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ANTISCHISTOSOMAL EFFICACY OF ARTEMETHER-LUMEFANTRINE WHILE ADMINISTERED AS CURATIVE TREATMENTS FOR MALARIA AMONG PATIENT IN KEMISE HEALTH CENTER, NORTH EASTERN OF ETHIOPIA

By Mulugeta Tilahun, B.Sc
Department of Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University

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Title: Antischistosomal Efficacy of Artemeter-Lumefantrine while Administered as Curative Treatments for Malaria among Patient in Kemise Health Center, North Eastern Ethiopia

Name of M.Sc Candidate: Mulugeta Tilahun (B.Sc, Department of Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University)

Advisors:

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

Mr. Solomon Mequanent (M.Sc, Department of Pharmacology, Faculty of Medicine, Addis Ababa University)

Mr. Abiy Habtewold (M.Sc, Department of Pharmacology, Faculty of Medicine, Addis Ababa University)

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ABBREVATIONS

ACT	Artemether-Lumefantrine combination therapy
AL	Artemether-Lumefantrine
DHA	DihydroArtemisinin
EPG	Egg per gram
FMOH	Federal minister of health
GMEC	Geometric mean egg count
MF-IRB	Medical Faculty Institution Review Board
CSA	Central statistics agency
PCR	Polymerase chain reaction
PfATP6	*Plasmodium* falciparum Ca^{+}-ATPase
PZQ	Praziquantel
SERCA	Sarco Endoplasmic reticulum Ca^{+}-ATPase
TCTP	Translationally controlled tumour protein
WHO	World health organization

ABSTRACT

Background: Schistosomiasis stands next to malaria in terms of extent of endemic areas. The distributions of both malaria and schistosomiasis show a large geographical overlap in tropical and subtropical environments, particularly in sub-Saharan Africa. This part of the world currently harbours more than 85% of the estimated global burdens due to malaria and schistosomiasis. Cross–sectional surveys carried out in different ecoepidemiological settings of Africa revealed high frequencies of *Plasmodium* and *Schistosoma* infections. Both experimental and clinical studies showed that the Artemisinin based therapy for the treatment of malaria do also have antischistosomal efficacies.

Objective: The main objective of this study was to investigate whether Artemether–Lumefantrine has antischistosomal efficacy when administered as curative treatments for uncomplicated *falciparum* malaria in Kemise, NorthEastern Ethiopia.

Method: Malaria cases age above 5 years with uncomplicated *falciparum* malaria, who (or their guardians consented) to participate in the study after fulfilling the inclusion criteria were enrolled in the study for follow up period of day 28, 29 or 30. 152 microscopically confirmed *P. falciparum* malaria cases were enrolled who attended the Kemise Health Center. Stool samples were collected from these malaria patients and diagnosed for schistosomiasis using Kato–katz technique. Co-infected patients were treated with Artemether-Lumfantrine. The cure rate, egg reduction and prevalence of co-infection were measured.

Result: Twenty eight of patients who fulfilled enrollment criteria completed follow up after treatment. The 28 co-infected patients were found stool-negative for *Schistosoma* eggs on day 28, 29 and 30. Twenty eight of the 152 (18.42%) were co-infected with *S. mansoni*. Cure rate and egg reduction rate were 100%.

Conclusion/Recommendation: Artemether-Lumefantrine was found to be highly efficacious (100%) for the treatment of *S. mansoni* and *P. falciparum* malaria co-infection. This study recommended a large scale study for better understanding of the efficacy of Artemether-Lumefantrine for treatment *S. mansoni* and *P. falciparum* co-infection.

Keywords: co-infection, Artemether-Lumefantrine, *S. mansoni*, *P. falciparum,* Kemise

1-INTRODUCTION AND LITRUTURE REVIEW

1.1. Introduction

Malaria has been a challenge to both public health and socio-economic development particularly in Sub-Saharan African countries (Deressa *et al.,* 2004). In Africa, *P. falciparum* malaria is a serious tropical disease that causes more than one million deaths each year. It is transmitted by a range of *Anopheles* mosquitoes and the risk of disease varies greatly across the continent (Kelly-hope, 2009).

Malaria is one leading cause of morbidity and mortality in Ethiopia, three quarters of the land mass is regarded as malaria affected (Negash *et al.,* 2004). Each year more than 5 million malaria cases are estimated to occur in Ethiopia (Senay, 2005). In Ethiopia, about 68% of the population is at risk of malaria, representing approximately 52 million people in 2007. Malaria is seasonal in most parts of Ethiopia, with unstable malaria transmission, rendering the country prone to epidemics. The transmission patterns and intensity vary greatly due to the large diversity in altitude, rainfall, and population movement; areas below 2000 meters are considered to be malarious or potentially malarious (FMOH, 2008).

It has been consistently reported as one of the leading causes of morbidity and mortality in the past years. The magnitude of the problem in 2002/2003 has even worsened and mortality accounting for 15.5% outpatient consultations, 20.4% admissions and 27% inpatient deaths (FMOH, 2004).

Five to six million clinical malaria cases and over 600,000 confirmed cases were reported in epidemic year from health facilities (FMOH, 2004). The number of malaria cases reported by health facilities is only a portion of the actual magnitude. *P. falciparum* and *P. vivax* are the two dominant parasite species with relative frequency of 60% and 40% respectively. This proportion varies from place to place and from season to season. *P. falciparum* is the dominant parasite species that causes severe manifestations and almost all malaria death happen due to infection by this parasite in malaria epidemic situations (FMOH, 2004).

Among parasites, in terms of the extent of endemic areas and number of people infected, *Schistosoma* holds second position in the world (Lescanoa *et al.,* 2004). The disease is a serious problem in Sub-Saharan Africa, South America, China and south East Asia. This disease is a lifelong chronic infection with debilitating symptoms mainly due to an immune reaction raised against parasite eggs trapped in the liver, spleen and gut (Abdulla *et al.,* 2007).

Among the 200 million people infected with schistosomiasis, an estimated 80% are in sub-Saharan Africa, and the annual mortality rate is estimated to be 280,000 in the region (Southgate *et al.,* 2005). Among 200 million people are infected of which 120 millions are symptomatic and 20 million have severe debilating disease. An estimated 85% of all cases, and most of the severely affected are concentrated in Africa (WHO, 1998a).

Schistosomiasis is caused by the blood fluke and leads to significant ill-health and economic burden. The disease is common in the tropics and subtropics and acquired through contact with freshwater bodies infested with the infective cercariae shed from the intermediate host snail. From a public health perspective, the three most important species are *Schistosoma mansoni* and *S. japonicum* (intestinal schistosomiasis) and *S. haematobium* (causing urinary schistosomiasis) (Danso-Appiah A, 2002). In some areas of sub-Saharan Africa there is an overlap in distributionof *S. mansoni* and *S. haematobium* resulting in mixed infections (WHO 2002).

In Ethiopia, the distribution of *S. haematobium* is limited to lowlands below **1000** m altitude. This is partly because of the limited distribution of the snail hosts, and partly because of environmental factors inhibiting the development of the parasite in the snail hosts (Kloos *et al.,* 1988). Water resources development projects in Africa have been often linked with an increase in schistosomiasis transmission (WHO, 1998a). The spread of endemic *S. mansoni* may be as result of particularly water resources development, resettlement, and refugee migration and population movements (Kloos *et al.,* 1988).

The public health importance of schistosomiasis is often underestimated for two reasons. First, like all helminthes infections, the distribution of worms in any community is widespread but uneven, *i.e.* few have heavy infections and severe

disease, while many have light infections and fewer symptoms. Some people with very few worms may have no symptoms. Second, severe disease usually follows after many years of silent or mildly symptomatic infection (WHO, 1993).

Some patients develop severe and intense manifestation of the disease, depending on cercariae burden and individual immune response (Martin *et al.,* 2007). The transmission cycle requires contamination of surface water by excreta, specific freshwater snails as intermediate hosts, and human water contact (Gryseels *et al.,* 2006). In non endemic areas, acute schistosomiasis usually develops in individuals who have their first contact with such contaminated water (Martin *et al.,* 2007).

In malaria endemic areas, co-infection with multiple parasites is common. *P. falciparum* and *Schistosoma* spp are co-endemic parasitic diseases worldwide (Snow *et al.,* 2005). Knowledge about the epidemiology of co-infection suggests that most at risk of *Plasmodium*–helminth co-infection are school-age children, rather than preschool children or adults (Brooker, 2007). Malaria and schistosomiasis are often geographically co-endemic in sub-Saharan Africa, and co-infections with these parasites are common in school-age children, the main target group for schistosomiasis control programmes (Mazigo *et al.,* 2010).

From clinical, diagnostic, preventive and therapeutic points of view, the co-endemicity of malaria and schistosomiasis and other helminthes infections has an important implication which is of increasing contemporary interest. Co-infections may increase the level of morbidity and immunological factors may alter responses to common treatment options (Booth, 2004a; Vennervald et *al*., 2004; Vennervalde *et al*., 2005).

Currently, Artemisinin and related derivatives are effective antimalarial drugs clearing parasitaemia and disease-related symptoms more promptly than any other available antimalarial drugs. Artemether, artesunate and arteether have short terminal half life times. Combination therapies with longer lasting antimalarial are being advocated as a strategy to prolong the length of time of Artemisinin (Xiao, 2005). Particularly within Artemisinin-based combination therapies, the Artemisinin have become key drugs for the treatment and control of malaria. In areas where malaria and schistosomiasis are co-endemic, the Artemisinin also exhibit antischistosomal properties, may have an

effect on both diseases and co-infection might alter drug efficacy (Shu-Hua *et al*., 2006).

The transmission of malaria and schistosomiasis takes place concomitantly in certain regions of the African continent and the use of Praziquantel for the treatment of schistosomiasis has been less promising than what was expected. In these circumstances, the use of Artemisinin derivatives with the aim of treating malaria cases may exert a significant influence on the reduction of the morbidity rate due to schistosomiasis (Lescanoa *et al*., 2004).

The malaria management guided one currently has been revised in Ethiopia. Now the Artemisinin base combination therapy is the first drug of choice for treatment of uncomplicated *falciparum* malaria. Particularly Quarterm- combination of Arthemether and Lumefantrine- is called on use used. Artemether has antischistosomial properties against several species of *Schistosoma* (Xiao *et al*., 2001).

There is, however, a debate regarding its use on a large scale to control schistosomiaisis worldwide. Because schistosomiasis and malaria have overlapping distributions, the risk that widespread use of Artemisinin for the treatment of schistosomiasis could favour the emergence of resistant strains of *Plasmodium spp*. must be seriously considered (Utzinger *et al*., 2001). However, there is a possibility that the use of Artemisinin to treat malaria could have, as a secondary effect, a beneficial effect on reducing burden of schistosomiasis (Lescanoa *et al*., 2004).

Schistosomiasis and malaria are co-endemic in study aimed to investigate antischistosomal efficacy of AL in Kemise. The present study was conducted to see the importance and side benefits of Artemether-Lumefantrine base therapy while administered for the treatment of malaria patients who were co-infected with *Schistosoma*. The result of this study attempted to be an input for further analysis of the efficacy of the ACT in large population. This encourages the use of single regimen to treat patients with both parasitic diseases, and this helps in saving the treatment cost.

1.2. Literature Review

The parasitic diseases affect people living in the poorest countries and these people are at high risk, not just of one serious infection but of many infected. The majority of `study focuses on a single disease. Malaria, schistosomiasis and intestinal helminthes infections are cause of high morbidity in most tropical parts of the world (Mazigo *et al.,* 2010).

Malaria remains a major public health threat to more than 600 million Africans. In Africa, it occurs in the endemic regions where the disease pathogen is continuously present in the community. These regions are characterized by an environment that is conducive to interactions between the *Anopheles* mosquito, malaria parasites and human hosts (Grover-koper *et al.,* 2006).

Periodic epidemics of malaria are a major public health problem for many sub-Saharan African countries. Populations in epidemic prone areas have a poorly developed immunity to malaria and the disease remains life threatening to all age groups (Grover-koper *et al.,* 2005). In Ethiopia, Malaria is the leading cause of morbidity and mortality, accounting for over five million cases and thousands of deaths annually. The risks of morbidity and mortality linked with malaria are characterized by spatial and temporal difference across the country (Yeshiwondim *et al.,* 2009).

Schistosomiasis continues to hold second position among parasites, after malaria, in terms of the extent of endemic areas and number of people infected (Lescanoa *et al.,* 2004; WHO, 1999). In tropical and subtropical area, the distributions of malaria and schistosomiasis show a large geographical overlap, mainly in sub-Saharan Africa. This part of the world currently harbours more than 85% of the estimated global burdens due to malaria and schistosomiasis (WHO, 1999). Cross-sectional surveys carried out in different ecoepidemiological settings revealed high frequencies of *Plasmodium* and *Schistosoma* infections are occurred in Africa (Shu-Hua *et al*., 2006). Across most parts of Africa, *Plasmodium-Schistosoma* co-infections, as well as-other infections, are common (WHO, 2005).

In micro dams, the prevalence of malaria, *S. mansoni* and Hook worm was higher at altitudes below 2,000 meters above sea level. *S. mansoni* was more prevalent 5 years before the survey (Alemayehu *et al.,* 1998). While at currently constructed micro-dams, *T. trichiura* was more prevalent. The widespread distribution of shistosomiasis and intestinal helminthes, and the presence of malaria infection during the dry season confirm that microdams create favourable conditions for the transmission of these parasitic diseases (Alemayehu *et al.,* 1998)

The study done in Ethiopia revealed that superinfected *S. mansoni* mice with *P. berghei* show that superinfection with *S. mansoni* enhanced *P. berghei* parasite development, increased parasitaemia and mortality and delayed reduction/clearance in parasitaemia. These suggest that co-infection with *Schistosoma* and malaria parasite would worsen malaria severity and delay parasite reduction or clearance after chemotherapy in human being (Legesse *et al.,* 2004).

Artemisinin is the active compound obtained from the leaves of a plant species that is widespread throughout China, and also grows naturally in central Europe, the United States and Argentina. The plant has been used as remedy to many different illnesses for centuries in the Chinese traditional medicine, but particularly for the treatment of febrile individual (Utzingera *et al*., 2001).

The unique structure of the active compound has been identified: it is a sesquiterpene lactone bearing a peroxide group and unlike most other antimalarial lack nitrogen containing heterocyclic ring system it is poorly soluble in water and decomposes in other polar solvents, probably by opening of lactose ring (Utzingera *et al*., 2001; Chris *et al.,* 2004). Following successful clinical testing, Artemisinin was approved by the Chinese Ministry of Public Health as a novel antimalarial drug in 1986/1987 (Li and Wu *et al*., 1998).

Several derivatives of Artemisinin showed improved solubility, chemical stability and enhanced antimalarial activity, the most important of which are Artemether, artesunate and arteether (Meshnick *et al*., 1996). These artimesinin have different solubility affinity either to water or lipid. All are ester-based compounds and lipid soluble, except Artesunate which is hydrophilic (Namdeo, *et al.,* 2006).

Coartem is a fixed–dose combination of Artemether-Lumfantrine. Its two components have different modes of action that provide synergistic anti-malarial activity (Makanga *et al.,* 2009). Artemether-Lumfantrine (AL) and its main active metabolites, dihydroArtemisinin (DHA) occur at approximately two hours post–dose, leading to a rapid reduction in asexual parasite mass and a quick resolution of symptoms. Lumfantrine is absorbed and cleared more slowly, and accumulates with successive doses, acting to prevent recrudescence by destroying any remaining parasites after Artemether and DHA have been cleared from the body (Djimde *et al.,* 2009).

Currently world health organization guideline recommended the use of artemether-based combination therapy for the treatment of uncomplicated *falciparum* malaria (Makanga *et al.,* 2009). It is useful for the treatment of infant, children and adults with acute, uncomplicated *P. falciparum* or mixed infections including *P. falciparum* (Makanga *et al.,* 2009). Food intake significantly enhanced the bioavailability of both Artemether-Lumfantrine, an effect which is more apparent for the highly lipophilic lumfantrine, and meal with only a small fat is also effective (Djimde *et al.,* 2009).

Artemisinin is the most potent antimalarial available, rapidly killing all asexual stage of *P. falciparum.* Artemisinin, but not quinine or chloroquine, inhibit the Sarco Endoplasmic reticulum Ca^{+}-ATPase (SERCA) orthologue *P. falciparum* Ca^{+}-ATPase (PfATP6) of *P. falciparum* in Xenopus oocytes with similar potency to thapsigargin (another sesquiterpene lactone and highly specific SERCA inhibitor) (Eckstein-Ludwig *et al.,* 2003).

Activated Artemisinin forms adducts with a variety of biological macromolecules, including haem, translationally controlled tumour protein (TCTP) and other higher molecular–weight proteins (Eckstein-Ludwig *et al.,* 2003). Artemisinin derivative are drugs approved and introduced for public antimalarial treatment. Even though their recommended for treatment of *P. falciparum* infection, these drugs also act against other parasites, as well as against tumor cells . The mechanisms of action attributed to Artemisinin include interference with parasite transport protein distribution of parasite mitochondrial function modulation of host immune function and inhibition of angiogenesis (Jacob *et al.,* 2006).

In Sudan, prospective clinical trial was done for treatment under five children with malaria, while show that 100% efficacy of Artemether-Lumfantrine with mild side effect in a few patients (Salah *et al.,* 2006). In Ethiopia, Artemether-Lumfantrine for the treatment of the children with uncomplicated *falciparum* malaria was 99% (Jima *et al.,* 2005). Artemisinin are new promising antischistosomal compounds, as are inhibitors of a *Schistosoma*-specific bifunctional enzyme, thioredoxin-glutathione reductase (Doenhoff *et al.,* 2008).

Around the world, the efficacy of the six-dose regime of Artemether –lumfantrine has been confirmed in many different populations, consistently achieving 28-day PCR (polymerase chain reaction) corrected cure rates of >95% in the evaluable population, rapidly clearing parasitaemia and fever, and demonstrating a significant gametocidal effect, even in areas of widespread parasite resistance to other antimalarial (Makanga *et al.,* 2009). In non immune population, efficacy of Artemether-Lumfantrine for the treatment of malaria is 96% (Hatz *et al.,* 2008).

In mice co-infected with *S mansoni*, Artemether showed excellent antimalarial efficacy with no indications of delayed clearance of *P. berghei* or recrudescence (Shu-hua *et al.,* 2006). In vitro and in experimental animal, Artemisinin derivatives are active against *S. mansoni* and *S. japonicum*. In vitro it was also active against *Leishmania major, Toxoplasma gondii,* and *Pneumocystis carinii* and *P.carinii* in vivo (Meshnick *et al*., 1996)

Artemether as part of integrated current control considerable potential to significantly reduce the burden of schistosomiasis in many part of the world (Utzinger *et al.,* 2001). Significant progress has been made with Artemether already widely used for the treatment of malaria. This led to the development of Artemether for chemoprophylaxis in schistosomiasis (Utzinger *et al.,* 2001).

Le *et al* 1982 confirmed that Artemether were antischistosomal properties for Mice or dogs infected with *S. japonicum* and treated with Artemether at various doses and routes of administration showed highly significant worm burden reductions ranging between 55 and 99%. The larval migratory stages of the parasite (schistosomula) were also susceptible to Artemether (Le *et al*., 1982). Hamster infected repeatedly treated with artemether at the dose 300 mg /kg, showed highly significant total and female

worm reduction rates of between 94 and 99% (Shuhua *et al.,* 2000a). Artemether exhibited modest in vitro and in vivo activities against adult *S.mansoni* but was twofold more active against schistosomula (Xiao, 1989).

Subsequent laboratory and clinical studies confirmed antischistosomal properties for other Artemisininin derivatives- artesunate (Le *et al*., 1983; Boulanger *et al*., 2007) and arteether (Yin *et al*., 1991; Xiao *et al*., 1992).Utzingera and his coworkers (2001) administered a 7-day regimen of Artemether at different concentrations to adult *S. mansoni* which is similar to what are recommended for malaria monotherapy (WHO, 1998b) and observed total worm burden reductions of 53-61%. PZQ resistance remains a threat and its prevention requires adequate monitoring of current mass drug administration programmes and development of new schistosomicides (Doenhoff *et al.,* 2008).

The above findings clearly indicate that it is important to investigate Artemether-Lumfantrine for treatment of *Schistosoma* infections in areas where *Schistosoma* and *falciparum* malaria are co-endemic. A small study in Sudan showed the antischistosomal efficacy of artesunate-sulfamethoxypyrazine-pyrimethamine and Artemether-Lumefantrine administered as treatment for uncomplicated *Plasmodium falciparum.* Stool Samples from 14 of the 306 patients screened on presentation were found to contain *Schistosoma mansoni* eggs. All the 14 patients were found stool-negative for *Schistosoma* eggs after being treated with Artemether–lumfantrine (Adam *et al*., 2008).

Artemether block the development of ovipositing adult *Schistosoma* worm because it selectively targets schistosomula. A clinical trial conducted in China that Artemether has benefit as an agent for chemoprophylaxis. In vivo studies in animal suggest that Artemether causes damage to the tegument and musculature of schistosomula. Artemether may exert its helminthotoxic effect through synergy with hemin or related heme-containing compound (Shuhua *et al.,* 2000b).

Artemisinin have become key drugs for the treatment and control of malaria, particularly within Artemisinin-based combination therapies. Since the Artemisinin also show antischistosomal properties, their use in areas where malaria and

schistosomiasis are co-endemic may have an effect on both diseases and co-.infection might alter drug efficacy (Shu-Hua *et al*., 2006).

Praziquantel is currently the drug of choice for the treatment of schistosomiasis. Selective treatment of *S. mansoni* infections in various endemic countries usually present cure rates of >70% when using the standard dose of 40 mg kg body weight of Praziquantel (Danso-Appiah *et al.,* 2002). The current reports on possible emerging drug resistance in human *Schistosoma* do not provide conclusive evidence for the increase of innately tolerant strains or for the appearance of newly mutated resistant strains. However, they strongly suggest that such tolerant or resistant strains can and do exist and that these strains may emerge more prominently under drug pressure or under specific circumstances (Geerts *et al.,* 2000).

Despite Praziquantel being widely used, there is no clinically relevant evidence for drug resistance, but in some studies in Africa low cure rate have been recorded (Doenhoff *et al.,* 2008). Higher treatment coverage increases evolutionary pressure on the *Schistosoma* population and hastens emergence of resistance (Smail *et al.,* 1996). Failure to monitor drug resistance developments of Praziquantel may have serious consequences in the longer term since it will be the only drug that is readily available for large-scale treatment of schistosomiasis (Doenhoff *et al.,* 2006)

The low cure rates obtained with Praziquantel in schistosomiasis can best be interpreted on the basis of epidemiological factors such as confounding by ongoing disease transmission and are unlikely to be connected with any drug resistance in the parasite (Cioli *et al*., 2003**).** Experimental development of drug resistance and the recent isolation of *S. mansoni* strains with a natural tolerance to high doses of PZQ have raised concerns over the adequacy of such a dose. Evidence from a mouse model suggests that some cases of PZQ resistance could be caused by host factors influence of host immunity in the efficacy of the drug, where as others could be attributable to the worm (King *et al.,* 2000; Alonso *et al.,* 2006).

However, unusually low cure rates have been reported from Senegal, raising fears for emergence of resistance to Praziquantel (Danso-Appiah *et al.,* 2002). *Schistosoma* isolates obtained in Egypt from uncured individual present evidence of lower susceptibility to the drug. Similarly, laboratory *Schistosoma* subjected to repeated

passages under drug pressure are partly insensitive to the drug (Cioli *et al*., 2003). The response of *Schistosoma isolate* worm to the drug was observed in vitro, in order to determine if the isolates obtained from these resistant infections are, in fact, less responsive to Praziquantel. The isolates from the resistant infections were less susceptible to Praziquantel-induced tegumental damage in vitro, suggesting that the worms are in some way less responsive to the drug (William *et al.,* 2001).

More is little field evidence that *Schistosoma* are becoming less sensitive to the drug. Meanwhile, the percentage of cure rates in endemic areas could be overestimated if one accounts for the sensitivity of most egg counting methods coupled with the limited faecal sampling (Botros *et al.,* 2007). The current sensitivity of *S. mansoni* infections to PZQ after 10 years of therapeutic pressure .It was assumed that the number of drug failures would have increased as continued drug pressure selected for worms with diminished sensitivity to PZQ (Botros *et al.,* 2005).

Some repots and treatment failure after multiple doses of Praziquantel that resistance to the drug may be present. This has been coupled with several in vivo and in vitro demonstrating a significant reduction in the drugs efficacy (Bortos *et al.,* 2007).

However, in certain regions of the African continent, the transmission of schistosomiasis and malaria takes place concomitantly, and the results from the use of Praziquantel have been less promising than what was expected. In these circumstances, the use of Artemisinin derivatives with the aim of treating malaria cases may exert a significant influence on the reduction of the morbidity rate due to endemic schistosomiasis (Danso-Appiah *et al.,* 2002). In Ethiopia, Artemisinin based combination therapy is drug of choice for treatment of uncomplicated *falciparum* malaria. Particularly, Quarterm- combination of Arthemether and Lumefantrine- is being used in Ethiopia. Artemether also has been proved to have activity against several species of *Schistosoma* (Xiao *et al*., 2001). There is, however, dispute regarding its use on large scale to control schistosomiaisis. Because of the overlapping distributions of schistosomiasis and malaria, the risk that widespread use of Artemisinin for the treatment of schistosomiasis could assist the emergence of resistant strains of *Plasmodium* spp and must be seriously considered (Utzinger *et al*., 2001).

Artemether, used to control malaria, is effective against immature *Schistosoma*, but is less effective against adult worms (Fenwick *et al.,* 2003). Artemether effectively targeting larval parasites than Praziquantel, which is effective against adult schistosomiasis (Utzinger *et al.,* 2003). Since Praziquantel is known to be effective only against mature *Schistosoma*, some parasite would survive and mature to egg producing stage (WHO, 2001).

Mainly within Artemisinin–based combination therapies, Artemisinin have become key drugs for the treatment and control of malaria. In areas where malaria and schistosomiasis are co-endemic, Artemisinin which exhibit antischistosomal properties, may have an effect on both diseases and co-infection might alter drug efficacy (Shu-Hua *et al*., 2006). Artemisinsinin derivative are effective against all *Schistosoma* species but only in the early stage of infection; these drug are therefore generally indicated only in specific circumstances, *i.e.* known recent exposure (WHO, 2001).

Therefore, a cross sectional study conducted in Kemise Health Center to evaluate coartem has an effect on *Schistosoma* during malaria co-infection.

2. STATEMENT OF THE PROBLEM AND SIGNIFICANCE OF THE STUDY

2.1. Statement of the Problem

In contrast with microbial infection, an appropriate definition of drug resistance is not easy to establish to parasitic infections. In different geographic area, susceptibility of the different maturation stages; sexes of the parasites and host immunity influence the efficacy of the drug (Alonso *et al.,* 2006). As a result of marked reductions in the price and very few unwanted side effects, Praziquantel is used in the majority area of the world. While in longer term, a failure to monitor drug resistance developments may have serious. Since the only drug that is readily available for large-scale treatment of schistosomiasis (Docnhoff *et al,* 2006; WHO, 1998c).

Emergence of Praziquantel resistance should be predictable within 10 to 20 years, and continued antischistosomal drug development should be pursued (King *et al.,* 2000). Emerging drug resistances in *Schistosoma* do not give conclusive evidence for the increase of innately tolerant strains or for the appearance of newly mutated resistant strains. However, tolerant or resistant strains can and do exist and that these strains may emerge under drug pressure (Geerts *et al.,* 2000).

Experimental development of drug resistance and the new isolation of *S. mansoni* strains with a natural tolerance to high doses of PZQ have raised concerns over the adequacy of such a dose. Evidence from a mouse model suggests that some cases of PZQ resistance could be caused by host factors (Alonso *et al.,* 2006). A few evidences exist in Egypt that some *Schistosoma* isolates may be less susceptible to Praziquantel (Smail *et al.,* 1996). Low cure rates are caused by the very high transmission as shown in field studies in Senegal. Since infected snails collected in the area indicated that particular parasite strain may possess some intrinsic insusceptibility to praziquantel to some extent refractory to treatment due to a slow maturation rate of the isolate (WHO, 1998b; Van Lieshout *et al.,* 1999).

Appearance of resistance speed up in higher treatment coverage increases evolutionary pressure on the *Schistosoma* population. Limited coverage may have reduced the tendency for resistance to emerge, as seen with the experience reported for *S. mansoni* in Egypt (Smail *et al.,* 1996; Southgate *et al.,* 2005). In human helminthes, the evidence for the emergence of drug resistance is equivocal in spite of the long-term widespread use of anti-helminthes. However, the risk of drug resistance is real. So treatment should be targeted only to high-risk groups such as school children, which ensures gene flow a worm population without drug pressure (WHO 1998c). In Ethiopia the unpublished data indicate that drug resistance to praziquantel may be emerging as result of increases the drug pressure on parasite populations.

2.2. Significance of the Study

Praziquantel is the cornerstone of current control programs for schistosomiasis. However, the low cure rates obtained with Praziquantel in a Senegalese focus of schistosomiasis can be interpreted on the basis of epidemiological factors such as confounding by ongoing disease transmission and are unlikely to be connected with any drug resistance in the parasite (Van Lieshout *et al.,* 1999). Also, *Schistosoma* isolates obtained in Egypt from uncured individual present evidence of lower susceptibility to the drug (Cioli *et al.,* 2000). These few studies serve as signal that resistance for Praziquantel may be emerging. With the ever increasing use of Praziquantel, there is a possibility of the development of resistance to the drug by *Schistosoma*, hence the necessity to explore the activities of other compounds (Southgate *et al.,* 2005)

Therefore, the aim of this research was to evaluate efficacy of Artemether-Lumefantrine to treat *falciparum* malaria and *S. mansoni* co-infection. This study aims to examine the importance and side benefits of Artemether-Lumefantrine base therapy in *falciparum* malaria with *S. mansoni* co-infected patients. This research will provide baseline data for further analysis of the efficacy of the ACT in large population.

3. HYPOTHESIS

Artemether-Lumefantrine base therapy given for the treatment of *falciparum* malaria has an efficacy of ≥90% for the treatment of shistosomiasis in *P. falciparum* - *S. mansoni* co-infected patients in Kemise, Northeastern Ethiopia.

4. OBJECTIVE OF THE STUDY

4.1. General Objective

To investigate whether Artemether–Lumefantrine has antischistosomal efficacy when administered as curative treatments for uncomplicated *falciparum* malaria

4.2. Specific Objectives

- To determine prevalence of *Schistosoma- falciparum* malaria co-infection

-To assess the antischistosomal activity of Artemether–Lumfantrine when it used for treatment of individuals with uncomplicated *P. falciparum* malaria.

- To see the effect of Artemether–Lumefantrine in reducing egg out put of schistosomiasis

5. MATERIAL AND METHOD

5.1. Study Area and Population

This cross-sectional study was conducted at Kemise Health Center in Kemise town, Northeastern Ethiopia. The town is found in the Oromia Zone of the Amhara Region. Kemise is located at 325 km Northeastern of Addis Ababa, Ethiopia and it has an altitude of 1424 m above sea level. The town has an estimated total population 18,897, of whom 10,151 are males and 8,746 are females (CSA, 2005).

In Kemise, malaria transmission is seasonal and unstable (Abeku *et al.,* 2003). The town is situated in an area where water collections, either temporary or semi-permanent are potential sites for the breading of *Anopheles* mosquito larva and infected snails resulting in a high level of both malaria and schistosomiasis burden in the community.

5.2. Study Subjects

Malaria cases age above 5 years with uncomplicated *falciparum* malaria, who or their guardians consented to participate in the study after fulfilling the inclusion criteria were enrolled in the study for follow up period of day 28, 29 or 30.

5.3. Study Design

Cross sectional study was conducted to determine Prevalence of *Shistosoma* and *falciparum* malaria co-infection and then efficacy of Artemether-Lumfantrine for treatment *Shistosoma* and *falciparum* malaria co-infection was determined using prospective longitudinal studies from September to November, 2009 at Kemise Health Center, in Northeastern Ethiopia. In addition patients with microscopically confirmed *falciparum* malaria were asked to provide stool and urine sample for determination of schistosomiasis. From all patients Socio-demographic variable (age, sex, residence, weight, educational status) were recorded. The study was designed to determine the efficacy of Artemether–Lumfantrine for treatment of *S. mansoni* and *falciparum* malaria co-infection as dose used for the treatment of *falciparum* malaria.

5.4. Sample Size Determination

The required sample size for the study was calculated using a formula for a single population proportion. 53% prevalence of schistosomiasis (Erko *et al.*, 2002) and 0.9% malaria (FMOH, 2007) were considered for the sample size calculation. However, for the final sample size determination, 0.9% prevalence of malaria used in preference to *Schistosoma* since malaria case visit the Health Center at any time when you had fever immediately for seeking medical treatment. Taking critical value at 95% confidence level ($Z\alpha/_2$ =1.96), degree of precision 0.05 and generate a minimum sample size is 132.

The study was based on purposive sampling method, adjusting the design effects by a factor of 2 and 10% should be added to account for the patient who likely to be either lost during follow up, withdraw or mising record; the minimum desire sample size for the study was 138 patients with uncomplicated *falciparum* malaria.

Single population proportion formula was used to calculate the sample size:

$$Z\ \alpha/_2 = \frac{P(1-P)}{D2} = \frac{(1.96)2^*\ 0.9(1-0.9)}{(0.05)\ 2} = 138$$

138 + (10%*138) = 152

5.5. Inclusion and Exclusion Criteria

5.5.1. Inclusion Criteria:

- Patients above 5 years age with uncomplicated *falciparum malaria*
- Presence of temperature $\geq$ 37.5 and <39.5 ºC at visit
- Absence of febrile conditions caused by diseases other than malaria
- Willing to sign on written consent and come for follow-up visits

5.5.2. Exclusion Criteria:

- Pregnant women and patients infected with species other than *P. falciparum*
- Had received Praziquantel three month before entry in the study
- Chronic, debilited clinical condition due to schistosomiasis

5.6. Study Variables

5.6.1. Independent Variable

- Clinical and laboratory state at the beginning of treatment
- .Socio-demographic variable (age, sex , residence, weight, educational status)

5.6.2. Dependent Variable

- Treatments out come

5.7. Data Collection Technique and Procedure

From each study subject, Stool and urine samples were collected at any time while attending Kemise Health Center from September to December 2009. These samples were used to determine the presence of *S. mansoni* and/or *S. haematobium* and *P. falciparum* co-infection and intensity of infection schistosomiaisis. The stool and urine were collected before treatment and only stool was examined on day 28, 29 and 30 since only *S. mansoni* were found. In patients who did not reported for the first

follow up visit, active searches were made to collect sample using sample collection materials and information collection format (Annex I).

5.8. Data Quality Control

Patients were recruited by Health officer and nurses, who have been working at Kemise Health Center, based on orientation given on the study protocol. Clinical examination was carried out on patient presenting with sign and symptom of malaria. Principal investigator had visited Health officers and nurses to assess quality of records by direct and indirect observation and to see overall performance as well as received feedback.

5.9. Laboratory Procedure

5.9.1. Detection of Malaria Parasite

Thin and Thick blood smears were prepared from each malaria case to detect malaria parasite. These smears were then stained using Giemsa staining method (WHO, 1991). Each stained smear was examined by experienced laboratory technicians and the principal investigator for detection of *P. falciparum* using light microscope.

5.9.2. Detection of *Schistosoma* Infection

As soon as a malaria case was confirmed, stool and urine was collected from each patient for *S. mansoni* and *S. haematobium* examinations. From each patient three consecutive stools and urine samples were examined (at day 0, 1 and 2). After treatment, only stool samples (at day 28, 29 and 30) were examined by Kato-katz method and sedimentation techniques, respectively (WHO, 1991) before any reinfection larva become an adult fertile *Schistosoma* (Martin *et al.,* 2007). We didn't attempt for urine examination after treatment as no ova of *S. haematobium* was detected from urine specimen during the study period.

The average numbers of egg per gram (epg) were determined. The overall mean (epg) was derived by averaging the egg count for the three individual specimens.

5.10. Treatment of Malaria and *Schistosoma* and *P. falciparum* Co-infected Patients

Microscopically confirmed for uncomplicated *P. falciparum* and *P. falciparum* - *S. mansoni* co-infection patients were treated with coartem using the national guideline.

5.11. Data Processing and Management

The laboratory result and clinical examination were recorded by principal investigator and Health officers/Nurses, respectively and then checked for completeness of record by the principal investigator. Intensity of infection of schistosomiasis was determined based on the microscopic examination of stool using 41.7 mg, kato-katz smears and expressed as the number of eggs per gm of stool.

At least two stool and urine samples were obtained before treatment and three stools were obtained after treatment day 28, 29 and 30. At least one of the pretreatment samples had positive before a case was including in the efficacy analysis. Cure rate was expressed as the percentage of *Schistosoma* and *P. falciparum* co-infected patient who became completely negative after treatment. The Egg output in each individual stool and/or urine was used to calculate the geometric mean egg count.

Data entry and clearance were done using Epi -info software version 6 and exported to SPSS software version 15 for analysis.

5.12. Data Analysis

The laboratory results were entered and analyzed using SPSS version 15 window software. Results of treatments was expressed as cure "for patients whose status changed from *Schistosoma* egg-positive to *Schistosoma* egg-negative after treatment," non-cure "for those whose status remain *Schistosoma* egg-positive after treatment. It was planned to assess effects of treatment by determining the change in geometric mean egg counts before and after treatment (day 28, 29 and 30). Group mean egg count was determined using average egg counts of each group. The geometric mean egg counts were calculated as anti-logarism of the mean of all log transformed egg counts +1. Egg reduction rate was calculated as [1-(GMEC per g after treatment /GMEC per g before treatment)] x 100. X^2 test was done along with P-value to see the presence of associations among categorical variables. P-value <0.05 was considered significant.

6. ETHICAL CONSIDERATION

The Study protocol was approved by the Department of Microbiology, Immunology and Parasitology and by Institutional Review Board of the Faculty of Medicine, Addis Ababa University. Permission was obtained from Kemise Health Center to conduct the study. Written and informed consents were obtained from each adult patient and from the parents/guardians of each child before enrolled to this study. The objective and benefit of the study were explained to each study subject. The Laboratory results of the study subject were reported to health officers and nurses in order to get appropriate treatment. All patients who were expected *Schistosoma* eggs on either days 28, 29 or 30 were consented to be treated with Praziquantel at a 40 mg/kg dose.

7. RESULT

This study aims to investigate the efficacy of Artemether-Lumfantrine for the treatment of *Schistosoma* when administered for the treatment of uncomplicated *falciparum* malaria in *P. falciparum/Schistosoma co-infected* patients was evaluated. The prevalence of *Schistosoma* and *falciparum* malaria co-infection in Kemise, Northeastern Ethiopia, has also determined from September to November 2009. 152 *falciparum* malaria cases age 6-65 years old were microscopically confirmed. Of 152 microscopically confirmed malaria case 28 of them found to be co-infected with *S. mansoni.*

Those co-infected with *S. mansoni* and *P. falciparum* malaria was given Artemether-Lumefantrine treatment as per the recommendation set by the national guideline for the treatment of *P. falciparum* malaria. During the study period, 28 (18.4%) of the 152 malaria cases were found to be co-infected with *S. mansoni* (Table-2). Efficacy of Artemether-Lumefantrine was determined and chi-square test was done along with P-value to see the presence of associations between groups and subsequent analyses was performed. The cure rate and egg reduction rate were measured at the time set.

Table-1: Efficacy of Artemether–lumfantrine against *S. mansoni* and *falciparum* malaria co-infected patients.

Variables	Group	Artemether–lumfantrine therapies
Cure rates	No of co-infected included	28
	No of cured co-infected	28
	% Cure	100%
	(X^2 = 152; df = 1)	P = 0.000
Geometric Mean egg counts	Before treatment	83.6
	After treatment	0.0
	% Egg reduction	100%

Cure rate after treatment were calculated as the percentages of individuals becoming *Schistosoma* egg free. Reduction in egg counts after treatment was planned to determine using the formula (1-[GMEC/gram after treatment/GMEC/gram before treatment]) x100. Twenty eight days later, stool sample were screened over three consecutive days and the treatment outcome was evaluated. Cure rate and percentage of egg reduction rate were 100% at the end of follow up period**.**

Table-2. *Schistosoma* and *falciparum* malaria co-infection stratified by sex, age, educational level and residence in Kemise Health Center, Oromia Zone of the Amhara Region, December 2009.

Variables		Sub category	No co-infected	No examined	No(%)co-infected
Age group		6-14	15	42	35.71 (15/42)
		15-24	8	50	16 (8/50)
		25-34	2	30	6.67 (2/30)
		>=35	3	30	10 (3/30)
Educational level		Illiterate	6	59	10.5(6/59)
		1-8 grade	18	59	30.5(18/59)
		9-10 grade	3	25	12(3/25)
		>10	1	9	11.1(1/9)
Sex		Female	5	60	8.3 (5/60)
		Male	23	92	25 (23/92)
Residence	Rural	6-14	12	27	44.4(12/27)
		15-24	3	25	12(3/25)
		25-34	2	14	14.3(2/14)
		>=35	1	17	5.8(1/17)
		Total	18	83	21.7(18/83)
	Urban	6-14	3	15	20(3/15)
		15-24	5	25	20(5/25)
		25-34	0	13	(0%)
		>=35	2	16	12.5(2/16)
		Total	10	69	14.5(10/69)
Total			28	152	28(18.4%)

Table-2. Show that the 152 malaria cases, the majorities were males 60.25% (92/152). The age groups 6-14 years old being the most co-infected 35.71 % (15/42), followed by age group 15-24 years 16% (8/50) and least has in age group 25-34 years olds 6.67 % (3/30). Grade 1-8 being the most co-infected 30.5% (18/59), followed by 9-10 grade12% (3/25) and least has illiterate 10.5% (6/59). Males were found to be more co-infected with *P. falciparum* and *Schistosoma* compared to females (25 % Vs 8.3 %).

Rural residence was more co-infection than urban (21.7% **Vs** 14.5%). Co-infection in rural residence classified by age show that 6-14 years old was the most co-infected 44.4(12/27), followed by age group 25-34 years old 14.3(2/14) and least was been in age group ≥ 35 years olds; 5.8%(1/17). Co-infection in urban residence classified by

age indicated that 6-14 years old and 15-24 years old was the most co-infected 20(3/15) and 20(5/25) and least was in age group 25-34 years old; 0.0%. The over all *Schistosoma* and *falciparum* malaria co-infection was 18.4% (28/152).

Table-3. Intensity of *S. mansoni* stratified by sex, age and educational level in Kemise Health Center, Oromia Zone of the Amhara Region, December 2009.

Variables	Sub category	No *Schistosoma* and malaria co-infected	Light infection (<100 egg/g)	Moderate infection (100-400 egg/g)
Age group	6-14	15	66.7(10/15)	33.3(5/15)
	15-24	8	50(4/8)	50(4/8)
	25-34	2	100(2/2)	0%
	>=35	3	100(3/3)	0%
Education level	Illiterate	6	83.3(5/6)	16.6(1/6)
	1-8 grade	18	61.1(11/18)	38.8(7/18)
	9-10 grade	3	66.7(2/3)	33.3(1/3)
	>10	1	100(1/1)	0%
Sex	Male	23	69.5(16/23)	30.4(7/23)
	Female	5	60(3/5)	40(2/5)
Over all		28	67.8(19/28)	32.1(9/28)

Table-3.Show that among the 28(18.4%) co-infected patients, 19 (67.8%) were shown light infection (<100epg), 9(32.14%) moderate infection (100-400epg). For classification of intensity of infection, "cut of value" was used according to WHO, 2001 [light 1-99epg, moderate = 100-399epg and heavily > 400epg].

Light infection was highest in the age group 25-34 years old and >=35; 100(2/2), 100(3/3) and then followed by 6-14 years old 66.7(10/15) and least was in 15-24years old 50(4/8). Light infection rate was peak in>10 grade; 100(1/1) followed by illiterate; 83.3(5/6); and the least was 1-8 grade; 61.1(11/18). Males have higher light infection rate 69.5(16/23) than females 60(3/5). Moderate infection was highest in the age group 15-24 years old; 50(4/8) and >=35; 100(2/2), 100(3/3) and then followed by 6-14 years old; 33.3(5/15) and least was in 25-34 and >=35 years old; 0%. Moderate infection rate was peak in 1-8 grade; 38.8 (7/18), followed by 9-10 grade; 33.3(1/3); and the least was >10 grade; 0%. Female have higher moderate infection rate; 40(2/5)

than male; 30.4(7/23). Over all, light infection was higher; 67.7% (19/28) when compared to moderate infections; 32.1% (9/28).

Table-4. Geometric means egg stratified by sex, age and educational level shown by in Kemise Health Center, Oromia Zone of the Amhara Region, December 2009.

Variables	Sub category	Geometric mean egg
Age group	6-14	71.6
	15-24	94.1
	25-34	79.4
	≥35	77.2
Educational level	Illiterate	82.3
	1-8 grade	146.2
	9-10 grade	94.2
	>10	72
Sex	Female	92.6
	Male	81.7
Over all		83.6

Table-4. Show that Geometric mean eggs count was range from 71.6 to 146.2. Geometric mean eggs count in the age group 15-24 years old (94.1epg) and then followed by the age group 25-34 years old (79.4epg) and least was in the age group 6-14 years old (71.6epg). Geometric mean egg count was peak in 1-8 (146.2epg), followed by 9-10 grade; 94.2epg and least was in > 10 grade; 72epg. Geometric mean egg higher in male (92.6epg) compared to that of male (81.7epg). The overall geometric mean egg counts were 83.6 epg.

8. DISCUSSTION

A number of studies have been conducted on the prevalence of schistosomiasis in Ethiopia. In Kemise, surveys have been done in intestinal parasite and malaria (Erko *et al.*, 2002; FMOH, 2007). Even if, malaria and schistosomias are often geographically co-endemic in sub-Saharan Africa, knowledge on the prevalence of *Schistosoma* and *P. falciparum* co-infection is limited in Ethiopia. Although there are studies conducted on the efficacy of Artemether–Lumfantrine against *P. falciparum* and *Schistosoma* co-infection in Sudan (Adam *et al.,* 2008), there is no such published data in Ethiopia. Hence this study was carried out for evaluation of efficacy of Artemether–Lumfantrine for treatment of *Schistosoma* and *P. falciparum* co-infected patients.

Artemether–Lumfantrine is the drug of choice for treatment *P. falciparum* infection as recommended by national guideline. These drugs also act against other parasite, as well as against tumor cells (Jacob *et al.,* 2006).

The evaluation of the Artemether–Lumfantrine for treatment of *Schistosoma* and *falciparum* co-infection were done at 28-30 day post treatment which is similar to previous studies conducted in Sudan (Adam *et al.,* 2008). Previous study was from sudan reported that a decrease of the number of *Schistosoma* eggs which nearly disappeared in 28-30 days after taking Artemether–Lumfantrine (Adam *et al.,* 2008). It was subsequently decided that 28-30 day after treatment would be the optimal period to observe the curable conditions. The cure rate was evaluated based on the number of pre-treatment positive subjects for *Schistosoma* eggs found to be egg-negative on post-treatment on examination of stool using a standard procedure at an optimal time (day 28, 29 or 30) and egg reduction was evaluated based on the percentage fall in egg counts. The present result was based on a decrease of number of *S. mansoni* egg which completely disappeared on days 28, 29 or 30. This is comparable to the study conducted in Sudan (Adam *et al.,* 2008).

The curable conditions were observed on day 28, 29 or30 and this was the optimal time to evaluate cure. These *S. mansoni* and *falciparum* malaria co-infected patients

were treated with Artemether-Lumefantrine as recommended by national guideline at the dose used for treatment of uncomplicated *P. falciparum* of malaria.

The result showed that both cure rate and percentage of egg reduction rate were 100%. This was similar to the study reported from Sudan (Adam *et al.,* 2008). The cure rate was similar as percentage of reduction in all Artemether–Lumfantrine treated co-infected patients irrespective of age, sex and intensity of infection. This indicated that the drug was efficacious for the co-infected individuals and this was statistically significant with very high cure and egg reduction rate as showed by Chi-square test (100%, $X^2 = 152$; df =1, P = 0.000).

Co-infection decreased from 6-14 to $\geq$ 35 years old; and highest of co-infection was found in age group 6-14 years old (school-age children). But, it was exceptional in age group $\geq$ 35 years old, which showed a relative increase. Stratification by age group indicated that there was statistically significant association among the different age age group ($X^2 = 13.88$; df = 4, P = 0.008).

But, the co-infection rate was found to be high among young age groups compared to old age group ($\geq$ 35 years old). This observation has also been found in other studies (WHO, 2005; Mazigo *et al.,* 2010; Yatich *et al.,* 2009). This observed decrease in co-infection with increase in age could be due to awareness about disease and availability of breeding sites for the intermediate host such as fresh water snail and *Anopheles* mosquito. The most co-infected with *S. mansoni* and *falciparum* malaria was age group 6-14 years olds (school age children). Similar finding was reported from others investigators (Mazigo *et al.,* 2010; Booth *et al.,* 2004b). Co-infected with *S. mansoni* and *falciparum* malaria were less common in this study area than reported from Tanzania (Mazigo *et al.,* 2010). The observed different could be due to different in exposure and endemicity of both parasite species.

Males were more co-infection than females. Similar finding was reported from Tanzania (Mazigo *et al.,* 2010). This difference might be exposure due to outdoor activities, occupation and exposed their bodies during hot weather. This indicated that there was statistically significantly association of co-infection among sex categories ($X^2 = 6.71$; df =1, P = 0.010).

A steady decrease in co-infection was observed from grades 1-8 to grade >10. But, the co-infection rate was found to be high among schoolchildren (grades 1-8) compared to illiterate patients (mostly old ages). This finding was similar to the study reported from Uganda (Woodburn *et al.,* 2009). The co-infection rate was lesser with increase in education level and this could be as a result of increased awareness about malaria and schistosomiasis found to be decrease risk of co-infection. Stratification of co-infection by educational background revealed that there was statistically significant association among educational categories (X^2 = 9.41; df =3, P = 0.024). But, slightly increase in illiterate individual when compared to literate and this could be poor knowledge and old age would have an impact on their exposure to the disease.

Co-infection was higher among age group 6-14 years old living in rural compared to urban residence. Similar finding was reported from Ghana (Hartgers *et al.,* 2008). This varies could be due distribution and density of intermediate hosts, *Anopheles* and fresh water snail high in rural than urban and this could lead to exposed to both infection. But this observed difference was not statically significant (X^2 = 1.29; df = 1, P = 0.255). The intensity of infection revealed that the peak for Geometric mean egg count in grade 9-10, female and in age group 15-24 and the difference was not statically significant difference in intensity of infection in age group (X^2 = 3.54; df = 3, P= 0.315), educational level (X^2 = 1.51; df = 3, P= 0.680) and sex (X^2 = 0.172; df =1, P = 0.678).

Besides, the intensity of infection for *S. mansoni* in the study subject was light to moderate and this finding has also been reported from Tanzania (Mazigo *et al.,* 2010). Light infection was considerably higher than moderate infection, but no statically significant difference in intensity of infection of *S. mansoni* between light and moderate infected individual (X^2 = 28; P = 0.358). In conclusion, this study revealed that high prevalence of *S. mansoni* and *falciparum* malaria co-infection and intensity *S .mansoni* infection were found among age group 6-14 years old, 1-8 grades and male in this study.

Very high cure rate and egg reduction were obtained (100%). Therefore, Artemether-Lumefantrine of the drug of choice for treatment of uncomplicated *falciparum* malaria

also showed highest efficacy against *S. mansoni* and *falciparum* malaria co-infection patients as doses used for the treatment of malaria.

The finding of the present study, support the need for large scale study by using a modified McMaster technique for better understanding of efficacy of Artemether-Lumefantrine for treatment of *Schistosoma* and *falciparum* malaria co-infection.

9. LIMITATION OF THE STUDY

The following limitations were identified

The number of co-infected patient found was small, making comparisons between stratified groups able to detect only major differences.

Since *S. haematobium* was not detected in the study, effectiveness of Artemether-Lumfantrine against *S. haematobium* could not be investigates

This study was generate data only on the efficacy of Artemether-Lumfantrine against *S. mansoni* and *falciparum* malaria co-infection; however, didn't generate data on the efficacy of Artemether-Lumfantrine against *falciparum* malaria

10. CONCLUSION AND RECOMMENDATIONS

10.1. CONCLUSION

Artemether-Lumefantrine was found to be highly effective (100%) for the treatment of schistosomiasis in uncomplicated *falciparum* malaria co-infected patients with dose used for the treatment of *falciparum* malaria.

Co-infection was highest in age group 6-14 years old, 1-8 grade and males. In general prevalence *P. falciparum- S. mansoni* co-infection was 18.42% among the study population.

Light infection was peak in the age group 25-34 years old, >10 grade and Males. How ever, moderate infection was highest in the age group 15-24 years old, in 1-8 grades female. Over all, light infection was higher than moderate infection.

10.2. RECOMMENDATION

Artemether-Lumefantrine would make possible the use of single regimen to treat patients with *Schistosoma* and *falciparum* malaria co-infection. This has also positive input in saving the treatment cost for integrated control for both infections.

Artemethere-Lumefantrine would serve as alternative treatment for schistosomiasis in region where Praziquantel are ineffective.

Large scale study would be required for better understanding of efficacy of Artemether-Lumefantrine for treatment of *Schistosoma* and malaria co-infection and/ or *Schistosoma* by using a modified McMaster method.

Finally, this study recommended for further study on the safety and possible adverse effects of Artemether-Lumefantrine while administered for the treatment of *P. falciparum* and *Schistosoma* co-infections.

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ANNEX-I: PRE- TEST QUESTIONER

I-PATIENT IDENTIFICATION

Serial No_____

Patient name_____

Age_____

Sex_____

Address _____

Weight _____ /kg

II-EDUCATION BACKGROUND

- Illiterate
- Primary school (1-8 grade)
- Secondary school (9-10 grade)
- > 10 grade

III-INCLUSION CRITERIA

- Patients above 5 years age with uncomplicated *falciparum malaria*
- Diagnosed to have mild schistosomiasis
- Presence of temperature ≥ 37.5 and <39.5 ºC at visit
- Absence of febrile conditions caused by diseases other than malaria()
- Willing to sign on Written consent form and to come for follow-up visits

IV- EXCLUSION CRITERIA

- Pregnant women and patients infected with species other than *P. falciparum*
- Denial of Written informed consent
- Had received Praziquantel treatment three month before enter in the study
- Had severe clinical signs/ symptom of the disease such as ascites and hepatosplenomegaly etc.

V-CLINICAL DATA

Date of entry in the study

_____

Date of arrival after treatment for follow up

_____

Date of loss to follow up

_____

Clinical manifestation

_____

_____

_____

IX-Laboratory data

Date of entry in the study

_____

Date of arrival after treatment for follow up

_____

Date of loss to follow up

_____

Blood smear: *P. falciparum* Positive /Negative _____

Stool examination: *S. haematobium* / *S. mansoni:*

_____

Before treatment: egg count /g of stool and egg count/ml of urine:

Day: 0_____1: _____ 2: _____

After treatment: egg count /g of stool and egg count/ml of urine

Day: 28 _____29: _____30: _____

IIX - COMMENTS

_____

_____

_____

_____

Name of Research: _____

Signature: _____

Date: _____

ANNEX-II: CONSENT FORM (English version)

I have been informed that the purpose of this study and it is necessary to investigate Artemether-Lumefantrine while administering as curative treatments for malaria and also has effect of reducing of schistosomiasis. Hence for the purpose of this study, I agreed to give blood, urine and stool sample for examination. I am also informed that laboratory results will be reported to health officer /nurse and will contribute to get an appropriate treatment. The investigator informed me if I include in the study, He will pay me transport expense based on our agreement. More over, as I have full right to participate with entirely voluntary, I also clearly understand that I am still free to withdrawn at any time, without any negative consequences for the medical care. Further more I am inform that the data will be use only for the propose of the study. Therefore I agree to participate in the study based on the above information.

I _____here agree to give samples and information stated above for examination with fully understood the purpose of this study.

Signature ----------Date----------

Contact address: Principal Investigator

Mobile: 0914127279 e- Mails: muluzem12@yahoo.com

Contact Address: AAU-MF IRB, e- Mails: AAU-MF IRB @yahoo.com

Tel No: 0115538734

ANNEX-III: CONSENT FORM (Oromiffa version)

Waraqaa Gaafannoo Walii Galtee

Qo'annaan kun qoricha koaartaam j edhamu dhukkuba busaatif jedhamee Y00 Kennamu Kandhukkuba bilhaariziyaa hirdh'isuqorannof akk a barbaachisu ta'uu isaaiuee hubadheera qorannon Kun waan naaf galeefdhiigaafifincaan keennauuf fedhii qabaachun ibsa.

Qarannoobu'aa laayibraatorii mana yaalaa nayaaluuf gabaasni akka godhamuufi yaaliiga'aakkan argadhu fi bu'aa gaariiakka kennu naafibsame.ra. Akkasumas mirgiqorannoo keessaa bauu koo akka naafibsameta'uu, kanas gochuu kootin kan adeemis marsaa yaatichaa gufuudhabuu issa haala' gaaridhaan hubadheera. dabalataannis qoranno mortaae qofaaf akka olu naaf ibsamera.

Qorannoo keessatti yoon makame gatiin geejjibaa akkanaak afalamu waliigatte keenya irratti ibsamera.dabalataannis qoranno mottaae qofaaf akka olu naaf ibsamera .Kanaafuu akka asii olitti ibsametti kauumsa odeefanno qoranno kessaatti hirmaachuuf fedhiin qaba.

Ani _____Qurannichi barbaachisaa tauusaa waanan argeef.Odeeffannoongaafatameef naamunaawwan kennuf walii galuu koo mallattookootiin nan mirkaneessa.

Mallattoo_____Guyyaa_____

Adraashaa Qoataa

L.S---------- IML----------

ANNEX-IV: CONSENT FORM (AMHARIC VERSION)

<u>የሰምምነት መጠየቂያ ቅፅ</u>

የዚህ ጥናት ጓላማ ኮላርተም የተባስ መድሀኒት ስወባ ተብሎ ሲሰጥ የብልሀርዚያ በሽታ የሚቀንስ መሆኑን ስማጥናት እንደተፈስገ ተረድቻስሁ :: የዚህ ጥናት ጓላማ ስስገባኝ ደም ሽንት እና ሰገራ ስመስጠት ፈቃደኛ መሆኔን እገልፃስሁ:: የሳብራቶሪ ምርመራ ውጤት ስሚያክመኝ ሀኪም ሪፖርት እንደሚደረግና ተገቢውን ህክምና እንዳገኝ ጓስተዋፅኦ እንደሚያደርግ ተገልፃልኝ እንዲሁም ከጥናቱ የመውጣት መብቴ የተጠበቀ መሆኑ የተገስጸልኝ ሲሆን ይህንን በማድረጌ ምክንያት የህክምናው ሂደት የማይደናቀፍ መሆኑን በሚገባ ተረድቻስሁ:: ስጥኚው በጥናቱ ከተካተትኩኝ የትራንስፖርት ወጪዬን በስምምነታችን መሰረት እንደሚከፈስኝ ተገልፃልኛል:: በተጨማሪ ናሙናው ስጥናቱ ጓላማ ብቻ እንደሚውል ተነግሮኛል:: ስስዚህ ከላይ በተጠቀሰው መረጃ መሰረት በጥናቱ ስመሳተፍ ፈቃደኛ ነኝ::

እኔ----------ጥናቱ ጠቃሚ ሆኖ ስሳገኘሁት የተጠየኩትን መረጃ እና ናሙናዎችን ስመስጠት መስማማቴን በፊርማዬን ጓረጋጣስሁ::

ፊርማ ---------- ቀን----------

የተመራማሪው ጓድራሻ
ስ.ቁ 0914 14 72 79

E-mail –muluzem12|@ yahoo.com

Annex–V: ETHICAL APPROVAL DOCUMENT

(IRB-AAU)

DECLARATION

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

M.Sc candidate: Mulugeta Tilahun G/medhin

Signature ----------

Date and place of submission ----------

Addis Ababa, Ethiopia

Supervisor: Nigus Fikrie

Signature ----------

Date and place of submission ----------

Addis Ababa, Ethiopia

Co-supervisor Solomon Mequant

Signature ----------

Date and place of submission ----------

Addis Ababa, Ethiopia

Co-supervisor Abiy Habtewelde

Signature ----------

Date and place of submission ----------

Addis Ababa, Ethiopia