



**SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
DEPARTMENT OF BIOLOGY**

**PEREVALANCE OF MALARIA AND ASSOCIATED FACTORS AMONG
PATIENT ATTENDING IN GUANGUA HEALTH CENTERE, ABAYA
WORDA, WEST GUJI ZONE.**

M.Sc. Thesis

BY

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Dilla, Ethiopia

**PEREVALANCE OF MALARIA AND ASSOCIATED RISK FACTOR
AMONG PATIENT ATTENDING IN WEST GUJI ZONE, ABAYA
WOREDA GUANGUA HEALTH CENTERE.**

**A THESIS SUBMITTED TO: SCHOOL OF GRADUATE STUDIES, DILLA
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I under signed, declare that this thesis is my original work and has not been presented for any or prize degree in any other university and all sources of materials or reference used for the thesis have been fully acknowledged.

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DEDICATION

This thesis is dedicated to my family for their persistent and boundless love, patience and strength that helped me to complete my MSc study.

BIOGRAPHICAL SKETCH

The author was born on May 4, 1986 E.C in West Guji Zone Abaya woreda Bunata Kebele, Ethiopia. She started her primary education at Odomike Primary School. She completed her secondary education at Guangua Secondary and Preparatory School in 2003 E.C. After completion of her Preparatory school education, she joined Mizan Teppi University, Biology Department in 2004-2006 E.C and had been awarded BSc degree in Applied Biology in 2006 E.C. Finally, she joined postgraduate program, Biomedical Science, Dilla University, 2011 E.C.

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ABBREVIATION

ACT	Artemisinin-based combination therapy
AL	Artemether-lumefantrine
AOR	Adjusted odds ratio
API	Annual parasite Incidence
CDC	Centre for Disease Control
CCM	Community-based Case Management
COR:	Crude odds ratio
CI:	Confidence interval
DALYs	Disability Adjusted Life Years
DBL-EBP	Duffy binding-like erythrocyte-binding protein
DDT	Dichloro-diphenyl-trichloroethane
DEET	Diethyltoluamide (insect repellent)
ENMIS	Ethiopia National Malaria Indicator Survey
FMoH	Federal Ministry of Health
GDP	Gross domestic product
HEWs	Health extension workers
IFA	Immunofluorescence antibody testing
IMNCI	Integrated management of neonatal and childhood Illness
IRS	Indoor residual spraying
ITN	Insecticide- treated mosquito nets
LLITNs	Long lasting insecticide-treated net
MOP	Malaria operational plan
PLDH	Plasmodium lactate dehydrogenase
PLos	Public library of science

RDT	Rapid diagnosis test
SP	Sulfadoxinpyrimethamine
TRAP	Thrombospondin- related a anonymous protein
WHO	World Health Organization

ABSTRACT

*Malaria is one of the most severe public health problems in the world today. It is caused by the infection of parasitic protozoa belonging to the genus Plasmodium. The main objective of this study was to assess the prevalence of malaria and associated risk factor among patients attending Guangua health centre. A cross-sectional study was conducted from September to October, 2023. Questionnaire was applied to get data about socio-demographic characteristics, Insecticide-treated bed nets condition and availability, Insecticide-treated bed nets utilization, Indoor residual spray, presence of stagnant water, outdoor stay at night, housing condition, and health information about malaria were collected by using structured questionnaire. Blood samples were collected from 227 systematically selected patients. Thin and thick blood smears were prepared, stained with 10% Giemsa and examined under light microscope for the presence of malaria parasite. Bivariate logistic regression analysis was used to assess the association of malaria cases with socio-demographic variables. The overall malarial prevalence in the study area was 33 (14.5%) with prevalence in the rural area significantly higher than urban ($p=0.006$) in which 23(10.1%) and 10(4.4%) malaria positive individuals in the rural and urban areas respectively. Malaria prevalence in males were higher than females ($p=0.05$) with the prevalence rate of 9.7% and 4.8% in males and females respectively. *P. falciparum* appeared to be the dominant Plasmodium species followed by plasmodium vivax and mixed. The study demonstrated that malaria remains a public health burden in the area. This would be an important indicative that the area needs due attention and further concerted malaria interventions. Therefore, community mobilization should be strengthen in order to improve implementation of malaria control activities and, hence, reducing the prevalence.*

Key words: *Plasmodium, prevalence, risk factor, prevention*

1. INTRODUCTION

1.1. Back ground of the study

Malaria is one of the most severe public health problems in the world today. It is caused by the infection of parasitic protozoa belonging to the genus *Plasmodium* with well-known five species: *P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae* and rarely *P. knowlesi* (Perrin *et al.*, 1984). It is one of the most severe public health problems worldwide with 300 to 500 million cases and about one million deaths reported, 90% of which were reported from Sub Saharan African countries (WHO, 2019).

According to WHO (2021) malaria report, an estimated 3.3 billion people are at risk of malaria, of which 1.2 billion are at high risk. In Ethiopia, 75% of the land are malaria's areas and An estimated 52% of the population is at risk of malaria infection (FMoH, 2020). *P. falciparum* and *P. vivax* are commonly known species in Ethiopia which cause malaria accounting for 60% and 40%, respectively (Tarekegn *et al.*, 2021). *P. malariae* and *P. ovale* are uncommon and account for <1% of confirmed malaria cases (Tafesse *et al.*, 2018).

The control of malaria and its *anopheline* vectors in Africa is less successful because of the occurrence of drug resistant of the parasites and insecticide resistant of the vectors in addition to change in the resting behavior of mosquitoes (from indoor to outdoor). As a result of frequent indoor insecticide sprays, lack of efficient infrastructure, shortage of trained man power, lack of equipment, lack of appropriate management, financial constraints and inability to integrate several methods it became hard to control malarial infection (Howard *et al.* , 2007).

Important risk factors such as house proximity to mosquito breeding sites or water drain, and malaria preventive measures at the household level have been identified in different studies. Additional factors such as number of bed nets per household, individual's age and residence, altitude, household wealth and peak monthly rainfall can affect malaria prevalence in Ethiopia (Tarekegn *et al.*, 2021).

The research done at Gedeo Zone Dilla Town, stated that highest prevalence of malaria found in the age groups between 15 and 24, because of high outdoor exposure to the mosquito vector bites, Living in the nearby stagnant water which exposed them to mosquito bites, because these

areas are suitable for breeding of mosquitoes around their homes (Molla and Ayele, 2015). There is scarcity of information about prevalence of malaria and their socio-demographic burdens in west Guji Zone Abaya District, Guangua kebele since there is no previous study done in the study area. Therefore, the objective of this study will be to fill the gap assessing the prevalence of malaria and associated risk factor among patient attending in Guangua health centre and to identify factors that contribute to the presence and severity of malaria.

1.2. Statement of the problems

Malaria is one of the most severe public health problems worldwide with 300 to 500 million cases and about one million deaths reported in the last decade, 90% of which were reported from Sub Saharan African countries (WHO, 2019). It is the fourth leading cause of death among children under the age of five years after pneumonia, non-bloody diarrhea and acute febrile illness in developing countries (Lo *et al.*, 2017). It is one of the major diseases of poor people in developing countries and one of the leading causes of avoidable death, especially in children and pregnant women (WHO, 2014).

Currently, malaria become one of the major tropical diseases adversely affecting the health of the peoples and the economic development of many developing countries (WHO, 2021). It is one of the main public health problems and causes of morbidity and mortality in Ethiopia (Tadele *et al.*, 2019). Despite the current efforts to control malaria in Ethiopia, the situation has not improved mainly due to the increasing problems of parasite resistance to the relatively cheaper anti-malarial drugs, vector resistance to insecticides, low coverage of malaria preventive services, poor access to health care and people living conditions (FMoH, 2019).

In Abaya Woreda malaria control measures such as the use of Artemisinin Combine Therapy (ACT), the use of insecticide treated bed net (ITNs), indoor residual spraying of insecticide (IRS) have been implemented. Despite of all these efforts yet malaria is the public health problem of the town. This verifies that there could be several reasons for this situation including the deficiencies in the health System that leads to lack of access to malaria control interventions. Therefore, the study will determine the prevalence of malaria patient attending in Guangua Health centre by identifying different potential factors, such as socio-economic, demographic, nutritional and other correlated factors in the study area.

1.3 Research Questions

In order to fill the gap in the objectives the following research question have been developed:

1. What are the risk factors that contribute to malaria disease in Guangua Health Center?
2. In what proportion malaria patients are attending Guangua Health Center for treatment?
3. What is the extent of infection of malaria species (*P.falciparum* and *P.vivax*,) in Guangua Health Center?

1.4. Objectives

1.4.1. General objective

The general objective of this study was to assess the prevalence of malaria and associated risk factor among patient attending in Guangua health centre, Abaya woreda West Guji zone.

Specific objectives

- To determine the prevalence of malaria disease among patients who attend Guangua health centre.
- To identify risk factors that contribute the presence and severity of malaria in the study area.

1.5. Significance of the Study

The result of this study is believed to benefit heath workers, the community and other researchers in the following ways:

It might help health professionals to be aware of the current prevalence of malaria among patients.

It might help community to have improved health and better awareness to be free from malaria.

Future researchers would also find the result of this study useful to conduct further studies.

1.7. Scope of the Study

Since malaria has caused a great health problem on community, the aim of this study was to identify the prevalence rate among patients attending in Guangua health center. The focus of this study was only on the prevalence of malaria because it is difficult for the investigator to cover all aspects of infection.

1.8. Organization of the Study

This study was organized in to five parts. The first part deals with the introductory part of the study that consists of background of the study, statement of the problem, objective of the study, significance of the study, scope of the study and definition of operational terms. In the second part, review of related literature from different recent sources was stated. In this part, epidemiology, morphology and life cycle, transmission, risk factors for transmission, pathogenesis and clinical manifestation, laboratory diagnosis, treatment and prevention method were discussed. Part three dealt about methodology used for this research. In this part, description of the study area, study design and period, study population and source, sample size and sampling technique, study variables, method of data collection including instruments and reagents to be used, quality control, method of data analysis and interpretation, ethical clearance were dealt about. In part four the result and discussion of the study were discussed. Part five was dealt about the conclusion and recommendation of the study. In the last two parts were the references and appendices of the study.

2. LITERATURE REVIEW

2.1. The Overview of Malaria

The word malaria comes from the Italian mal'aria meaning spoiled air (Garnham, 1963). Scientific studies only became possible after the discovery of the parasites by Charles Louis Alphonse Laveran in 1880 and the incrimination of mosquitoes as the vectors first for avian malaria by Ronald Ross in 1897 (Lawn *et al.*, 2016). Malaria is caused by infection with protozoan parasites belonging to the genus *Plasmodium* transmitted by female *Anopheles* species mosquitoes. Malaria parasites begins in 1880 with the discovery of the parasites in the blood of malaria patients by Alphonse Laveran (Cox, 2010). The sexual stages in the blood were discovered by William MacCallum in birds infected with a related haematozoan, *Haemoproteus columbae*, in 1897 and the whole of the transmission cycle in culicine mosquitoes and birds infected with *Plasmodium relictum* was elucidated by Ronald Ross in 1897 (Garnham, 1963). In 1898 the Italian malariologists, Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava demonstrated conclusively that human malaria was also transmitted by mosquitoes (Cox, 2010).

Malaria is a life threatening disease caused by parasites that are transmitted to people through the bites of infected female mosquitoes (Zerihun *et al.*, 2007). Most deaths are caused by *P. falciparum* because *P. vivax*, *P. ovale* and *P. malariae* generally cause a milder form of malaria. The species *P. knowlesi* rarely causes disease in humans (WHO, 2017). About 3.2 billion people almost half of the world's populations are at risk of malaria (WHO, 2018). Young children, pregnant women and non-immune travelers from malaria-free areas are particularly vulnerable to the disease when they become infected. Malaria is preventable, curable and increased efforts are dramatically reducing the malaria burden in many places (Simon *et al.*, 2017).

Between 2000 and 2015, malaria incidence among population at risk (the rate of new cases) fell by 37% globally (WHO, 2016). In that, the same period malaria death rates among populations at risk fell by 60% globally among all age groups and by 65% among children under five years. Sub-Saharan Africa carries a disproportionately high share of the global malaria burden (WHO, 2017).

2.2. Malaria in Ethiopia

About 75% of the land and 60% of the population is exposed to malaria in Ethiopia (Berhe *et al.*, 2019). Ethiopia is generally, considered as a low-to-moderate malaria transmission intensity (Tarekegn *et al.*, 2021). Due to the unstable and seasonal transmission of malaria in the country, protective immunity of the population is generally low and all age groups are at risk. Prevalence of malaria currently estimated to be 1.3 (FMoH, 2021).

Malaria poses no risk to inhabitants at altitudes above 2000 meters, mostly in the Northern and Eastern Highlands (Yared *et al.*, 2007). The lower elevations of the Northern and Eastern Highlands are also known areas of malaria endemicity (FMoH, 2018). The western, central and eastern highlands, as well as the highland-fringe areas along the Rift Valley are especially vulnerable to epidemics (Alelign *et al.*, 2018).

The Dega zone of Ethiopia (altitude above 2,500 meters) with a mean annual temperature of 1015 degree Celsius are malaria-free. Much of the Woina Dega zone (Altitude 1500 – 2500 meters) is also malaria free, especially the zone in the 2000 – 2500 meters above sea level (FMoH, 2018). Malaria in Ethiopia often occurs below 2000 meters, with short-lived transmission following the rains (Legesse *et al.*, 2007). However, malaria epidemics have been recorded up to 2400 meters during periods when increased temperature and adequate precipitation are conducive for both vector survival and parasite development within the vector (Tamiru *et al.*, 2014).

2.2.1. History of Malaria Epidemics In Ethiopia

Malaria epidemic occurred in most highland as well as lowland areas in the country. Many localized but severe outbreaks of malaria occurred in Oromia, Amhara and SNNP Regional States, leading to widespread epidemic malaria in highland and highland fringe areas (Aschalew and Tadesse, 2016).

The epidemiology of malaria in Ethiopia is well described in national documents demonstrating the threat to larger number of the population from both *P. falciparum* and *p. vivax*; the major *Anopheles arabiensis* vector and the high variability across different transmission strata. This variability is produced in part by geography and climate and in part by recent scale up of control

measures (Legesse *et al.*, 2007). This variability requires that the country address very different situations with prevention and control tools, but it also provides the opportunity to actively create and extend malaria free areas (Nigatu *et al.*, 2014). Malaria is pervasive to Ethiopia; 70% of the landscape areas below 2000 m above sea level is malarious which is fertile low land areas and suitable for agriculture. More than 54 million populations live in these areas and are at risk of malaria. The transmission is unstable and seasonal from September to December and April to May, which is coincide with major planting and harvesting season for farmers (Nigatu *et al.*, 2014).

2.2.2. Causes of Malaria Epidemics

Malaria epidemics can occur because of variability or changes in the rate of infection and population immunity. Generally, epidemics occur in places where there is low and unstable malaria transmission, and where people have low or no immunity (Esayas *et al.*, 2020). However, there could be epidemics in high transmission areas if there is deterioration of health system, interruption of anti-malarial measures or migration of non-immune individuals, such as population movement in search of labor to these areas (Yemane *et al.*, 2006).

Other triggering factors include unusual local weather phenomena and activities resulting in environmental modification that increase vector population; Increased vulnerability of population from famine and malnutrition; Interruptions of anti-malarial measures which have kept malaria under control (Tadele *et al.*, 2019).

2.3. The Malaria Vector (The Anopheles Mosquito)

Temperature and humidity have a direct effect on the longevity of the mosquito. Each species can thrive at an optimal level as a result of ecological adaptation. The spread of malaria requires that conditions are favorable for survival of both mosquito and the parasite. Temperatures from approximately 21°-32°C and relative humidity of at least 60% are most conducive for maintenance of transmission (FMoH, 2021). In tropical regions, temperature and humidity are often mediated by altitude. Altitude is significant in determining the distribution of malaria and its seasonal impact on many regions of the world. In Africa, for example, altitudes above 1,000-1,500m are considered safe from malaria (WHO, 2021).

The mosquito density (number of female mosquitoes per human inhabitants) is a critical determinant of the intensity of infection (Yemane *et al.*, 2006). Transmission is directly proportional to density. Mosquito longevity is also a critical factor. The malaria vector requires water to complete its life cycle: egg, larva, pupa, and the adult. While between 200-1000 eggs can be laid, the quantity is influenced by the amount of blood taken in. Blood feeding usually starts at dusk and continues until dawn (Ashley *et al.*, 2008).

2.4. Some Malaria Transmission Enhancing Factors

Malaria transmission intensity, along with its temporal and spatial distribution in Ethiopia is determined by the diverse eco-climatic conditions. Climatic factors such as temperature, rainfall and humidity show high variability mainly as a function of altitude and are the most important variables that influence malaria transmission (Tamiru *et al.*, 2014). Malaria transmission varies among communities largely due to environmental factors, such as proximity to breeding sites (Tirados *et al.*, 2006).

Many water resources development and management projects result in local outbreaks of malaria and other vector-borne diseases such as schistosomiasis, lymphatic filariasis and Japanese encephalitis (Legesse *et al.*, 2007). These outbreaks can be attributed to an increase in the number of breeding sites for mosquitoes, an extended breeding season and longevity of mosquitoes, relocation of local populations to high-risk reservoir shorelines and the arrival of migrant populations seeking a livelihood around the newly created reservoirs (Delenasaw *et al.*, 2009).

The following three factors affect malaria transmission and illness. Breeding sites: Anopheles mosquitoes need stagnant or slow-flowing bodies of non-contaminated water to use as breeding sites to lay their eggs. These sites which may increase during the rainy season include: Small ponds, ditches, pits, and canals, swamps, reservoirs, and rice fields, pools of water after rain, uncovered water tanks along the banks of slow-flowing streams, water-filled animal hoof prints and objects that collect water, such as empty tins and containers, parasites: Enough parasites must exist in the human population to infect the mosquito (FMoH, 2019). The Climate: The temperature must be an average of at least 18–20°C and the humidity above 60% for the mosquito to survive and for the parasite to develop and become infective and The warmer the

weather, the faster the development of the parasite. Parasites in the blood of an infected person are drawn into the stomach of the mosquito as it feeds (FMoH, 2018).

2.5. Life Cycle of *Plasmodium* species

The malaria parasite has a complex, multistage life cycle occurring within two living beings, the vector mosquitoes and the vertebrate hosts. The survival and development of the parasite within the invertebrate and vertebrate hosts, in intercellular and extracellular environment is made possible by a toolkit of more than 5000 genes and their specialized proteins that help the parasite to invade and grow within multiple cell type and to evade host immune responses (Laurence *et al.*, 2008). Mosquitoes are the definitive hosts for the malaria parasites where in the sexual phase of the parasites life cycle occurs. The sexual phase is called sporogony and results in the development innumerable infecting forms of the parasite within the mosquito that induce disease in the human host following their injection with the mosquito bite (Figure:1). When the female anopheles draws, a blood meal from an individual infected with malaria the male and female gametocytes of the parasite find their way into the gut of the mosquito. The molecular and cellular changes in the gametocytes help the parasite to quickly adjust to the insect host from the warm-blooded human host and then to initiate the sporogonic cycle (Carolina and Sanjeev, 2005).

The male and female gametes fuse in the mosquito gut to form zygotes, which subsequently develop into actively moving ookinates that burrow into the mosquito mid gut wall to develop into oocysts. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After the sporogonic phase of 8-15 days, the oocyst bursts and releases sporozoites into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary glands. When the mosquito thus loaded with sporozoites takes another blood meal, the sporozoites get injected from its salivary glands into the human blood stream, causing malaria infection in the human host (Tamiru *et al.*, 2014). It has been found that the infected mosquito and the parasite mutually benefit each other and there by promote transmission of the infection. The plasmodium infected mosquitoes have a better survival and show an increased rate of blood feeding, particularly from an infected host (Carolina and Sanjeev, 2005).

With the mosquito bite, tens to a few hundred invasive sporozoites are introduced into the skin, following the intradermal deposition some sporozoites are destroyed by the local macrophages, some enter the lymphatics, and some others find a blood vessel (Ashley *et al.*, 2008). The sporozoites that enter a lymphatic vessel reach the draining lymph node where in some of the sporozoites partially develop into exoerythrocytic stage (Ashley *et al.*, 2008) and may prime the T cells to mount a protective immune response (Michael and Denise, 2007).

The sporozoites that find a blood vessel reach the liver within a few hours. It has recently been shown that the sporozoites travel by a continuous sequence of stick-and-slip motility, using the thrombospondin-related anonymous protein (TRAP) family and an actin-myosin motor. The sporozoites then negotiate through the liver sinusoids, and migrate into a few hepatocytes, and then multiply and grow within parasitophorous vacuole. Each sporozoite develops into a schizont containing 10,000-30,000 merozoites (Kebaier, *et al.*, 2009).

A favorable environment created by the circumsporozoite protein of the parasite facilitates the growth and development of the parasite in the liver cells. The entire pre-erythrocytic phase lasts about 5-16 days depending on the parasite species: on an average 5-6 days for *P.falciparum*, 8 days for *P.vivax*, 9 days for *P. ovale*, 13 days for *P. malariae* and 8-9 days for *P. knowlesi* (Malcolm and Michael, 2006).

The merozoites that develop within the hepatocyte are contained inside host cell-derived vesicles called merosomes that exit the liver intact thereby protecting the merozoites from phagocytosis by Kupffer cells. These merozoites are eventually released into the blood stream at the lung capillaries and initiate the blood stage of infection thereon. In *P.vivax* and *P. ovale* malaria, some of the sporozoites may remain dormant for months within the liver. Termed as hypnozoites, these forms develop into schizonts after some latent period, usually of a few weeks to months. It has been suggested that these late developing hypnozoites are genotypically different from the sporozoites that cause acute infection soon after the inoculation by a mosquito bite and in many patients cause relapses of the clinical infection after weeks to months (Olivier *et al.*, 2008).

Red blood cells are the center stage for the asexual development of the malaria parasite. Within the red blood cells repeated cycles of parasitic development occur with precise periodicity and at

the end of each cycle, hundreds of fresh daughter parasites are released that invade more number of red blood cells. The merozoites released from the liver recognize, attach and enter the red blood cells (RBCs) by multiple receptor-ligand interactions in as little as 60 seconds. This quick disappearance from the circulation into the red cells minimizes the exposure of the antigens on the surface of the parasite there by protecting these parasite forms from the host immune response (William, 2007).

The invasion of the merozoites into the red cells is facilitated by molecular interactions between distinct ligands on the merozoite and host receptors on the erythrocyte membrane. *P. vivax* invades only Duffy blood group positive red cells, using the Duffy-binding protein and the reticulocyte homology protein, found mostly on the reticulocytes. The more virulent *P. falciparum* uses several different receptor families and alternate invasion pathways that are highly redundant. Varieties of duffy binding like (DBL) homologous proteins and the reticulocyte binding – like homologous proteins of *P. falciparum* recognize different RBC receptors other than the duffy blood group or the reticulocyte receptors (Tilley *et al.*, 2011). Such redundancy is helped by the fact that *P. falciparum* has four duffy binding-like erythrocyte-binding protein genes, in comparisons to only one gene in the DBL-EBP family as in the case of *P. vivax* allowing *P. falciparum* to invade any red blood cell (Ghislaine *et al.*, 2009).

The process of attachment, invasion and establishment of the merozoite into the red cells is made possible by the specialized apical secretory organelles of the merozoite, called the micronemes, rhoptries and dense granules. The initial interaction between the parasite and the red cells stimulates a rapid wave of deformation across the red cell membrane, leading to the formation of a stable parasite-host cell junction (Figure:1). Following this, the parasite pushes its way through the erythrocyte bi layer with the help of the actin myosin motor, proteins of the thrombospondin-related anonymous protein family (TRAP) and aldolase, and creates a parasitophorous vacuole to seal itself from the host cell cytoplasm thus creating a hospitable environment for its development within the red cell, at this stage the parasite appear as an intracellular ring (Laurence, *et al.*, 2002).

Within the red blood cells the parasite numbers expand rapidly with a sustained cycling of the parasite population. Even Though the red blood cells provide some immunological advantage to

the growing parasite, the lack of standard biosynthetic pathways and intra cellular Organelles in the red blood cells tend to create obstacles for the fast-growing intra cellular parasites. These impediments are overcome by growing ring stages by several mechanisms: by restriction of the nutrient to the abundant hemoglobin, by dramatic expansion of the surface area through the formation of a tub vesicular net work, and by export of a range of remodeling and virulence factors into the red blood cell (Olivier *et al.*, 2008).

Hemoglobin from the red blood cell, the principal nutrient for the growing parasite, is ingested in to a food vacuole and degraded. The amino acids thus made available are utilized for protein biosynthesis and the remaining toxic heme is detoxified by heme polymerase and sequestered as hemozoin (malaria pigment). The parasite depends on anaerobic glycolysis for energy, utilizing enzymes such as PLDH, *plasmodium* aldolase *etc.* As the parasite grows and multiplies within the red blood cell the membrane permeability and cytosolic composition of the host cell is modified (Virgilio *et al.*, 2003).

These new permeation pathways induced by the parasite in the host cell membrane help not only in the uptake of solutes from the extra cellular medium but also in the disposal of metabolic wastes, and in the origin and maintenance of electro chemical ion gradients. At the same time, the premature hemolysis of the highly permeabilized infected red blood cells is prevented by the excessive ingestion, digestion and detoxification of the host cell hemoglobin and its discharge out of the infected RBCs through the new permeation pathways, thereby preserving the osmotic stability of infected red blood cells (Kieran, 2001).

The erythrocyte cycle occurs every 24 hours in case of *P. knowlesi*, 48 hours in cases of *P. falciparum*, *P. vivax*, *P. ovale* and 72 hours in case of *P. malariae*. During each cycle, each merozoite grows and divides within the vacuole into 8-32 (average 10) fresh merozoites, through the stages of ring, trophozoite and schizont. At the end of the cycle, the infected red blood cells rupture, releasing the new merozoites that in turn infect more RBCs. With unbridled growth, the parasite number can rise rapidly to levels as high as 10¹³ per host (Brian, *et al.*, 2008). Figure 1 below shows the life cycle of malaria growth and development occurring within mosquito and human beings.

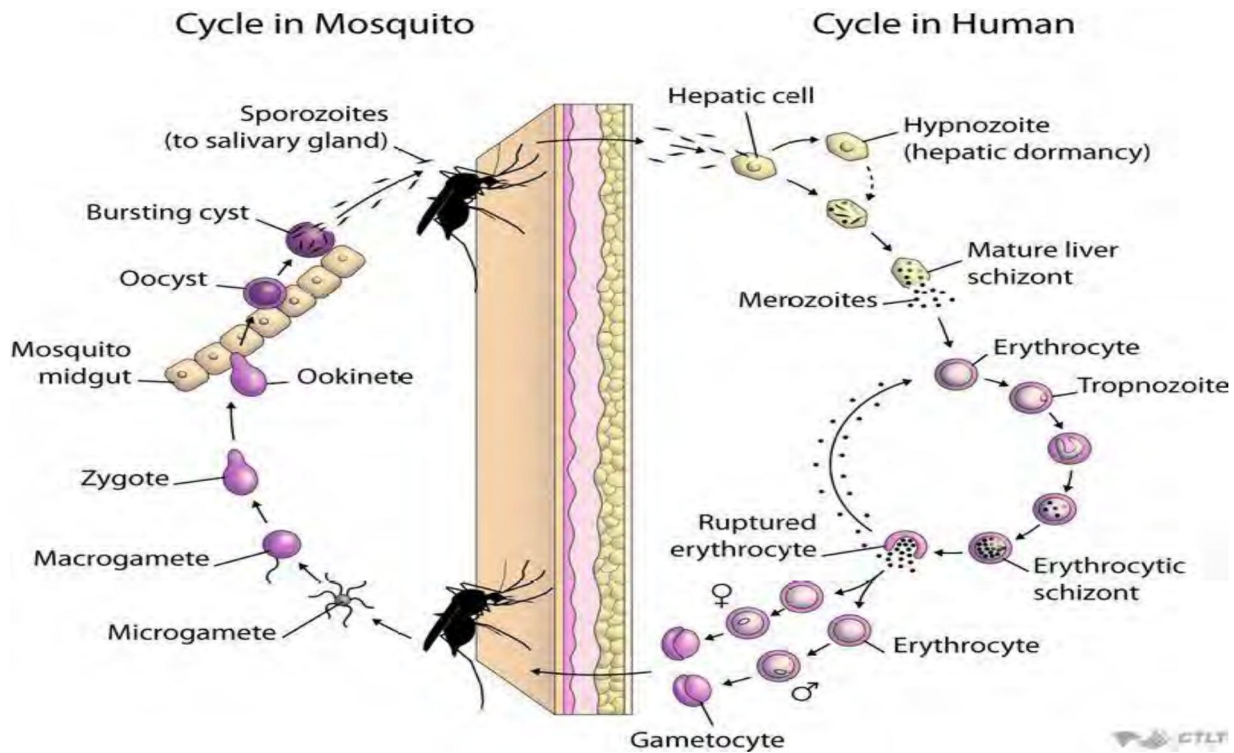


Figure 1: Complex, multistage life cycle of malaria growth and development occurring within mosquito and human (William, 2000)

2.6. The Role of Human Factors in the Spread of Malaria in Ethiopia

Human factors in Ethiopia contributing to the spread of malaria include population growth and movements, urbanization, water development schemes, agricultural development, conflicts, and improper use of drugs and the attendant consequences of the emerging drug-resistant malaria parasites (Yared *et al.*, 2007).

Seasonal migration from highlands to lowlands for agriculture work has increased since 1991 with the growth of large-scale agricultural development projects. Western lowland district populations have increased by 20-30% with the arrival of tens of thousands of agricultural workers during the harvest season, which is also the high transmission season for malaria (Fasil *et al.*, 2021). Laborers often work during the cooler evening hours when vector-biting rates are high, and often sleep in the fields. Land scarcity in the highlands resulted in establishment of new villages for settlement in western malarious lowlands (Ayele *et al.*, 2013).

The low level of urbanization in Ethiopia (16%) masks recent trends marked by substantial increases in urban growth rates. The chapter on migration and urbanization showed a number of localities with a growth rate of 6% (twice the national rate of population growth) or higher (Abebe *et al.*, 2011). Urban areas have also traditionally not been included in spray operations or bed-net distribution programs because of resource constraints and the good access of urban dwellers to health institutions. When favorable climatic conditions for transmission exist, the high urban population density, the presence of multiple breeding sites, the lack of indoor residual spray, and lower household bed-net coverage, allow for rapid spread of malaria (Ayele *et al.*, 2013).

Successive governments have sought to harness the power of Ethiopian rivers for electric generation as well as develop irrigation agriculture to counter the impacts of drought and the resulting famines. The Awash River Valley is the most notable for developments in the irrigation front. As in most tropical countries river-fed agriculture has also led to the resurgence of malaria even in areas where its threats have been receding. What is good for crops like cotton and sugar cane – high temperature and plenty of water is also a heaven for the malaria vector (Yared *et al.*, 2007). The relation between irrigated crops and the presence of malaria has long been noted in the Awash Valley plantations of sugarcane, fruit, and cotton and the introduction of irrigated rice cultivation in parts of Gambella has been suggested as an important contributing factor in malaria transmission (Solomon *et al.*, 2011).

The low educational level of malaria sufferers most of whom live in the countryside and have never been to school or received adequate guidance regarding dosage, fail to adhere to prescription requirements, or stop medication all together up on feeling well. As is the case elsewhere where malaria is endemic drug-resistance has been the inevitable outcome in many parts of Ethiopia (Ayele *et al.*, 2013). There is no national study on drug resistance in Ethiopia but isolated studies have shown drug resistance to SP to be less than 5% in Humera and as high as 18% in Ziway (1998), and even higher in another study of Ziway (30%) conducted by the Institute of Pathology in 2000 (Aschalew and Tadesse,2016). The use/misuse of antimalaria such as SP (Sulfadoxine-Pyrimethamine) led to drug resistance. Other anti malaria drugs in Ethiopia that parasite has developed resistance include AL (Artemether-Lumefantrine) (Tsige *et al.* , 2009).

2.7. Symptoms of Malaria

The sign and symptoms of malaria typically begin 8-25 days following infection, However symptoms may occur later in those who have taken anti malarial medication as prevention. Initial manifestations of the disease common to all malaria species are similar to flu-like symptom, and can resemble other conditions such as gastroenteritis and viral diseases. The presentation may include headache, fever, shivering, joint pain, vomiting, hemolytic anemia, jaundice, hemoglobin in the urine, retinal damage and convulsion (Beare *et al.*, 2006).

The classical symptom of malaria is paroxysm- a cyclical occurrence of sudden coldness followed by shivering and then fever and sweating, occurring every two days in *P. ovale* infection and every three days for *P. malariae* and *P. falciparum* infection can cause recurrent fever every 36-48 hours, or a less pronounced and almost continuous fever (Tilley *et al.*, 2011). Almost all severe forms and deaths from malaria are caused by *P. falciparum* rarely *P. vivax* or *p.ovale* produce serious complications, debilitating relapses and even death. The major complications of sever malaria include cerebral, pulmonary edema, acute renal failure, sever-anemia and bleeding. Acidosis and hypoglycemia are the most common metabolic complications (Bartoloni and Zammarchi, 2012).

Symptoms of malaria can recur after varying symptom-free periods. Depending up on the cause, recurrence can be classified as recrudescence, relapse or re-infection (WHO, 2017). Recurrence is due to one of the following; a) therapeutic failure resulting from non adherence to treatment, resistance of the parasite to the drugs used, poor quality of the medication; b) reactivation of hypnozoites and c) exposure to new infection by the mosquito vector. Recrudescence is when symptoms return after a symptom-free period. It is caused by parasites surviving in the blood because of inadequate or ineffective treatment. This is occurs more frequently in malaria from *P. falciparum*, *P. vivax* and rarely with *P. malariae* (White, 2011).

Relapse is resurgence of parasitemia and clinical manifestations due to reinvasion of the erythrocytes by merozoites from dormant hypnozoites in the liver. Relapses are believed to occur 21 to 140 days after treatment of the tropical strain and 180 to 420 days after treatment of the temperate strain. The main cause is treatment failure. Based on clinical observation alone, it is very difficult to distinguish recrudescence from relapse or relapse from re-infection. In some

situation the distinction can be made by identifying a parasite genotype in the relapse which is identical to that of the primary infection. Relapse commonly seen with *P. vivax* and *P. ovale* infections (White, 2011).

Re-infection means the parasite that caused the past infection was eliminated from the body but new parasite was introduced. In genotyping, re-infection can be defined by finding a parasite that is genetically different from the one that cause the primary infection (Markus, 2011). Almost all severe forms and deaths from malaria are caused by *P. falcipareum*. Rarely, *P. vivax* or *P. ovale* produce serious complications, debilitating relapses and even death. The major complications of severe malaria include severe anemia and bleeding. Acidosis and hypoglycemia are the most common metabolic complications. Any of these complications can develop rapidly and progress to death within hours or days (Mehta *et al.*, 2001).

In many patients, several of these complications exist together or evolve in rapid succession within a few hours. In clinical practice patients must be assessed for any of these signs or symptoms that suggest an increased risk for developing complications and must be treated immediately. In various studies risk factors for severe malaria and death include age greater than 65 years, female sex (especially when associated with pregnancy), non immune status, coexisting medical conditions, no malarial prophylaxis, delay in treatment, and severity of the illness at admission (Brunneel *et al.*, 2003).

2.8. Malaria diagnosis

A diagnosis of malaria is usually based on the patient's signs and symptoms, clinical history, and physical examination. If available, laboratory confirmation of the malaria parasite is recommended. Prompt and accurate assessment will lead to improved differential diagnosis of fever during pregnancy, improved management of non-malarial illness, and effective case management of malaria (Lemma *et al.*, 2021).

Subsequent advances in diagnosis and treatment of malaria, including the operationalization of multispecies RDTs, and accumulating clinical evidence for several effective options for improved preferal care as documented in other African countries, prompted the revision of the 2021 National Malaria Diagnosis and Treatment Guidelines again in 2021 (FMoH, 2021). At the hospital and health center levels, clinicians are expected to make an accurate diagnosis of

malaria based on the result of microscopic examination of patient blood smears rather than relying on clinical assessment alone (Wilson, 2012).

Laboratory technicians should undertake microscopic investigation to identify species of malaria and density of parasite (WHO, 2018). At the health post level, HEWs are expected to make a diagnosis of malaria by multi-species RDT rather than by clinical assessment alone. For patients needing referral to health facilities with more advanced capabilities because of severe malaria, a pre-referral treatment should be given by the HEW (Williams *et al.*, 2016).

2.8.1. Clinical diagnosis

A clinical diagnosis entails making a clinical assessment by taking an accurate history of the illness and performing a physical examination. Clinical diagnosis of malaria is made in a patient who has fever or history of fever in the last 48 hours and lives in malaria-endemic areas or has a history of travel within the last 30 days to malaria-endemic areas (Renia *et al.*, 2012) Basing the diagnosis on clinical features alone is not recommended, as this often has low specificity and increases the chances of the patient being misdiagnosed (FMoH, 2021).

2.8.2. Parasitological Diagnosis

Microscopic diagnosis and RDTs are the methods employed for confirmation of malaria etiology (Cutchan M *et al.*, 2008). Currently, malaria microscopy is only available in health centers and hospitals, and in higher private health facilities; generally these facilities have electrical power and fresh water. Light microscopy using thick blood films can be very sensitive, detecting as few as 20 parasites/ μ l of blood. Thin blood film stained with Giemsa is useful for identifying the malaria parasite species and has a sensitivity of 20 parasites/ μ l (FMoH, 2017).

The recommended method to determine parasite load is by quantifying the percentage of parasitized red blood cells. In 2005, single-species RDTs were introduced at health posts in Ethiopia, greatly improving access to accurate *P. falciparum* malaria diagnosis at peripheral levels. Currently multi-species RDTs capable of specifically detecting both *P. falciparum* and *P. vivax*, are being supplied by FMoH to health posts, enhancing malaria diagnosis by species at the periphery and reducing the need for empiric treatment and wastage of anti-malarial drugs. It also provides the opportunity to accurately identify parasite-negative patients in whom another cause

of fever (diagnosis) must be sought immediately. Patients who test negative by malaria RDT or microscopy do not need anti-malarial medications (FMoH, 2016).

2.8.3 Serological tests

Diagnosis of malaria using serological methods is usually based on the detection of antibodies against asexual blood stage malaria parasites. Immunofluorescence antibody testing (IFA) has been a reliable serologic test for malaria in recent decades (She *et al.*, 2007).

Although IFA is time-consuming and subjective, it is highly sensitive and specific (Sulver *et al.*, 1969). The literature clearly illustrates the reliability of IFA, so that it was usually regarded as the gold standard for malarial serology testing (Doderer *et al.*, 2007).

IFA is useful in epidemiological surveys, for screening potential blood donors, and occasionally for providing evidence of recent infection in non-immunes. Until recently, it was a validated method for detecting *Plasmodium*-specific antibodies in various blood bank units, which was useful for screening prospective blood donors, so avoiding transfusion-transmitted malaria (Reesing *et al.*, 2005).

2.9. Prevention and Control of Malaria

Methods used to prevent malaria include medications, mosquito eliminating and the prevention of bites (WHO, 2011). The presence of malaria in an area requires a combination of high human population density, high anopheles mosquito population density and high rate of transmission from humans to mosquitoes and from mosquitoes to humans. If any of these is lowered sufficiently, the parasite will eventually disappear from that area (Mangistu and Solomon, 2014).

Vector control refers to methods used decrease malaria by reducing the levels of transmission by mosquitoes. For individual protection, the most effective insect repellents are based on DEET or picaridin. Insecticide- treated mosquito nets (ITNs) and indoor residual spraying (IRS) have been shown to be highly effective in preventing malaria among children in areas where malaria is common (Tanser *et al.*, 2012).

Mosquito nets help keep mosquitoes away from people and reduce infection rates and transmission of malaria. Nets are not a perfect barrier and often treated with an insecticide

designed to kill the mosquito before it has time to find a way past the net World Health Organization (WHO, 2018). Insecticides treated nets are estimated to be twice as effective as untreated nets and often greater than 70% protection compared with no net (Esayas *et al.*, 2020).

Indoor residual spraying is the spraying of insecticides on the walls inside a home (WHO, 2015). After feeding, many mosquitoes rest on a nearby surface while digesting the blood meal, so if the walls of houses have been coated with insecticides, the resting mosquitoes can be killed before they can bite another person and transfer the malaria parasite (WHO, 2019). As of 2006, the world health organization recommends 12 insecticides in IRS operations, including DDT and the pyrethroids cyfluthrin and delta methrin (WHO, 2017). There are a number of other methods to reduce mosquito bites and stop the spread of malaria. Efforts to decrease mosquitoes larva by decreasing the viability of open water in which they develop or by adding substances to decrease their development is effective in some locations (WHO, 2016).

Community participation and health education strategies promoting awareness of malaria and the importance of control measures have been successfully used to reduce the incidence of malaria in some areas of the developing world (Desai *et al.*, 2016). Recognizing the disease in the early stages can stop the disease from becoming fatal (Esayas *et al.*, 2020). Education can also inform people to cover areas of stagnant, still water such as water tanks that are ideal breeding grounds from the parasite and mosquito, thus cutting down the risk of the transmission between people (Esayas *et al.*, 2020).

Individuals can protect themselves against malaria by wearing protective clothing and using insect repellents and bed nets. According to Zerihun (*et al.*, 2007). field trials indicate that insecticide treated bed nets and curtains have the potential to reduce childhood mortality by 15 percent to 35 percent. Drugs are used to prevent (chemoprophylaxis) and treat infection in individuals. However, given increasing levels of chloroquine-resistant malaria, new drugs are needed (Chico *et al.*, 2017). User-friendly drug packaging helps ensure that patients take their medicine correctly. Better compliance helps prevent the development of drug-resistant malaria (Williams *et al.*, 2016). While many new antimalarial drugs have been developed in the last 20 years (mefloquine, halofantrine, artemisinin, malarone, atovaquone and proguanil, co-artemether), there is still need for an affordable, effective, safe alternative to chloroquine (Yared *et al.*, 2007).

2.9.1. Traditional Malaria Prevention

These are eating garlic with green paper, drink gourd juice and avoiding eating vegetables such as tomato during rainy season to prevent malaria. These have both curative and preventive effect. There is also a home remedy called haregresa, this is a root of a small plant, they drink its juice (Aschalew and Tadesse 2016). There are also local plants such as Dumuga and Endode used to prevent malaria (Banchiamlak and Young, 2019). When malaria epidemic arise they smoke Dombaya and Goetzenni leaf (Legesse *et al.*, 2007)

2.9.2. Environmental Management Activities

Environmental management was the most popular malaria prevention method in Ethiopia. Communities are destroying mosquito breeding sites; clearing stagnant water, covering spring water, eradicating dirt from the compound (Mohammed *et al.*, 2015). If accumulated water could not be removed, spraying used motor oil has preventive effect is disposed off waste, by burning or burying it, in holes dug far from their house. Preparing toilet properly, as a toilet, which was not dug properly, could accumulate water and thus be a good breeding site for mosquito (Hassen *et al.*, 2014). In addition, separation of house from their cattle's/livestock since fecal matter from animals results in favorable environment for mosquito breeding. In addition to environmental management, some considered keeping personal hygiene as a good malaria preventive mechanism (Aschalew and Tadesse, 2016).

2.9.3. Malaria Vector Control

In Ethiopia, controlling malaria vector has a long history of more than 50 years, but malaria remains a major cause of morbidity and mortality in Ethiopia (Tsige *et al.*, 2009). Currently the main goal of vector control in Ethiopia is to reduce the level of malaria transmission. The main focus is: Improved targeting of localities for coverage and quality of indoor residual spraying, introduction, expansion and scaling up the use of ITNs and application of environmental management and chemical larval control in areas where it could be cost effective (Hassen *et al.*, 2014).

Public and individual measures include wearing long sleeves and pants during the dusk-to-dawn period. Sanitary improvements, such as filling and draining areas of impounded water; installing

screens and using bed nets; particularly the use of impregnated bed nets increases the effectiveness of the bed net; larvicides and biological control, for example using larvivorous fish; and nightly spraying of screened living and sleeping quarters with insecticides (Howard *et al.*, 2007).

According to Ethiopian Federal Ministry of Health malarias are defined as being located <2,000 m altitude. Of 5,083 surveyed households, 3,282 (65.6%) owned at least one ITN (FMoH, 2018). In ITN-owning households, 53.2% of all persons had slept under an ITN the prior night, including 60.1% children <5 years of age, 60.9% of women 15 - 49 years of age and 65.7% of pregnant women. Overall, 20.0% households reported to have had IRS in the past 12 months. Of 747 children with reported fever in the two weeks preceding the survey, 131 or 16.3% sought medical attention within 24 hours. Of those with fever, 11.9% took an anti-malarial drug and 4.7% took it within 24 hours of fever onset (FMoH, 2016).

2.9.4. Insecticide-Treated Bed Nets (ITNS)

Insecticide-treated bed nets are nets dipped in a pyrethroid insecticide solution. This treatment creates a physical barrier, or a halo around the net, repelling or killing the mosquitoes (Daddi *et al.*, 2010). Each ITN can last up to 12 months before needing to be re-treated with insecticide. Long-lasting insecticide treated nets (LLITNs) are increasingly popular, as they last longer than traditional ITNs, repelling mosquitoes for up to four years. With LLITNs, the insecticide is woven into the fabric of the nets, causing it to self-replenish with each wash, by bringing the insecticide to the surface of the net (Hassen, *et al.*, 2014). Mosquitoes that carry malaria are most active at night and in the early morning; bed nets are particularly vital during these times. Studies show that sleeping under a bed net can reduce child mortality from malaria by as much as 20%. The repellent in the nets can also reduce the number of mosquitoes in the surrounding area (Mohammed, *et al.*, 2015).

Ethiopia has produced tremendous results in its fight against malaria. The country's national malaria control program conducted mass distributions of LLITNs in 2020 and 2021. By the end of 2020, 55 million LLITNs had been distributed (FMoH, 2021). Approximately, 20 million was financed by the Global Fund. This distribution resulted in an estimated coverage of almost two LLITNs per household in the malaria endemic regions of the country (WHO, 2021).

There are two types of ITNs, long lasting : Effectiveness lasts the lifetime of the net (2 to 3 years) and re treatable : Effectiveness is limited to 6 months to 3 years, depending on the formulation of the chemical and the environment (FMoH, 2017). Of all the methods of preventing mosquito bites, sleeping indoors under an ITN is probably the most effective, as mosquitoes bite at night, when people are asleep. ITNs reduce human contact with mosquitoes by acting as a barrier, killing them if they land on the net or by repelling them and driving them away from where people are sleeping (WHO, 2021).

2.9.5. Indoor Residual Spraying

The main purpose of IRS is to lower malaria transmission by reducing the survival of mosquitoes entering houses and sleeping areas (Tanser *et al.*, 2012). To be effective, IRS operations should be country-specific and must include: Adequate commitment and social acceptance Spraying of at least 80% of homes and barns in an area and Enough health system capacity to deliver quality, well-timed, and high coverage (Yemane *et al.*, 2006). Credible information about local vectors, especially their insecticide susceptibility, as well as indoor versus outdoor feeding and resting behaviors (Olivr *et al.*, 2008).

Currently, IRS is contributing to malaria prevention. Most IRS programs have specially trained staff to do the spraying. Providers should stay updated about any local IRS programs in their areas and educate clients accordingly. A new approach to improve the effectiveness, efficiency, soundness, and sustainability of IRS is called integrated vector management (WHO, 2021).

2.10. Medications and Treatment of Malaria

Malaria treatment success relies on prompt diagnosis and recommendations of the most appropriate treatment regimen. Artemisinin-based combination therapy (ACT) is recommended by the WHO for treatment of uncomplicated *P. falciparum* malaria and chloroquine are recommended for *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* malaria infections (Krishnaa *et al.*, 2004). Primaquine is recommended to prevent the relapse of *P. vivax* and *P. ovale* infections. These recommendations are modified for special groups such as pregnant or lactating women, patients with other comorbidities, individuals with glucose-6-phosphate dehydrogenase (G6PD)

deficiency individuals and young children and infants. The early diagnosis of malaria can prevent further progression and lower the severity of the disease (WHO, 2014).

The WHO Global Technical Strategy for Malaria aims, by 2030, to reduce malaria case incidence and mortality rates globally by 90%; to eliminate malaria from 35 countries in which malaria was transmitted in 2021; and to prevent the re-establishment of malaria in all countries that became malaria-free (WHO, 2021). Countries such as, Algeria, Argentina, Paraguay, Sri Lanka and the Maldives have been declared malaria free by WHO in recent years (WHO, 2021).

The treatment is an antibiotic (clindamycine or azithromycine) that, when administered to mice at the same time as they are infected with malaria, protects against the development of parasites in the blood. Treatment in this manner also generates a long-term immunity against the parasite. This treatment is very encouraging and could potentially be administered at regular intervals as a needle-free natural vaccination (Esayas *et al.*, 2020).

2.11. The Socio-Economic Impacts of Malaria

The overall cost of malaria in Africa is an estimated 300 million cases of acute malaria occur each year globally and 2 million malaria deaths occur in Africa, mostly in young children and especially in sub-Saharan Africa (WHO, 2017). Malaria is the leading cause of mortality in African children under-five year. *Plasmodium falciparum* is most commonly found in Africa. The economic cost of malaria is estimated to be more than \$12 billion a year in lost productivity. Throughout Africa, resistance to chloroquine, the cheapest and most widely used antimalarial drug is common (WHO, 2018).

Sulfadoxine-pyrimethamine (SP), often seen as the first and least expensive alternative. Chloroquine is also among drugs to which resistance has developed, especially in East and Southern Africa. ITNs around sleeping areas may reduce malarial deaths in young children by an average of 20 percent. The World Health Organization has recently backed a new, limited policy to spray DDT inside homes (WHO, 2021). Malaria is ranked as the leading communicable disease in Ethiopia, accounting for approximately 30% of the overall Disability Adjusted Life Years (DALYs) lost and causes about 70,000 deaths each year. It is estimated that over five million episodes of malaria occur each year in Ethiopia (FMoH, 2018).

The burden of malaria has been increasing due to a combination of large population movements, increasing large scale epidemics, mixed infections of *Plasmodium vivax* and *P. falciparum*, increasing parasite resistance to malaria drugs, vector resistance to insecticides, low coverage of malaria prevention services, and general poverty (FMoH, 2021).

Infection during pregnancy, particularly among new mothers, increases the risk of maternal mortality, neonatal mortality, and low birth weight. In addition to loss of life malaria places an economic burden on African nations (WHO, 2015).

3. MATERIALS AND METHODOLOGY

3.1 Description of the Study Area

The study was conducted in Guangua health center. It is found in the west Guji Zone Abaya woreda. It is located at 100 km away from the zone capital town Bule hora and 366 km away from Addis Ababa. The town has a latitude and longitude of 6⁰40N and 38⁰10 E with an elevation of 1750 meters above sea level Guangua health centre located as latitude and longitude of 6⁰21'31"N and 38⁰17'38" (AWGHCO, 2018). The mean annual temperature of the District Were from 16 to 36⁰C and the mean annual rain falls were 900mm to 1600mm. (AWARDO 2018). The total Population size of the town is 8983 (CSA, 2018). It has 1 health centers and 3 private clinics. (AWHO, 2018). Malaria is endemic in the area, but it shows seasonal variation. The major malaria transmission periods occur from September to December, following the rainy summer seasons and from March to May (AWHO, 2018).

3.2 Study design and period

Health centered cross-sectional study was conducted from September to October 2022 to determine the prevalence of malaria among patients in Guangua health centre, Oromia Ethiopia. The study was involved administration of a structured questionnaire and laboratory blood sample.

3.3. Population

3.3.1. Source of population

Patients attending in Guangua health center were used as the source of population.

3.3.2. Study population

All patients who came for health care service during the study period that fulfill the inclusion and exclusion period were included in the study as study participants.

3.4. Eligibility Criteria

3.4.1. Inclusion criteria

All patients who attend in Guangua health center were included in the study period.

3.4.2. Exclusion criteria

Study participants who were absent during the data collection period or unable to give blood Sample would be excluded from the study.

3.5. Sampling technique and Sample size

3.5.1. Sampling technique

Study participants who visited the health center laboratory for blood film examination and met the eligibility criteria were selected by non probability consecutive sampling technique.

3.5.2. Sample size

The sample size was computed using the general formula for a single population proportion. Since there was no previous malaria prevalence (p) report from the study town, 16.0 % prevalence of malaria used from other studies within Dilla (Molla and Ayele, 2015), 5% marginal error and 95% confidence interval (CI). Accordingly, the final sample size became 227. Sample size (n) was determined using the following statistical formula (Naing *et al.*, 2006).

$$n = \frac{Z^2 P(1-P)}{d^2}$$

$$n = \frac{1.96^2 \times 0.16(1-0.16)}{(0.05)^2}$$

$$n = \frac{1.96^2 \times 0.16(1-0.16)}{0.0025} = 3.84 * 0.1344 / 0.0025 = 0.5161 / 0.0025 = 206$$

By adding 10% for non- response the final sample size was=227, d = margin of error between the sample and the population. n = sample size. Z = 95% confident interval. P = prevalence rate of malaria based on previous study.

3.6. Study Variables

3.6.1. Dependent variable:

- ✓ malaria positive/negative

3.6.2. Independent variable:

- ✓ Demographic factors: sex, age, level of education and number of house hold member.
- ✓ Presence of stagnant water
- ✓ House condition and ITN condition

3.7. Method of data collection

The main instruments used to collect data for this study was primary data and secondary data such as, questionnaire, blood sample collection, books and scientific journals.

3.7.1. Socio-demographic data

Data about socio-demographic characteristics, ITN condition and availability, ITN utilization, IRS, presence of stagnant water, outdoor stay at night, housing condition, and health information about malaria were collected by using structured questionnaire. It was used to obtain information on age, gender, occupation, educational background (Nigatu *et al.*, 2014). Participants who could not read or write was interviewed in local language by the researcher. The original questionnaire, which were prepared in English language will be translated into Afan oromo version in order to avoid language barrier.

To ensure confidentiality, participants' data were linked to a code number and the data collection procedure was anonymous, the name of the participants and any participants' identifier was not be written on the questionnaire and also during the interview to keep the privacy, they were interviewed alone.

3.7.2. Blood sample collection and Laboratory processing

Capillary blood specimen was collected from finger prick using sterile blood lucent to prepare thick and thin blood smears. Blood specimen collection and processing was done by Laboratory professional of Guangua health centre. The smears was air dried and only thin films was fixed with methanol and both thin and thick films were stained with 10% Giemsa stain for 15 minutes. All dried slides were placed in slides box and examined by laboratory technologist at the Guangua health centre. The presence of malaria parasites on thick blood smear and the identification of plasmodium species from smear was done through oil immersed objectives

(100x) at 1000x magnification. The thick smear was used to determine whether the malaria parasite were present or absent and thin smear used to identify the type of plasmodium species (Moody and Chiodini, 2001).

3.8. Data analysis and interpretation

The generated data were coded, entered and analyzed using Statistical Package for Social Science (SPSS) version 20.0. Data was analyzed using descriptive statistics to show the distribution of malaria cases with respect to sex, age and plasmodium species. The proportion of *P.falciparum* and *P.vivax* were expressed as the total number of each species per total number of confirmed malaria cases. Frequencies and proportions (weighted) were reported. Pearson's Chi-square (χ^2) assess the data quality issues in terms of missing data (data incompleteness), consistency and diagnostic performance issues. Similarly, bivariate logistic regression analysis was used to assess the association of malaria cases with socio-demographic variables. The results of regression analysis was presented by crude/unadjusted odds ratio (OR) and adjusted odds ratio (AOR) with 95% confidence intervals (CIs). A variable with a p-value <0.05 in the bivariate analysis were considered statistically significant.

3.9. Ethical clearance

The study protocol was approved by research ethics review committee of Dilla University before commencement of the study. Permission to conduct the research was obtained from the Abaya Woreda Guangua health center authorities after giving them recommendation letter written by Dilla University. Informed written consent was obtained from participants with age ≥ 18 years old. For participants of age < 18 years old, informed written consent was obtained from their parents or guardians.

4. RESULT AND DISCUSSION

4.1. Socio demographic characteristics of the respondents.

A total of 227 study participants with 118(51.9%) of the respondents were males and the remaining 109(48.1%) of the study participants were females. Of the total study participants, 72(31.7%) and 155(68.3%) urban and rural residence respectively were included in the study.

The age distribution of the participants also indicates the majority 96(42.2%) were found on the age range 16-30 followed by 60(26.4%) and 29(12.8%) found at the age range 6-15 and 31-40 years old respectively. The other 21(9.3%) were below 5 years old and above 40 years old.

Of the total study participants 75(33%) were farmers, 65(28.7%) were students and 30 (13.2%) were daily laborers respectively. The other 23(10.1%) were merchants, 29(12.8%) had no specific occupation and only 5(2.2%) were government employers.

The majority 91(40.2%) of the study participants had no income and 63(27.7%) and 53(23.3%) were an income level below 2000 and 2001-5000 respectively. The other 16(7%) had an income level between five and ten thousands and only 4(1.8%) of the had an income level beyond ten thousands.

Regarding their marital status, majority of them 128(56.4%) were married followed by 89(39.2) and 10(4.4%) were single and divorced respectively.

The majority 95(41.9%) of the study participants had family member greater than 6 and followed by 80(35.2%) family member with 4-6 and 52(22.9%) had a family member 1-3 respectively.

Moreover the educational status of the study participants indicated that, 94(41.4%) and 73(32.2%) were at primary school and illiterate level respectively. The remaining 20(8.8%), were at secondary school level 20(8.8%) were degree holders, 10(4.4%) were diploma holders and the other 10(4.4%) were following their education at higher institutions (Table 1).

Table 1: Scio demographic characteristics of the study participants (n=227)

Variables	Categories	Frequency	Percentage
Sex	Male	118	51.9
	Female	109	48.1
Age	Below 6	21	9.3
	6-15	60	26.4
	16-30	96	42.2
	31-40	29	12.8
	Above 40	21	9.3
Marital status	Single	89	39.2
	Married	128	56.4
	Divorced	10	4.4
Educational status	Illiterate	73	32.2
	Primary school	94	41.4
	Secondary school	20	8.8
	Higher education	10	4.4
	Diploma	10	4.4
	Degree	20	8.8
Residence	Urban	72	31.7
	Rural	155	68.3
Level of income	< 2000	53	23.3
	2001-5000	63	27.7
	5001-10,000	16	7.0
	>10001	4	1.8
	None	91	40.2
Occupations	Students	65	28.7
	Farmers	75	33.0
	Merchants	23	10.1
	Day workers	30	13.2
	Government employee	5	2.2
	None	29	12.8
Family size	1-3	52	22.9
	4-6	80	35.2
	>6	95	41.9

4.2. Prevalence of malaria in the study area.

The overall malarial prevalence of the study area was 33 (14.5%) and prevalence in the rural area were significantly higher than urban ($p= 0.006$) with 23(10.1%) and 10(4.4%) malaria positive individuals in the rural and urban areas respectively. This may be due to reasons, related in improper utilization of malarial prevention mechanisms and lack of awareness about the nature and cause and transmission of the disease in rural areas than urban. This result was noticeably lower than 48%, 36.1%, 33.8%, 21.8% and 17% reported from Woreta town, northwest Ethiopia; Addi Arkay health center, northwest Ethiopia; Abeshge, South-central Ethiopia; Gondar, northwest Ethiopia and Metema hospital northwest Ethiopia, respectively.

On the other hand, it was higher than the studies conducted in Ataye, North Shoa, Ethiopia (8.4%), Bahirdar city (5%) and Kombolcha town (7.5%) (Chala, 2015). Similarly the finding of Molla and Ayele, (2015) showed that malaria prevalence in rural kebeles was more (53.6%) than in the town (46.4%) indicating that a less effective malaria control measures were applied in the rural areas.

Malaria prevalence in males were higher than females with the prevalence rate of 9.7% and 4.8% in males and females respectively. Such higher malaria prevalence in males might be attributed to the fact that males are usually engaged in outdoor activities that put them at greater risk of mosquito biting.

Similarly, Tesfahunegn *et al.*, (2019) Most of the patients (72%) were males, This could be due to the reason that males were more likely engaged in agricultural, livestock keeping and traditional gold mining activities than females in his study area than the other studies. Most of male adolescent population in those Kebeles engaged on traditional gold mining, most of the summer period stays outdoor overnight.

In this rural area, males in the reproductive age groups (≥ 15 years old) are commonly breadwinners of their families, spending most of their time especially evenings outdoors when the peak biting activity of the infective mosquito is observed (Alkadir *et al.*, 2020). On the contrary, a study conducted at Hallaba Health Center reported that females were more affected than males with (57.40%) 97 and (42.6%) 72 respectively (Girum Tefera, 2014). The variation

was may be due to nature of occupation and outdoor activities among males and females might bring such differences (Ahmed *et al.*, 2021).

Regarding the age group malaria case was relatively higher on the age group between 16-30 and 6-15 having 4.8% and 3.9 % malaria cases respectively followed by the age group below 6 with 2.6% malarial cases. Although the malaria case indicates a high prevalence rate on children's and the economically active group of the community the difference was insignificant ($p= 0.53$) among the age groups. This may be attributed to the engagements of these age groups to different types economical activities so that their work place, the time of working activities and working conditions exposes them to malaria transmission easily than the elders and children's who were relatively saved their home.

Similarly, the finding of Alkadir *et al.*, (2020) showed that, majority of malaria prevalence were found on the age group of subjects greater 15 years and above, followed by the age group 5–14 years, and under five children. The greatest malaria prevalence was seen among 15-24 years of age with 35.7% (Molla and Ayele, 2015).

The family size of the sampled individuals and malaria prevalence also indicates the prevalence was significantly higher ($p=0.02$). (Table2). Accordingly the higher percentage (7.9%) of individuals with malaria case were found on families having above six family members followed by 4.4% with a family size 4-6 individuals. However the malarial case was 2.2% in families having family members fewer than three. This implies with small family size the malaria prevention mechanisms like ITNs were properly utilized and unable to provide ITN to all the family members or in properly utilized in families with a large family size.

The odds of malaria infection were higher among children whose family is with two under-five children than children with a family having one under-five children. This might be related with the number of ITN in the household and mothers' care. As if the number of ITN is not enough, other children can't access ITN and mothers tend to sleep with the youngest child in the household. Children who utilize ITN had a reduced risk of malaria infection compared to those who do not utilize ITN (Ahmed *et al.*, 2021).

Table 2: Malaria prevalence on socio economy of the study area (n= 227)

Variables	Categories	Malaria case		C.OR(95%CI)	A.OR(95%CI)	P-value
		Positive(%)	Negative(%)			
Sex	Male	22(9.7%)	96(42.3%)	1.45(0.78-2.70)	1.06(0.62-1.78)	0.05*
	Female	11(4.8%)	98(43.2%)	1	1	
Age	Below 6	6(2.6%)	15(6.6%)	1.5(0.17-14.03)	0.52(0.24-2.78)	0.053*
	6-15	9(3.9%)	51(22.5%)	1.0(0.15-7.3)	0.75(0.27-2.04)	
	16-30	11(4.8%)	85(37.4%)	1.6 (0.34-3.58)	0.8(.34-2.29)	
	31-40	4(1.7%)	25(11%)	0.6(0.17-2.28)	0.52(.17-1.65)	
	Above 40	3(1.3%)	18(7.9%)	1	1	
Residence	Urban	10(4.4%)	62(27.3%)	1.59(0.82-3.06)	1.16(0.66-2.03)	0.006*
	Rural	23(10.1%)	132(58.1%)	1	1	
Income level	< 2000	9(3.9%)	44(19.4%)	0.69(0.21-2.21)	0.9(0.45-1.77)	0.05*
	2001-5000	5(2.2%)	58(25.6%)	0.76(0.23-2.47)	1.1(0.57-2.08)	
	5001-10,000	1(0.4%)	15(6.6%)	1.8(0.42-8.00)	1.2(0.41-3.50)	
	>10001	1(0.4%)	3(1.3%)	0.13(0.01-0.97)	0.31(0.03-3.1)	
	None	17(7.5%)	74(32.6%)	1	1	
Family size	1-3	5(2.2%)	47(20.7%)	1.0(0.14-7.02)	1.14(0.57-2.31)	0.02*
	4-6	10(4.4%)	70(30.8%)	0.8(0.12-5.2)	0.88(0.47-1.65)	
	>6	18(7.9%)	77(33.9%)	1	1	

*P value less than 0.05, AOR: Adjusted Odds Ratio, CI: Confidence interval, "1" in COR and AOR indicate the reference category

4.3. Factors associated with malaria prevalence in the study area

Factors associated with malaria prevalence were analyzed and assessed as indicated in the table 2. The multi and bivariate regression analysis of the factors indicates, Environmental factors like stagnant water, outside stay at night IRS, and ITNs were significant predictors of malaria infection in the study area.

The odd ratio for malaria was higher AOR= 3.55 95%CI: (2.25- 5.63 for those who didn't use insecticide treated bed nets than those who utilizes ITNs. Similarly Molla and Ayele (2015) in their finding indicates Individuals having and using insecticide treated nets were 0.2 times less likely to get malaria parasites than those of not having adequate bed nets (OR=0.16, 95%CI: 0.08-0.29), ($p < 0.05$).

Malaria was significantly associated with factors like IRS and outside stay at night. The odd ratio for malaria for IRS was OR= 5.8 95% CI: (3.8- 15.8) higher for those who didn't have hoses sprayed with IRS than sprayed houses. But, malaria was not significantly associated with factors like Living in the nearby stagnant water. However, it is difficult to justify why this factors was not associated because the study has small number of sample size. Moreover, the collected data was health centered which was not supplemented with observation to increase the data quality like distance from stagnant water around home of study participants.

More so, participants whose house has not been sprayed with insecticide in the past 6 months are two times more likely to get malaria infection (OR=2.45, 95% CI: 2.20-3.99), $p=0.02$. According to Ahmed *et al.*, (2021), outdoor stay at night (AOR = 3.9, 95% CI = 1.14–13.8), and presence of stagnant water near to house (AOR = 4.0, 95% CI = 1.14–14.6) infection. Utilization of ITN (AOR = 0.22, 95% CI = 0.06–0.61) and health information about malaria (AOR = 0.29, 95% CI = 0.06–0.65) were found to reduce the odds of malaria.

Mosquitoes that carry malaria are most active at night and in the early morning; bed nets are particularly vital during these times. Studies show that sleeping under a bed net can reduce child mortality from malaria by as much as 20%. The repellent in the nets can also reduce the number of mosquitoes in the surrounding area (Mohammed, *et al.*, 2015).

The main purpose of IRS is to lower malaria transmission by reducing the survival of mosquitoes entering houses and sleeping areas (Tanser *et al.*, 2012). To be effective, IRS operations should be country-specific and must include: Adequate commitment and social acceptance Spraying of at least 80% of homes and barns in an area and Enough health system capacity to deliver quality, well-timed, and high coverage (Yemane *et al.*, 2006). Credible information about local vectors, especially their insecticide susceptibility, as well as indoor versus outdoor feeding and resting behaviors (Olivr *et al.*, 2008). Environmental management was the most popular malaria

prevention method in Ethiopia. Communities are destroying mosquito breeding sites; clearing stagnant water, covering spring water, eradicating dirt from the compound (Mohammed *et al.*, 2015).

Table 3: Malaria prevalence and associated risk factors in the study area (n= 227)

Factors	Categories	Malaria		COR (95% CI)	AOR(95%CI)	P-value
		Yes	No			
having insecticide treated bed nets (ITN)	Yes	9	95	3.55 (2.25- 5.63)	2.10 (1- 4.2)	0.006*
	No	24	99	1	1	
Sleeping under insecticide treated bed nets	Yes	3	68	3.39(1.1- 5.210)	2.79 (0.9- 8.1)	0.003*
	No	30	126	1	1	
stagnant water around your home	Yes	21	98	1.0 (0.3- 2.7)	0.9 (0.3- 2.6)	0.08*
	No	12	96	1	1	
housing sprayed with insecticide residual spray(IRS)	Yes	5	47	5.8(3.8- 15.8)	2.38(0.5-3.3)	0.02*
	No	28	147	1	1	
working outside at night	Yes	25	110	2.85 (1.2- 6.7)	1.22(0.9-1.6)	0.005*
	No	8	84	1	1	

*P value less than 0.05, AOR: Adjusted Odds Ratio, CI: Confidence interval, "1" in COR and AOR indicate the reference category.

4.4. Composition of malaria species in the study area

The *Plasmodium* species distribution of the study area were shown in Figure 2. Accordingly *Plasmodium falciparum* was the predominant species, accounting for 54.5%(18), followed by *Plasmodium vivax* 27.3% (9) and mixed 18.2%(6). Similarly the findings of Tesfaye *et al.*, (2021) revealed high prevalence of *P. falciparum* and a relatively high proportion of *P. vivax*. Alkadir *et al.*,(2020) also indicates *P. falciparum* constituted the most predominant malaria

infections (75.2%) while markedly lower *P. vivax* was also present (24.5%). In addition, 0.28% of mixed malarial infection of both *P. falciparum* and *P. vivax* was detected. Tesfahunegn *et al.*, (2019) in his study also indicates most of the cases were caused by *Plasmodium falciparum* (86.9%). Although the two *Plasmodium* species, *P. falciparum* and *P. vivax*, are important parasites in malaria related problems in Ethiopia, *P. falciparum* was the most predominant species accounting for about 76% of all malaria infections among children assessed in this study (Getachew and Tsige, 2016).

However the finding of Molla and Ayele, (2015) showed that, *plasmodium vivax* was the predominant species, accounting for 62.5% (n=35), followed by *Plasmodium falciparum* 26.8% (n=15) and mixed infection with 10.7% (n=6). According to Fasil *et al.*, (2021) *P. falciparum* and *P.vivax* have nearly equal contributions to the disease, malaria which contradicts with the current study. The result of the current study agrees with different types of studies conducts else were in Ethiopia and also contradicts with others as indicated above. This variation in epidemiological distribution of *plasmodium* species might be due to variation in environmental and climatic factors in different parts of the country. Figure 2 below show malarial species composition in the study area.

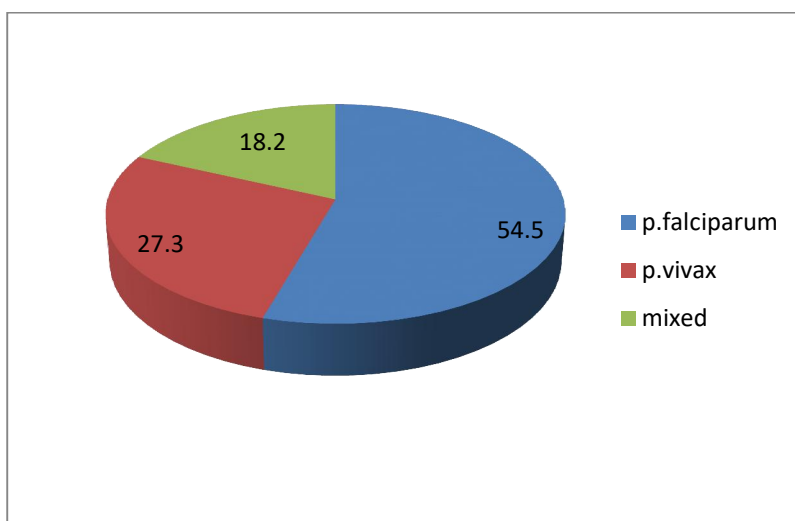


Figure 2: Malaria species composition in the study area.

5. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

The study was aimed at assessing the prevalence of malaria among patients attending in Guangua health centre. Accordingly the prevalence malaria in study area were 14.5%. higher than studies done in some parts Ethiopia and lower than other findings else were in the country. The prevalence was higher in rural areas (10.1%) than urban (4.4%) as well as it was higher in males (9.7%) than females (4.8%). More over the prevalence of malaria was significantly greater with a larger family size among the study participants.

The study indicated that there is association between prevalence of malaria parasites and ITNs bed net ownership and utilization, Outdoor stay at night, presence of mosquito breeding areas and IRS. The deadly *P. falciparum* (54%) appeared to be the dominant *Plasmodium* species followed by *plasmodium vivax* (27.3%). The study demonstrated that malaria remains a public health problem in the area. This would be an important indicative that the area needs due attention and further concerted malaria interventions.

5.2. RECOMMENDATIONS

Based on the research results, the following recommendations were forwarded:

The local health workers and decision makers better to empower the community and create prevention modalities like suitable cloths and insecticidal repellents for outdoor conditions at the night.

Regular health education must be provided to raise individuals and community awareness about the mode of malaria transmission, prevention and control especially in the rural kebeles.

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DILLA UNIVERISTY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF BIOLOGY
APPENDECES

APPENDECES I

Questionnaire

I am biomedical science student in biology department at Dilla University. I am conducting a research on the prevalence of malaria among patient attending in Abaya woreda Guangua health center.

I need your genuine response for each question.

Dear respondent to participate, withdraw or refuse in the participation in the study, but your real honest participation is very important for introducing intervention programs, controlling, preventing and elimination of the disease progression. The questionnaires will take about 25 minutes and all of your answers will be kept confidential. I will assure you that there will be no problem regarding with the participation of this study. Therefore, you are kindly requested to provide consent of participation.

Thank you for giving your time and agreeing. If there is any question that you want to ask, please do not hesitate to ask at any point/time.

A. Personal information

Code of patient -----

1. Age (in years) A) <6 B) 6-15 C) 16-30 D) 31-40 E) >45
2. Sex A) Female B) Male
3. Residence A) Urban B) Rural

B. Socio demographic factor

4. Occupation A) Student B) Merchant C) Farmer D) Day worker E) Government employee F) Others

5. Monthly income A) >2000 B) 2001-5000 C) 5001-10,000 D) <10,001 E) None
6. Educational status A) Illiterate B) Primary school C) High school
D) Higher education E) Degree F) Diploma
7. Marital status A) Single B) Married C) Divorce D) Other
8. The number of household A) 1-3 B) 4-6 C) >6

C. Information on malaria prevalence and related conditions

9. Have you heard of malaria?. A) Yes B) No
10. Where did you hear, see or receive the information about malaria?
A) Television/Radio B) Church C) Health workers C) School D) Kebele meeting
11. Did you every encounter malaria disease? A) yes B) No
12. Does your family member infected with malaria? A) Yes B) No
13. How frequent it was? A) more frequent B) less frequent C) no frequent D) None
14. What are symptoms of malaria? A) Fever with shivering B) Body pain C) Loss of appetite D) Don't know
15. What did you do first when the fever started ? A) Went to health facility B) Use herb/natural remedies C) Went to religious leaders D) Went drug store to get drug D) We have done nothing. E) None
16. How malaria can be transmitted?. A) Through mosquito bite B) Contaminated water C) Other
17. How can someone protect themselves against malaria? A) Sleep under Insecticide treated nets B) Use mosquito repellent C) Avoid mosquito bites
D) Spray house with insecticide E) Take preventive medication
18. Which group of people is more affected by malaria? A) Adult B) Children
C) Pregnant women D) All are equally affected E) I do not know
19. What are the main malaria transmission seasons? A) Before main rainy season B) Main rainy season C) After main rainy season D) All year round E) Unknown
20. Do you have Insecticide treated bed nets ? A) Yes B) No
21. Do you sleep under Insecticide treated bed nets? A) Yes B) No

22. When do you sleep under Insecticide treated bed nets? A) Sometimes B) Often
C) None
23. Is your house sprayed with Insecticide residual spray? A) Yes B) No

D. Environmental factor

24. Is there any stagnant water around your home? A) Yes B) No
25. Do you work outside at night? A) Yes B) No
26. Do you have knowledge about prevention of mosquito breeding site? A) yes B) No
27. What are there? A) Cleaning household surround B) cleaning bushes/tall grass C)
drainage of stagnant water D) do not know
28. When mosquito bite? A) During night time B) During day time C) Any time D)
Don't know
29. What do you do to avoid mosquito bites?

Appendices II : Malaria diagnosis result

No	Code of patient	Malaria diagnosis result			Negative
		Positive			
		Pf	Pv	mixed	