helminthic infection was among the top ten infectious diseases, and high prevalence of helminthic infection was detected

in malaria patients in the study area (Degarege *et al.*, 2010), which may also have contributed to the high prevalence of anemia in spite of the treatment of malaria.

However, the relative improvement in mean haemoglobin level, following effective Coartem® treatment, is in agreement with previous reports from the same area (Kefyalew *et al*., 2009) and from other African countries (Falade *et al.*, 2005; Kobbe *et al*., 2008).

The mild adverse events, following Coartem® treatment, recorded in the present study were also reported in other studies (Stohrer *et al*., 2004; Mulenga *et al* 2006; Fanello *et al*., 2007; Assefa *et al*., 2010; Faye *et al*., 2010, Hamde, 2011). This confirms the safety of Coartem® use in Halaba Special Woreda.

Although Coartem® demonstrated high clearance rate of fever, parasitemia, gametocytemia and other symptoms of malaria, the luck of compliance observed among the screened patients is a problem that could lead to the development of drug resistance in the area. This can only be minimized through health education of the population and adequate follow up of their drug use behaviour.

6. CONCLUSION

Based on the findings of the present study, the following conclusions can be drawn:

- The six-dose regimen of Coartem® showed high therapeutic efficacy (98.7%) in the treatment of uncomplicated *falciparum* malaria in Halaba Special Woreda, Southern Ethiopia.

- Rapid clearance of fever, asexual parasitemia and gametocytemia was observed after Coartem® treatment of uncomplicated *falciparum* malaria.

- Improvement in mean haemoglobin level was achieved following Coartem® treatment.

- Coartem® was safe and tolerable with no serious adverse events.

7. RECOMMENDATION

- Since Coartem® is freely distributed for the treatment of uncomplicated *falciparum* malaria by health posts and health centers in the Woreda, the luck of compliance observed among the patients screened for malaria is a source of concern for the emergence of resistant parasite. Therefore, the Health Office of the Woreda must create awareness on the proper use of the drug and the consequences of default in scheduled dose consumption.

- The 1.3% LCF detected in the study is an additional reason for *in vivo* assessment of the efficacy of Coartem®, including PCR correction, every two years as per WHO recommendation.

- As more than half of the study participants were anemic, studies on the nutritional status of the general population must be undertaken to determine the extent of nutritional anemia in the Woreda.

- The high *P.falciparum* and *P.vivax* malaria prevalence observed in the study area necessitates strong malaria intervention measures in the Woreda.

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Annex I. Calculating Kaplan-Meier survival curves by hand

D	N	TF	ex	IR	SCI	FCI
0	89	0	0	1.000	1.000	0
1	89	0	0	1.000	1.000	0
2	89	0	0	1.000	1.000	0
3	89	0	2	1.000	1.000	0
7	87	0	3	1.000	1.000	0
14	84	0	1	1.000	1.000	0
21	83	0	1	1.000	1.000	0
28	82	1	2	0.987	0.987	0.013
Total		1	9			

D= day of test (D-1 the day before)

N_D = number of subjects remaining at risk = $N_{D-1} - TF_{D-1} - ex_{D-1}$

TF = incident cases of therapeutic failure

ex = excluded due to loss to follow-up, withdrawal or protocol violation

IR_D = interval risk (at Day 0, IR = 1) = $[(N_D - ex_D) - TF_D]/(N_D - ex_D)$

SCI_D = cumulative incidence of therapeutic success (at Day 0, SCID = 1) =I R_D x SCI_{D-1}

FCI_D = cumulative incidence of therapeutic failure =1 – SCI_D

Annex II. **Written Consent Form**

Anti malarial drug called Coartem® has been used for five years as the first line treatment for uncomplicated *Plasmodium falciparum* malaria in Ethiopia. Therefore, the federal ministry of health (FMOH) wants to monitory the efficacy of Coartem® in six selected sentinel sites. Halaba Special Woreda is among those selected sentinel sites. So, You/your children are sincerely required to participate in the study. In this study, patient with uncomplicated *falciparum* malaria beginning from six months of age are allowed to participate. The study will be carried out for 28 days in Halaba Health Center. But, this doesn't mean that you will be required to come each day. You are requested to come to Halaba Health Center in the 1^{st}, 2^{nd}, 3^{rd}, 7^{th}, 14^{th} , 21^{st} and 28^{th} days of follow up. In each day of follow up, finger prick blood will be taken to check the clearance of malaria parasite, and additional drop of blood will be taken at day 0, day 14 and day 28 to measure the haemoglobin level. Coartem® will be given for three consecutive days, twice a day. The morning dose will be administered by the help of a clinician in the health center and the evening dose will be give to take it at home (given to guardians or parents in the case of children). If you fail to be better, it is possible to come back on any other day. We want you to know that Coartem® as other drugs has its own side effect but this doesn't have any connection with the study that we will be carried out, so if this drug causes a serious adverse event on you/your child during the study period, you have full right to stop the medication, and we are ready to treat you/your child with other antimalarial drug.

This study wants to recruit those patients who are willing to participate. Thus, you have a right not to participate (not to let your children to participate) in the study. We want you to be sure that there is nothing you/your children will loss by not participating in the study. You/your child also have a right to cease participating at any time while you/your child participate.

For those patients that will be enrolled in the study, transportation fee will be covered by the project during each follow up day. In addition, we want you to know that any report of the study doesn't include the name of you/ your child. Thus, the information in your records is strictly confidential. If this is not clear for you, you can ask for further explanation.

This consent form has been read out for me in my own language and I understand the content and I am voluntarily agreed to participate (to let my child participate) in the study.

The name of the study participants _____

(The name of the guardian or parents in the case of children)

Signature _____

Date _____

The name of the witness _____

Signature _____

Date _____

The name of the investigator _____

Signature _____

Date _____

Annex III. **Weight-based Administration of Coartem®**

Weight	20mg of Artemether and 120mg of Lumefantrine (Coartem®)					
	0 hours	8 hours	24 hours	36 hours	48 hours	60 hours
5-14kg	1	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