PREVALENCE AND ASSOCIATION OF MALARIA AND ANEMIA AMONG PATIENTS VISITING ALABA HEALTH CENTER, ALABA KULITO TOWN, SOUTHERN ETHIOPIA

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KALEAB TEREFE

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We here by certify that we have read and evaluated the thesis prepared under our guidance, by Kaleab Terefe entitled **"Prevalence and Association of Malaria and Anemia among Patients Visiting Alaba Health Center, Alaba Kulito Town, Southern Ethiopia"**. We recommend that it be submitted as fulfilling the thesis requirements.

Sisay Menkir (PhD) _____ _____

Major Advisor Signature Date

Ameha Kebede (PhD) _____ _____

Co-Advisor Signature Date

As members of the Board of Examiners of the M.Sc. thesis Open Defense Examination, we certify that we have read, and evaluated the thesis prepared by Kaleab Terefe and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Biology (Microbiology).

_____ _____ _____

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DEDICATION

This manuscript is dedicated to my brother Dr. Ermiyas Terefe , who passed away without seeing my success.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work .I have followed all ethical and technical principles of scholarship of principles in the preparation, data collection ,data analysis and compilation of this thesis. Any scholarly matter that is included in this Thesis has been given recognition through citation.

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Name: Kaleab Terefe Signature: _____

Date: June 2017

School /Department: Haramaya University, Haramaya

BIOGRAPHICAL SKETCH

The author was born on March 16, 1986 in Adama town, Eastern Showa Zone of Oromia Regional State. He attended his elementary school education at Adama Primary School and Secondary School education at Hawas Secondary and Preparatory School at Adama. Then, he joined Jimma University in 2005 and graduated with Bachelor of Education Degree in Biology with minor Chemistry in July, 2007. After graduation, he was employed by the Addis Ababa Educational Office to work at Yekatit 23 Secondary School as a Biology Teacher. In 2010 he joined the Department of Biology at Haramaya University to study his M.Sc. degree in Microbiology.

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ACRONYMS AND ABBREVIATIONS

ACT	Artimesian-Based Combination Therapy
CDC	Center for Disease Control
Hb	Hemoglobin
IRS	Indoor Residual Spray
ITN	Insecticide Treated Net
ITM	Insecticide Treated materials
LLIN	Long Lasting Insecticide Net
MOH	Ministry of Health
NSP	National Strategic Plan
RBM	Roll Back Malaria
SMA	Severe Malarial Anemia
SNNPR	Southern Nations Nationalities and Peoples' Region
SPSS	Statistical Package for Social Sciences
SSA	Sub-Saharan Africa
WHO	World Health Organization

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PREVALENCE AND ASSOCIATION OF MALARIA AND ANEMIA AMONG PATIENTS VISITING ALABA HEALTH CENTER, ALABA KULITO TOWN, SOUTHERN ETHIOPIA

ABSTRACT

Malaria is a vector-borne infectious disease caused by protozoan parasites of the genus Plasmodium. It is ranked as the leading communicable disease in Ethiopia; it accounting to 17% of all outpatient visits in 2011/2012.The aim of this study was to determine the prevalence and association of malaria and anemia among patients visiting Alaba Health Center, Alaba Kulito town Southern Ethiopia. The study was a Health Center based cross sectional survey and conducted from November to December 2015. Blood sample were collected from 384 participates and used for determination of malaria and anemia. Malaria detection and identification of ***Plasmodium*** *species were based on microscopic examination of thick and thin Giemsa stained blood film. Determination of the hemoglobin concentration was carried out using portable hematology analyzer (Mission Hb). Moreover, structured questionnaires were used to access socio demographic characteristics participants and risk factor in relation to malaria. SPSS version (16) software was used to analyze the data and chi square test was calculated to determine association between malaria and anemia . The overall prevalence of malaria and anemia were found to be 43(11.2%) and 87 (22.65%) respectively. Major Plasmodium species detected were* ***P. vivax*** *and* ***P. falciparium*** *with the prevalence of 38(9.9%) and 5 (1.3%) respectively. Prevalence of malaria was high among male 25 (16.3%) and anemia 64 (27.7%) among female and high 13 (19.1%) prevalence of malaria and anemia 44(24.2%) obtained in age groups of ≤15 and 16-30 respectively. From malaria infected participants,12(27.9%) were anemia positive and the rest 31(72.1%) were anemia negative and the association was not statisticallysignificant (χ^2=0.76, P =0.383). On basis of the findings simultaneous combat against the malaria Infections and accessing other etiologic factors for anemia such as nutrition, parasitic infection is very crucial to reduce the relative burden of malaria and anemia infection in this study area.*

Key words: Anemia, Haemolglobin, Malaria, *Plasmodium* species.

1. INTRODUCTION

Malaria is a vector-borne infectious disease caused by protozoan parasites of the genus *Plasmodium*. There are four distinct *Plasmodium* species infect humans' namely: *P.falciparium, P.vivax, P.malariae,* and *P.ovale* (WHO, 2007). The three human malaria parasites *P.vivax*, *P. malariae* and *P. ovale* are contribute to fewer infections and to more moderate disease and relatively few deaths. However, *P. falciparum* is the commonest species of *plasmodium* that causes severe morbidity and mortality (Ojukwu *et al.,* 2002). This parasite is transmitted among humans by female mosquitoes of the genus Anopheles (Alonso *et al.,* 2005).

Malaria is the most serious tropical disease of human kind and the cause of much death and morbidity in areas where it is endemic. Globally, the annual disease burden in endemic countries is estimated as 225 million clinical cases and 781,000 deaths with the greatest impact in sub-Saharan Africa (WHO, 2010 b). The disease occurs in 109 countries worldwide and just 35 of those countries are responsible for the majority of the annual global deaths from malaria infection. Most of these high-risk countries lie in SSA and Southeast Asia. While Nigeria, Democratic Republic of Congo, Uganda, Ethiopia and Tanzania has the highest infection rates and account for 50 percent of the total global deaths (RBM P, 2009).In Ethiopia malaria is ranked as the leading communicable disease, accounting for about 30% of the overall Disability Adjusted Life Years lost. Approximately 57.3 million (68%) of the 84.3 million population of Ethiopia live in areas at risk of malaria (PMI, 2010).

Malaria is still a serious threat and burden on the economic and health systems of the highly affected countries. The WHO estimates that malaria can reduce the gross domestic products of countries with high infection rates by as much as 1.3 percent, while accounting for up to 40 percent of annual public health expenditure. A high malaria infection rate within a country is a poverty promoting condition, as malaria can impair childhood growth; adversely affect intellectual development and education opportunities, all of which can ultimately diminish worker productivity (Hotez *el al*., 2006).

Anemia is also a common finding in malaria (Grobusch and Kremsner, 2005). Anemia is one of an important public health problem worldwide with prevalence of 43% in the developing countries and 9% in the developed nations (WHO and CDC, 2008). WHO estimated the prevalence of anemia in Ethiopia to be 62.7% and 52.3% among pregnant and non-pregnant women respectively (WHO and CDC, 2008). Anemia is usually multi factorial in origin; a WHO report outlined the main causes of anemia as: dietary deficiencies of micronutrients, genetic disorder and infectious diseases such as malaria, hookworm infections or schistosomiasis (WHO, 2015).

The anemia of malaria has several causes namely: rupture of parasitized red blood cells in tissue avenues, destruction of parasitized and un parasitized red blood cells in the reticulo-endothelial system especially the spleen, haemolysis due to the presence of malaria antigen, antibodies and marrow suppression (Roberts *et al.,* 2005). Severe malarial anemia (SMA) is the most common clinical manifestation of severe *P. falciparum* malaria in infants and young children and accounts for the greatest amount of global malaria-related morbidity and mortality (WHO, 2010 a). In particular, malaria is responsible in Uganda alone for up to100, 000 deaths per year among preschool-aged children, over half of which are caused by severe malaria anemia (Uganda Ministry of Health, 2010).

In Ethiopia the fight against malaria is governed by a five year strategic plan based on malaria control interventions that include distribution of insecticide treated nets (ITNs), indoor residual spraying (IRS), and prompt and effective treatment with artemesian-based combination therapy (ACT). Despite the presence of a control program, the majority of the Ethiopian population is still at risk from malaria. Malaria remains one of the major determinants of ill health in many parts of Ethiopia. According to the MOH of Ethiopia; malaria was the leading cause of outpatient visits and health facility admissions, in 2010/2011 accounting for 17% of reported outpatient visits and 8% of health facility admissions among all age groups. Altitude and climatic factors are the main determinants for malaria epidemiology in the country and areas below 2000 meters above sea level are classified as malarious (PMI, 2015).

In Southern Nation's Nationalities and Peoples Regional State (SNNPR), more than 65% of the total population is at risk of malaria and around 80% of the total districts in the region are malarious From 2001- 2005 there were 3,406,568 malaria cases, 53,795 admissions and 4,397 malaria deaths in the region(Frehywot,2006).Therefore, determining the prevalence of malaria and establishing the relative contribution of malaria to anemia is essential for both clinical management and development of prevention strategies (Grantham and Ani, 2001).

In Ethiopia, most studies have been carried out on prevalence and association of malaria and anemia.Very little progress has, however been made in understanding prevalence and association of malaria and anemia .In other words, less attention has been given to the relative contribution of malaria to anemia . In addition to this, the current burden of malaria after the implementation of the national strategic plan on malaria control and prevention is not known in the study area. Therefore, this study was conducted to determine the current burden of malaria and anemia infection and to assess the association between malaria infection and anemia among patients visiting Alaba Heath Center, Southern Ethiopia. The outcome of this research would have significance primarily for health centers and other health institutions in understanding the prevalence and intensity of malaria and anemia infections and in giving emphasis for those infections most prevalent in the study area. It also gives an understanding of the major risk factor in relation to the malaria and anemia infections and it enhances the stakeholders to design and implement appropriate prevention and control measures. In addition, the finding of this research would also serve as a starting point to enable other researchers to conduct further study on the epidemiology of malaria and anemia infections in the study area.

Objectives

General objective

To determine the prevalence and association of malaria and anemia among patients visiting Alaba Health Center, Alaba Kulito town, southern Ethiopia.

Specific objectives

- To determine the prevalence of malaria parasite infections among patients visiting Alaba Health Center, Alaba Kulito town, Southern Ethiopia.
- To determine the prevalence of anemic conditions among patients visiting Alaba Health Center, Alaba Kulito town, Southern Ethiopia.
- To determine association between malaria and anemia among patients visiting Alaba Health Center, Alaba Kulito town, Southern Ethiopia.
- To assess association of major risk factors for malaria and anemia infections among patients visiting Alaba Health Center, Alaba Kulito town, Southern Ethiopia.

2. LITERATURE REVIEW

2.1. Malaria Parasite and its Vector

Malaria is caused by a single-celled parasite from the genus *Plasmodium.* More than 100 different species of *Plasmodium* exists. They produce malaria in many types of animals and birds, as well as humans. There are four distinct *Plasmodium* species infect humans' namely: *P. falciparium, P.vivax, P.malariae* and *P.ovale (*WHO, 2007). However ,*P. falciparum* is the most common cause of infection and is responsible for about 80% of all malaria cases, and is also responsible for about 90% of the deaths from malaria (Mendis *et al*., 2001).These human *Plasmodium* species are found in tropical and sub-tropical regions throughout the world and exhibited overlapping geographical distribution (CDC, 2004).Though each of the species belongs to the same genus, each one has a distinctive appearance under the microscope and each one produce a different pattern of symptoms. Two or more species can live in the same area and infect a single person at the same time (DPD, 2010). *P.falciparium, P.vivax, P.malariae,* and *P.ovale* are known to occur in Ethiopia. However, *P. falciparum* and *P. vivax* are the dominant malaria parasites distributed all over Ethiopia and account for about 60%and 40% of malaria cases, respectively (PMI, 2010).

There are about 380 species of *Anopheles* mosquitoes (WHO, 2002). 70 species can transmit malaria under natural condition and of these 40 is of major importance as vectors. Some *anophelines* prefer to bite animals and thus either does not normally transmit malaria parasites to humans or do so very. Some others do not live long enough for the parasite to develop in the mosquito or the parasite does not seem to be able to develop (WHO, 2002).The most efficient vectors in the world are those such as *A.gambiae, A.arabiensis* and *A.funestus* (Phillip, 1992). In Africa members *A. gambiae* complex and *A. funestus* are widely distributed and are responsible for the transmission of malaria in the region. *A.gambiae* species is the most anthropophagic species in the complex and the most important, probably the world's most efficient malaria vector with characteristic indoor and outdoor resting (Githeko, 2001).

The major malaria vector in Ethiopia is *A.arabiensis*. It is widely distributed in the country and is usually the vector of epidemic malaria. The second most frequent vector species in the country is *A. pharoensis* (WHO, 2002). Other vectors are *A. funestus* and *A. nili*, which were

in the past important vectors of malaria in limited areas in Ethiopia; however, they are now extremely scarce and much localized in their distribution (MOH, 2002).

2.2. Life Cycle of *Plasmodium* Species

The malaria parasite exhibits a complex life cycle involving an insect vector *Anopheline mosquito* and a vertebrate host (human). *P. falciparum, P. vivax, P.malariae* , and *P. ovale* are species of *Plasmodium* that invade human *(*WHO, 2007). All this species of *palamodium* undergo two forms of replication: sexual and asexual (Aynalem *,* 2009).

Malaria infection is most frequently initiated with the bite of an infected female *Anopheles* mosquito, which injects the sporozoite stage of the parasite with its bite. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. In case of *P. vivax* and *P. ovale* a dormant stage (hypnozoites) can persist in the liver and cause relapses by invading the bloodstream, weeks, or even years later (CDC, 2006).

The next stage of development, called the erythrocytic or blood stage, is initiated when exo-erythrocytic merozoites from the liver invade red blood cells (RBCs). Merozoites of *P. falciparum* can infect RBCs of all ages, whereas those of *P. vivax* and *P. ovale* infect reticulocytes and those of *P. malariae* invade only older RBCs (CDC, 2006).The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes).The gametocytes; male (micro gametocytes) and female (macro gametocytes) are ingested by an *Anopheles* mosquito during a blood meal (CDC, 2006).

The parasites' multiplication in the mosquito is known as the sporogonic cycle .Once in the mosquito's stomach, the gametocytes develop into sperm-like male gametes or large, egg-like female gametes. Fertilization produces an oocyst filled with infectious sporozoites. When the oocyst matures, it ruptures and the thread-like sporozoites migrate, by the thousands, to the mosquito's salivary(saliva-producing) glands .The cycle starts over again when the mosquito bites its next victim (WHO, 2007).

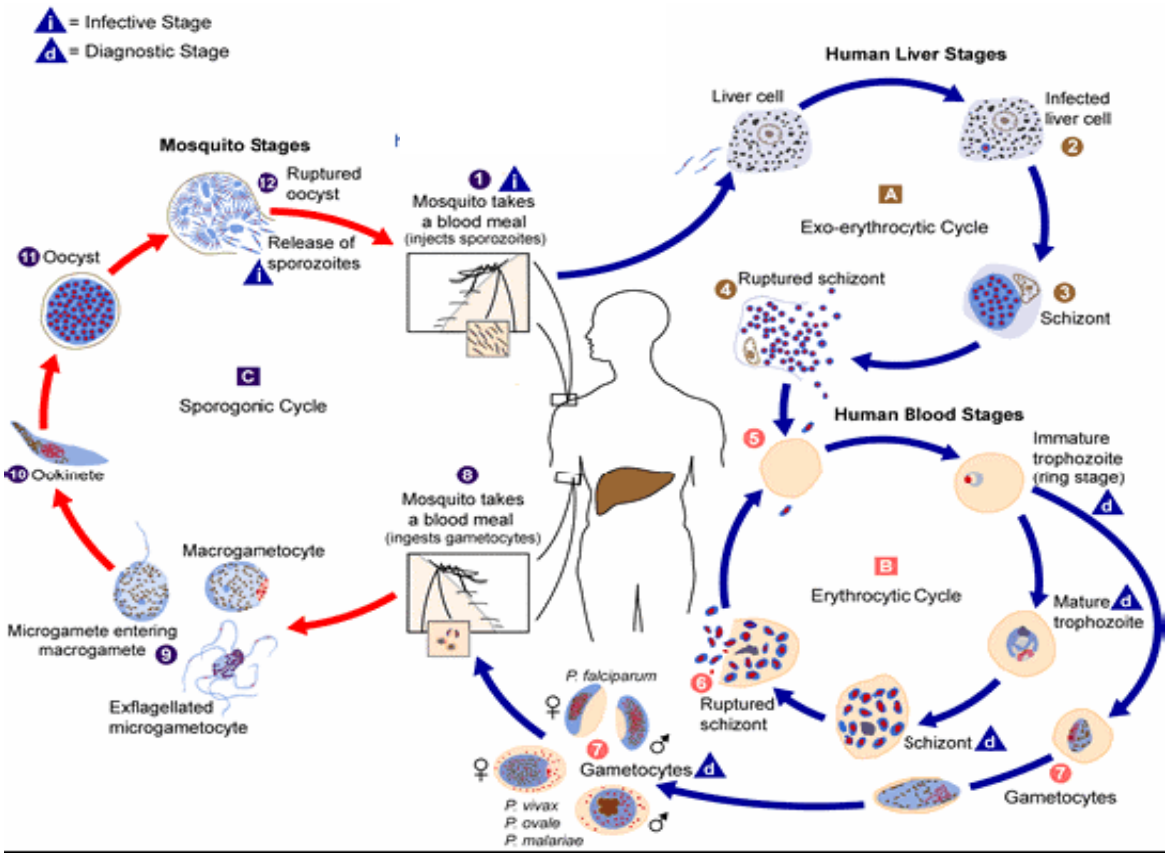

Figure 1. Life cycle of *Plasmodium* species

Source: CDC (2006)

2.3. Pathogenesis and Clinical Manifestation of Malaria Infection

The pathology and clinical manifestations associated with all malarial species is related to the rupture of infected erythrocytes and the release of parasite material, metabolites, hemozoin (i.e., malaria pigment) and cellular debris. In addition, there is an increased activity of the reticulo-endothelial system as evidenced by macrophages with ingested infected and normal erythrocytes and hemozoin. In particular the liver and spleen are often enlarged during malaria. *Plasmodium* infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occurring at either 48 or 72 hour intervals .The severity of the attack depends on the *Plasmodium* species as well as state of immunity and the general health and nutritional status of the infected individual(Weatherall *et al.,* 2002).

Malaria also develops into a chronic disease which has a tendency to relapse or recrudesce over months or even years. As discussed above, pro inflammatory cytokines, and especially TNF-α, are believed to play a role in the disease manifestations. Higher levels of TNF-α and other pro inflammatory cytokines are also associated with severe anemia, cerebral malaria and respiratory distress. However, the precise role that pro inflammatory and anti-inflammatory immune responses play in the resolution of the disease and its pathogenesis is not clear (CDC, 2004).

The signs and symptoms of the malaria parasite are diverse with a wide range of outcomes and pathologies (Weatherall *et al.,* 2002).The malaria parasites pass through human blood then into the liver, where it develops. After it has completed its development, it goes back in to the blood stream and a person then develops symptoms of malaria. The signs and symptoms of malaria typically begin 8–25 days following infection (Fairhurst and Wellems, 2010).The most common first symptoms of malaria are similar to those of Flu. The patient may experience headache, aching muscles, stomach ache and weak or lack of energy. After a day or so the body temperature may rise (up to 40^{o}C) and the patient may have fever, shivers, severe headache, diarrhea, loss of appetite, nausea,vomiting, back pain and increased sweating (Bupa, 2009).These symptoms may be seen in all types of malaria and the malaria paroxysm is typically accompanied by sudden shaking chills. This may last 10 to 15 minutes or longer. During this stage, the patient complains of feeling extremely cold, despitea stead elevation of body temperature. Chills may be followed by severe frontal headache and myalgia in the limbs and back. This stage lasts 2-6 hours in *P. vivax* and *P. ovale* infections, 6 hours or more in *P. malariae* infection and considerably longer in *P. falciparum* malaria. Finally, the patient starts to sweat profusely for several hours and usually begins to feel better until the onset of the next paroxysm. In fact, the spectrum of the disease may range from mild asymptomatic infections (reflecting the ability of adaptive immune mechanisms to prevent disease) through symptomatic (fever), to severe disease and this is greatly influenced by the infecting parasite, host genetic, geographic, nutritional and social factors (Miller *et al.,* 2002). The initial symptoms of the disease are also quite variable, particularly in children and pregnant women and symptom include irregular fever, malaise, headaches, muscular pains, sweats, chills, nausea and vomiting attributed to their weaker immunity (Lalloo *et al .,*2006).

2.4. Epidemiology and Geographical Distribution of Malaria

2.4.1. Global epidemiology and geographical distribution of malaria

Malaria remains the single most important infection causing morbidity and mortality in the world and is second only to *M. tuberculosis* as the single most important infection agent. One hundred and nine countries were endemic for malaria in 2008, 45 within the WHO African region (WHO, 2008).It is widespread in tropical and subtropical regions, including parts of America, Asia and Africa. The WHO estimates that in 2010 there were 219 million cases of malaria resulting in 660,000 deaths (WHO, 2012). Others have estimated the number of cases at between 350 and 550 million for *P .falciparum* malaria and deaths in 2010 at 1.24 million (Murray *et al.,* 2012) up from 1.0 million deaths in 1990 (Lozano *et al.,* 2012).

The majority of cases (65%) occur in children under 15 years old (Murray *et al*., 2012). Malaria morbidity can involve anemia, hypoglycemia, jaundice, hepatic dysfunction, splenomegaly, pregnancy Complications including miscarriage, premature birth, and low birth weight, and pulmonary edema, with cerebral malaria characterized by convulsions and coma. Severe malarial anemia may be responsible for as many deaths as cerebral malaria, with peak incidence occurring in children less than two years of age (Menendez *et al.,* 2000).

Malaria is abundant problem in Africa, in the endemic countries of Africa, children under the age of five and pregnant women bear the brunt of the burden of malaria disease, this is because they have lower immunity to the disease compared to other people in the same environmental locations. About 125 million pregnant women are at risk of infection each year in SSA, maternal malaria is associated with up to 200,000 estimated infant deaths yearly (Hartman, 2010).

Malaria causes severe anemia which leads to risk of spontaneous abortion, premature delivery or stillbirth, low birth weight babies and contributes to the death of 10 000 pregnant women each year in Africa (WHO, 2009). WHO estimates indicate that close to 45 million disability-adjusted life years (DALYS) are lost due to malaria in Africa about 30-40 percent of all fevers seen in health centers in Africa are due to malaria with huge seasonal variability between rainy and dry seasons(WHO, 2001).

2.4.2. Epidemiology and geographical distribution of malaria in Ethiopia

In Ethiopia, malaria is one of the most important public health problems, with more than three-quarters of the land mass (altitude <2000 m) of the country is either malarious or potentially malarious, and Over 50 million (60%) of the total Ethiopian population of 84.2 million live in areas at risk of malaria as of 2014, generally at elevations below 2,000 meters above sea level (RBMI, 2015). Ethiopia is thought to experience some 10 million cases per year, the fourth highest case number in sub-Saharan Africa (behind Nigeria, the DRC, Tanzania, and Uganda .In Ethiopia, the estimated incidence rate for malaria (i.e., the estimated probability of con-tracing the disease in a year) is 15%, which is low relative to the rest of sub-Saharan Africa (where the average incidence rate is 0.33), but higher than any other country outside of sub-Saharan Africa (Korenromp, 2005).

Annually, half a million microscopically confirmed cases of malaria are reported to the Federal Ministry of Health (FMOH) from basic health services (WHO, 2006). In 2012/2013, there were 57,503 public sector malaria hospitalizations, 4,984,266 malaria outpatient cases, and 2,942,031 laboratory-confirmed *P. falciparum* out patient malaria cases, and 1,258,131 *P. vivax* cases according to the annual micro-plan. According to the FMOH, in 2011/2012, malaria was the leading cause of outpatient visits, accounting for 17% of all outpatient visits, and 8% of health facility admissions among all age groups. Malaria is one of the top ten causes of inpatient deaths among children aged less than five years and in older individuals according to the Health Management Information System (HMIS) data (RBMI, 2015). Ethiopian villages vary considerably in their exposure to malaria and even villages in close proximity to one another can have starkly different malaria incidence problems. Many districts have variable topographical features, with some households within communities located above and below 2,000 meters. Due in part to household locations at various altitudes and distances from efficient malaria vector breeding sites, malaria risk is unevenly distributed within many districts (PMI, 2015).

A high malaria infection rate within a country is a poverty promoting condition, as malaria can impair childhood growth; adversely affect intellectual development and education opportunities, all of which can ultimately diminish worker productivity (Hotez *et al.,* 2006).

2.4.3. Factors affecting the epidemiology of malaria infection

2.4.3.1. Climate factors

Malaria, like most vector-borne diseases, is highly influenced by environmental and ecologic factors due to the habitat requirements of the mosquito vectors species. Perhaps the most important environmental factors in disease transmission are temperature and humidity (Gilles, 1993). Climate affects both parasites and mosquitoes. Mosquitoes must live long enough for the parasite to complete its development within them. Therefore, environmental factors that affect mosquito survival can influence malaria incidence. Rainfall expands breeding grounds, and in many tropical areas, malaria cases increase during the rainy season (Patz *et al.,* 2000).

Malaria parasites require a minimum temperature for development and mosquito vectors require temperature and humidity conditions that allow a long enough life span to permit parasite development and multiple blood meals. Larval habitats for mosquito vectors are influenced by both climatic factors such as rainfall and temperature (Mbogo 2003). Studies have shown that rain fall, temperature and humidity have been associated with the dynamics of malaria vector population and hence with the spread of the disease. Ambient temperature plays a major role in the life cycle of the malaria vector. The development of the parasite *(plasmodium*) within the mosquitoes (sporogenic cycle) is dependent on temperature It takes about 610 days at temperature of 28^{o}c, but stops at temperature below 16^{0}C.The daily survival of the vector mosquito is dependent on temperature as well. For range of temperature between 16^{o}C-36^{o}C, daily survival is about 90%; while with a temperature above 36^{0}C survival shown to drop rapidly. Humidity and rainfall have been shown to regulate malaria vector survival. Relative humidity below 60% was related to a decrease in vector life span, hence, which means ultimately low rate of malaria transmission. In contrast, at a humidity level above 60%, the infection rate increases substantially, this can be explained by improved vector survival (Ye *et al*., 2007).

2.4.3.2. Non climatic factors

The malaria parasite is capable of becoming resistant to the action of anti-malaria drugs. This is due to small changes in the parasite DNA (point mutations), Over-prescription of anti-malaria (due to confusion with other febrile diseases) and the uncontrolled selling of poor

quality drugs contribute to the increase in drug-resistant parasites. The widespread and increasing occurrence of *P. falciparum* resistant against affordable anti-malarial drugs, such as chloroquine and sulphadoxine-pyrimethamine is increasingly hampering the fight against malaria. Chloroquine and sulphadoxine-pyrimethamine are still the most widely used drugs for treatment in most of Africa, because of low cost and availability (CDC, 2006).

City conditions, such as stagnation of water facilitates, open wells and uncovered water tanks, and water logging at construction sites can create new places for mosquito larvae to develop (ESS, 2010).The household environment must also be considered in this discussion. Housing factors such as the presence of open eaves, covered windows or doors, screens and bed nets are important in determining an individual's risk of exposure. Presence of potential breeding sites in the vicinity of the house may also be influenced by human behaviors such as clearing of vegetation, agricultural practices, sanitation practices and house hold water use behaviors (Mbogo ,2003).In Ethiopia man-made breeding places including quarries, blocking natural drainage systems, road building, irrigation agriculture, water conservation or storage etc., are among some of the activities that enhance the incidence of malaria (MOH, 2002).

2.5. Diagnosis of Malaria

Accurate diagnosis is an important part of good malaria management and minimizes the risk of over use. Several different diagnostic techniques are available, out of which microscopic diagnosis and rapid diagnostic tests are mostly used today (WHO, 2000).Microscopy is the most commonly used method to detect the malaria parasite about 165 million blood films was examined for malaria in 2010(WHO, 2012) A thick and thin blood film is used when using a microscope to diagnosing malaria infection.The samples are stained, often using the Giemsa,Wright's or Field's staining techniques. Parasitaemia at 50 parasites/micro liter blood can be found if someone with experience performs the examination. Differentiating the *Plasmodium* species is easiest in thin blood films. In practice, several slides must be read systematically before a negative result (WHO, 2012).

Rapid diagnostic tests (RDT) have recently been recommended because they are easy to use, give fast results and are increasingly affordable (Moonen *et al.,* 2010). RDTs based on aldolase have been more dependent on higher parasite densities. These can detect all

Plasmodium species (Moody, 2002).Commercially available RDTs are often more accurate than blood films at predicting the presence of malaria parasites, but they are widely variable in diagnostic sensitivity and specificity depending on manufacturer, and are unable to tell how many parasites are present (Wilson ,2012).

PCR-based methods have high sensitivity and specificity, with good ability to make the diagnosis even with low parasite densities (Moonen *et al.,* 2010).Although polymerase chain reaction-based tests have been developed, they are not widely used in areas where malaria is common as of 2012, due to their complexity .In addition this method is also demanding in regard of equipment and staff, which limits its use (Nadjm and Behrens, 2012).

2.6. Prevention and Control of Malaria

Malaria morbidity and mortality can be reduced by preventing the infection in the first place. Most endemic countries have implemented a double pronged approach to malaria control, with effective case management using artemisinin based combination therapy (ACT) and reducing vector-human contact with vector control (Kleinschmidt *et al.*,2007).Chemoprevention is a term referring to the prophylactic use of anti-malarial drugs in high risk groups (IPT), most often pregnant women (IPTp) or infants (IPTi) (WHO ,2012).

Malaria vector control is to significantly reduce the incidence and prevalence of both parasite infection and clinical malaria by controlling the malaria-bearing mosquito and thereby reducing and/or interrupting transmission (WHO, 2010).Malaria vector control essentially achieved through two main vector control interventions: indoor (house) residual insecticide spraying (IRS) and insecticide-treated (mosquito) nets (ITNs). Reduction of man-vector contact can also be achieved by wearing protective clothing that covers the arms and legs especially in the evenings, covering windows with wire mesh and closing doors to prevent mosquitoes from entering the house, using repellents and spraying rooms with insecticide before going to bed. Other preventive strategies mentioned include boiling of drinking water, improved sanitation, clearing of bushes around the compound, avoiding cold weather, good nutrition, burning mosquito coils, screening of buildings, taking anti-malarias regularly and closing windows early(Fergusson and Fergusson, 2007).

The recent malaria control and prevention in Ethiopia has been governed by a five-year strategic plan. The 2011-2015 National Strategic Plan (NSP) provides a detailed account on the status and direction of the major malaria prevention and control strategies that include community empowerment and mobilization, early diagnosis and treatment, selective vector control, surveillance and epidemic control, as well as supporting strategies that include Monitoring and Evaluation, human resources development and operational research (MOH ,2010).

2.6.1 .Treatment of malaria infection

Malaria is treated with anti-malarial medications; the ones used depend on the type and severity of the disease. Prompt treatment is recommended for all symptoms of the disease, within 24 hours if possible as well as a appropriate drugs should be given in adequate dosage and correctly administered during the period of time recommended (Fergusson and Fergusson, 2007).The emergence of resistance to commonly used anti malarial drugs, mainly 4 aminoquin olones and sulphonamides (sulfadoxine), has been a major obstacle in the fight against malaria for several decades, and has necessitated a change in the recommended treatment guidelines in areas with endemic malaria. In order to prevent the development of resistance, artemisinin and its derivatives should be used in combination with other drugs, so- called ACTs.

ACTs now constitute the basis for first-line treatment against malaria in. essentially all malaria endemic countries ACT are about 90% effective when used to treat uncomplicated malaria (WHO, 2010 a). The most effective treatment for *P. falciparum* infection is the use of artemisinins in combination with other anti-malarials (known as artemisinin-combination therapy, or ACT), which decreases resistance to any single drug component .Treatment of *P. vivax* requires both treatment of blood stages (with chloroquine or ACT) as well as clearance of liver forms with primaquine. Infection with *P. vivax, P. ovale* or *P. malariae* is usually treated without the need for hospitalization (WHO, 2009).

Treatment of severe malaria involves supportive measures that are best done in a crucial care unit. This includes the management of high fevers and the seizures that may result from it. It also includes monitoring for poor breathing effort, low blood sugar, and low blood potassium (Sarkar *et al.,* 2012).

Recommended treatment for severe malaria is the intravenous use of anti-malarial drugs. For severe malaria, artemisinin is superior to quinine in both children and adults (Sinclair *et al.,* 2012).To treat malaria during pregnancy, the WHO recommends the use of quinine plus clindamycin early in the pregnancy (1st trimester), and ACT in later stages (2nd and 3rd trimesters).In highly endemic areas, preventive doses of sulfadoxine–pyrimethamine should periodically be given to pregnant women to clear placenta of parasites (WHO, 2009). Treatment of resistant strains became increasingly dependent on this class of drugs. The treatment is very potent and eradicates almost all of the parasites in the body. The cost of artemisinins limits their use in the developing world (Howitt et *al.,* 2010).

2.6.2. Vector control

2.6.2.1. Insecticide-treated bed nets (ITNS)

ITNS have been widely accepted as an effective intervention for malaria prevention. ITNs affect malaria transmission by killing and/or diverting infected mosquitoes away from individual net users and households with treated nets. There are several categories of bed nets used to protect against mosquitoes: untreated nets and nets impregnated with either short-lasting or long-lasting insecticides. Mosquito nets without treatment are not as effective as insecticide-treated nets (Goodman *et al.,* 2009).Currently, only insecticides of the parathyroid class are recommended for treating LLINs (WHO, 2012).

The World Health Organization's Rollback Malaria Partnership and the Millennium Development Goals have popularized ITN intervention programs; both aimed to achieve 80 percent ITN usage among pregnant women and children less than 5 years of age in Africa A simple mosquito net treated with an insecticide is a proven and cost effective way to repel or kill mosquitoes carrying the parasite that causes malaria (WHO, 2006). Consistent use of ITNs can reduce malaria transmission by up to 90 percent .Night-time use of mosquito nets treated with long-lasting insecticide (LLIN) to prevent mosquito bites is recommended by the WHO (WHO, 2009).

Mosquito nets have been advocated for as the most preventive tools against malaria especially in sub-Saharan Africa. A review of studies from areas with endemic *P. falciparum* malaria

estimated that ITNs to have a protective efficacy of 17% on malaria prevalence in children. They also found ITNs to reduce the incidence of uncomplicated malaria among children by half (Eisele, 2010). There is also evidence that if more than 80 percent of households in an area sleep under an ITN, malaria transmission is significantly reduced, which can benefit people who do not use an ITN themselves (CDC ,2008).

2.6.2.2. Indoor residual spraying (IRS)

Indoor spraying is one of the most valuable tools in malaria vector control. It was the strategy used in the most successful eradication programs of the 50's and 60's .As the name implies, IRS involves coating of the walls and other surfaces of houses with a residual insecticide. For several months, the insecticide will kill mosquitoes and other insects that come in contact with these surfaces (CDC, 2008).

The World Health Organization (WHO) recommends a number of insecticides for individual residual spraying: DDT wet table powder(WP); malathion WP; fenitrothion WP; pirimiphos-methylWP and emulsifiable concentrate (EC); bendiocarbWP; propoxurWP; alpha-cypermethrin WP and suspension concentrate (SC);cyfluthrin WP; deltamethrin WP; etofenprox WP; lambda-cyhalothrincapsule suspension (CS) and WP (WHOPES ,2007). The safety and efficacy of these are constantly being evaluated by WHO. The different insecticide classes also have different duration of insecticidal effects(WHO, 2006).Organophosphates and carbonates have the shortest duration, which in turn means that they require frequent spray cycles in order to achieve optimal coverage(WHO, 2006).

The costs associated with the different insecticides when used in IRS also differ, with DDT and pyrethroids being the cheapest. IRS has the advantage of being able to make use of a much wider range of insecticide products in comparison to ITNs, for which pyrethroids are the only class of insecticide currently used. Insecticide spraying is often done on a very large scale in order to maximize the mass effect of the insecticide however ,IRS is thought to be only effective if a large proportion of the population is protected(WHO, 2006).

2.7. Anemia

Anemia is defined as a reduction in the concentration of hemoglobin in the blood (measured in grams per liter of blood, or g/L), primarily caused by disruption of erythrocyte level equilibrium (Hoffbrand *et al.,* 2006; de Benoist *et al.,* 2008). Anemia adversely affects people worldwide and occurs when there is inadequate number of red blood cells or inadequate amount of hemoglobin for the body to function properly. Hemoglobin is a protein in red blood cells that carries oxygen to the brain, muscular system, immune system, and other parts of the body. Without adequate oxygen, the physical and mental capacities of individuals are reduced (MOST, 2004). Generally speaking, anemia is a public health problem that affects populations in both rich and poor countries. It affects an estimated two billion people worldwide are thought to be affected with this condition, and an increased demand on the body for growth in preschool aged children (4-6 years) means anemia is particularly prevalent in this group (WHO and UNICEF, 2004).

Severe anemia may exist alone or in combination with other complications particularly cerebral malaria and respiratory distress in which it portends worse prognosis. It is a significant cause of morbidity and mortality in children below five years of age. Children below 3 years are predominantly affected with a mean age of 1.8 years (WHO, 2004).

Strong evidence links anemia to health and development problems. Anemia is related to reduce work capacity, reduced ability to execute activities of daily living, poor pregnancy outcomes and reduced cognitive function (Krause, 2000). Anemia in pregnant women results in lower birth weight babies who have a higher Iron deficiency with or without anemia reduces work productivity in adults and limits cognitive development in children, thus limiting their achievement in school and ultimately reducing investment benefits in education. In addition, iron-deficiency leads to impaired gastrointestinal functions. Iron deficiency anemia also has adverse effects on the immune system because it results in reduced resistance to infections (Stoltzfus, 2001).

In Ethiopia, there is a high prevalence of anemia. However, the magnitude and importance of iron deficiency anemia as a public health problem is still under investigation because in several developing countries the intake of iron from diet is more than adequate. In spite of the

high intake of iron in teff-consuming communities, there is high prevalence of anemia. Therefore, the cause of iron deficiency in Ethiopia may not be the inadequate dietary intake of iron i.e. other etiologic factors like iron mal-absorption and iron loss might play a role in iron deficiency anemia. In such communities with an already high intake of iron, the conventional supplementation of iron might not be an effective method of intervention or might even be harmful as iron overload in the body damages organs such as liver and heart (Kohgo *et al.,* 2008). Therefore, all important risk factors have to be identified and their role in causing anemia should be evaluated.

2.7.1. Causes of anemia

Anemia is a multi-factorial health problem in which the risk factors could be nutritional (iron, folate, and vitamin B12 deficiencies), clinical (infectious diseases such as malaria, helminthes infections, tuberculosis, HIV/AIDS and general inflammatory disorders), socioeconomic factors (educational levels of parents and low household income), and demographic factors (age, gender, and family size) (Al-Mekhlafi *et al.,* 2008). Assessing the causes of anemia is complex, especially where many different etiologic agents are at play simultaneously, as is the case in much of the developing world (Friedman *et al.,* 2005).

Nutritional deficiencies are regarded as the most important cause of anemia in the world and a major potential contributor to adolescent anemia in sub-Saharan Africa (Leenstra *et al.,* 2004). According to Krivienė and Ragelienė, half of all reasons for anemia are iron deficiency. Besides specific nutrient deficiencies, parasitic diseases, including helminthes infections and *P. falciparum, have* long been recognized as important contributors to anemia in endemic countries (Krivienė and Ragelienė, 2006). There are also many other rarer causes of anemia, the most common being genetic disorders such as the sickle cell anemia (McDevitt *et al.,* 2004).

The risk of anemia increases when individuals are exposed to malaria and helminthes infections. Malaria, especially due to the protozoan *Plasmodium,* causes anemia by rupturing red blood cells and by suppressing the production of new red blood cells (Roberts *et al.,* 2005). Helminthes such as hookworms can cause blood loss and therefore iron loss. The number of adult hookworms and the fecal egg count, which is an indirect estimate of the

number of worms, are strongly correlated with the amount of blood lost which, if chronic, can result in iron deficiency anemia. The nematode *Trichuris trichiura* can cause anemia when the worm burden is heavy. Heavy infections also cause inflammation and dysentery, which in turn can cause further blood loss (Koukounari, *et al.,* 2008).

2.7.2. Anemia and malaria

Anemia is a common finding in malaria and the degree correlates with severity of parasitaemia (Grobusch and Kremsner, 2005). *P.falciparum* is the commonest species of *plasmodium* that causes malaria and contributes significantly to high prevalence of severe anemia among paediatric patients (Ojukwu, 2002).

According to the WHO, severe malarial anemia is defined as a haemoglobin(Hb)concentration <5g/dL or a haematocrit (Hct) <0.15 in the presence of a *P. falciparum* parasitaemia >10,000 parasites per µl, with a normocytic blood film (WHO, 2004). Sevier malarial anemia is thought to arise from mechanisms involving increased destruction of nonparasitized and parasitized RBCs as well as a decreased production of RBCs. Severe anemia develops rapidly in children and the rate is directly proportional to the degree of parasitaemia in any cases (WHO, 2004).Children with SMA may present with malaise, fatigue and dypsnoea or respiratory distress, defined by tachypoea, deep gasping breathing (Menendez *et al.,* 2000). It also has been found to be associated with about 6.7% reduction in total body water, a loss slightly more than mild dehydration such that overzealous volume expansion may be detrimental (Planche *et al,* 2004).

The prevalence of anemia, defined as a haematocrit<0.33 as measured in community surveys in malaria endemic areas of Africa, varies between 31 and 91% in children and between 60 and 80% in pregnant women. Its prevalence and or severity is determined by a number of interacting factors including: the species of the infecting parasite; the intensity of transmission (endemicity); age and pregnancy status of the host; associated host genetic factors; and causes of anemia other than malaria, e.g. hookworm infections. In particular, malaria is responsible in Uganda alone for up to100, 000 deaths per year among preschool-aged children, over half of which are caused by severe malaria anemia (Uganda Ministry of Health, 2010).

Studies have also shown a direct negative impact of malarial infection on hemoglobin levels on children in Kenya and Ethiopia (Koukounari *et al*., 2008; Amare *et al*., 2010).It is a significant cause of morbidity and mortality in children below five years of age (Krause, 2000).The mortality rate of malaria-related anemia is between 5.6% and 16% for children 4-6 years of age and 6% for pregnant women, especially in primigravidae (Chang *et al.,* 2004). It accounts for between 26 and 62% of severe malaria admissions in malaria endemic countries (Mockenhaupt *et al.,* 2004) and up to 29% of total hospital admissions as reported in Ilorin (Ernest, 2002) and Kenya (Lackritz *et al.*, 1992).

Hospital based data of deaths from anemia ranges between 11.2% in Sierra Leone and 14% in Kenya for children below 5years (Brabin *et al.*, 2002). Therefore, establishing the relative contribution of malaria infection to anemia is essential for both clinical management and development of prevention strategies. The severity of this condition should not be underestimated; it is considered the second most common cause of disability in the world and being chronically anemic early in life can have far-reaching consequences, impairing both physical and cognitive development (Grantham and Anni, 2001).

2.7.3. Epidemiology of anemia

Anemia continues to be an important public health problem worldwide with prevalence of 43% in the developing countries and of 9% in the developed nations (WHO and CDC, 2008). It is estimated that around 1.62 billion people are affected by anemia globally. This absolute number of cases is translated into prevalence rate of 24.8% (95% CI: 22.9-26.7%) worldwide. Particularly preschool-age children and women of reproductive age take the disproportionate burden. Globally, almost half of all preschool children (47.4%) and pregnant women (41.8%) and close to one-third of non-pregnant women (30.2%) are anemic (de Benoist *et al*., 2008).

The highest prevalence of anemia occurs in the developing world. It is estimated that in sub-Saharan Africa 23 million pregnant women are exposed to malaria infection annually and approximately 400,000 pregnant women develop moderate or severe anemia (haemoglobin< 80 g/L or hematocrit <0.25) each year in sub-Saharan Africa as a result of malaria infection (Guyatt *et al*., 2001). Among African countries, national prevalence levels in preschool aged children range from 48-54% in Zimbabwe (Midzi *et al.*, 2010).

To 61-66% in Nigeria (Tohon *et al.,* 2008) and an alarmingly high 73-74% in Zanzibar. Uganda appears to be no exception with 64-71% of under five-year olds classified as anemic in 2001, translating into 3·98million children (de Benoist *et al.,* 2008).Other studies report even higher prevalence levels, reaching 80% among children less than 10 months old, one third of whom were diagnosed with severe forms of anemia with hemoglobin levels dropping under 80 g/L of blood, well below the threshold of 110 g/L used to classify anemia in this particular age group (Crawley, 2004). According to WHO 12.8% and 3.7% of maternal mortality in Asia and Africa respectively are directly attributable to anemia. Globally, Iron Deficiency Anemia (IDA) alone causes 841000 deaths and 35057000 Disability Adjusted Life Years (DALYs) lost (WHO and CDC, 2008).

In Ethiopia anemia is a common problem which has been recognized for decades. Using mathematical models, WHO (World Health Organization) estimated the prevalence of anemia in Ethiopia to be 62.7% and 52.3% among pregnant and non-pregnant women respectively (WHO and CDC, 2008). However, the DHS of 2005 reported anemia prevalence of 26.6% among women in Ethiopia (CSA, 2006).

In addition to this reasonable number of national level surveys determined the prevalence of anemia in women of reproductive age and came up with figures ranging from 16.6 to 30.4% .Concerning pregnant women, two large scale surveys reported 30.6% (CSA, 2005) and 18.4 % (Haidar *et al.,* 1999) prevalence's. Small scale studies in Awassa and Jimma (Belachew and Legesse, 2006) towns also found 15.1% and 38.2% prevalence. All the studies consistently witnessed the public health significance of anemia in the country.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Alaba Health Center, in Alaba *wereda* which is located 313 km south of Addis Ababa and about 85 Km south west of the Southern Nations Nationalities and Peoples Regional (SNNPR) state capital of Hawassa. The *wereda* is geographically located $70^0$17' N latitude and $38^0$06' E longitude and the altitude of the *Wereda* ranges this from 1700 to 2200 meters above sea level, but most of the population in the *Wereda* is found at about 1800 meters above sea level. The annual rainfall varies from 100 to 120 millimeter, while the annual mean temperature is between 18 to 23°C.

Alaba Kulito is the administrative center of the Alaba special *wereda*. Based on figures obtained from the CSA, this *wereda* has projected population, as of July 2007 E.C was 235,835 of whom 144,307 were males and 142,821 were females. Regarding residency around 231,400 of the population dwells in rural parts of the *Wereda* and the rest 51,293 reside in urban (CSA, 2013). The *Wereda* 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. Malaria transmission in Alaba 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: Aalaba Special *Wereda* Health Office).

3.2. The Study Design

The study design was a Health Center based cross-sectional survey on the prevalence and association of malaria and anemia among patients visiting Alaba Health Center from November to December 2015.

3.3. The Study Population

The study population was patients visiting Alaba Health Center for medical examination from November to December 2015. Subjects were screened for malaria and anemia infection.

Structured questionnaires were used to assess the socio-demographic characteristics of the participants and major risk factor in relation to malaria.

3.4. Sample Size Determination and Sampling Techniques

3.4.1. Sample size determination

The sample size for this study was estimated by using the statistical formula provided by (Naing *et al.* 2007). The estimation of population proportion was calculated using the following formula. Where p is the average prevalence p = 0.5 was used, as this value gave us sample size sufficiently large to guarantee an accurate prediction, at 95% confidence interval and 5% error of estimate.

$$n = \frac{(z\alpha/2)2 * p (1-p)}{d^2}$$

Where:- n= sample size

p= 0.5 (average prevalence)

Zα/2=1.96 (Z=score corresponds to 95% confidence interval.)

d= 0.05 (Margin of error)

$$n = \frac{(1.96)2 (0.5) (0.5)}{(0.05)^2} = 384$$

To minimize errors arising from the likelihood of non-compliance, 2 % of the sample was added to the normal sample. Therefore, a total of 390 patients were selected from Alaba Health Center to participate in the study.

Inclusion criteria:

Randomly selected participants who were willing to participate in the study during sample collection period.

Exclusion criteria:

Individuals who were unable to give blood sample due to health related problems and who are not willing to participate in this study were excluded.

3.4.2. Sampling techniques

Participantes were selected using simple random sampling. In Alaba Health Center around 80 patients were visiting every day ,for this study out of 80 out patients visiting Alaba Health Center around 30 patients were selected each day using simple random sampling via lottery method for about 13 working days from November to December 2015 .A total of 390 participants were involved in this study but only 384 participants data were analyzed and presented on the result and the rest 6 participants were excluded as a result of incomplete data and examination procedure errors during blood sample collection ,staining and microscopic examination.

3.5. Methods of Data Collection

3.5.1. Questionnaire survey

Structured and pre-tested questionnaires were used to gather relevant information on socio demographic characteristics of study participants and to identify the major risk factors of malaria in the study area. The questionnaire was first developed in English and then pre- tested on randomly selected participants to refine any problem on the questionnaire. Finally, the questionnaire was interviewed to randomly selected study participants on the study area from November to December 2015 (Refer to the Questionnaire sample in Appendix).

3.5.2. Blood sample collection

The participants selected for the study were briefed about the purpose of the study and the laboratory examination procedures to be followed by the senior laboratory technicians. The finger was first cleaned with an alcohol-moistened swab, dried with a piece of dry cotton before blood sample collection. Finally blood sample was collected from each participant by puncturing their finger-tips using sterilized disposable blood lancet with the help of a laboratory technician.

3.5.3. Blood films preparation and staining

Peripheral smear examination of well-prepared and well-stained blood film is the gold standard for confirming the presence of malaria parasite. Using the drop of blood, thin and

thick blood smears were made on the same slides side by side and properly labeled per individual. The smears were air-dried and the thin smear was fixed with 100% methanol for 30 seconds. Following this, the smears were stained with 3 % Giemsa for 30 minutes.

3.5.4. Microscopic examination and identification of malaria parasite

Microscopic examination of thick and thin films of blood is the method of choice for confirming the clinical diagnosis of malaria and identifying the specific species responsible for disease (Dawit *et al.,* 2004). Microscopic examination of thick films using high power magnification for the presence of parasites and parasite species identification using thin films under 100x oil immersion objective was done. Malaria parasites take up stain in a special way in both thick and thin blood films that enables to distinguish the various parts of the parasite. Chromatin (part of the parasite nucleus) was usually round in shape and stains deep red and cytoplasm occurs in a number of forms, from a ring shape to a totally irregular shape and stains blue.

In a thick blood film with 100 x oil immersion objectives and 7x ocular, no red blood cells were seen. The malaria parasites were seen along with the white blood cells; the parasites appeared smaller in the thick film than in the thin blood films. So it was difficult to distinguish *plasmodium* species. In thin films the red blood cells were fixed and the morphology of the parasitized cells were seen easly.Species identification was made based upon the size and shape of the various stages of the parasite, the presence of stippling (i.e. bright red dots) and fimbriation (i.e. ragged ends). To ensure accuracy, all positive slides and a random sample of 5% of the negative slides was re-examined by a separate microscope, which was blinded to the diagnosis of the first slide-reader.

3.5.5. Determination of Hemoglobin Concentration

Determination of the hemoglobin concentration was carried out using hematologyanalyzer
The indicator was placed on hematology analyzer (Mission *Hb*) for determination of hemoglobin level .A single drop of blood sample from each study participant was taken and dropped on the hemoglobin determining indicator paper.

Finally, the concentration of hemoglobin level was quantitatively determined within 5 minutes and read in g/dl at Alaba Kulito Health Center laboratory.

Anemia is defined by a Hgb concentration

- Less than 11 g/dl in children < 6 years.
- Less than 12 g/dl (in children 6-14 years and in female adults above the age of14 years.
- Less than 13 g/dl in male adults above the age of 14 years.
- Severe anemia less than 7 g/dl (WHO, 1989).

3.6. Data Analysis

At the end of the study clinical, socio-demographic and risk factor data were obtained. Data from both the laboratory and survey was checked for completeness. To ensure the validity and clarity of these data, each data was recompiled and recounted in order to fill some missing information. Coded data was exported into the appropriate SPSS version 16 software package. Frequency distribution and percentages calculated to give a clear picture of participants socio demographic characteristics such as age, sex, marital, distribution of *Plasmodium* species and anemia and chi square test were used to test the association between malaria and anemia. P value less than 0.05 was considered as significant.

3.7. Data Quality Control

To ensure accurate and reliable results, quality control was applied to laboratory procedures and for diagnosing parasites. Before blood sample collection, slides were properly soaked in hot water, washed with distilled water, rinsed in denatured alcohol and cleaned with gauze. In addition, the glass slides were labeled to match with the file of particular participant. For blood sample collection one sterile lancet was used per person.

During data collection, all the activities of the work was carefully monitored and supervised by the researcher. The quality of the Giemsa staining solution, fixation chemical (methanol) and the microscope were checked before using directly. In addition, to ensure general safety, disposable gloves were worn and universal bio-safety precautions were followed at all times.

3.8. Ethical Considerations

Ethical clearance was obtained from the ethical clearance committee of Alaba *Wereda* Health Bureau. The objectives of the study were explained to the study participants and their agreement was obtained. Diagnosis was done using sterile and disposable materials. Only a laboratory technicians were allowed to take blood samples and do all other activities related to clinical examination while diagnosis was supervised by health personnel.Participants diagnosed positive for malaria was treated free of charge with appropriate drug which was provided by the researcher. The prescription how to use the drug was given by health center pharmacist.

4. RESULTS AND DISCUSSION

4.1. Some socio-demographic Characteristics of Study Participants visiting Alaba Health Center

The socio-demographic characteristics of participants are summarized and presented in (Table 1). A total of 384 participants were included in this study, out of which 153 (39.8%) were males and the rest 231 (60.2%) were females. About 68 (17.7%) of the respondents were ≤15 years old, 182 (47.4 %) were 16-30 years old, 108 (28.1 %) were 31-45 years old and 26 (6.8%) were 46 and above years old (Table 1). Most of the participants were married 238 (61.9%) followed by single/ unmarried 127(33.1%) and rest were widowed 4(1%) and divorced 15 (4%) (Table 1).

Regarding livestock availability in the house, 57 (14.84%) of the participants kept livestock in their house and 327 (85.16%) did not kept livestock in their house (Table 1). Among the participants, 282 (73.44%) were rural residents and the rest 102 (26.56%) were urban residents (Table 1).About 178 (46.3%) of the participants had no formal education whereas, 64 (16.7 %) can read and write, 92 (24 %) were completed primary education, 33 (8.6%) secondary education and 17 (4.4%) of participants had higher education level (Table 1).

As depicted in (Table 1) 151(39.32%) were farmers, 22(5.73%) were house wives, 106 (27.6%) merchants, 63 (16.4 %) were students, 27 (7.03%) employees, 3(0.8%) were has no job and 12 (3.12%) daily laborer. The monthly income of the majority of the participants 132(34.6 %) were in between 501-1000 ETB, whereas 130 (33.3%) were in between 151-500 ETB per month, 91(23.7%) had monthly income of 1001-1500 ETB and 31(8.1%) had grater than1500 ETB (Table 1).

Table 1. Some socio-demographic characteristics of study participants visiting Alaba Health Center

Variables		Number	Percent (%)
Sex	Male	153	39.84
	Female	231	60.2
Age	<=15	68	17.7
	16-30	182	47.4
	31-45	108	28.1
	>45	26	6.8
Marital status	Single	127	33.1
	Married	238	61.9
	widowed	4	1
	Divorced	15	4
Educational; status	No education	178	46.3
	Read and write	64	16.7
	Primary education	92	24
	Secondary education	33	8.6
	Higher education	17	4.4
Occupation	Farmer	151	39.32
	Merchant	106	27.6
	Student	63	16.4
	Employees	27	7.03
	House wife	22	5.73
	Has no job	3	0.8
	Daily labor	12	3.12
Residence	Urban	102	26.56
	Rural	282	73.44
Monthly income	151-500	130	33.6
	501-1000	132	34.6
	1001-1500	91	23.7
	>1500	31	8.1
Presence of live stock in the house	Yes	57	14.84
	No	327	85.16

4.2. Prevalence of *Plasmodium* species by Age and Sex of Patients who visited Alaba Health Center from November –December 2015

In the present study, an overall parasite rate of 43 (11.2%) was obtained from a total 384 study participants examined for malaria infection (*P. falciparum* or *P. vivax*) during the study period (November - December, 2015) (Table 2). The current result was higher than that reported from the three largest regions of Ethiopia, i.e. Oromia, Amhara and SNNPR (Graves *et al*., 2008) were the prevalence was 4.1%. But the result was lower than the 2011/2012 report of the (FMOH), malaria was the leading cause of outpatient visits, accounting for 17% of all outpatient visits, and 8% of health facility admissions among all age groups (RBMI, 2015). Similarly lower than earlier reports made from this Health Center in 2007; the estimated incidence rate for malaria was 27.9% (Abraham *et al.,* 2010). This difference could be explained by the fact that variation in age and sex proportion of study participants and subjects selecting criteria between the current and privies studies as well as the implementation of National Strategic Plan for malaria such as early diagnosis and prompt treatment, selective vector control and environmental management likely to play a significant role in the lower parasite rates of the studied participants.

In this study, out of the total positive slides 43 (11.2%), *P. vivax* accounts for 38 (88.37%) and *P. falciparum* for the remaining percentage 5 (11.63%).There was no case of mixed infection observed in the present study (Table 2). Similarly there were high prevalence of *P.vivax* than *P. falciparium* on the privies study on this study area, the prevalence of *P. falciparium*, *P. vivax* and both *P. falciparium* and *P. vivax* were shown to be 13.3%, 81.77% and 4.9%, respectively (Abraham *et al.,* 2010). Similarly, Yasinzai and Kakarsu (2008) while studying prevalence of malaria parasite species in Pakistan, observed high prevalence of *P. vivax* (88.69%) and a low prevalence of *P. falciparum* (11.3%). In contrast to this *P. falciparum* and *P.vivax* are the main species accounting for 60% and 40% of malaria cases in Ethiopia respectively (PMI, 2010).The difference in parasite species occurrence may represent variations in geographical location, climatic factor such as temperature, humidity and rain fall and vector species abundance. The above factors have effect on transmission of *plasmodium* species, on the development of parasites as well as on the activity and survival of *anopheline* mosquitoes (Asnakew *et al.,* 2009).

Furthermore, these differences may also relate to differences in clinical manifestation and treatment of infections (more severe symptom or the duration of infection) which affect parasite prevalence in the study (Bodker *et al*., 2006).

Although malaria infection was observed in all age groups, a relatively higher prevalence 13 (19.1 %) of *Plasmodium* species infections were detected in the age groups ≤15 years and followed by 23 (12.63%) in 16-30 age group, 6 (5.6 %) in 31-4 5 age group and 1 (3.8%) in the age group above 45 (Table 2). In this finding, statistically significant difference (P= 0.023) of was seen between malaria infection and age. This was in line; with a study conducted in some selected rural villages around Arba Minch town, southern Ethiopia found that the difference in prevalence of *Plasmodium* species among the age groups was significant (Yarcho, 2011). In contrast with this the report made for malaria infection in Amhara, Oromia and SNNP regions of Ethiopia had shown that the difference in prevalence of *Plasmodium* species among the age groups was not significant (Graves *et al*., 2008). As the results showed in (Table 2), in the current study, the highest prevalence of *Plasmodium* species was observed in the age group ≤15 years and which fit into the conventional characterization of the epidemiology of malaria based on age stratification. That is conventionally in areas of high endemicity, prevalence of malaria infection is known to peak at an early age and continuously show a slow decline with increasing age (WHO, 2000). This pattern of prevalence is a reflection of the age related state of anti malaria immunity that is developed as a result of repeated malaria infections under established malaria endemicity (WHO, 2000).

Out of the total malaria positive individuals, 25 (16.3%) were male and 18 (7.8 %) were female (Table 3). In this finding, statistically significant difference was seen between malaria infection and sex (p=0.034). In line with this, a study conducted on the prevalence of malaria in rural villages around Arba Minch town showed that statically significant difference of malaria infection with sex (Yarcho, 2011). Unlike this studies reported by Graves *et al* (2008) shows that no significant difference of malaria parasite with sex. The contrarily possible explanation for the discrepancy could be that men have a greater occupation risk of contracting malaria than women if they work in fields or forest of peak biting times and other behavioral risk factors (Anne *et al*.,2010).

Table 1. Prevalence of malaria Plasmodium species by age and Sex of Patients

Age group in year and sex		Total examined %	Total number of malaria positive%	*P.F* Number of positive %	*P.V* Number of positive %	Total number of malaria negative	DF	χ^2	P value
≤15	Male	31	9(29)	3(9 .7)	6(19.4)	22(71)	2	5.110	.078
	Female	37	4(10.81)	0(0)	4(10.8)	33(89.2)			
	Total	68	**13(19.1)**	3(4.4)	10(14.4)	55(80.9)			
16-30	Male	70	14(20)	0	14(20)	56(80)	**2**	**9.014**	**.011**
	Female	112	9(8.04)	2(1.8)	7(6.25)	103(91.96)			
	Total	182	**23(12.63)**	2(1.1)	21(11.5)	159(87.37)			
31-45	Male	43	2(4.65)	0(0)	2(4.7)	41(95.35)	1	.111	.739
	Female	65	4(6.1)	0(0)	4(6.2)	61(93.9)			
	Total	108	**6(5.6)**	0(0)	6(5.6)	102(94.4)			
>45	Male	9	0(0)	0(0)	0(0)	9(100)	1	.551	.458
	Female	17	1(5.88)	0(0)	1(5.88)	16(94.22)			
	Total	26	**1(3.8)**	0(0)	1(3.8)	25(96.2)			
All age groups							**3**	**9.539**	**0.023**
sex Male		153(39.8)	**25 (16.3)**	3 (1.96)	22 (14.37)	128 (83.67)	**2**	**6.771**	**0.034**
Female		231(60.2)	**18 (7.8)**	2 (0.86)	16 (6.92)	213(92.2)			
Total		384(100)	**43(11.2)**	**5(1.3)**	**38(9.9)**	**341(88.8)**			

PV= *Plasmodium vivax*, PF= *Plasmodium falciparum ,* DF=degree of freedom, χ^2=chi square

4.3. Prevalence of Anemia by Age and Sex of Patients who visited Alaba Health Center from November –December 2015

The Prevalence of anemia with age and sex are summarized and presented in (Table 3). The overall prevalence of anemia from the total 384 individuals examined in Alaba health center, from November -December, 2015 was showed that, 87 (22.66%) participants were anemic (Table3). Anemia prevalence obtained from this study was lower than global and developing countries but higher than developed nation anemia prevalence based WHO and CDC (2008) report. The report stated that anemia continues to be an important public health problem worldwide with prevalence of 43% in the developing countries and of 9% in the developed nations it is estimated that around 1.62 billion people are affected by anemia globally. This absolute number of cases is translated into prevalence rate of 24.8% worldwide (WHO and CDC, 2008).

Regarding the prevalence of anemia between different sexes, 23 (15%) of males and 64 (27.7%) females were anemic .The prevalence was significantly higher among females than males. The differences in sex were statistically significant (P=.004) (Table3). The findings of this study were in line with DHS and CDC 2006 report, which stated that anemia prevalence of 26.6% among women in Ethiopia (CSA, 2006). In contrast with this result, other study reported by WHO using mathematical models estimated the prevalence of anemia in Ethiopia to be 62.7% and 52.3% among pregnant and non-pregnant women respectively .On the other hand in this study the prevalence of anemia among males were higher than reported by WHO and CDC, (2008) which is 12.7%(WHO and CDC, 2008).

Moreover anemia prevalence was also vary with different age groups, anemia prevalence was high 44 (24.2%) in the age group 16-30, followed by 16 (23.5%) in age groups ≤15, 23 (21.3%) in 31-45 and 4 (15.4%) >45 age groups. In this study the prevalence of anemia is not statistically significant with age (P=0.761) (Table 3).Moreover, the prevalence of anemia among females were higher than male in16 -30 age groups and which was also statistically significant (P=0.014) (Table 3). The observed increase in prevalence of anemia with increasing age and significantly higher prevalence in adult females than males can be explained by the fact that children during growth spurts and menstruating women have higher

physiological demands for iron (Viteri, 1997). In line with this result in 2011, WHO report high prevalence of anemia among children 42.6%, and for all women of reproductive age was 29.4 % (WHO, 2015). Also some studies suggest that even low hemoglobin levels may be associated with poor performance on mobility tests and physical function (Eisenstaedt *et al.,* 2006). In healthy older individuals between 60 and 98 years of age, hemoglobin levels do not change significantly (Balducci, 2003).

Table 2. Prevalence of Anemia by Age and Sex of Patients who visited Alaba Health Center from November –December 2015

Age group in year and sex		Total examined %	Number of anemia positive %	Number of anemia negative %	χ^2	DF	P value
≤15	Male	31	**6(19.4%)**	25(80.6%)	.552	1	.458
	Female	37	**10(27%)**	27(73%)			
	Total	68	**16(23.5%)**	52(76.5%)			
16-30	Male	70	**10(14.3%)**	60(85.7%)	6.070	1	.014
	Female	112	**34(30.4%)**	78(69.6%)			
	Total	182	**44(24.2%)**	138(75.8%)			
31-45	Male	43	**6(14%)**	37(86%)	2.298	1	.13
	Female	65	**17(26.2%)**	48(73.8%)			
	Total	108	**23(21.3%)**	85(78.7%)			
>45	Male	9	**1(11.1%)**	8(88.9%)	.193	1	.660
	Female	17	**3(17.6%)**	14(82.4%)			
	Total	26	**4(15.4%)**	22(84.6%)			
All age groups					1.168	3	.761
Sex	Male 153(39.8)		**23(15%)**	130(85%)	8.436	1	.004
	Female 231(60.2)		**64(27.7%)**	167(72.3%)			
	Total 384 (100)		**87(22.66%)**	297(77.34%)			

DF=degree of freedom, χ^2=chi square

4.4. Association of the Prevalence of Anemia with the Prevalence of Malaria Parasitic Infection

As depicted in (Table4). From the total 384 study participants ,12 (3.125%) were both anemia and malaria positive, 31(8.07 %) were malaria positive and anemia negative, 75 (19.53%) were anemia positive and malaria negative and the rest 266 (69.27%) were both malaria and anemia negative. In this study the association of malaria infections with anemia was not statistically significant (P =0.383). Similarly, other studies have also shown a direct negative impact of malarial infection on hemoglobin levels on children in Kenya and Ethiopia (Koukounari *et al.,* 2008; Amare *et al.,* 2010). Unlike this, study made in this study area have showed that high anemia prevalence for malaria infection was observed (p <0.001) (Abraham *et al.,* 2010). The finding of the present study was sustained by the fact that there is national malaria control program in Ethiopia, this includes effective malaria control such as early diagnosis and anti malarial drug treatment to reduce malaria morbidity and acute infection. Therefore, early diagnosed and treatment of patients before they develop high paracitemia may be reason for contradictory report on the effect of paracitemia on hemoglobin concentration. Similarly Korenromp in 2004 suggests that in malaria endemic regions, malaria control can reduce anemia and severe anemia by over a quarter and by 60%, respectively (Korenromp *et al.,* 2004).

As depicted in (table 4), out of 87 (22.65) anemia and 43 (11.2%) anemia infection, only 12 (3.125%) were infected with both malaria and anemia and the rest 75(19.53%) of anemia positive and 31(8.07 %) of malaria positive patients were free from malaria and anemia infection respectively. In other word, from the total 43 (11.2%) malaria positive cases only 12 (27.9%) were anemia positive and the rest 31 (72.1%) were anemia negative (Table 4). It is higher than the privies study in this study area which reported that 23.15% malaria positive participants were anemia positive (Abraham *et al.,* 2010). In relation with this varies studies also reported that malaria-related anemia accounts for 26 % of severe malaria admissions in malaria endemic countries (Mockenhaupt *et al.,* 2004). In contrast to this high prevalence were reported among African countries, national prevalence levels in preschool aged children range from 48-54% in Zimbabwe (Midzi et al., 2010).To 61-66% in Nigeria (Tohon et al., 2008) and an alarmingly high 73-74% in Zanzibar. Uganda appears to be no exception with 64-71% of

Less than five-year olds classified as anemic in 2001,(de Benoist *et al.,* 2008).

Table 4. Association between malaria and anemia infection

		Anemia		
		Positive	Negative	Total
Malaria	Positive	12(3.12%)	31(8.1%)	43(11.2%)
	Negative	75(19.53%)	266(69.3%)	341(88.8%)
	Total	87(22.65%)	297(77.34%)	384(100%)
χ^2(chi square) = 0.76, DF (degree of freedom) =1 P (P value) =0.383				

As showed in (Table 5) there is also variation on the prevalence of malaria and anemia cases with respect to sex and age. In relation to sex, from the total 18 (7.8%) females and 25(16.3%) males malaria positive cases, 7 (38.9%) females and 5 (20%) males were anemia positive (Table 5). Similarly study conducted in this study area have showed that *Plasmodium* infected females were found to have significantly higher anemia infection than males (Abraham *et al.* 2010).

Regarding malaria and anemia infection with different age groups , from the total 13 (19.1%) in age group ≤15 ,23 (12.6%) in age group 16-30, 6 (5.6%) in age group 31-45 and 1 (3.8%) in age group >45 malaria positive cases, 4 (30.8%), 6 (26.1%) and 2 (33.3%) in age group ≤15, 16-30 and 31-45 were anemia positive respectively (Table 5) . In addition to this from the total *Plasmodium* infection prevalence of anemia became higher in male in the age group ≤15 and in female in the age group 16-30 (Table 5). This might be due to the fact that the prevalence and severity is determined by a number of interacting factors including: the species of the infecting parasite; the intensity of transmission (endemicity); sex, age and pregnancy status of the host; associated host genetic factors; and causes of anemia other than malaria, e.g. hookworm infections (Uganda Ministry of Health, 2010).

Table 5. Prevalence of malaria and anemia infection between different sex and age groups

Age	Sex	Total examined	Anemia positive %	Malaria positive %	Malaria and anemia positive%	Malaria positive and anemia negative %
≤15	Male	31	6(19.4)	9(29)	3(33.3)	6(66.7)
	Female	37	10(27)	4(10.8)	1(25)	3(75)
Total		68	**16(23.53)**	**13(19.1)**	**4(30.8)**	**9(69.2)**
16-30	Male	70	10(14.3)	14(20)	2(14.3)	12(85.7)
	Female	112	34(30.4)	9(8)	4(44.4)	5(55.6)
Total		182	**44(24.2)**	**23(12.6**	**6(26.1)**	**17(73.9)**
31-45	Male	43	6(14)	2(4.7)	0(0)	2(100)
	Female	65	17(26.2)	4(6.2)	2(50)	2(50)
Total		108	**23(21.3)**	**6(5.6)**	**2(33.3)**	**4(66.7)**
>45	Male	9	1(11.1)	0(0)	0(0)	0(0)
	Female	17	3(17.6%)	1(5.9)	0(0)	1(100)
Total		26	**4(15.4%)**	**1(3.8)**	**0(0)**	**1(100)**
Sexes	Male	153(39.8)	23(15)	25(16.3)	5(20)	20(80)
	Female	231(60.2)	64(27.7)	18 (7.8)	7(38.9)	11(61.1)
	Total	384(100)	**87(22.66)**	**43(11.2)**	**12(27.9)**	**31(72.1)**

4.5. Association of Major Risk Factors for Malaria Parasitic Infections of Examined Individuals in Alaba Health Center

As shown in Table 6. Analysis was done to assess the association between malaria infections with selected risk factors. From the total 384 sample participants who have provided blood samples, only 43 (11.2%) were infected with *plasmodium* parasite. Among the positives, 15(11.7%), 26 (10.9) and 2 (13.3%) were single, married and divorced respectively. In this study the malaria prevalence was not statistically associated with marital status (P=0.88) (Table 6).

Regarding educational status of study participant 30 (16.9%) illiterates, 8 (12.5%) who can read and write and 5 (5.4%) who completed primary education were infected with malaria (Table 6). In this finding large number of illiterate and who can read and write individuals were infected by malaria than literate. In the present study, statistically significant difference was observed between educational status of participants and occurrence of malaria infection (P=.004). In line with this, a multicenter study conducted in African region indicated that literacy was significantly associated with malaria incidence (WHO and RBM, 2002). Another study conducted in the Sudan also revealed that risk of malaria attack was significantly associated with grade of household education (El-Gayoum *et al*., 2009). Unlike earlier reports, finding from rural Tanzania demonstrated that educational level was not associated with malaria parasite infections (Somi *et al*., 2007).This can be explained by the levels of education which may affect treatment seeking and prevention behaviors of infected individuals.

According to the study result, out of 384 total study participates 17 (13.1%) with monthly income 150-500 EB, 20 (15.2%) with monthly income 501-1000 and 6 (6. 6%) with monthly income 1001-1500 EB were infected with malaria. In this finding, monthly income was significantly associated with the occurrence of malaria infection (P=.039)(Table 6). In agreement to this various Studies revealed that poor people are at increased risk of becoming infected with malaria; possible explanation for this observation is that poor people are less likely to be able to pay either for effective malaria treatment or to afford for purchasing insecticide-treated nets (WHO-UNICEF, 2004)

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Table 6. Association of major risk factors for malaria parasitic infections of examined Individuals in Alaba Health Center.

Risk factor		Total no of examined	Total no of malaria positive	χ^2	DF	P value
Marital status	Single	127	15(11.8%)	.639	3	.887
	Married	238	26(10.9%)			
	windowed	4	0(0%)			
	Divorced	15	2(13.3%)			
Educational status	No education	178	30 (16.9%)	15. 21	4	. 004
	Read and write	64	8 (12.5%)			
	Primary education	92	5 (5.4%)			
	Secondary education	33	0 (0%)			
	Higher education	17	0 (0%)			
Occupation	Farmer	151	23(15.2 %)	8.269	6	.219
	Merchant	106	11(10.3%)			
	Student	63	5(7.9%)			
	Employees	27	0(0%)			
	House wife	22	1(4.5%)			
	Has no job	3	0 (0%)			
	Daily labor	12	2(16.7%)			
Residence	Urban	102	4(3.9%)	7.39	3	.007
	Rural	282	39(13.8%)			
Monthly income	151-500	130	17(13.1%)	8.386	3	.039
	501-1000	132	20(15.2%)			
	1001-1500	91	6(6.6%)			
	>1500	31	0 (0%)			
ITN possession and utilization	Yes	233	19(8.2%)	5.519	1	.019
	No	151	24(15.9%)			

DF=degree of freedom χ^2=chi square, Significant difference at 0.05 level

Concerning occupation, the sample participants were categorized into seven groups. These were farmers 151 (39.3%), merchants106 (27.6%), students 63 (16.4%), employees 27 (7%), house wife 22 (5.7%), has no job 3 (0.8%) and daily laborers 12 (3.1%).

Among these, 23 (15.2%) of farmers, 11 (10.3) merchants, 5 (7.9%) students, 1 (4.5%) house wife and 2 (16.7%) daily labor participants were positive for malaria. The association of malaria infection with occupation of the sample populations was not statistically significant (Table 6). Similarly, reports made from Thailand (Sudathip, 2002) and Vietnam (Barker, 2002) pointed that no significant relationship was shown between occupation and malaria prevalence. In contrast to these occupations may bring people into contact with infected *Anopheline* mosquitoes (Pat *et al.*, 2005). Report made from the Sudan indicated that risk of malaria attack was significantly associated with occupation of household (El-Gayoum *et al*., 2009). In addition to this the study also revealed that out of 384 sample participants, 39 (13. 8%) from rural and 4(3.9%) from urban were infected with malaria. The malaria prevalence was higher in the rural area than the urban area. The association of malaria with the residence was also statistically significant (P=0.007) (Table 6). Similarly study conducted by Coene (1993) has shown that, generally malaria transmission is negatively associated with urbanization inKinshasa.This is mainly due to overcrowding, elimination of breeding sites due to construction or pollution of the breeding places resulting in lower vector densities in urban centers.In relation to the presence of livestock, 6 (12.28%) of the respondents who keep live stock in their house and 36 (11.01%) who do not keep live stock in their house were malaria positive (Table 6). This finding also revealed indirect relationship between keeping live stock and malaria infection observed. In agreement to this finding, a study conducted in Burkinafaso keeping domestic animals in the house was not associated with either an increased or decreased risk of malaria (Shelby *et al*., 2009).Apart from this study conducted in northern and southern Ethiopia, keeping cattle in the household compound was found to be a risk factor for the occurrence of malaria (Franco *et al*., 2008). Similar observations were recorded in the Ziway and Adami Tulu, were the highest prevalence of malaria was reported among individuals who kept cattle in the house (Wakgari *et al*., 2007).The possession and utilization of ITN from the total 384 study participants have showed that, 19 (8.2 %) participants who have and use ITN and 24 (15.9%)of participants who have no ITN were malaria positive .In the present study, possession of ITNs was associated with a lower risk of malaria infection (Table 6). In line with this, a study conducted in Ethiopia demonstrated that mosquito net ownership in the study population was associated with a low prevalence of febrile illness in children (Wakgari *et al*., 2007). Similarly, in Benin (Nahun *et al*., 2010) found that bed net use

was associated with a lower of malaria infection. Unlike this, Study conducted in rural Tanzania investigated that use of mosquito net the night before survey was negatively and significantly associated with malaria parasite infection (Somi *et al*., 2007). This discrepancy can be explained by improper use of ITNs and nature of socio-economic activity of the study population. There were a number of malpractices like not using the nets at night.

4.6. Association of Major Risk Factors for Anemia of Examined Individuals in Alaba Health Center

As indicated on Table 7 the overall 87 (22.65%) anemia prevalence was obtained from the total 384 study participants., in which, anemia prevalence was higher among married 56 (23.5%) participants than 28 (22.2%) single (Table 7). However it is not significant (P=.829). Similarly study conducted among women showed that anemia was also found to be more prevalent amongst married mothers (97.18%) than single women (2.82%), but this relationship was not statistically significant (Ndukwu *et al*,. 2012).

The educational status of 178 (46.4%) of the sample participants was illiterate, out of this 51 (28.7%) of them were anemic, whereas out of 92(24%) participants who have completed primary schooling 20 (21.7%) were anemic.On the other side only 1 (5.9%) participants who has completed higher education was anemic. This was statistically significant (P=0.034) (Table 7). Illiterate individuals were highly infected with anemia than literate ones. The findings of this study were in line with the results of a study conducted in a primary health centre in Rivers state, Nigeria (Ndukwu *et al*., 2012), which found a statistically significant association between the severity of anemia and educational status.

The prevalence of anemia ranges from a level of 30 (25.4%) in the 150-500 monthly income groups to 3(6.5%) among the richest wealth index group (Table7). It is evident from this study prevalence of anemia declines with increase in income status, however the linear trend analysis through chi^2 test showed that there was no statistically significant trend of association between monthly in came and prevalence of anemia (P= 0.06). The observation of the present study was in line to the study reported by Kapur *et al*. (2002) and (Ndukwu *et al.*, 2012), this study has shown that the prevalence of anemia was observed to be increasing as the socio-economic status reduced. And also anemia infection was significantly associated with socio-

economic status.The prevalence of anemia also differ with respect to occupation of study participants34 (24.5%) farmers, 22 (20.8%)merchants,12 (19.%) students,3 (11.1%)employees ,11(50%) housewife and 2 (16.7%) daily labors were anemic .It was statistically significant (P=0.35)(Table 7). On the other hand out of the total 384 examined individual 70 (24.8%) of r ural residents and 17 (16.7.8%) of urban residents were anemic. There was no statistically sign ificant difference of anemia infection with places of residence (P=.092) (Table 7). In contrast with this Manmeetkaur and Kamaljit (2009) also conducted a similar kind of study in an urban area of Chandigarh and as per their result the overall prevalence of anemia among reproductive age women was 73.3%.

Regarding anemia infection with utilization of ITN participants, 42 (18 %) participants who have and use ITN and 45 (29.8 %) of participants who have no ITN were anemia positive (Table 8). In the present study, utilization of ITNs was associated with a lower risk of anemia infection (P=.007) (Table 7).In line with this, ITN use was significantly associated with moderate-to-severe anemia in countries grouped by low national-level ITN use (<20%), as well as in countries grouped by high national-level ITN use (>20%). In pooled analyses of countries with more than one survey, ITN use increased significantly and prevalence of moderate-to-severe anemia decreased significantly (Ye et al. 2007). In unadjusted models, significant protective effects of ITNs were seen in Benin 2001, Benin 2006, Cameroon 2004, DRC 2007, Ethiopia 2005, and Tanzania 2004-05 .Associations were marginally significant in Malawi 2010 and Niger 2006. A marginally significant inverse association was seen in Madagascar 2008-09, in which ITN use was associated with increased odds of anemia (WHO, 2011).

Table 7. Association of major risk factors with prevalence of anemia of examined individuals in Alaba Health Center

Risk factor		Total no of examined	Total no of anemia positive	χ^2	DF	P value
Marital status	Single	127	28(22.2%)	.887	3	.829
	Married	238	58(23.5 %)			.
	windowed	4	1(25%)			
	Divorced	15	2(13.3%)			
Educational; status	No education	178	51(28.7%)	10.44	4	. 034
	Read and write	64	12(18.8%)			.
	Primary education	92	20 (21.7%)			
	Secondary education	33	3(9.1%)			
	Higher education	17	1(5.9%)			
Occupation	Farmer	151	37(24.5%)	13.545	6	.035
	Merchant	106	22(20.8%)			.
	Student	63	12(19%)			
	Employees	27	3(11.1%)			
	House wife	22	11(50%)			
	Has no job	3	0 (0%)			
	Daily labor	12	2(16.7%)			
Residence	Urban	102	17(16.7%)	2.844	1	. 92
	Rural	238	70(24.8%)			
monthly income	151-500	130	30(23.1%)	3.524	3	.060
	501-1000	132	31(23.5%)			
	1001-1500	91	(25.3%)			
	>1500	31	3 (9.7%)			
ITN possession and utilization	Yes	233	42(18%)	7.25	1	.007
	No	151	45 (29.8%)			

DF=degree of freedom, χ^2 = chi square

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

Malaria is still a serious threat to individuals and burden on the economic and health systems of highly affected countries. Globally, the annual disease burden in endemic countries is estimated as 225 million clinical cases and 781,000 deaths with the greatest impact in sub-Saharan Africa (WHO, 2010 b). In Ethiopia over 50 million (60%) of the 84.2 million people are exposed to malaria as of 2014, generally at elevations below 2,000 meters above sea level (RBMI, 2015). The main objective of the study was to assess the prevalence and association of malaria and anemia among patients visiting Alaba Health center, Alaba kulito town. The design of the study was health center based laboratory investigation. Additionally, structured and pre-tested questionnaires were administered to randomly selected patients visiting Alaba Health Center to assess the socio-demographic characteristics and the level of knowledge and awareness of the respondents related to malaria infection.

To determine the prevalence of malaria and anemia cases blood samples were taken from 384 participantes by finger-prinking using safety lancet. Thick and thin blood smears were prepared and examined microscopically after staining with 3% Giemsa solution for malaria and a drop of blood samples were also used to determine blood hemoglobin level using hematology Analyzer (Mission *Hb*).

Major *Plasmodium* species detected with microscope were *P. vivax* and *P. falciparium* with the prevalence of 9.9% and 1.3% in the sample participants respectively. Although malaria infection was observed in all age groups, a relatively higher prevalence (19.11%) of *Plasmodium* species infections was detected in the age groups of ≤15 years. In other age groups *plasmodium* infections were detected 23 (12.6%), 6 (5.6%), and 1 (3.8%) in the age groups, 16-30, 31-45 and >45 respectively, the difference was statistically significant.

Regarding hemoglobin concentration and prevalence of anemic condition, by WHO criteria, Anemia is defined by a Hgb concentration less than 11 g/dl (in children < 6 years), less than 12 g/dl (in children 6-14 years and in female adults above the age of 14 years) and less than 13 g/dl in male adults above the age of 14 years while severe anemia is defined by a Hgb

concentration of less than 7 g/dl (WHO, 1989). Hemoglobin concentration and prevalence of anemic condition in examined 384 individuals in Alaba Health Center, during November–December, 2015 was 87 (22.65%) .The results also showed no statistically significant difference of malaria and anemia infection (X^2=0.76, P =0.383).

5.2. Conclusion

Based on the findings from survey the following conclusions may be drawn about the malaria and anemia situation in Alaba kulito town:

- The two *Plasmodium* species namely P. *vivax* and *P. falciparum* were the species that cause malaria in Alaba Health Center, Alaba town.
- A total of 43 (11.2%) malaria positive ,87 (22.65%) anemia positive and 12 (3.125%) anemia and malaria positive individuals were obtained from 384 study participantes .
- Prevalence of malaria was high among male 25 (16.3%) and anemia 64 (27.7%). among female and high 13 (19.1%) prevalence of malaria and anemia 44(24.2%) obtained in age groups of ≤15 and 16-30 respectively.
- The results also showed no statistically significant difference of malaria and anemia infection (X^2=0.76, P =0.383).

5.3. Recommendation

- Further study are still needed to assess the relative impact of other etiologic factor such as nutrition, pregnancy or parasitic diseases on anemia mainly for children , pregnant and reproductive age women.
- Government should fosse on simultaneous combat against malaria infections and improving the quality and accessibility of malaria diagnosis and treatment service in health care system to reduce relative burden of malaria and its impact on the development of anemia.
- Government should focus to wards reducing the prevalence and risk of anemia infection by creating awareness ,facilitate diagnosis and treatment service and

accessing the local etiologic factor of anemia in a given setting specially for children and reproductive age women

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7. APPENDIX

Appendix 1.Questionnaire

Part 1. Socio -demographic characteristics of study participants

1. Sex Male_____ Female_____

2. Age_____

3. What is your marital status? A/ Single ----- B/ Married ----- C/ Widowed --

D/Divorced/separated -----

4. What is your residence? A. Rural B. Urban

5. What is your educational status?

A. No education ---- B. Only read and writes ------

C. Primary education ----- D. Secondary education---- E. Higher education -----

6. What is your occupation?

A. Farmer____ B. Merchant _____ D. Student____ E. Employee

F. House wife____ G. Daily laborer____ H. Has no job____ I

7. What is your monthly income?

A.151-500….. B.501-100 …… C.1001-1500 …….. D.>1500 …….

Part 2. laboratorial examination result recording format

1. Malaria parasitima A .positive…….. B. negative……..

If positive, *plasmodium* species is? A .*Plasmodium vivax* ☐ B. *Plasmodium falciparum* ☐

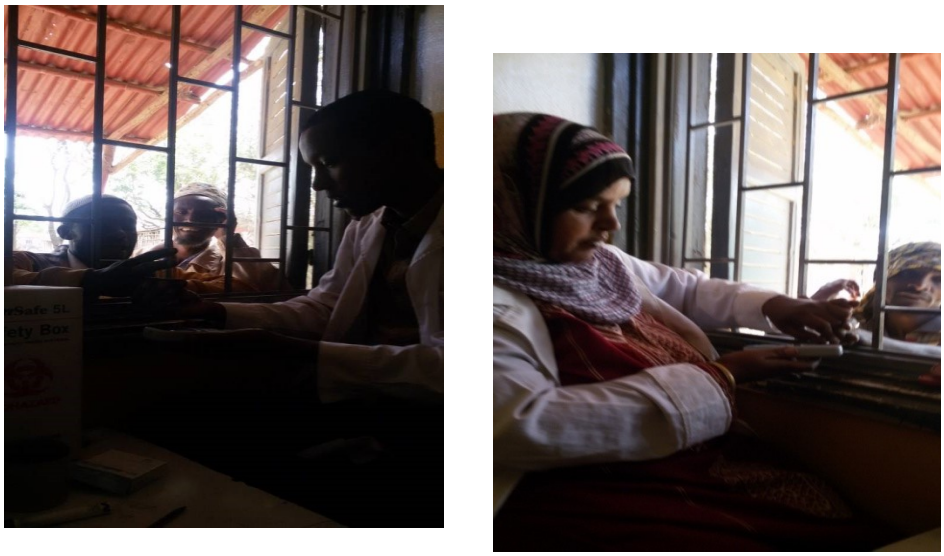
2 Hemoglobin concentration ……

A. Anemic……..

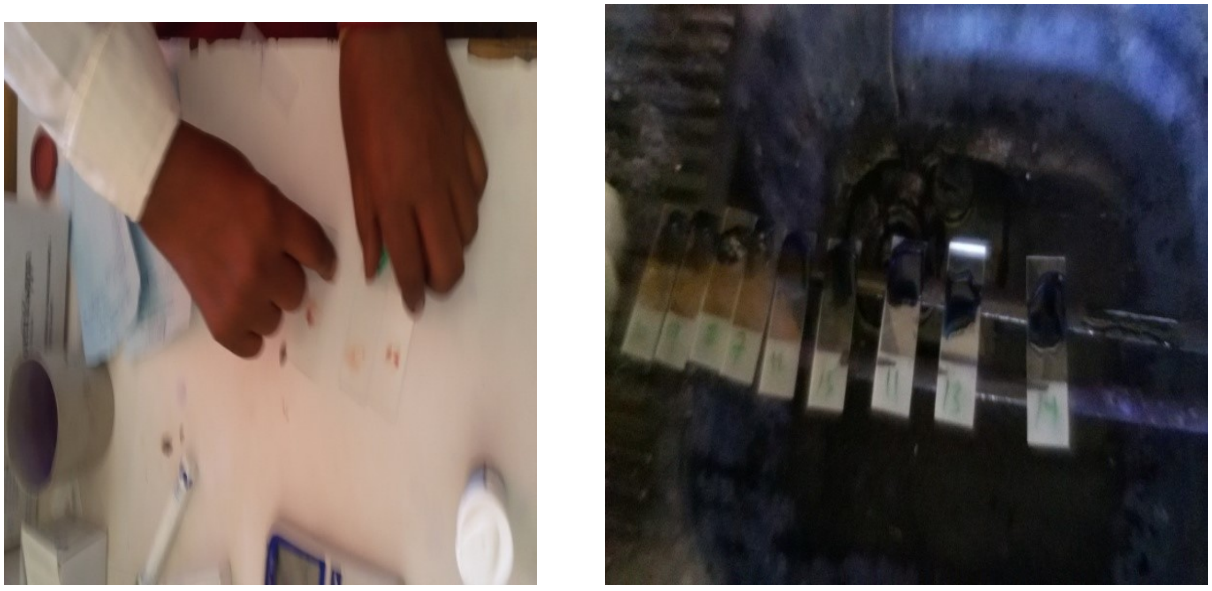
B. Normal……

Appendix II. Figures

Appendix Figure1. Blood sample collection from each participant by puncturing their finger-tips using sterilized disposable blood lancet with the help of a laboratory technician.



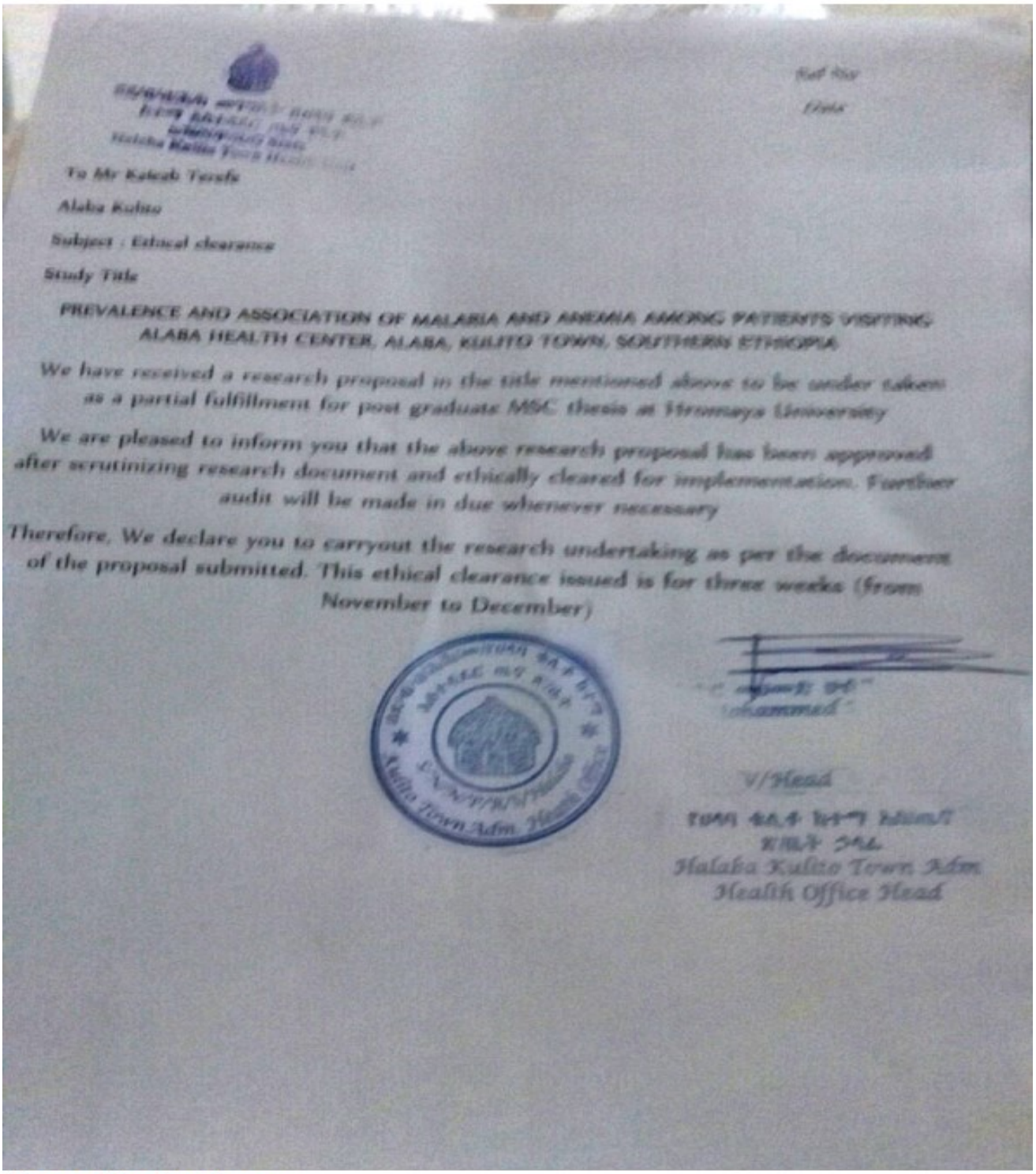
Appendix Figure 2. Preparation of thick and thin blood films on class slides and staining for detection of malaria parasite and *Plasmodium* species



Appendix Figure 3. Blood sample used for hemoglobin concentration determination using hematology analyzer (Mission *Hb*)

Appendix Figure 4. Microscopic examinations of thick films using high power magnification for the presence of parasites and parasite species identification using thin films under 100x oil immersion objective lens.

Appendix III. Ethical Clearance



To Mr Kaleab Tesfa

Alaba Kulito

Subject : Ethical clearance

Study Title

PREVALENCE AND ASSOCIATION OF MALARIA AND ANEMIA AMONG PATIENTS VISITING ALABA HEALTH CENTER, ALABA, KULITO TOWN, SOUTHERN ETHIOPIA

We have received a research proposal in the title mentioned above to be under taken as a partial fulfillment for post graduate MSC thesis at Hromaya University

We are pleased to inform you that the above research proposal has been approved after scrutinizing research document and ethically cleared for implementation. Further audit will be made in due whenever necessary

Therefore, We declare you to carryout the research undertaking as per the document of the proposal submitted. This ethical clearance issued is for three weeks (from November to December)

V/Head

Halaba Kulito Town Adm
Health Office Head