PREVALENCE OF MALARIA AND ITS ASSOCIATION WITH HEMOGLOBIN AMONG PATIENTS VISITING PAWE GENERAL HOSPITAL, NORTH-EAST ETHIOPIA

MSc THESIS

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As Thesis Research advisors, wehereby certify that we have read and evaluated this thesis prepared, under our guidance, by Habtamu Amsalu, entitled "Prevalence of Malaria and Its Association with Hemoglobin Level among Patients Visiting Pawe General Hospital North-west Ethiopia. We recommend that it can be submitted as fulfilling of the thesis requirement.

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DEDICATION

I dedicate this thesis manuscript to my brother Adane Amsalu and my best friend Yibrehu Bogale for their encouragement and nursing me with affection, love and for their dedicated partnership in the success of my life.

STATEMENT OF THE AUTHOR

I declare that this thesis is the result of my own work and that all sources or materials used have been duly acknowledged. This thesis is submitted for partial fulfillment of the requirements of MSc. degree in Microbiology at Haramaya University and to be made available at the University's Library under the rules of the Library. I confidently declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

The author was born in 1989GC in Amhara Regional State, Enjibara town. He completed his elementary and secondary education at Askune and Enjibara Comprehensive High Schools, respectively. In 2013 he joined Gondar University and obtained BSc. in biology through regular program in 2015. Then, he directly sponsored by MOE and joined to Haramaya University to pursue his MSc in Microbiology in 2015/16.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACT	Artemesinin-Based Combination Therapy
CBT	Complete Blood Count Test
CDC	Center for Disease Control
CRPF	Chloroquine Resistant *Plasmodium falciparum*
CSA	Central Statistical Authority
Hb	Haemoglobin
HIV	Human Immuno Deficiency Virus
IDA	Iron Deficiency Anemia
IRS	Indoor Residual Spray
ITM	Insecticide Treated Materials
ITN	Insecticide Treated Net
MMWR	Morbidity and Mortality Weekly Report
MOH	Ministry of Health
RBM	Rolls Back Malaria
RDT	Rapid Diagnostic Test
SPSS	Statistical Package for Social Sciences
SSA	Sub-Saharan Africa
UNICEF	United Nations International Children's Education Fund
WHO	World Health Organization

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ABSTRACT

Human malaria is a common life-threatening disease in many tropical and sub-tropical areas. It is one of the most important causes of morbidity and mortality resulting in serious socio-economic problems particularly in developing countries and one of the major tropical diseases adversely affecting the health of the peoples and the economic development of many developing countries, particularly in Sub-Saharan Africa. The aim of the study was to assess the prevalence of malaria and its association with haemoglobin level among local patients visiting Pawe General Hospital in Pawe Town North-west Ethiopia during peak malaria transmission season, in 2016. Clinical records of malaria for the last five years were obtained from the Hospital were analyzed. Malaria diagnosis was carried out based on microscopic examination of thick and thin Giemsa stained blood films from randomly selected samples of 402 participants during the peak malaria transmission season, October - December 2016. Additionally, structured and pre tested questionnaires were used to assess the socio-demographic characteristics and the level of knowledge and awareness of the respondents related to malaria. The data from the clinical records showed that malaria was dominant diseases in the study area. Results of parasitological survey showed that the most dominant Plasmodium species was P. falciparum. Among the overall prevalence of 40.0% malaria parasite among studied population, P. falciparum (86.9%) and P. vivax (13.0%) respectively. Out of the 161 malaria patients, 81 respondents were an anemic and there were significance variations of anemic prevalence among age groups (P=0.025) and haemoglobin concentration was statistically associated with age status (X^2=22.60). Regular health education must be provide to raise individual and community awareness about the mode of malaria transmission, prevention and control.

Key words and Phreses: Anemia, Hemoglobin, Malaria, Pawe, Prevalence, Risk factor

1. INTRODUCTION

Malaria is a major public health and medical concern in many parts of the world, especially in countries of tropics and subtropics such as in Africa, South East Asia, Hispaniola (Haiti and Dominican Republic), and the Indian subcontinent, the Middle East, Oceania and Latin America. I t also rarely occurs in temperate climate and estimation has shown that 1.2 billion people are at ris k of malaria; of this 300-500 million people are infected and more than 1 million are dying each ye ar globally. The majority of these deaths occur in young children in Sub-Saharan Africa (SSA) where one out of five deaths of children is due to malaria (Stratton et al., 2008).

Previous reports (eight years ago) showed that about 40% of the world's populations, particularly those in the poorest countries are affected by malaria infection (247million) and about 90% of all malaria deaths (881000) in the world occur in Africa south of the Sahara. But, according to World Malaria Report (2009), 243 million cases of malaria estimated to occur globally, the highest proportion (85%) were in Africa. Similarly, from the estimated global death (863000) due to malaria, 89% were in Africa.

Malaria is a systemic disease caused by infection of the red blood cells with intracellular protozoan parasites of the genus *Plasmodium* (WHO, 2007a). These are *Plasmodium falciparum, P. vivax, P. malariae and P. ovale*. Only four species of *Plasmodium* infect humans in nature. In addition to these, *P. falciparum* has caused the most deadly, wide spread and severe malaria. *Plasmodium knowlesi* has been recently defined as the fifth human malaria species (White, 2008.

The huge malaria cases and deaths have been due to the fact that *P. falciparum* causes the majority of infections in Africa. The severity of *P. falciparum* malaria is due to the organism's ability to invade young and old red blood cells, which is not characteristic of the other Plasmodium species. In addition, lack of strong and effective malaria prevention and control services, health serve deterioration, parasite resistance to antimalarial drugs, resistance of vector mosquitoes to insecticides, civil unrest coupled with population movement and economic development programs in wetlands, desert fringes and highlands have contributed to the huge malaria cases and deaths in malarious areas. The distribution of *Plasmodium* species varies across

the world and even among localities in malarious areas. For example, *P. falciparum* predominates in Haiti, Papua New Guinea, and Sub-Saharan Africa, while *P. vivax* is more common in Central America and the Indian subcontinent and causes more than 80 million clinical episodes of illness yearly. The prevalence of these two species is approximately equal in the Indian subcontinent, eastern Asia, Oceania, and South America. *P. malariae* is found in most endemic areas, especially throughout SSA, but it is much less common than the other species. *P. ovale* is unusual outside Africa, and where it is found accounts for less than one percent of isolates (Breman *et al.,* 2006).

Malaria transmission varies among communities largely due to environmental factors, such as proximity to breeding sites. Many water resources development and management projects result in local outbreaks of malaria and other vector-borne diseases such as schistosomiasis (Steinmann *et al.,* 2006), lymphatic filariasis (Erlanger *et al*., 2005), and Japanese encephalitis (Keiser *et al*., 2005). 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 (Ghebreyesus *et al*., 1999).

In Ethiopia, approximately 75% of the total area is estimated to be malarious, with 68% of the total population (52 million people) being at risk of infection. According to the National Health Services Statistics, malaria is among the top 10 leading causes of morbidity. Proximity to micro-dams which were constructed for small irrigation development schemes is considered as one of the risk factors for increased malaria incidence (Ghebreyesus *et al.,* 2000). The actual malaria cases that occur annually throughout the country are estimated to be 4–5 million (WHO, 2000). Malaria is responsible for 30–40% of outpatient visits to health facilities, 10–20% of hospital admissions and 10–40% of severe cases in children under five years of age (MOH, 2000). In Ethiopia, malaria transmission peaks bi-annually from September to December and April to May, coinciding with end of short and main rainy seasons, respectively, and harvesting season, with serious consequences for the subsistence economy of Ethiopia's countryside and for the nation in general. Malaria is frequently referred to as a disease of the poor. At a macro level, there is clear evidence that the burden of malaria is greatest among the poorest countries of the world,

especially those in SSA (Gallup & Sachs, 2001). In Ethiopia it is estimated that three-fourths of the land is below 2000 meters above sea level, which is malarious with two-thirds of the country's population at risk (Kassahun, 2004).

One of the most inevitable manifestations of malaria is anemia (the reduction of hemoglobin concentration below the normal range for all age and sex groups). World Health Organization defines anemia as hemoglobin below 11.0g/dL (grams per deciliter) and hemoglobin concentration of less than 12 g/dL for women and less than 13 g/dL for men (WHO,2010). Malaria may cause anemia through a number of different mechanisms including excess removal of parasitized erythrocytes, immune destruction of parasitized red cells, and impaired erythropoietin as a result of bone marrow dysfunction (Ekvall, 2003). Anemia is usually multifactorial in origin and although malaria is an important contributor for nutritional deficiencies (iron and folate), other infectious diseases (hookworm, schistosomiasis and genetic red blood cell disorders (sickle cell and thalassaemias) are other important contributing factors (Vanden,1998). Malaria-associated anemia is a major cause of morbidity, admission, and mortality among children in malaria endemic areas of SSA. Because anemia presents itself with non-specific signs and symptoms, the condition is often unrecognized and under-treated. If left untreated, anemia is a major risk factor for mortality (Schellenberg *et al*., 2003).

Keizer *et al. (*2004) reported that Ethiopia and other countries in SSA are characterized by rapid population increase particularly in areas where the highest rates of *Plasmodium falciparum* are common. Ethiopia is also characterized by poor housing, lack of proper sanitation, poor drainage of surface water, inadequate health services and wide-spread economic disparity. The current study area, Pawe district is the place where the *woreda* is located and it is one of the special Woreda administrations under Benishangul Gumuz Regional State in western Ethiopia. However, there is no previous documented study reported on association of malaria infection with hemoglobin level among local inhabitants in the study area. This was what motivated the researcher to conduct this MSc. thesis research to generate epidemiological information on the status of malaria infection and malaria-associated anemia. Thus, the general objective of the study was to determine the prevalence of malaria and its association with hemoglobin level among patients visiting Pawe General Hospital, North-west Ethiopia.

The specific objectives were:

1. To determine the prevalence of malaria and the predominant *Plasmodium* species among patients visiting Pawe General Hospital.
2. To determine the level of hemoglobin among malaria patients visiting Pawe General Hospital.
3. To examine the association between malaria and haemoglobin concentration in the study population.

2. LITERATURE REVIEW

2.1. Malaria

Malaria is one of the most prevalent parasitic infections in the world and certainly the most detrimental. Each year, over two million people die from the disease, with the vast majority of the deaths in children under five years old in SSA (Snow *et al*., 2005). The spread of drug-resistant malaria parasites threatens to compound the problem even more. The situation is so dire that economists have determined that malaria is a definitive cause of poverty in many afflicted regions (Sachs and Malaney, 2002). Furthermore, in malaria-endemic regions, the effect of the disease is also manifested by its lasting influence on human genetics, resulting in the preservation of potentially harmful variants of human genes (i.e., sickle cell trait), largely because of their advantage in heterozygotes protected from severe, complicated, and fatal malaria.

Human malaria is one of the most important causes of morbidity and mortality resulting in serious socio-economic problems particularly in developing countries. It is one of the major tropical diseases adversely affecting the health of the peoples and the economic development of many developing countries, particularly in SSA. Each year, between 300 – 500 million malaria cases and up to three million deaths occur throughout the world, Africa accounting for more than 90% of the burden. Over 80% of malarial deaths occur in Africa with nearly 90% of those deaths being among young children and pregnant women in the developing countries of SSA, while less than 15% of the deaths occur in Asia and Eastern Europe (WHO and UNICEF, 2005).

2.2. Malaria Parasite (*Plasmodium* species)

Malaria parasites are micro-organisms that belong to the genus *Plasmodium* (Phylum Apicomplexa). There are more than 400 species of *Plasmodium*, which can infect many animal species such as reptiles, birds, and various mammals. Only four species of *Plasmodium* infect humans in nature (Mueller *et al*., 2007)**.** In addition there is one species (*P. knowlesi*) that

naturally infects macaques which has recently been recognized to be a cause of zoonotic malaria in humans (Baird, 2009). The species infecting humans are:

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

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

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

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

P. knowlesi- Non-human anthropoid malaria is recognized as the fifth species of *Plasmodium* infecting humans. It is found throughout Southeast Asia as a natural pathogen of long-tailed and pig-tailed macaques. It has recently been shown to be a significant cause of zoonotic malaria in that region, particularly in Malaysia (Baird, 2009).

2.3. Malaria Vectors

There are about 380 species of *Anopheles* mosquitoes (WHO, 2002). 70 species can transmit malaria under natural condition, and of these 40 is 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). In Africa, members of *Anopheles gambiae* complex and *Anopheles funestus* are widely distributed and are responsible for the transmission of malaria in the region.

2.4. The Life Cycle of *Plasmodium* Species

The malaria parasite exhibits a complex life cycle involving an insect vector *Anopheles* mosquito and a vertebrate host (human). All four species exhibit a similar life cycle with only minor variations. When an infected female *Anopheles* mosquito bites a human, it takes in blood. At the same time, it injects saliva that contains the infectious form of the parasite, the sporozoite, into a person's bloodstream. The thread-like sporozoite then invades a liver cell. There, during the next week or two (depending on the *Plasmodium* species), each sporozoite develops into a schizont, a structure that contains thousands of tiny rounded merozoites (another stage of the parasite). When the schizont matures, it ruptures and releases the merozoites into the bloodstream. Alternatively, some *P. vivax* and *P. ovale* sporozoites turn into hypnozoites, a form that can remain dormant in the liver for months or years. If they become active again, the hypnozoites develop into schizonts that then cause relapses in infected people (WHO, 2007b). Merozoites released from the liver upon rupture of schizonts rapidly invade RBCs, where they grow by consuming haemoglobin. Within the RBC, most merozoites go through another round of asexual reproduction, again forming schizonts filled with yet more merozoites. When the schizont matures, the cell ruptures and merozoites burst out. The newly released merozoites invade other RBCs, and the infection continues its cycle until it is brought under control, either by medicine or the body's immune system or defenses (WHO, 2007b).

The *Plasmodium* parasite completes its life cycle through the mosquito when some of the merozoites that penetrate RBCs do not develop asexually into schizonts, but instead change into

male and female sexual forms known as gametocytes. These circulate in the person's blood stream, until they are picked up by blood-seeking female *Anopheles* mosquito (WHO, 2007a). When a female mosquito bites an infected person, it sucks up gametocytes along with blood. Once in the mosquito's stomach, the gametocytes develop into sperm-like male gametes or large, egg-like female gametes. Fertilization produces an oocyte filled with infectious sporozoites. When the oocyte 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, 2007a).

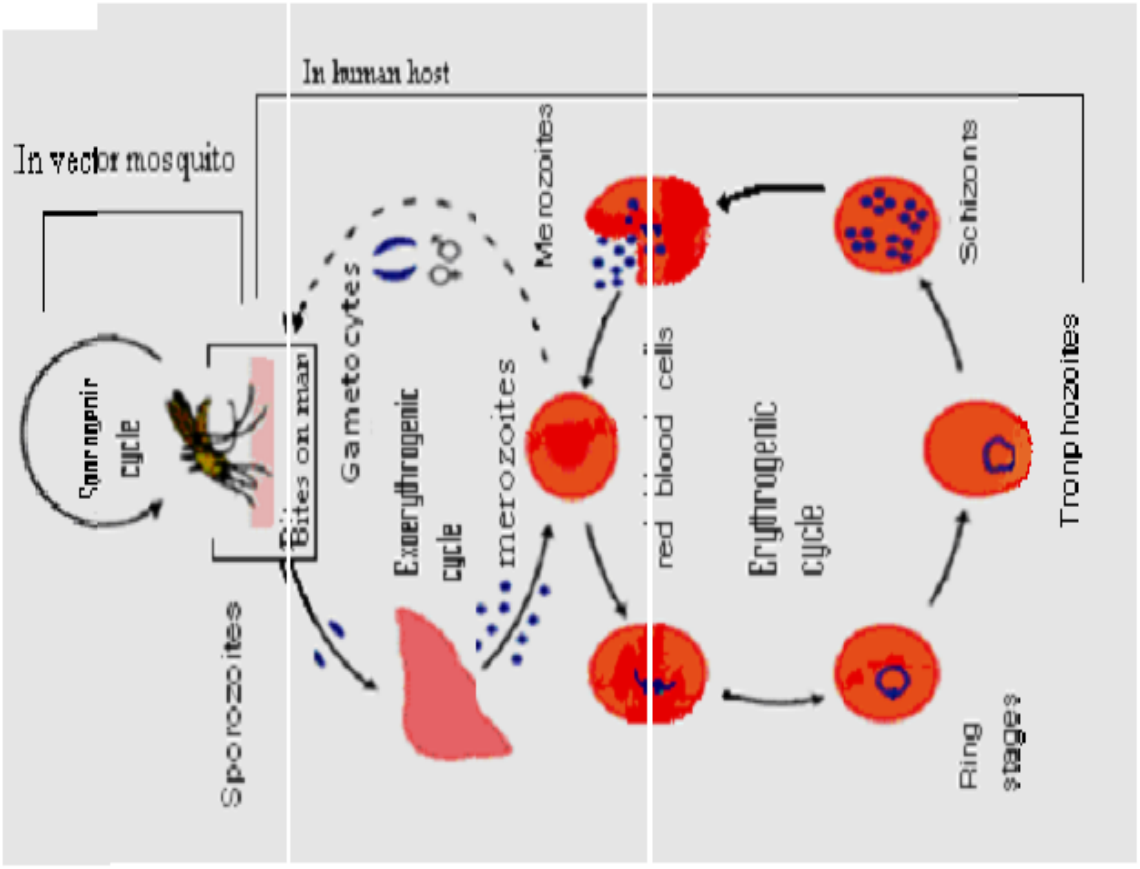

Figure 1. Life cycle of plasmodium species (Source: Lamb *et al*., 2006)

In summary, malaria parasites undergo three distinct asexual replicative stages: exoerythrocytic schizogony, blood stage schizogony, which occurs in humans and sporogony, which occur in mosquito, resulting in the production of the invasive forms of merozoites and sporozoites. Sexual

reproduction occurs with the switch from vertebrate to invertebrate host and leads to the formation of the invasive ookinete (CDC, 2006).

2.5. Clinical Manifestation of Malaria Parasite Infections

The most characteristic symptom of malaria is fever other common symptoms include chills, head ache, nausea, and vomiting (Garcia, 2008). As the disease progresses, some patients may develop the classic malaria paroxysm with bouts of illness alternating with symptom free period. The malaria paroxysm comprises three successive stages. The first is a 15 to 60 minutes cold stage characterized by shivering and a feeling of cold. Next comes the 2 to 6 hour hot stage, in which there in fever, sometimes reaching 41°C, flushed, dry skin, and often headache, nausea, and vomiting, finally there is the 2 to 4 hour sweating stage during which the fever drops rapidly and the patient sweats. The most frequent and serious complication of malaria are cerebral malaria and severe anemia kidney that can result death (CDC, 2008).

2.6. Pathogenesis

The disease remains one of the major killers of humans worldwide, threatening the lives of more than one-third of the world's population (Smith and McKenzie, 2004). Humans contact malaria from the bite of a plasmodial-infected female *Anopheline* mosquito (Killeen *et al*., 2000). As the mosquito inserts its proboscis into a human to take its blood meal, it injects the plasmodial sporozoite at the same time through its saliva (Killeen *et al.*, 2006). The sporozoite begins the asexual cycle by the preerythrocytic development of merozoites in the parenchymal cells of the liver. The merozoites can repeat the pre-erythrocytic cycle in the liver cells, or they can enter the erythrocyte cycle. Once the merozoites penetrate the erythrocytes, the parasite undergoes several morphological changes. First, a ring form develops, which enlarges to become a mature amoeboid trophozoite filling most of the parasitized red blood cell (Philip, 2011). Next, asexual multiplication takes place by the splitting of nuclear material and cytoplasm of the amoeboid appearing parasite to form more merozoites.

2.7. Global Epidemiology of Malaria

The epidemiology of malaria is highly dependent on the transmission pattern of the parasite (Figure 2). An area supporting active malaria transmission is termed endemic whereas sporadic outbreaks determine epidemic areas (WHO, 2000). These are conductive environments for the transmission, the presence of suitable *Anopheles* mosquitoes, the presence of *Plasmodium*, and the presence of a reservoir of the parasite.

The variation of malaria epidemiology is not limited by continents or between countries. There is also variation in the distribution of *Plasmodium* in a single country. As MOH (2002a) stated two extreme occurrences of malaria were seen in different parts of a country. In one extreme malaria might be unstable, occurring in epidemics separated by intervals of low incidence of malaria. Unstable malaria occurs when there is sudden development of circumstances which are conductive for the transmission of infection at levels far above the usual period of occurrence. In this case it occurs as an acute febrile illness and it affects all age groups and result in high mortality and morbidity.

2.8. Epidemiology and distribution of malaria in Ethiopia

Ethiopia is a tropical country which is located in the horn of Africa, between 3^0 25' and 14^0 54' North latitudes and between 33^0 and 48^0 East longitude. Moreover, Ethiopia has now become one of the land-locked countries, since the independence of Eritrea. Due to higher altitudes in most parts of the country the physical and biotic environments as well as the type of food production are similar with that of temperate regions (Ashenafi, 2008).

Ethiopia has a total area of 1.14 million km^2 and a country of great geographic diversity (http://www.ethemb.se/ee_eth.html). It has high and rugged mountains, flat-topped plateau, and deep gorges, incised river valleys, and rolling plains. About half of all the highlands of Africa above 2000 m are found in Ethiopia (MOH, 2002a). Besides, altitudes of the country range from the highest peak at *Ras Dejen* (4620 meter above sea level) to the depression of *Kobarsink* (110 meters below sea level).

At low temperatures (14-19°C) a small increase in temperature can greatly increase the risk of malaria transmission. However, high temperature (>40°C) is lethal to mosquito and the parasite. In areas where mean annual temperature is close to the physiological tolerance limit of the parasite, a small temperature increase would be lethal to the parasite, and malaria transmission would therefore decrease (Teklehaimanot *et al*., 2004).

Altitude and climate (rainfall and temperature) are the most important determinants of malaria transmission in Ethiopia. Transmission is seasonal and largely unstable in character ranging from less than three months to more than six months duration. The major transmission season of malaria follows the June-September rains and occurs between September and December while the minor transmission season occurs between April and May following the February-March rains (CDM5, 2017).

Related to health and disease, rivers have important feature, for instance, seasonal fluctuation. As a result of seasonal variation in rainfall, the rivers and lakes of Ethiopia have seasonal characteristics. During the rainy season both the rivers and lakes are full and sometimes they flow over their banks and these lead to flooding. During the dry season their volume is decreased and they create different pockets of water body that is favorable ground for the breeding of different disease vectors such as mosquito. Most Lakes of Ethiopia are found in the rift valley (Teklehaimanot *et al*., 2004).The distribution of malaria in Ethiopia varies from place to place due to the above factors directly or indirectly affecting the pattern of malaria transmission. For example, the distribution of malaria in Ethiopia is largely determined by altitude. Altitude affects the pattern of malaria distribution in Ethiopia through its effect on temperature. Risk of malaria is highest in the western lowlands of Oromia, Amhara, Tigray and almost the entire regions of Gambella and Benishangul Gumuz. The midlands of Ethiopia between 1,000 and 2,200 meters altitude experience seasonal transmission of malaria with sporadic epidemics every few years. In the eastern lowlands of Ethiopia (primarily Afar and Somali), malaria is endemic only along the rivers, as this part of the country is largely dry away from rivers. Transmission is limited by the lack of water collections for mosquito breeding and low humidity due to low rainfall and sparse

vegetation. The central highlands of Ethiopia are free of malaria mainly due to the low temperatures, which slows the development of the vector and the parasite (CDM5, 2017)

2.9. Factors that Affect the Epidemiology of Malaria

2.9.1. Climatic Factors

As Ethiopia is located in the tropical region, most parts of the country have high temperature throughout the year. High amount of seasonal rainfall in most parts of the country and perennial rain falls in some areas is also the result of the location of the country. The seasonal rainfall with high temperature is possible for the occurrence of unstable, seasonal malaria transmission after the onset of the rainfall in most part of the country (Ashenafi, 2008). Several field studies have reported the impact of ambient temperature on malaria outcomes. For example, in South Africa, Craig and colleagues (2004) identified that a significant correlation between temperature and the number of malaria cases. In the same way, in Ethiopia, minimum temperature was associated with malaria in a cold district (minimum temperature below 12°C); while in a hot district (minimum temperature above 12°C) the effect was not significant (Teklehaimanot *et al*., 2004).

In Ethiopia the three elements of climate i.e. temperature, rainfall and humidity are strongly associated with altitude. Moreover, location contributes to seasonal variation of rainfall and temperature in the country. A minimum monthly rainfall of 50 to 80mm sustained over a period of months is thought to be necessary for endemic malaria transmission. However man can make breeding sites such as those created through irrigation (MOH, 2002). Rainfall also influences malaria transmission by providing mosquito breeding places and increasing humidity, which improves mosquito survival rates (Reid, 2000). The relationship between malaria and rainfall can be complex. According to Aron and Patz (2001), water is necessary for larval development; however, heavy rainfall during the wet season may flush mosquito larvae away. Heavy rainfall followed by drought can result in ephemeral pools which may also provide mosquito habitats. In contrast, a prolonged dry season followed by flooding can increase malaria incidence if it leaves remnant mosquito breeding places. A prolonged dry season can decrease mosquito numbers by reducing breeding sites and also reduce malaria incidence (Dennis, 1999).

2.9.2. Non- Climatic Factors

Malaria parasite is capable of becoming resistant to the action of anti-malaria drugs. Drug resistance has been confirmed in only two of the four human malaria parasite species, *P. falciparum* and *P. vivax* (CDC, 2004). Studies revealed that chloroquine resistant *P. falciparum* (CRPF) first developed independently in three to four foci in Southeast Asia, Oceania, and South America in the late 1950's and early 1960's. Since then, chloroquine resistance has spread to nearly all areas of the world where *falciparum* malaria is transmitted (Sanchez and Lanzer, 2000. *P. falciparum* has also developed resistance to nearly all of the other currently available antimalarial drugs, such as sulfadoxine/ pyrimethamine, mefloquine, halofantrine, and quinine. Although resistance to these drugs tends to be much less widespread geographically (Sanchez and Lanzer, 2000). 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-malaria drugs, such as chloroquine and sulphadoxinepyrimethamine is increasingly hampering the fight against malaria. Chloroquine and sulphadoxine -pyrimethamine are still the most widely used drugs for the treatment of malaria in most of African countries because of low cost and availability (CDC, 2006).

2.10. Prevention and Control Strategies of Malaria

The recent malaria control and prevention in Ethiopia has been governed by a five-year strategic plan for 2006–2010 include: I) Early diagnosis and effective treatment, II) Selective vector control mainly through the use of ITNs and IRS, III) Epidemic prevention and control IV) Human resource development, V) Health Management and Information System VI) Monitoring and evaluation VII) Operational Research VIII) distribution and prompt treatment of malaria patients using ACT based on diagnosis of patients (WHO African Region, 2007). Every year, malaria continues to claim over a million lives around the globe. Attempts have been made to control the disease by eliminating the parasite. However, with a hundred *Plasmodium* species known to cause malaria, eradication of the parasite remains a daunting task. As a result, increased efforts and

resources have been channeled towards finding ways of minimizing human-vector contact, thereby controlling the disease (MOH, 2002b).

2.10.1. Vector Control

Vector control is an important part of the global malaria control strategy. The idea behind vector control is to reduce levels of mortality and morbidity by reducing transmission of the disease (Toure, 2001).

2.10.2. Insecticide Treated Materials (ITMs)

Insecticide treated materials is one of the most upcoming malaria control strategy, which combines a physical barrier with an insecticide against adult vectors. The netting material is treated with synthetic pyrethroid insecticide, which is relatively safe. The insecticide repels mosquitoes and inhibits them from enjoying their blood meal even when there are large holes in the nets. The most commonly used pyrethroids are permethrin, deltamethrin and lambda cyhalothrin (MOH, 2002a). The evidence from several studies show that use of insecticide-treated materials reduced severe malaria cases in children by about 45% and all-cause mortality by about 20% (MOH, 2002a).

2.10.3. Indoor Residual House Spraying

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 (RBM, 2003). 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).

2.10.4. Biological Control

Biological control of mosquito vectors involves the introduction of natural enemies into mosquito breeding sites. These could be in form of parasites or predatory animals e.g. fish, insects, fungi, nematodes etc. Use of biological control agents require a good understanding of the agents and

the mosquitoes to be controlled as well as their local environment. The most widely employed biological control agents are the larvivorous fish.

2.10.5. Medical Treatment

There are a number of drugs that can help prevent or interrupt malaria in travelers to places where infection is common. Many of these drugs are also used in treatment. Chloroquine may be used where chloroquine-resistant parasites are not common (Jacquerioz and Croft, 2009). In places where Plasmodium is resistant to one or more medications, three medications mefloquine (Lariam), doxycycline (available generically), or the combination of atovaquone and proguanil hydrochloride (Malarone) are frequently used when prophylaxis is needed (Jacquerioz and Croft, 2009). Doxycycline and the atovaquone plus proguanil combination are the best tolerated; mefloquine is associated with death, suicide, and neurological and psychiatric symptoms (Jacquerioz and Croft, 2009).

The protective effect does not begin immediately, and people visiting areas where malaria exists usually start taking the drugs one to two weeks before arriving and continue taking them for four weeks after leaving (except for atovaquone/proguanil, which only needs to be started two days before and continued for seven days afterward) (Freedom, 2008). The use of preventative drugs is often not practical for those who live in areas where malaria exists, and their use is usually only in pregnant women and short-term visitors. This is due to the cost of the drugs, side effects from long-term use, and the difficulty in obtaining anti-malarial drugs outside of wealthy nations (Fernando *et al*., 2011). During pregnancy, medication to prevent malaria has been found to improve the weight of the baby at birth and decrease the risk of anemia in the mother (Radeva-petrova *et al*., 2014). The use of preventative drugs where malaria-bearing mosquitoes are present may encourage the development of partial resistance (Turschner and Efferth, 2009).

2.11. Hemoglobin Level and Anemia and its Association with Malaria

Haemoglobin is a protein found in red blood cells that carries oxygen and gives blood its red color and frequently abbreviated as Hb. Most people have the type of haemoglobin called haemoglobin '**a**' (also called normal or adult haemoglobin). However there are many different

types of haemoglobin found in people throughout the world. Haemoglobin '**c**' is one type; sickle haemoglobin is another type and a blood test is required to determine haemoglobin type. Normal range of haemoglobin levels is defined by the World Health Organization as between 12 and 16 g/dL. By WHO criteria, anemia is defined as a haemoglobin concentration lower than 13 g/dL in men and lowers than 12 g/dL in women. Severe anemia is characterized as Hb < 10 g/dL. Haemoglobin, which is contained in red blood cells, serves as the oxygen carrier in blood. The name haemoglobin comes from heme and globin, since each subunit of haemoglobin is a globular protein with an embedded heme (or haem) group. Each heme group contains an iron atom, and this is responsible for the binding of oxygen. The presence of haemoglobin in blood increases the oxygen carrying ability of a liter of blood from 5 to 250 ml. Hemoglobin also plays a major role in the transport of carbon dioxide from the tissues back to the lungs. Myoglobin, on the other hand, is located in muscle, and serves as a reserve supply of oxygen and also facilitates the movement of O_2 within muscle (Streitweiser and Heathcock, 1981).

2.11.1. Haemoglobin Degradation in the Human Malaria Parasite

Pathogens may enter host cells and thus escape the immune system. Within the host cell the pathogens may replicate until they exit in order to infect other cells. Host cells may be forced to support the pathogen by increased nutrient supply and waste disposal across the cell membrane (Gulbin and Lqng, 2001). The intraerythrocytic malaria parasite develops within a cell that contains a single major cytosolic protein, haemoglobin. The organism avidly ingests host haemoglobin and degrades it in a specialized proteolytic organelle called the digestive vacuole.

2.11.2. Anemia

Iron is a major component of haemoglobin that carries oxygen to all parts of the body. Iron also has a critical role within cells assisting in oxygen utilization, enzymatic systems, especially for neural development, and overall cell function everywhere in the body. Thus, iron deficiency affects all body functions, not only through anemia, which appears late in the process of tissue iron deficits (MMWR, 1998). The main contributory causes of anemia are infectious diseases, nutritional disorders, and haemoglobinopathies (Phillips and Pasvol, 1992). Some investigators cite an iron-deficient diet, hookworm infection, or schistosomiasis as important causes for anemia

(Brooker *et al*., 1999), while others have identified malaria as the primary cause. Pediatric anemia is a major public health problem in malaria-endemic areas of SSA. Up to 75% of children in SSA are estimated to be anemic (hemoglobin <11g/dL), mainly due to malaria and ir on deficiency. The etiology of anemia in SSA is multifactorial, but infection with *Plasmodium falc iparum* is considered the major cause of anemia in children below 2 years of age. Some maternal factors in the antenatal period (e.g., placental malaria, poor nutrition, HIV infection) are also known to predispose African children to the development of anemia (Reed *et al.,* 1994). Several factors including, the infecting malaria parasite species, intensity of transmission, age of the patient, host-genetic factors and presence of other non-malarial causes of anemia determine the prevalence and severity of malarial anemia. The pathogenesis of malarial anemia is multifactorial, complex and incompletely understood: postulated mechanisms include excessive destruction or defective production of red blood cells or a combination of both processes. Malaria-associated anemia may present as an acute episode or as a chronic process following repeated, often, asymptomatic infection (Phillips, 1992).

2.11.3. Iron Deficiency

Iron deficiency anemia (IDA) are estimated to be the most widespread of all nutritional deficiencies (Chaparro, 2008), iron is a major component of haemoglobin that carries oxygen to all parts of the body. It also has a critical role within cells assisting in oxygen utilization, enzymatic systems, especially for neural development, and overall cell function everywhere in the body. Thus, iron deficiency affects all body functions, not only through anemia, which appears late in the process of tissue iron deficits. Iron deficiency can exist with or without anemia. Iron is found in the body in two forms, as functional iron (iron that serves a metabolic function) and as storage iron. When a person has depleted their stores of iron, they are said to be “iron deficient.” When the depletion progresses; the haemoglobin concentration in red blood cells falls below the normal range (MMWR, 1999).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The present study was conducted at Pawe General Hospital, Pawe district, Benishangul Gumuz Regional State, North-west Ethiopia (Figure 3). It is located 570 km away from Addis Ababa, the capital city of Ethiopia.

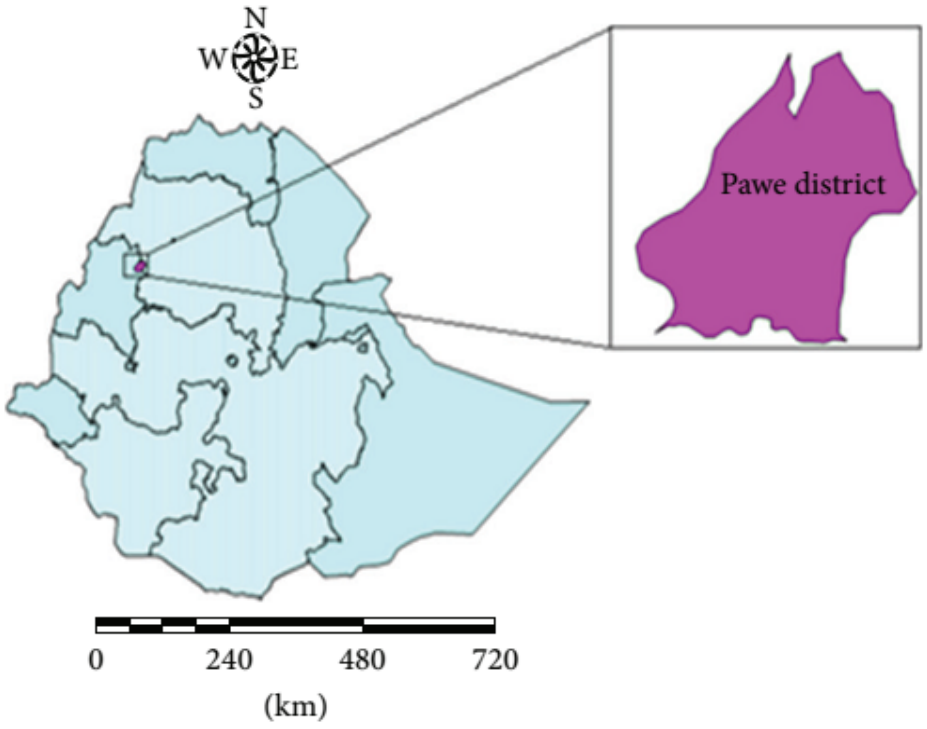

Figure 2. Map of the study site.

The study site is geographically located at $11^{0}.009'$ N latitude, $36^{0}.003'$ E longitude, and an altitude of 1050 meters above sea level. In this district, the major and minor malaria transmission peak seasons are from September to December and from April to May, respectively, coinciding with the major harvesting and planting seasons. The peak rainfall occurs from July to August. The mean annual rain fall and maximum temperature of the area are 1555.1 mm and 32(with mean monthly values ranging in 27–37), respectively. Pawe district has a total population of 45,552, of whom 23,265 were men and

22,287 were women. About 10,068 (22.1%) of the population are urban inhabitants (CSA, 2007). According to the growth rate of Ethiopian population in 2013 i.e. (2.58%), total population of the Pawe district were estimated to be 52,603 of whom 26,866 were men and 25,737 were women. As the hospital has all facilities for malaria diagnosis, standard microscopic investigations were carried out for all patients following standard procedures recommended by WHO, (2010).

3.2. Design of the Study

A hospital based cross-sectional survey was carried out in malaria transmission season i.e. from October-December 2016.

3.3. Study Population

Study population was categorized in to sub populations of different age groups. These are young (≤15 years old), adults (16-40 years old) and elders (≥40 years old). **Inclusion criteria: C**lients with suspicion of having malaria symptoms and patients visiting the hospital during the observation period was randomly selected. **Exclusion criteria:** Those patients who were receiving anti-malaria treatments at the time of study were excluded.

3.4. Sample Size Determination

The sample size of the study was estimated using the formula shown by Naing *et al*. (2007) and assuming 50% prevalence since there was no reported study in the area.

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

$n=z^2\ P\ (1-P)/d^2$ Where, n= sample size

$n= (1.96)^2(.5)\ (1-0.5)/\ (0.05)^2$ P=prevalence of the disease

n=384 Z=95% confidence interval

d= precision (0.05)

To minimize an error from the likelihood of occurrence of non-compliance and non-responsive study participants, 5% of the sample size was added to the normal sample. Therefore, four hundred two (402) study participants were included in the present study.

3.5. Sampling Techniques

Subjects with suspicion of having malaria symptoms were recruited into the study until the optimal sample size was reached.

3.6. Method of Data Collection

3.6.1. Clinical examination of study subjects

The physical examination of each study subject for manifestation of signs and symptoms of Malaria was carried out by physician.

3.6.2. Blood sample collection

Blood samples from 402 persons were collected by pricking their finger-tips with disposable lancet by the help of an experienced laboratory technician. The finger was first cleansed with an alcohol-moistened swab, dried with a piece of dry cotton and punctured with a disposable blood lancet.

3.6.3. Blood film preparation and staining

Using drops of blood, thin and thick blood smears were made on the same slide side by side and properly labeled for each 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. Staining and blood film examination was performed by following the standard protocol of World Health Organization (Garcia, 2001). Experienced laboratory technician examined the slides for parasites under the microscope. The presence of malaria parasites on thick blood smear was examined using the high power magnification objective (40x) and the identification of

Plasmodium species from the thin blood smear was done using the oil immersion objective (100x). The thick smear was used to determine the presence and absence of malaria parasites and the thin smear was used to identify the type of *Plasmodium* species.

3.6.4. Questionnaire survey

A structured questionnaire was used to collect information related to the socio-demographic, characteristics of the respondents. These were age, sex, level of education and work status, family size and house type, possession of domestic animals in and around the home, experience to malaria infection, use of preventive measures, use of anti-malarial drugs, and other malaria related issues, so the questionnaire was distributed to 402 respondents.

3.6.5. Collection of malaria records

For secondary data analysis, the Malaria data (i.e., Malaria positive cases) during the period of five years from 2012 to 2016 were collected from the hospital records and documentation.

3.6.6. Collection of Weather Data

Weather data were collected from meteorological station of the study area from Pawe Agricultural Office during the study period (October - December, 2016). The average rainfall (mm), average temperature (^{0}C) and average relative humidity (%) of the study area during the study period was recorded (Appendix I).

3.7. Laboratory Examination Procedures

Examination of well-prepared and well-stained blood films is the gold standard for confirming the presence of malaria parasite (Payne, 1988). For the positivity test, 100 fields were examined in longitudinal and vertical directions. To ensure accuracy, all positive slides and a random sample of 10% of the negative slides were re-examined by a second specialized laboratory technician who is blinded to the diagnosis of the first slide-reader.

Microscopic examination of thick films were done using high power magnification to detect the presence of parasites and the parasite species identification was done using examination of the thin films under 100x oil immersion objective. The method recommended for staining thick blood was Field's Stain which is made from two components. Field's A is a buffered solution of azure dye and Field's B is a buffered solution of eosin. Both Field's A and B were supplied ready for use by the manufacturer. In thin films the red blood cells were fixed so the morphology of the parasitized cells could be seen. Species identification can be made, based upon the size and shape of the various stages of the parasite and the presence of stippling (i.e. bright red dots) and fimbriation (i.e. ragged ends). However, malaria parasites may be missed on a thin blood film when there is a low parasitaemia. Therefore, examination of a thick blood film is recommended. With a thick blood film, the red cells are approximately 6-20 layers thick which results in a larger volume of blood being examined. In examining stained thick blood films, the red blood cells are lyses (destroyed), so diagnosis is based on the appearance of the parasite. In thick films, organisms tend to be more compact and denser than in thin films (Garcia, 2001).

3.7.1. Identification of malaria parasites

Plasmodium species were identified by their morphological characteristics observed from Giemsa stained thin blood smears. They are also their clinical course, including incubation period path physiology and associated morbidity and mortality (WHO and UNICEF, 2005).

3.7.2. Determination of blood hemoglobin concentration

Haemoglobin concentration was measured using the hematology analyzer (Cell-Dyn 1800).The Cell-Dyn 1800 is a new hematology analyzer with a throughput of 60 specimens per hour. It provides a basic blood count with a 3-part white blood cell differential. Due to its capacity the Cell-Dyn 1800 is well suited in a small clinical laboratory as a primary analyzer or a backup analyzer in a medium-size clinical laboratory. Each blood sample was placed into the test tube then, Complete Blood count Test was carried out using the machine (CBT) (Kendall R, 2003).

3.8. Data Analysis

At the end of the study clinical data and prevalence data of the malaria parasite of the peak malaria transmission seasons were obtained, SPSS version 16.0 was used for statistical data analysis. Descriptive statistics were used to give a clear picture of population characteristics such as age, sex, and distribution of *Plasmodium* species and Hemoglobin level. Association of sex with proportion of malaria parasites was made using chi-square test. Statistical significance were defined at P-values less than 0.05 ($P<0.05$). To ensure the validity and clarity of these data, each data was recompiled and recounted in order to fill some missing information. Pearson's coefficient of correlation analyses were also conducted to determine the association between malaria prevalence data and hemoglobin level.

3.9. Data Quality Control

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 in the field in such a way that the slide code was match with the file of that particular individual. During blood sample collection one sterile lancet was used per person. The quality of the Giemsa staining solution, fixation chemical (methanol) and the microscope was checked before using directly. To ensure quality, the staining techniques and blood film examination was conducted according to standard protocols of World Health Organization (Garcia, 2001).

3.10. Ethical Consideration

The study received ethical clearance from the Institutional Review Board of the College of Health Sciences of the Haramaya University, and from local Pawe special Woreda health bureau. All the study participants were clearly informed about the purpose of the study and kindly asked to participate and permission were obtained before the actual investigation.

4. RESULTS AND DISCUSSION

4.1. Socio-Demographic Characteristics of Study Participants

The socio-demographic characteristics of respondents are summarized and presented in Table 1. A total of 402 respondents were included in the present study. 210(52.2%) were males and the rest 192 (48%) were females. Among the respondents, 18 (4.5%) were temperate residents, 10 (2.5) were high land residents and the rest 374(93.0%) were low land residents (Table 1).

As depicted in Table 1, 68(17%) were students, 42(10.4%) were government employer, 130(32.3%) were farmer, 38 (9.5%) were house wives, 24(6.0%) has no job, 22(5.5%) were merchant and 26 (6.5%) were private sector employee and 52(13%) daily laborer. Regarding the educational level 70 (17.4%) of the respondents had secondary school education, 146 (36.3%) completed Elementary school, 92 (23%) were illiterate and 34 (8.5%) were can only read and write whereas, 36 (9%) had higher education and 24 (6%) were under school age (Table 1).

Regarding livestock availability in the house, 134(33.3%) of the respondents kept livestock in their house and 268 (66.7%) didn't possess livestock at all. The average income of the respondent were 1875.00 ETB per month and 100(24.9%) had no specified income at all. The maximum and minimum monthly income was 3600.00, and 150.00 ETB per month, respectively. 206 (51.2%) of the respondents were living in improved type of house and 196 (49%) had conventional housing units. Regarding to the respondents' duration of stay in the study area, 48 (12%) lived since birth, 94 (23.4%) stayed above 10 years, 46 (11.4%) were stayed less than 1year, 103 (26%) were 1-5 year and 55 (14%) stayed in the area 6-10 years (Table 1).

Table 1. Some socio-demographic characteristics of study participants in Pawe General Hospital North-west Ethiopia, 2016

Character	Number (%)	Character	Number (%)
Sex		Occupation	
Male	210 (52.2)	Farmers	130 (32.33)
Female	192 (48)	Merchants	22 (5.47)
Age Group		Government employee	42 (10.44)
≤15	70 (17.4)	Students	68 (16.91)
16-40	314 (78.1)	Private sector employee	26 (6.46)
≥40	18 (4.4)	House wife	38 (9.45)
Marital Status		Daily laborer	52 (12.93)
Married	202 (50.2)	Un employed	24 (5.97)
Single	160 (39.8)	Presence of Livestock	
Divorced	28 (6.96)	Yes	134 (33.33)
Widowed	12 (2.98)	No	268 (66.66)
Family Size (No of persons)		House Type	
1-3	170 (42.28)	Conventional	196 (48.75)
4-6	150 (37.31)	Improved	206 (51.24)
7-10	68 (16.91)	Current Place of Residence	
>11	14 (3.48)	High land	10 (2.48)
Educational Level		Lowland	374 (93.03)
Illiterate	92 (22.88)	Temperate	18 (4.47)
Only read and write	34 (8.45)	Duration of stay in the area	
Elementary education	146 (36.31)	<1 years	56 (13.93)
Secondary education	70 (17.41)	1-5 years	116 (28.85)
Diploma and above	36 (8.95)	6-10 years	75 (18.65)
Under school age	24 (5.97)	>10 years	97 (24.12)
Average Monthly Income		Since birth	58 (14.42)
100-500	50 (12.43)		
600-1000	80 (19.90)		
>1000	172 (42.78)		
Not specific income	100 (24.87)		

4.2. Respondents' Level of Knowledge and Awareness about Malaria

In relation to the transmission of malaria, 250 (62.18%) of the respondents replied that it was transmissible, 52 (12.93%) replied that malaria cannot be transmitted from person to person and 100 (24.87) had no idea about transmission of malaria at all (Table 2). The majority of the respondents said that malaria could be transmitted from infected person to a healthy one. The result of this study was high compared to the central Ethiopian studies where 12% of the respondents reported malaria to be transmitted (Mengistu and Wakgarii, 2009). Also this proportion was lower than that reported from the central Ethiopian study where 74% of the respondents were found to know that malaria was transmitted from person to person (Yeneneh *et al.,* 1993). The difference in the results might be attributed to the difference in level of awareness and knowledge about sources of malaria infection among the study population.

Regarding modes of malaria transmission 212 (52.73%) of the respondents correctly associated malaria with mosquito bites (Table 2). This was relatively higher figure compared to the reports made (48%) from other parts of country (Mengistu and Wakgari, 2009).This showed that the presence of a better knowledge about the method of malaria transmission in the present study. However, a study from residents of Pawe general hospital and Pawe district, north-west Ethiopia showed that a large figure (98.2%) of the respondents reported mosquito bite as the cause of malaria. By Ayalew and Amsalu,(2009) indicating that the population had at greater awareness on the modes of transmission of malaria than the present study population.

In addition, respondents mentioned other mode of transmission of malaria like body contact 12 (2.98%), drinking from unprotected water sources 8 (1.99%), dirty environment 20(4.97%), from cold and rain 10 (2.48%), from rain water 12(2.98%) and 128 (31.84%) were didn't know about transmission of malaria (Table 2). Mosquitoes mainly bite people during night time. The majority of participants 316 (78.60%) knew that mosquitoes bite mostly at night and 24 (5.97%) responded that mosquitoes bite at day time and night, 14 (3.48%) replied that mosquito bite at day time, whereas 48 (11.94%) had no idea about the time when mosquito bite humans (Table 2).

This knowledge in the present study was relatively higher than the level reported in the study carried out in central Ethiopia where 42.6% knew that mosquitoes bite most at night (Mengistu and Wakgari, 2009).

As the result shown in Table 2, the knowledge regarding the breeding site of mosquitoes was also high, with 292 (72.63%) of the respondents mentioned stagnant water as the main breeding site of the malaria vector, 22 (5.47%) said that running water was suitable for malaria breeding, 14 (3.48) said soil as breeding site; whereas 74 (18.40%) of the respondents had no idea about the breeding site of mosquito. From this study 132 (32.83%) had good knowledge about malaria followed by 270 (67.16%) had poor knowledge about malaria (Table 2).

Most of the respondents 204 (50.74%) had access to protected water source and 198 (49.25%) had no protected water source. Regarding the bed net possession 132(32.83%) were had one net per house, 142 (35.32%) were using two nets per house, 52 (12.93%) were using three nets per house and 76 (18.90%) had no bed net at all.

As the result shown in table 2, knowledge about treatment was high in which 200 (49.75%) of the study subjects stating that they would seek treatment in Hospital, 14 (3.48%) prefer to go to traditional healers, 166 (41.29%) prefer health center, 8 (1.99%) prefer just to go to pharmacy/drug shop, 14 (3.48%) had no response at all. The majority of respondents reflect issues of accessibility and quality in the health facilities of the Hospital. Similar results were found in a study conducted in northern Ethiopia (Johan *et al*., 2009). The reasons mentioned for their preference of these sources were; 174 (43.28%) said effectiveness of treatment followed by 180 (44.77%) who said because of the closeness to home and low cost was mentioned by 48 (11.94%) respondents. Moreover, in the same study the most frequently mentioned reason for preferring the mentioned sources of treatment was effectiveness of treatment (83.3%) (Yeneneh *et. al.*, 1993).

Regarding knowledge about preventability of malaria among the study participants, 358 (89.05%) said that malaria can be prevented, 7(1.74%) replied malaria cannot be prevented and 36(8.95%) have no idea about malaria preventivness (Table2). This finding was in agreement with studies done in southern Ethiopia (Wakgari *et al*., 2002). This could be explained by the fact that the

activities of Malaria Control Program to prevent and control the disease in the area might have enhanced their knowledge on the preventability of malaria and its preventive measures.

The most frequently preventive methods of malaria mentioned by respondents were use of bed net or ITN 164 (40.79 %). ITN is a malaria prevention tool known by the community as “Agober” or “Zanzira” to mean bed net in local language. Almost all participants heard about or seen ITN; it is distributed by the government. Most reported that they have ITN, some use it for its primary purpose and some use it inappropriately (ACIPH, 2009).The other preventive method of malaria mentioned by this study were taking tablets 44 (10.94%),environmental sanitation 144 (35.82%), using local cotton sheets 6(1.49%) and smoke from burning leaves and cow dung 4 (0.99%),spraying the house hold with insecticides 8 (1.99%) and the remaining 32 (7.96%) of the participants did not know method of prevention at all.

Table 2. Level of knowledge and awareness about malaria by respondents in Pawe General Hospital North-west Ethiopia, 2016

Items (Variables)	Number (%)	Items (Variables)	Number (%)
Is malaria transmitted		Receiving health extension-service	
Yes	2,50 (62.18)		
No	52 (12.93)	Yes	142 (35.32)
I don't know	100 (24.87)	No	260 (64.67)
Mode of transmission		First choice of treatment-centers	
Mosquito bite	212 (52.73)		
Body contact	12 (2.98)	Traditional healers	14 (3.48)
Drinking unprotected- water	8 (1.99)	Hospital	200 (49.75)
From marshy area	20 (4.97)	Health centers/ clinics	166 (41.29)
From cold environment	10 (2.48)	Pharmacy/drug shops	8 (1.99)
From rain water	12 (2.98)	Don't know	14 (3.48)
I don't know	128 (31.84)	Reasons for choosing the-center	
Mosquito biting time			
Night time	316 (78.60)	Treatment is effective	174 (43.28)
Day time	14 (3.48)	Low cost drugs	48 (11.94)
Day and night	24 (5.97)	Closeness to home	180 (44.77)
I don't know	48 (11.94)	Malaria is preventive disease	
Mosquito breeding site		Yes	358 (89.05)
In the stagnant water	292 (72.63)	No	7 (1.74)
In the running water	22 (5.47)	Don't know	36 (8.95)
In the soil	14 (3.48)	Preventive methods	
I don't know	74 (18.40)	Taking tablets	44 (10.94)
Knowledge about malaria		Spraying the household- with insecticide	8 (1.99)
Yes	132 (32.83)		
No	270 (67.16)	Environmental sanitation	144 (35.82)
Source of water		Use of bed nets	164 (40.79)
Protected	204 (50.74)	Using local cotton sheets	6 (1.49)
Unprotected	198 (49.25)	Using smoke (burning- leaves) and cow dung	4 (0.99)
Bed net possession			32 (7.96)
Absent	76 (18.90)	Don't know	
One per house	132 (32.83)		
Two per house	142 (35.32)		
Three per house	52 (12.93)		

Figure 3 shows level of respondents' awareness about signs and symptoms of malaria in the study area. As it is shown in the figure 3, knowledge about the symptoms of malaria was very high. Majority respondents identified fever, vomiting, loss of appetite, shivering and chills as main symptoms both in adults and children. Some also stated back pain, headache and joint pain. Most of the study subjects had knowledge of at least one of the classical symptoms. This finding was in agreement with study done in Ganta Kanchama, Ganta Meche and Zigti Merche around Arba Minch town (Yarcho Yaya, 2011). In contrast, in a study conducted in western Kenya only 30% of the respondents were aware of malaria symptoms. The most frequently reported symptoms of malaria were fever 107 (26.61%), chills 29 (7.21%), shivering 57 (14.17%), headache 40 (9.95%), loss of appetite 24 (5.97%), joint pain 35 (8.7), backache 19 (4.72%), vomiting 74 (18.4%), and 17(4.22%) have no idea about symptoms and signs of malaria (Figure 3.)

Figure 3. Reported signs/ symptoms of malaria by studied respondents in Pawe Hospital, Pawe District, North-west Ethiopia, 2016

4.3. Trend of Prevalence of Malaria among Patients Admitted at Pawe General Hospital during 2012-2016

According to the retrospective clinical record from Pawe General Hospital (2012-2016), the prevalence of malaria was 2979 (24.89%) in year 2012, 2521(17.59%) in year 2013,1792(20.79%) in year 2014, 3288(17.13%) in the year 2015 and 3273 (17.19%) in year 2016(Table 3).The major *Plasmodium* species identified in examined patients at Pawe General Hospital during 2012-2016 were *P. falciparum* and *P. vivax* 79.15% and 20.84%,respectively. The highest cumulative prevalence of malaria out of the whole clinical record of the past five years (2012-2016) was detected in the 2012 and 2014 (Appendix; Table2).This was higher than the prevalence recorded in 2013 and 2015. The slight increment in the prevalence of malaria infection in the year 2012 and 2014 compared to 2013, 2015 and 2016 might be related with increase in the average annual rainfall and it might also be associated with improper use of bed nets and lack of sanitation. In agreement with this entomological surveys carried out in Kenya found that recent urbanization and construction activities were positively associated with female *Anopheline mosquito* breeding activity (Jacob *et al.*, 2003). This might be due to increasing number of population, improper use of bed net utilization and climate change in the area and the retrospective data analysis indicated that the seasonal malaria transmission had been a major cause of outpatient morbidity in Pawe.

Additionally, the record shows annual prevalence of malaria positive, *P. falcifarum* and *P. vivax* in the years 2012 to 2016. As the results shown in Figure 4 in 2012 the highest (437) and lowest (76) malaria positives were reported on May and January respectively. In the same way, February and October were the months reported with low (60) and high (376) malaria positives in 2013.

Figure 4 shows yearly prevalence of malaria, P. falicparum and P. vivax in the years 2012 to 2016. Malaria transmission occurs throughout the years and the maximum monthly prevalence reported in the year 2012 to 2016 was May and the minimum monthly prevalence was March. This shows that the main seasons were from July to August and the short rainy seasons were from January to

March. Regarding to P. falcifarum and P. vivax, the higher prevalence was reported in May and November respectively.

The records of Pawe general hospital also showed that there was a predominance of *P. falciparum* over *P. vivax*. Similar to the present study, several reviews showed that *P. falciparum* is the dominant species in Ethiopia, followed by *P. vivax*, accounting for 60% and 40% of all malaria cases, respectively (Tulu, 1993). The variation between these two species might be due to the seasonality in characteristics of the two important parasites. It is generally known that *P. falciparum* is the dominant species during the peak malaria transmission season in September and October, while *P. vivax* tends to dominate during the dry season in Ethiopia.

Moreover, parasitological community based study conducted by Gebreyesus,(1999) on the impact of small irrigation dams on malaria burden in Northern Ethiopia revealed a predominance of *P. falciparum*. The prevalence of malaria infections varies seasonally, with *P. vivax* dominating in the dry season (March-June) and *P. falciparum* peaking in September- October, after the end of the main rainy season. Hence, the proportion of malaria cases due to the two parasites species can vary across seasons and localities (Figure 4).

Figure 4. Distribution of the monthly total prevalence of malaria parasites from 2012 to 2016

4.4. Prevalence of Malaria among the Study Participants

Out of a total of 402 samples examined 161 (40.0%) were infected with malaria parasites (Table 3). Alternatively, the accessibility of the community to early treatment was likely to play a significant role in the lower prevalence in present study area. In addition; it could be explained by the difference in geographic location and in socio-demographic characteristics of the study population (Leonard *et al*., 2008).

Regarding to malaria infection with age groups observed, a relatively high prevalence 18 (47.4%) of *Plasmodium* species infections were detected in the age group ≤ 15 years old male, 12 (37.5%)

in age group≤ 15 years old female,73 (44.5%) in age group16-40 years old male,53 (35.3%) in age group16-40 years old female, 2 (25.0%) age group ≥40 years old male,3 (30.0%) age groups ≥40 years old female (Table 3).This difference in prevalence of *Plasmodium* species was statistically significant (P =0.0001) among age groups. This was in contrast with the report made from Ethiopia where the difference in prevalence of *Plasmodium* species among the age groups was not significant (Graves *et al*., 2008). However, similar result was reported in Nigeria where the difference in prevalence of *Plasmodium* species among the age groups was significant *(*Ilozumba and Uzozie, 2009).

As the results show in Table 4, in the current study, There was no statistically significant difference (x^2=0.69, p=0.279) and (x^2=0.055, p=0.618) in prevalence of malaria among male and female within the age groups of ≤ 15 years old, and ≥40 years old study participants respectively. However, the age groups of 16-40 years old participants were statistically significant within male and female (x^2=3.116, p=0.049) value.

The highest prevalence of *Plasmodium* species was observed in the age group ≤ 15 years old and which does fit into the conventional characterization of the epidemiology of malaria based on age stratification. That is conventionally in areas of high endamicity, prevalence of malaria infection is known to peak at an early age with an increase up to the age of 5 years; followed by a sharp fall in age groups 10-15 years and continuing on a slow decline with increasing age (WHO, 2000). This pattern of prevalence is a reflection of the age related state of antimalarial immunity that is developed as a result of repeated malaria infections under established malaria endemicity (WHO, 2000).

Out of the total malaria positive individuals, 44.3% were males and 35.4% were females (Table3). This finding, statistically shows significant variation of malaria infection between sexes (p =0.0001). A similar report was made from Nigeria which shows that the prevalence of malaria parasite was significantly higher in males than in females (Ilozumba and Uzozie, 2009).In contrary with this, Graves *et al* (2008) reported that there was no significant difference in the prevalence of malaria parasite between sexes. The possible explanation for the discrepancy could be that men have a greater occupation risk of contracting malaria than women if they work in mines, fields or

forest during peak biting times or migrate to areas of high endemicity for work and other behavioral risk factors (Anne *et al*., 2010).

Table 3. Proportion of malaria by age and sex of patients visiting Pawe general Hospital during October-December 2016

Age group in Years	Male		Female		Both Sexes		X^2	P-Value
	No. Examined	No. Positive (%)	No. Examined	No. Positive (%)	No. Examined	No. Positive (%)		
≤15 years	38	18 (47.4)	32	12(37.5)	70	30(42.9)	0.69	0.279
16-40	164	73(44.5)	150	53(35.3)	314	126(40.1)	3.12	0.049*
≥40	8	2(25)	10	3(30.0)	18	5(27.8)	.055	0.618
All age groups	210	93(44.3)	192	68(35.4)	402	161(40.0)	32.7	0.0001*

4.5. Major *Plasmodium* Species Identified Among Study Participants

The major *plasmodium* species identified were p*lasmodium falciparum* (86.9%) and *P. vivax* (13.0%)*(*Table 4). There was no case of mixed infection observed in the current study. *P. falciparum* and *P. vivax* are the main species accounting for 60% and 40% of malaria Cases respectively (MOH, 1999). In a similar study, *P. falciparum* was more frequently (69.4%) observed than *P. vivax* 30.6% earlier report (Graves *et al*. 2008), showed that there were 1.3 times as many people infected with *P. falciparum* 2.5% as compared to *P. vivax* 1.9% infections, with 0.3% infections being mixed. On the contrary to this, prevalence of malaria parasite species in Pakistan, observed high prevalence of *P. vivax* 88.69% and a low prevalence of *P. falciparum* 11.3%. The difference in parasite species occurrence may represent seasonal variations in the epidemiology of the parasite. Apart from seasonal variation, these differences might 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 (Bødker *et al*., 2006).Furthermore*, P. falciparum* pre dominates *P. vivax* during the period of increased

transmission or epidemic years, while *P. vivax* is the predominant species during low transmission or non-epidemic years. Such temporal variations in the relative frequency of the two parasite species might be related to a decrease in temperature and the effect of antimalarial drugs used (Yeshiwondim *et al.,* 2009).

Table 4. Major Plasmodium species identified in examined patients at Pawe General Hospital during October-December 2016.

Age group(Years) and Sex	No. Examined	No. Positive (%)	*Pf*	*Pv*
			No. Positive (%)	No. Positive (%)
≤15				
Male	38	18(47.4)	15(83.3)	3(16.7)
Female	32	12(37.5)	11(91.7)	1(8.3)
16-40				
Male	164	73(44.5)	62(84.9)	11(15.1)
Female	150	53(35.3)	48(90.5)	5(9.4)
≥40				
Male	8	2(25)	2(100)	0(0)
Female	10	3(30)	2(66.7)	1(33.3)
All Age groups				
Male	210	93(44.3)	79(84.9)	14(15.1)
Female	192	68(35.4)		7(10.3)
			61(89.7)	
Total	402	161(40.0)	140(86.9)	21(13.0)

Pf= Plasmodium falciparum, Pv= Plasmodium vivax

4.6. Haemoglobin Concentration and Prevalence of Anaemic Condition among Examined Study Participants

Normal range of haemoglobin levels is defined by the World Health Organization as between 12 and 16 g/dL. By WHO criteria, anemia is defined as a hemoglobin concentration lower than 13 g/dL in men and lowers than 12 g/dL in women. Severe anemia is characterized as Hb < 10 g/dl (Charves *et al*., 2004)**.**

According to the Haemoglobin concentration and prevalence of anemic condition in examined 161malaria patients in Pawe general hospital, during October-December, 2016, 81 (50.3%) respondents were anemic and out of these40(43.0%) were males and 41 (60.2%) were females. In this study there were significance variations of anemic prevalence among age groups P=0.025 (Table 5).

Malaria contributes to reduce haemoglobin concentrations through a number of mechanisms, principally by destruction and removal of parasitized red cells and the shortening of the life span of non-parasitized red cells, and decreasing the rate of erythrocyte production in the bone marrow (McDevitt *et al.,* 2004). Some of the mechanisms that cause anemia during malaria are associated more with the acute clinical states, whereas chronic or repeated infections are more likely to involve dyserythropoiesis (Menendez *et al.,* 2000).The prevalence of anemia decreases with age (Guralnik *et al*., 2004).

Table 5. Haemoglobin concentration and prevalence of anaemic conditions by age and sex of examined study participants in Pawe General Hospital, during October-December 2016

Age group (Years) and Sex	No. Examined	Mean±SEM Hb (g/dl)	Anaemic cases (Hb<12g/dl) Frequency (%)	X^2	P-Value
≤15					
Male	18	12.1±0.3	11 (61.1)		
Female	12	11.8±0.4	9 (75.0)		
16-40					
Male	73	12.1±0.2	29 (39.7)		
Female	53	12.8±0.6	31 (58.4)		
≥40					
Male	2	14±0.9	0 (0)		
Female	3	13.2±0.2	1 (33)		
All age groups					
Male	93	12±0.2	40 (43.0)		
Female	68	12.7±0.5	41 (60.2)		
Total	161	12.7±0.7	81 (50.3)	22.60	0.025*

4.7. Association between Malaria and Haemoglobin Concentration among Malaria Patients in study area

This study has also analyzed association between haemoglobin concentration and malaria parasites (Table 6). A significant association was found between Hb concentration of 6-8 g/dl and 8.1-10 g/dl with (p=0.06, x^2=8.05, r=-0.14 and p=0.07، x^2=15.13, r=-0.29) respectively. However, no significant association was observed malaria parasite and haemoglobin concentration of 10.1-12g/dl and 12.1-18g/dl with (P=0.45, r=-0.58, x^2=6.95 and P=0.25, r=-0.61, x^2=7.09)

consecutively. This could suggest that other factors such as poverty, poor health and sanitary conditions, limited knowledge of nutritional matters among certain households and fluctuations in incomes which affect the nutritional status are may be predominant among the study subjects as reported by (Chaparro, 2008).

Table 6. Association between malaria and haemoglobin concentration among malaria patients

Hb Concentration (g/dl)	Pf	Pv	R	X^2	P-value
6-8	50	7	-0.14	8.05	0.06*
8.1-10	35	9	-0.29	15.13	0.07*
10.1-12	30	3	-0.58	6.95	0.45
12.1-18	25	2	-0.61	7.09	0.25

Key: Hb= Haemoglobin, Pf= Plasmodium falcifarum, Pv= Plasmodium vivax, r= Correlation, x^2 =chai-square,

4.8. Relation of Major Risk Factors with prevalence of Malaria among the Study Participants

As shown in Table 7 analysis was done to assess the relation between malaria positivity and selected socio-demographic factors. In this study there were 402 study participants and 161 (40.0%) were malaria positive. Among these 95 (47.0%) of the study participants were married, 53 (33.1) were single, 8 (28.6) were divorced and lastly 5(41.7%) were widowed. The malaria prevalence was statistically related with marital status (P = .031).

This study was in agreement with a study by Adugna *et al*. (2004) on age specific malaria infection prevalence in Akaki town Addis Ababa and found that more malaria prevalence in the age group 15 years and above were 6.9% followed by 5-14 age group 3.4% and 0-4 age group 0.7%, respectively. However, in a study conducted by Fentaw (2010) more parasite prevalence was 20% detected in the age group 0 - 4, followed by 0.89% in the age group 15 years and above. Therefore, in this study there was significant relation between age and malaria prevalence (P = 0.0001).

Regarding to bed net possession, out of malaria positive participants, 35(46.1%) of the respondents were not had bed net to prevent malaria infection, 63 (47.7) had One per house, 48 (33.8) had two per house, and 15 (28.8) had three per house. The malaria prevalence was statistically related with bed net possession (P = 0.024). In line with this studies on Insecticide Treated Nets (ITN) undertaken in different African and Asian countries have consistently documented significant reduction in the rate of malaria parasitaemia and malaria morbidity. On the other hand, the study of (ACIPH, 2009) in Oromia and Amhara region showed that some respondents used the ITN as a sheet, scarf and curtain .According to this report they did not like to sleep under the bed net because they did not feel comfortable. Moreover, in some studies also show that elevation has long been recognized to be related with malaria due to its relation with temperature and humidity (Salehi *et al*., 2008). In this finding, higher malaria infections were observed in households living at warm arid low land than in households either living at warm and sub moist low land or warm semiarid low land (Table 7).And also statically significant difference (P =0.001) in the malaria infections was observed between residence places (Table 7). In line with this, the effect of high altitude in reducing malaria infection was revealed on reports made by Graves *et al*. (2008), Bødker *et al*. (2006). This was attributed to high altitude in reducing malaria infection was revealed on reports made by the lower ambient temperature in highland areas (Devi and Jauhari, 2006). Further factor to consider is that the altitude limit of transmission in an area may be due to lack of breeding sites, rather than unfavorable climatic conditions, as evidenced in the highlands of Uganda (Niringiye and Douglason, 2010).

In relation to the duration of stay in the study area, 51 (44%) of the respondents lived 1-5years, 30(30.9%) lived >10 years, 29 (50%) who lived since birth, 26(46.4%) lived <1year, and 25(33.3%) lived 6-10years (Table 6). There is no direct relationship between duration of stay and malaria infection observed (p =0.065).

Table 7. Relation of major risk factors with malaria parasite infections of examined individuals in Pawe general hospital during October-December 2016

Major risk factors	No Examined	Malaria parasite infection Positive Freq (%)	Df	X^2 test	P-Value
Age group			2	32.73	0.001*
≤15	70(17.4)	30(42.9)			
16-40	314(78.1)	126(40.1)			
≥40	18(4.5)	5(27.8)			
Marital status			3	8.84	0.031*
Married	202(50.2)	95(47.0)			
Single	160(39.8)	53(33.1)			
Divorced	28(7.0)	8(28.6)			
Widowed	12(3.0)	5(41.7)			
Bed net possession:			3	9.41	0.029*
Absent	76(18.9)	35(46.1)			
One per house	132(32.8)	63(47.7)			
Two per house	142(35.3)	48(33.8)			
Three per house	52(12.9)	15(28.8)			
Duration of stay in the area			4	8.85	0.065

<1year	56(13.9)	26(46.4)			
1-5year	116(28.9)	51(44)			
6-10year	75(18.7)	25(33.3)			
>10 years	97(24.1)	30(30.9)			
Since birth	58(14.4)	29(50)			
Current Place of residence			2	34.24	0.001*
High land	55(13.7)	15(27.3)			
Low land	229(57.0)	120(52.4)			
Temperate	118(29.4)	26(22.0)			

DF =degree of freedom

4.9. Relationship between Malaria with Educational status and Occupation among Study Participants

As shown in Table 8 the number and percentage of study subjects were 92 (22.9%) illiterate, 34 (8.5%) of participants were able to read and write, 146 (36.3%) had completed primary education, and those who completed secondary education were 70 (17.4%),36 (8.9%) of the study participants were those participants who had earned diploma and above and finally 24 (6.0%) of the participants were under school age. However there was no significant association between educational status and malaria infection prevalence in this study (P= 0.421).

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). Unlike this, in this no statically significant (P = 0.15) difference was observed in malaria and different occupation (Table 8).

Table 8.Malaria experience vs. educational status and occupation among study participants in Pawe

Major risk factors	Frequency (%)	Prevalence of Malaria (%)	Df	X^2	P-Value
Educational Level			5	4.96	0.421
Illiterate	92(22.9)	40 (43.5)			
	34(8.5)	12 (35.3)			
Read and write	146(36.3)	57 (39)			
Primary education	70(17.4)	25 (35.7)			
Secondary education	36(8.9)	13 (36.1)			
Diploma and above	24(6.0)	14 (58.3)			
Under school age			7	10.86	0.15
Occupation	130 (32.2)	58 (44.6)			
Farmer	22 (5.5)	5 (22.7)			
Merchant	42 (10.4)	11 (26.2)			
Government employee	68 (16.9)	24 (35.3)			
Student	26 (6.5)	10 (38.5)			
Private sector employee	38 (9.5)	18 (47.4)			
House wife	52 (12.9)	22 (42.3)			
Daily laborer	24 (6.0)	13 (54.2)			
Unemployed					

Df =Degree of freedom

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

Malaria is still a major public health and medical concern in many parts of the world, especially in countries of tropics and subtropics such as in Africa, South East Asia, and Latin America. In Ethiopia an estimated 65% of the 100 million people are exposed to malaria. The main objective of the study was to assess the prevalence of malaria and its association with hemoglobin level among patients visiting Pawe General Hospital, Pawe district. The study involved cross-sectional survey for monthly malaria prevalence cases, use of retrospective Hospital malaria records and use of laboratory results. The study was carried out during October-December, 2016. Additionally, structured and pre-tested questionnaires were administered to randomly selected patients visiting Pawe Hospital to assess the socio-demographic characteristics and the level of knowledge and awareness of the respondents related to malaria. To determine prevalence of malaria cases blood samples were collected from 402 respondents by finger-prinking. Thick and thin blood smears were prepared and examined microscopically after staining with 3% Giemsa solution.

The results of questionnaire survey indicated that the study participants had better knowledge on the method of malaria transmission; majority of the participants correctly associated malaria with mosquito bites. The results showed that knowledge about the symptoms of malaria was also very high. Most of the study subjects had knowledge of at least one of the classical symptoms.

Knowledge regarding the breeding site of mosquitoes was also high with 72.6% of the respondents mentioned stagnant water as the breeding site. The results showed that the knowledge about preventability of malaria among the study participants was high. This could be related to experience of inhabitants about breeding site of malaria and the results of the activities of malaria control program to prevent and control the disease in the area. A total of 402 blood samples were examined of these (40%) were infected with malaria parasites. The overall prevalence of malaria parasite infection was161 (40%); out of this the major *plasmodium* species identified were *Plasmodium falciparum* (86.9%) and *P. vivax* (13.0%). There was statistically

significant association between age groups. Relatively more malaria infections were detected in age groups of ≤ 15 years. This does fit into the conventional characterization of the epidemiology of malaria based on age stratification.

Regarding to hemoglobin concentration and prevalence of anemic condition, participants who were less than 15 ages had 12.1 g/dl and 11.8 g/dl of Hb level, 12.1 g/dl and 12.8 g/dl of Hb concentration was obtained from the age groups between 16-40 and lastly, 12 g/dl and 13.2 g/dl were detected within the age groups of ≥40.

Out of the 161 malaria patients, 81respondents were anemic and there were significance variations of anemic conditions among age groups. The overall mean of hemoglobin concentration among the participants were 12 g/dl in males and 12.7 g/dl in females.

5.2. Conclusion

The present study was an initial step to the understanding of the epidemiology of malaria in study area. Based on the findings and retrospective studies, the following conclusions may be drawn about the malaria situation in Pawe: Community members know about causes, symptoms, mode of transmission and prevention methods of malaria as well as most of them know that malaria is transmitted by mosquito bite. The two *Plasmodium* species namely *P. falciparum* and *P. vivax* were the species that cause malaria in Pawe town and the results also provide more evidence on the existence of a relationship between some socio demographic and the burden of malaria in the area; these relationships are, however, complex and complicated by other environmental and socio-economic factors.

Out of the total 161 malaria patients,81 respondents were anemic and high prevalence of anemia was seen in the age group of ≤ 15 years and the prevalence of anemia decreases with age increases. Finally it can be concluded that; there was uneven distribution of monthly malaria cases in the population appears to be an indication of unstable epidemiological situation in the area, which is suggestive of an epidemic transmission of malaria in the area.

5.3. Recommendations

The findings of the present study showed that malaria parasitic infections were prevalent in the surrounding area of Pawe district. According to the results obtained from the current study, the following recommendations are forwarded

- The use of impregnated bed nets should be introduced to the community through education
- The community must clean or destroy the breeding site of mosquito

- Regular health education must provide to raise individual and community awareness about the mode of malaria transmission ,prevention and control

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7. APPENDIX

Appendix I. prevailing meteorological conditions in Pawe town 2016

Table 1. Meteorological conditions prevailed in Pawe town in the year 2016

Month	Rainfall(mm)	Max.Temp (^{O}c)	Min. Temp (^{O}c)	RH (%)
January	0.0	35.8	31.5	83
February	0.0	40	34.2	83
March	40.6	41.5	35.5	83
April	0.0	40	35	84
May	238.1	37.5	28.5	92
June	189.5	36.8	26.5	90
July	332.9	31.0	22.6	93
August	231.1	31.5	23	93
September	172.3	31	27	93
October	109.2	34.5	27.5	87
November	5.8	35.5	31.5	82
December	3.2	36	32	86
Annual average	110.23	35.93	29.679.8	

Year	Parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Overall	(%)
2012	No. examined	1021	1331	475	726	832	716	2543	793	668	653	1424	785	11967	(100)
	No. Positive	76	78	234	292	437	343	399	193	266	207	301	153	2979	(24.89)
	P.falcifarum	54	60	164	232	148	224	285	176	234	129	250	123	2220	(74.52)
	P.vivax	22	18	70	60	289	119	114	17	32	78	51	30	759	(25.47)
2013	No. examined	1179	1136	568	1274	1170	1036	996	231	903	1318	1021	1406	14325	(100)
	No. Positive	87	60	359	247	249	137	122	8	189	376	201	118	2521	(17.59)
	P. f alcifarum	81	59	343	240	231	108	109	376	173	347	172	103	2313	(91.74)
	P.vivax	6	1	16	7	18	29	13	347	16	29	29	15	208	(8.25)
									29						
2014	No. examined	1212	629	436	1583	617	637	801	515	1114	596	661	1028	8616	(100)
	No. Positive	107	157	132	90	160	78	108	110	207	151	212	280	1792	(20.79)
	P.falcifarum	92	138	124	73	146	67	88	89	187	141	196	266	1607	(89.67)
	P.vivax	15	19	8	17	14	11	20	21	20	10	16	14	185	(10.32)
2015	No. examined	1050	1126	1105	1215	1204	1410	1520	165	2136	2302	2420	2055	19193	(100)
	No. Positive	86	72	119	153	269	227	233	0	425	545	659	384	3288	(17.13)
	P.falcifarum	46	50	86	141	230	192	211	116	312	330	459	240	2387	(72.59)
	P.vivax	40	22	33	12	39	35	22	90	113	215	200	144	901	(27.40)
									26						
2016	No. examined	1070	1102	1025	1220	1310	1455	1520	162	2115	2150	2315	2130	19038	(100)
	No. Positive	132	193	147	280	320	228	255	6	314	433	513	319	3273	(17.19)
	P.falcifarum	105	124	98	204	252	208	224	139	218	295	388	246	2438	(74.48)
	P.vivax	27	69	49	76	68	20	31	76	96	138	125	73	835	(25.51)
									63						

Table 2. Trends of malaria prevalence rate among patients visited Pawe General Hospital from 2012-2016.

Appendix II: Consent Form (English Version)

PIN ----------

Name of study participant ---------- Age---------- Sex----------
Laboratory technician Name---------- Site /Health center ----------

Hello my name is _____ and I work in Haramaya University.
I'm here to collect information about the Prevalence of malaria and its association with hemoglobin level among patients in Pawe town, Northwest Ethiopia. The purpose of the study is to understand the community's awareness, perception and practice towards malaria prevention and control in the area.

Therefore I will ask you some questions related to malaria and examine your children and you for signs of malaria and will take blood sample to examine for malaria parasites. Please be assured that the information will be confidential since participation is based on your willingness besides; you can withdraw from the study anytime. However your kindly participation would play key role in the success of this study. In addition, no personal identification will be written and we assure you that whatever information you are providing will only be used for the research purpose and the data will be handled only by the researcher.

Are you willing to participate in the study?
Agreed _____
Not Agreed _____

Thank you

Name Data collector _____ signature _____
Date of data collection _____

Appendix III: Consent Form (: Amharic Version)

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Appendix V: Questionnaire (English version)

Prevalence of malaria and its association with hemoglobin level among patients in Pawe town, Northwest Ethiopia

Part I. Household Identification

Date _____ Woreda _____

Village _____ Household number_____

Part II. Socio-demographic characteristics of household.

1. Sex Male_____ Female_____
2. Age_____
3. What is your current marital status?

   A. Married_____ B. Single_____ C. Widowed _____
4. What is total number of family members?
5. Where is your current place of residence?

A. warm and sub moist low land
B. warm and arid low land
C. warm semiarid low warm

6. How long have you lived in the area?

   A. <one year B. 1-5 years C. 6-10 years

   D. > 10 years E. Since birth
7. What is the highest level of educational status you have completed?

   A. Illiterate___ B. Only read and write____ C. Elementary school (grade1-6) ____

   D. Secondary school (grade 7-12) __ E. Higher education (Grade 12 +) ____

   F. Under school age

8. What is your occupation?

   A. Farmer____ B. Merchant _____ C. Government employee_____

   D. Student____ E. Employee (private sector) ____ F. House wife____

   G. Daily laborer____ H. has no job____ I. Others (specify) ____
9. Do you have live stock in your house? Yes-------No------

10. What is your average monthly income? _____

11. What is the type of housing unit? A) Conventional B) Improved

Part III. Questions about knowledge and awareness related to malaria and treatment seeking behavior.

12. Is malaria transmissible disease? Yes__ No___

13. How is malaria transmitted from person to person?

A. Through mosquito bite B. Through bodily contact with patients

C. use of unprotected water D. From cold environment

E. From rain water F, Marshy area G. Don't know

14. When do mosquitoes bite most?

A. Day time B. Night time C .Day and night D. I don't know

15. Where do mosquitoes breed?

A. In the stagnant water B. In the running water C On the soil D. I do not know .

_____

16. What are the main symptoms of malaria?

A. Fever B. Chills C. Shivering D. Headache

E. Backache F. Joint pian G. Loss of appetite H. Vomiting

17. Where do you and your family go to seek treatment for malaria?

A. Traditional healer B. Health center/ clinic C. Hospital

D. Pharmacy (drug shop) E. don't know

18. Why do you prefer these sources?

A. Treatment is effective B. Low cost of drugs

C. Closeness to home E. Others (specify) _____

19. Is there health post in your locality? No-------- Yes---------

20. Is malaria can be cause of death? Yes----------No----------

21. Is malaria can be preventable disease?

A. Yes B. No C. I do not know

22. If yes, what kind of methods you know to prevent malaria?

A. Take tablets B. House hold spray with insecticides

C. Environmental sanitation D. Use of mosquito net (bed net)

E. Use local cotton sheets F. Smoke from burning leaves and animal products (cow dung)

G. I do not know

24. Is there health post in your locality? No-------- Yes---------

25. How many beds net do you have in your house?

A. absent B. one per house C. two per house D. three per house

26. How is the availability of health extension service in your area? A. good B. poor

27. If good, have you ever seen or heard any health education messages pertaining to malaria?

Yes------ No------

Appendix VI. Amharic Version of Questionnaire

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Ethical Clearance

To: Miss Habtamu Amsalu

Ref. No. 0077/08

Pawe

Date 11/12/16

Subjecet: **Ethical Clearance**

Study Title

"Prevalence of Malaria and its association with Hemoglobin Level among patients visiting Pawe General Hospital, Northwest Ethiopia."

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

At its fourth session, the hospital management committee(HMC) of Pawe Hospital(PH) on 11th October, 2016 has discussed over the issue.

We are pleased 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 course whenever necessary.

Therefore, we declare you to carry out the research undertaking as per document of the proposal submitted. This ethical clearance issued is valid for three(3) months.

Regards,

Dr. Abyssinia Hailu

Chief Clinical Office

Management director, HMC-PH

Pawe General Hospital

Cc:

- Office of Research Core process
- College of Medicine and health Sciences
- Hospital Management Committee (HMC)
- Pawe General Hospital

Appendix VII: Ethical Clearance