Jimma University

College of Natural Sciences

School of Graduate Studies

Frequency of Severe malaria associated with *Plasmodium vivax* and *Plasmodium falciparum* among pregnant women and children in Pawe Hospital, Benishangul Gumuz Regional State, North Western Ethiopia

By: Getachew Geleta

September 2014

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By: Getachew Geleta

Advisor: Tsige Ketema (Assistant Professor)

Thesis submitted to the Department of Biology, College of Natural Sciences, and Jimma University in partial fulfillment for the requirement for the degree of Master of Science in Biology (Ecological & Systematic Zoology).

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Declaration

I hereby, declare that this thesis is my original work, that it is the documentation of work carried out by me and that it has not been submitted to any previous application for higher degree. I announce that I have developed and written the enclosed master thesis completely by myself, and have not used sources or means without declaration in the text. Any thoughts from others or literal questions are clearly marked. The master thesis was not used in the same or in similar version to achieve an academic grading or is being published elsewhere. This has been submitted in partial fulfillment of the requirement for M.Sc. Degree at Jimma University. I declare that this thesis is not submitted to any other institutions of others are involved, every efferent is made to indicate this clearly, with due reference to the literature and acknowledgment source made.

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Acronyms

BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
EDTA	Ethylene Demine Tetra acetic Acid
EHNRI	Ethiopian Health and Nutrition Research Institute
FMOH	Federal Ministry of Health
HB	Hemoglobin
PMI	President's Malaria Initiative
UNICEF	United Nation Children's Fund
RBC	Red Blood Cell
RBM	Roll Back Malaria
SSA	sub-Saharan Africa
SPSS	Statistical Package for Social Science
WBC	White Blood Cell
WHO	World Health Organization

Abstract

Malaria is still a top public health concern in tropical and subtropical regions of the world and causes significant mortality and morbidity every year. Majority of deaths due to severe malaria complications occur in sub-Sahara Africa among biologically risked groups. Thus, this study was designed to assess the incidence of severe malaria syndromes among children less than ten years and pregnant women in Pawe hospital, Benishangulgumuz region, Northwestern Ethiopia. Children aged <10yearsand pregnant women, seeking medication for malaria infection during the study period in the hospital were recruited for the study. Socio-demographic characteristics, physical, hematological parameters and clinical features of uncomplicated and complicated malaria were assessed following standard parasitological, and clinical procedure. Data was analyzed using SPSS statistical soft ware. Chi-square test was the main statistical test employed to compare variables and significant level was considered at P<0.05.During the study period, a total of 384(children<10 years, n=263, and pregnant women n=121) were malaria positive. Of these 200 and 89 children and pregnant women were infected with Plasmodium falciparum respectively. The rest were positive for P. vivax and mixed infections. Most severe malaria symptoms such as respiratory distress, persistent vomiting, splenomegaly, and confusion were significantly higher (P<0.05) among children, while severe anemia, hyperparasitemia, prostration, and Hemoglobinuria were commonly encountered among malaria infected pregnant women. Most of the severe malaria complications were also appeared with P. vivax infected children and pregnant women. The study revealed that significant number of children and pregnant women assessed were developed severe life threatening malaria complications. This urge prompt early detection and effective treatment to reduce death associated with malaria in the study site.

1. INTRODUCTION

Malaria is the most common tropical disease, remaining widespread throughout the tropical and subtropical regions, including parts of Africa, Asia and America. It is a major cause of illness and death in large area of the developing world, especially Africa. According to the World Health Organization report (WHO, 2008), at the end of 2007 there were 109 malaria endemic countries and 3.3 billion people were at the risk of malaria. Malaria causes at least 300 million and possibly as many as 500 million cases of acute illness each year, which result in 1to 3 million deaths each year. Ninety percent of deaths occur in sub-Sahara Africa. The majority of these deaths are among children less than five years of age and pregnant women (Mitiku, 2011).

These estimates are not reliable because of inadequate malaria case reporting in most endemic countries and lack of national wide malaria distribution pattern. Accurate estimates of malaria distribution are required for planning, implementation and evaluation of malaria control programs. Hence, there is need for precise estimates about the number of people at risk of malaria to optimize the use of limited resources in high-risk areas (Mitiku, 2011).

The President's Malaria Initiative (2010) of Ethiopia, national malaria plan indicated that malaria is ranked as the leading communicable disease in Ethiopia, accounting for about 30% of the overall disability adjusted life years lost. Approximately 75% of the country is malarious with about 68% of the total population living in areas at risk of malaria. Malaria is reported to cause thousands of deaths each year. According to Ethiopia's Federal Ministry of Health (FMOH), in 2009, malaria was the first cause of outpatient visits, health facility admissions and in-patient deaths, accounting for 12% of out-patient visits and 9.9% of admissions. Thus, this study was

designed to assess incidence of severe malaria syndromes associated to *P.vivax* and *P. falciparum* among biological risked groups in one of malaria endemic areas of Ethiopia

1.2. Statement of the problem

Plasmodium vivax is the second important parasite of human malaria in Ethiopia and widely perceived as causing mild and self-limited illness. However, *P. vivax*, previously thought benign parasite, is recently reported to cause life threatening complications among children from endemic regions. Some of the reported severe malaria complications are cerebral malaria, dysfunction of different organs, hypoglycemia, jaundice, thrombocytopenia, renal impairment, hepatic dysfunction, acute kidney injury and hypotension. Unlike other countries in Africa, prevalence of *P. vivax* infection in east Africa, particularly in Ethiopia is higher. In some areas of the country, the prevalence rate exceeds 70% of total malaria infections. This was previously accounted to high Duffy blood group positivity of most population of the country, but recently contradictory reports are coming.

Although *P. vivax* malaria is highly prevalent in some parts of Ethiopia and its risk due to drug resistance is increasing, except the first very recent severe malaria complications syndromes in children reported from the country, its actual effect on other group of population was not studied. Moreover, effects in other biological risk groups, specifically pregnant women also need to be addressed. In comparison to *P. falciparum, P. vivax* current challenge should also be addressed. Thus, it is rational to assess the current status of *P. vivax* and *P. falciparum* associated severe malaria complications among pregnant women and children in malaria endemic areas.

1.3. Objectives

1.3.1 General objective

To assess incidence of severe malaria syndromes associated to *P.vivax* and *P. falciparum* in Pawe hospital, Benishangulgumuz, Northwestern Ethiopia

1.3.2 Specific objectives

To assess the current prevalence of malaria in the study area among children <10years and pregnant women.

To estimate the incidence of severe malaria among pregnant women and children <10 years in the study area.

To compare severe malaria complications occur due to *P. vivax* and *P. falciparum* in the study area.

1.4. Significance of the study

This study will provide significant information on the level of current situation of malaria in Pawe district; malaria associated severe malaria complications (*P. vivax* and *P. falciparum*). Furthermore, it will provide evidences for incidence of severe malaria associated to *P. vivax* malaria in Ethiopia. Moreover, future research in the district may use the result of this study as base line data.

2. LITRATURE REVIEW

2.1. Malaria epidemiology and life cycle of the parasite

Malaria is a vector borne disease caused by the parasitic protozoa of the genus *Plasmodium*. Humans infected with malaria parasites can show a wide range of symptoms including fever, shaking chills, headache, muscle aches, tiredness, nausea, vomiting, and diarrhea. Malaria may also cause anemia and jaundice because of the loss of red blood cells (Alemu *et al*., 2011)

There are five known species of *Plasmodium* that cause malaria in humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and a fifth species, *P. knowlesi*, is a zoonosis that causes malaria mainly in non-human primates but can also infect humans (Walker *et al*., 2009). *P. vivax* and *P. ovale* have dormant liver stage parasites, called hypnozoites, which can relapse, and cause malaria several months or years after the infecting mosquito bite. *P. malariae* can produce long-lasting infections and, if left untreated, can persist asymptomatically in the human host for years, even a lifetime. *P. falciparum* is the most common cause of severe, potentially fatal malaria since it is the only one which severe complications such as cerebral malaria, severe anemia, renal failure and pulmonary affection are frequently seen (Heddini, 2002).

It is responsible for about 80% of all malaria cases, and also responsible for about 90% of the deaths from malaria (Mends *et al*., 2001). The features important for severity and pathogenesis of *P. falciparum* are its ability to invade erythrocytes of all ages, causing hyperparasitemia and adherence of the infected erythrocyte to the endothelium of the capillaries via parasite-derived proteins expressed on the surface of infected cell, this sequestration enables the parasite to escape host immune system (Houdini, 2002).

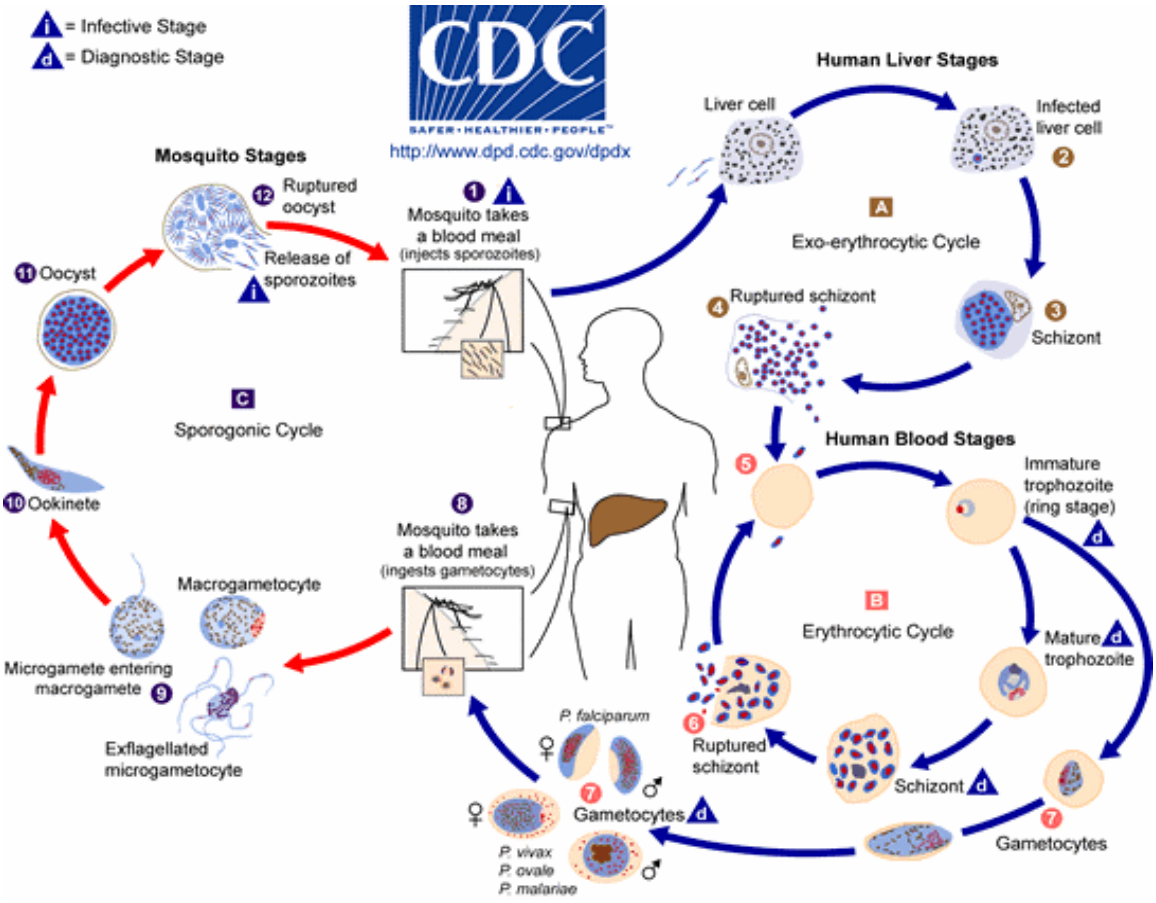

Figure 2 Life cycle of *Plasmodium* species.

Source: CDC http:// www.dpd.cdc.gov/dpdx/HTML/Malaria.htm.

Infection starts when the female anopheles mosquito inoculates motile sporozoite into the blood stream. Half an hour later most are destroyed by phagocytes from the blood but the few that enter in the liver parenchyma cells transform themselves to the next stage called the trophozoites. It divides to give rise to pre-erythrocyte schizonts. Six to sixteen days later, the schizonts rupture to release extra-erythrocyte merozoites in to the surrounding tissue and the circulating system where they invade RBC. With a periodicity characteristic of the *Plasmodium* species, the erythrocyte schizonts mature and rupture 48 or 72 hrs, liberating 10-20 erythrocyte merozoites. The merozoites attach to specific receptors on the surface of red blood cell membranes to initiate

further invasion.In a period of about ten days, the newly invading merozoites differentiate into male and female gametocytes through purely asexual replication. Then, sexual reproduction takes place following the ingestion of gametocytes into the gut of the appropriate species of anophelesmosquito (Deans and Cohen, 1983; Tine and Paoletti, 1996).

There are many factors affecting malaria transmission. These include the type and density of Anophelesspecies mosquito vector and the longevity of the vector to allow the sporozoite cycle to be completed. This in turn depends on physical factors such as altitude (<2000m above sea level) and temperature (about 28 ^{o}C) of the environment. The proportion of infected. A person carrying the gametocyte in the community and the number of gametocytes in the infected person is also another factor, which determines malaria transmission (Greenwood, 1997).

Epidemiologically, *P. falciparum* malaria covers 85% of all malaria cases worldwide. Almost all deaths are caused by this species. It occurs mainly in the hotter and more humid regions of the world, (Schofield, 2000). It is the most pathogenic of all the human malaria species. The following are believed to contribute to its malignancy: the high level of parasitemia as the result of invading young and old RBCs; erythrocyte schizogony that takes place in the capillary beds of the internal organs such as the spleen, bone marrow, brain, kidney, intestine, heart and placenta. It also causes changes on the surface of parasitized red blood cells and form knobs, which cause sequestration of infected red blood cells with mature parasites in vital organs, particularly the brain and the heart. Symptoms of acute complications such as cerebral malaria, diarrhea, and symptoms of chronic complications such as tropical splenomegaly syndromes, falciparum recrudescence and latent malaria are also known during *P. falciparum* malaria (Parija, 1996).

P. vivax malaria is the second most important public health problem. It is more common in the temperate than in the tropics before its eradication (Gillers and Warrels, 1993; Parija, 1996). It is characterized by the occurrence of true malaria relapses due to the re-activation of hypnozoites in the liver cells. Although the complication of *P. vivax* malaria is relatively less, rupture of an enlarged spleen occasionally occurs and could be life threatening (Schofield, 2000).

Unlike *P. falciparum*, all the life cycle stages can be found in the peripheral blood (Parija, 1996). Fever is a key clinical manifestation and a characteristic feature of malaria infection. It usually occurs shortly after the rupture of malaria infected RBCs (Parija, 1996).

When erythrocyte schizonts ruptured, they release parasites, erythrocyte debris, and especially protein toxins, glycosulphatidylinositol (GPI) of parasite origins, which activate tissue macrophages to produce pro inflammatory cytokines such as IL-1 and TNF, which cause fever, chill, sweat and other pathological effects (Clark and Schofield, 2000).

In *P. vivax* malaria, for example, fever occurs every 48 hours following erythrocyte raptures, while TNF rises shortly an hour or so before the onset of rigors (Greenwood, 1996). Children infected with *P. falciparum* and treated with anti-TNF monoclonal antibodies (Moab) were known to have their fever reduced, showing that TNF to be the main causative agent of fever in malaria infection (Greenwood, 1996). It is, therefore, practically as well as theoretically important to understand the relationship between malaria parasitemia and fever. Direct correlation of fever with *P. falciparum* parasitemia has been reported (Lisse *et al.*, 1994; Greenwood, 1996; Rogier *et al.*, 1996). This is mainly due to the increase in TNF level, a factor strongly associated with high parasitemia (Kwiatkowski *et al.*, 1990).

Attempts have also been made to identify the level of parasitemia that can be used to define the clinical episode of malaria. Using logistic regression methods, the threshold of trophozoite count was estimated to be in the range of 2-20000 parasites/µl of blood that has optimum sensitivity and specificity for the definition of a clinical episode of *P. falciparum* malaria (Greenwood, 1996). However, this approach is complicated by the fact that the threshold of trophozoite count to induce fever is age specific. In *P. falciparum* malaria, for example, the maximum threshold at one year of age was found to be 2-45 trophozoites per leucocytes, and a minimum of 0.5 trophozoites per leukocyte, at 60 years of age. When the parasite density of a person crosses the threshold level corresponding to his or her age, the individual's risk of fever was to be multiplied by 44%(Rogier *et al.,* 1996).

2.2. The global malaria situation

Malaria is one of the leading causes of illness and death in the world. Nine out of ten of these deaths occur in Africa and the rest occur in Asia and Latin America. Being the world's most prevalent vector-borne disease, it is endemic in 92 countries, with pockets of transmission in an additional eight countries (Alemu *et al*., 2011). With respect to the endemic altitudinal range, the greatest altitude at which malaria occurs differs very much from place to place in the tropics, from nearly 2500m in Kenya to under 1000m in Sri-Lanka (Bruce-Chwatt, 1980; Gilles and Warrell, 1993).

Throughout most of sub-Saharan Africa (SSA), malaria shows a high endnemicity with Average altitude of 1400m, but a low epidemic potential except in some areas (Gilles and Warrell, 1993). On the other hand, within the endemic altitudinal range, the movement of infected people from

malaria endemic to areas where the disease had been eradicated was reported for the resurgence of the disease (Martens and Hall, 2000).

Such resurgence was observed in Armenia, Azerbaijan, Chechnya, Russia, Tajikistan, Turkey, Madagascar, South Africa, and Zanzibar where the disease was previously eradicated using effective control programs (Lambert, 2005). It has been estimated in the 1950s that worldwide, the annual incidence of the disease was of the order of 250 million cases with 2.5 million people dying of malaria every year (Bruce-Chwatt, 1980).

In 1990, 80% of cases were in Africa, with the remainder clustered in nine countries: India, Brazil, Afghanistan, Sri-Lanka, Thailand, Indonesia, Vietnam, Cambodia and China. Malaria has been estimated to account for 2.3% of global disease after pneumococcal acute respiratory infections (3.5%) and tuberculosis (TB) (2.8%); annually it kills one in 20 children less than 5 years of age (WHO, 2004).

According to WHO estimates in 1998 up to 500 million clinical malaria cases occurred with nearly 3 million people dying of the disease (WHO, 2000). Currently, about 6-10% of the world population harbor malaria parasites in their bloodstream (Mayxay *et al*., 2004).

Almost 3% of disability adjusted life years are due to malaria mortality globally (Breman, 2001). Cerebral malaria is estimated to be responsible for a fatality rate of more than 20% of malaria cases even in urban areas (Warrell *et al*., 1990).Worldwide prevalence of the disease is estimated to be 300-500 million clinical cases each year (Breman, 2001).

2.3 .Malaria in Africa

Malaria is the largest single component of disease burden; epidemic malaria in particular, remains a major public health concern in tropical countries. In many developing countries, and especially in Africa, malaria exacts an enormous toll in lives, in medical costs, and in days of labor lost (Lambert, 2005). Especially, the physical consequence of Infection with *P. falciparum* is the main causes of malaria morbidity and mortality in Africa (Snow *et al*., 1999).

The epidemiological situation with respect to malaria has worsened in Africa over the last decade and the disease has occurred in areas previously free of malaria. Outbreaks have also been reported in some locations of Africa that had been previously thought to be at elevations too high for malaria transmission due to changes in climate (Lindsay and Martens, 1998) and human migration (Martens and Hall, 2000).

On the other hand, compared to the malaria situation between the 1920s and 1950s, the current pattern of malaria in the highlands is characterized by increased frequencies, expanded geographic areas, and increased case-fatality rates (Zhou *et al*., 2004). It has been reported that about 18% of the people in Africa live in areas prone to epidemic malaria due to unstable and seasonal characteristics of malaria transmission (WHO, 1993).

East African highland chains, from Ethiopia in the north to South Africa in the south are the most affected parts of Africa (Lindsay and Martens, 1998). In recent years, disastrous malaria epidemics were reported from Botswana, Mozambique, and South Africa, Zambia, and Zimbabwe. Statistical assumptions suggest that about 110,000 People die every year, in sub-Saharan Africa, during malaria epidemics (Brinkmann, 1991; Nchinda and WHO, 1998) suggested that approximately 60% of the population of SSA lives in areas of stable malaria

transmission where protective immunity develops from about the age of five. 30% live in areas of seasonal transmission where protection is gained rather later (at age of 10); and 10% live in areas of unstable transmission where epidemics may occur with substantial consequences for adult morbidity and mortality. Studies revealed that a recent upsurge of malaria in endemic areas with explosive epidemics in many parts of Africa is caused by several interacting factors, including rapid climatic changes, population movements and spreading resistance to anti-malaria drugs (Nchinda and WHO, 1998).

Climatologically a change such as increased temperatures, humidity, and unusually prolonged heavy rainfall is considered to aggravate malaria, particularly at higher altitudes (Gebre-Mariam et *al.*, 1988). In particular, it has been reported that increasing temperature could be part of the reason why malaria can now survive at higher altitudes although many other confounding factors exist (Patz *et al*., 2000). Seasonal migration of workers has been associated with epidemics in Kenya (Boland and Williams, 2003).

The disease is directly responsible for one in five childhood deaths in Africa and in directly contributes to illness and deaths from respiratory infections, diarrheal disease and malnutrition (WHO, 1999). In SSA alone, an estimated 0.9-2.3 million annual child deaths are attributed to malaria (Villamor *et al*., 2003). About 1 millions of those who die from malaria are children below five years of age. Every 30 seconds malaria kills an African child (RBM, 2006) and about 25% of the child mortality in SSA is due to malaria (WHO, 2000; UNICEF, 2004).

Apart from the health impact, the disease is a major impediment to socio-economic development as the main transmission seasons coincide with peak agricultural harvesting times. There is a striking correlation between a country's per-capita gross domestic Product (GDP) and malaria

that demonstrates lower rates of economic growth of endemic countries. It impedes the development of a country's population in a complex manner, restricting population growth, reducing savings, economic investment and the productivity of the workforce (Sachs and Maloney, 2002; Greenwood *et al*., 2005).

In addition to the impact on GDP, there is also significant cost to the health sector that in it will impact on the macro-economics of individual countries (Sachs and Maloney, 2002). Likewise, Gallup and Sachs (2001) showed the impact of malaria on the economy of the endemic countries.

Sauer born *et al*. (1995) provided a detailed specification of the wage rate method of assessing the time costs of illness as the sum of the opportunity cost of wages forgone by the sick individual due to illness, and the opportunity costs of healthy household members' time spent on treating or attending to the sick person or accompanying them for treatment. According to the report of the Commission on Macroeconomics and Health, currently, a total US$ 600 million is spent annually for malaria control. Overall, malaria accounts for 10% of Africa's disease burden and it is estimated that malaria costs the continent US$ 10 to 12 billion in lost GDP growth (UNICEF, 2004).

2.4 Malaria situation in Ethiopia

Like other African countries, malaria is a major public health problem in Ethiopia with an average of 5-6 million cases per year (FMOH, 2007). In Ethiopia, the epidemiological pattern of malaria transmission is generally unstable and seasonal; the level of transmission varying from place to place because of differences in altitude and rainfall patterns (Alemu *et al*., 2011). Changes have been observed in the epidemiology of malaria through time. Previously, malaria was known to occur in areas below 2000 m but currently it has been documented to occur indigenously even in areas above 2400 m, such as Addis Ababa, Akaki (Woyessa A *et al*., 2004). Malaria is a major public health problem in Ethiopia where an estimated 68% of the population

lives in malarious areas. *P. falciparum* and *P. vivax* are the two dominant parasite species with relative frequency of 60% and 40%, respectively (FMOH, 2004). A major challenge for malaria epidemiologists is to evaluate the strengths and weaknesses of both methods in estimating malaria incidence and time trends, especially as malaria control programmes are intensified worldwide (Woyessa A *et al*., 2004).

The major transmission of malaria follows the June – September rains and occurs in the period from September – December, while the minor transmission season occurs in April – May following the February – March rains. The bimodal malaria transmission pattern is limited to areas that receive the small "*Belg"* rains and are mainly located in the eastern part of Ethiopia, while the major malaria transmission occurs in all areas at risk of malaria (FMOH, 2006).

2.5. Severe malaria complications due to *P. falciparum* and *P. Vivax*

Severe under reporting by patients and insufficient worldwide surveillance hampers epidemiological studies on the toll of malaria. The World Health Organization (WHO) estimates that malaria caused 300–500 million infections, 100 million clinical cases (*Plasmodium falciparum*), and 1.5–2.7 million deaths in 1994(WHO, 1997). Malaria manifests in a variety of disease forms. Acute infections can lead to cerebral malaria (CM), anemia, respiratory distress, or hypoglycemia; acute complicated malaria infections sometimes have long-term neurological consequences. Repeated infections contribute to severe anemia (Newton *et al*., 1998). Malaria during pregnancy reduces birth weights and contributes to maternal, fetal, and infant mortality. Compared with complicated malaria other forms of acute malaria, severe malarial anemia, prenatal manifestations, and long-term consequences of complicated malaria receive much less attention (Newton *et al*., 1998).

However, the connection between malaria and anemia is difficult to define because some people experience parasitemia in the absence of malarial disease (Newton *et al*., 1997). Whereas others become anemic as parasites are cleared. Differential diagnosis of malarial anemia is also difficult

because malnutrition, hemoglobinopathies, and other common disorders contribute to anemia in Africa (Newton *et al*., 1998).

In the study by Snow and others, the annual admission rate for severe malarial anemia was 7.6 per 1,000 children. In a 1990–1992 study of health clinics in rural Malawi, the annual admission rate for this condition was 54.7 per 1,000 children < 5 years old; in urban areas, the rate dropped to 5.3 per 1,000 children (Craig *et al*., 1999; Slutsker *et al*., 1994). Less than one quarter of all people with malaria visit health clinics; however, those with severe malarial anemia are more likely to seek attention (Brinkman *et al*., 1991).

Childhood mortality of severe malarial anemia was estimated from fatality rates and disease incidence. In general, very low hemoglobin is associated with death shortly after admission (Lackritz *et al*., 1992).

A review of severe malarial anemia transfusion risks identified several studies that documented mild and severe anemia according to WHO guidelines. During pregnancy, malaria infections of the mother, the placenta, and the fetus adversely affect fetal and newborn survival, especially through low birth weight (LBW; delivery weight < 2,500 g). Maternal malaria is reported during pregnancy if clinical signs and symptoms reflect infection or if parasitemia is demonstrated in peripheral maternal blood (Steketee *et al*., 1996; Redd *et al*., 1996). Fetal infection is detected by placental or umbilical cord-blood parasitemia (Redd *et al*., 1996).

The vast majority of reports on severe *P. vivax* malaria are from south East Asia and India, there are few published data on severe *P. vivax* from Africa (Ketema and Bacha, 2013). The current study was conducted at New Halfa hospital in the eastern Sudan during the period of September 2009-December 2011 to investigate manifestations of severe *P. vivax* among children so as to

add to the previous studies on severe malaria and its treatment in Sudan (Adam *et al*., 2002 and Mirghani *et al*., 2011). Such data is of paramount importance for the care givers, health planners and for controlling the disease e.g. by using an effective drug and eradicating this species. *P. falciparum* (95%) was the main species in the area and *P. vivax* was rare and constituted only 3% of the species in the area (Himeidan *et al*., 2005)

The recent study documented the severe manifestations of *P. vivax* malaria (anemia, jaundice, hypotension, thrombocytopenia, repeated convulsions, cerebral malaria, epistaxis, renal impairment and hypoglycemia) in an area characterized by *P. falciparum* malaria (Himeidan *et al*., 2005).

Perhaps there is an increase in the *P. vivax* malaria due to influx of Ethiopian and Eritrean populations through the border following the peace and construction of Asphalt roads between these countries and Sudan. Recent reports showed that most of the malaria infections in Ethiopia were *P. vivax* infections and even with reported treatment failure (Woyessa *et al*., 2012; Ketema *et al*., 2011) the findings of some authors, who also believe that *P. vivax* in not a rare disease in Africa and might use receptors other than Duffy to invade erythrocytes (Woyessa *et al*., 2012; Mendes *et al*., 2011) Severe *P. vivax* malaria is an existing entity in eastern Sudan. Further studies involving clinical and molecular research are required to understand emergence of severe *P. vivax* malaria (Mahgoub *et al*., 2012). The number of children with severe malaria complications was higher among those children who visited Halaba Kulito health center, the site where the highest (13%) treatment failure to chloroquine (the first line drug) by *P.vivax* malaria was documented (Ketema and Bacha, 2013)

There are some distinct differences between *P.vivax* and *P.falciparum* malaria which are responsible for the benign nature of *P.vivax* malaria. Firstly, *P.vivax* can only invade young red blood cells i.e. reticulocytes. Hence the density of parasites is lower than *P.falciparum* that invades all stages of RBCs causing high density of parasites (Gilles and Warrell, 1996). Secondly, the pyrogenic threshold of *P. vivax* is 150~200 parasites/μl which is much lower than that of *P.falciparum* (1 500~2 000 /μl). Therefore, patients with *P.vivax* infection develop fever earlier than *P.falciparum* mono infection resulting in early medical attention and treatment reducing the chance of development of severe disease (Mohapatra *et al*., 2012). Thirdly resistance to drugs particularly, chloroquine is not commonly found in vivax malaria (Mohapatra *et al*., 2002; Tjitra *et al*., 2008). Fourthly, *P. vivax* does not sequester within the microvasculature of different internal organs (Gilles and Warrell, 1996).

As the previous study indicated the same manifestations of severity that were due to *P. falciparum* malaria in the same pediatric ward as well as in the different regions of Sudan (Adam, *et al*., 2002; Mirghani, *et al.*, 2011). Only 3% of malaria cases (99/1539 blood films from 190 individuals) in the same setting were *P.vivax* while 95% were *P. falciparum* malaria giving the rate of *P. vivax/P. falciparum* malaria of 0.03 (Hamadan, *et al*., 2005).

2.6. Prevalence of malaria in Ethiopia

The problem of malaria is very severe in Ethiopia where it has been the major cause of illness and death for many years According to records from the Ethiopian Federal Ministry of Health, 75% of the country is malarious with about 68% of the total population living in areas at risk of malaria (FMOH, 1999; Adhanom, 2006).

That is, more than 50 million people are at risk from malaria (Lesaffre *et al.*, 2001) and four to five million people are affected by malaria annually (FMH, 2004).The transmission of malaria in Ethiopia depends on altitude and rainfall with a lag time varying from a few weeks before the beginning of the rainy season to more than a month after the end of the rainy season (Tulu, *et al*., 1993; Deressa *et al*., 2003). Epidemics of malaria are relatively frequent involving highland or highland fringe areas of Ethiopia, mainly areas 1,000-2,000 m above sea level (Tulu, *et al*., 1993; Adhanom, 2006; FMH, 2006).

Positive malaria diagnosis rate decreased with age. But, for households, the risk of malaria increased per unit increase in family size. Generally, malaria parasite prevalence differed between age and gender with the highest prevalence occurring in children and females. The findings of the association between socio-economic factors and malaria prevalence are similar to some of the results from previous studies (Sintasath *et a*l., 2005). The following socio-economic factors are related to malaria risk: construction material of walls, roof and floor of house; main source of drinking water; time taken to collect water; toilet facilities and availability of electricity. Besides socio-economic factors, there are demographic and geographic factors that also had an effect on the risk of malaria. These include gender, age, family size and the region where the respondents lived.

In addition to the main effects, there were interactional effects between the socio-economic, demographic and geographic factors that also influenced the risk of malaria. Most notable of these were the interaction between the main source of drinking water and the main construction material of the room's roof; the time taken to (Ayele *et al.* 2012)

In most African countries including Ethiopia, the number of cases reported annually fall by at least a quarter and, in some instances, by more than a half, between 2000 and 2010 (Ayele *et al* 2012).

Despite considerable progress in malaria control over the past decade, it is the most important public health problem in Ethiopia where an estimated 68% of the population lives in malarious areas and three quarters of the total land mass is regarded as malarious (FMOH, 2008). *Plasmodium falciparum* and *Plasmodium vivax* are the two predominant malaria parasites, distributed all over the country and accounting for 60% and 40% of malaria cases, respectively. Reports indicate that clinical malaria accounts for 10%-40% of all out patient consultations, with corresponding proportional morbidity among children under 5 years in age being 10% - 20%(FMOH, 2008).

Males were more affected than females by malaria parasites but vary year to year. In relation to Plasmodium species and age groups in the study area *P.falciparum* was the predominant parasite in all age groups (Alemu *et al*., 2012). Malaria and anemia are associated with serious mortality and morbidity among pregnant women (Ofili and Okojie, 2005). The incidence of the disease in most parts of the country is unstable, mainly due to the country's topographical and climatic variability (Abose *et al.,* 2003).

Rainfall and temperature are the most important determinants of malaria transmission (Senay and Virdin, 2005). Although areas below 2,000 meters are considered malarious (Tulu, 1993), malaria epidemics have been recorded up to 2400 meters (Negash *et al*., 2004), during periods when increased temperature and adequate precipitation are conducive for both vector survival and parasite development within the vector. The disease is one of the country's leading health problems in terms of morbidity, mortality and impediment to socioeconomic development and

top ranking in the list of common communicable diseases, consistently ranking in the top 10 causes of outpatient visits, admissions, and deaths at health centers and hospitals (FMOH, 2004; Adugna, 2010).

Since a significant proportion of the population is out of reach of the health service coverage, these figures could under estimate the actual burden of malaria in Ethiopia (PMI, 2008). According to WHO, the number of reported malaria cases decreased by 41% from an annual average of 3.2 million between 2000 and 2005 to 1.75 million in 2009. During the same period, 33% decline in malaria admission, and 60% reduction in the death of under five children was also reported (WHO,2010).

3. Materials and methods

3.1 Description of the Study area

The study was conducted in Pawe hospital, Benishangulgumuz regional state, Northwestern Ethiopia (Figure 2). Geographically the study site is located at 11.009' N latitude, 36.003' E longitude and an altitude of 1050 meter above sea level (masl). The area is malarias and has uni-modal rainfall which mostly concentrates between March and September. The peak rainfall occurs from July to August. The mean annual rain fall and maximum temperature of the area is about 1555.1mm and 32^{o}C, where the mean monthly values are 27-37^{o}C (Abayneh Esayas, 2003) respectively. Pawe Woreda has a total population of 45,552, of whom 23,265 were men and 22,287 women. 10,068 or 22.1% of population were urban inhabitants (CSA, 2007).

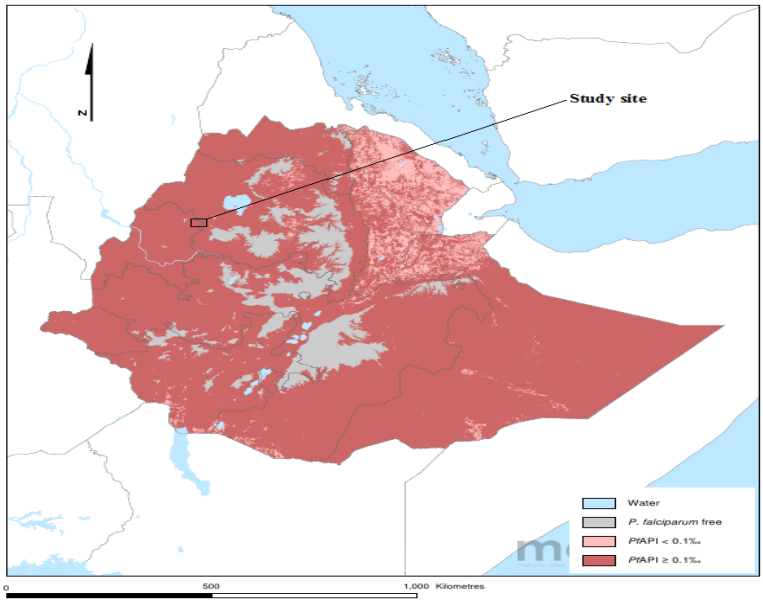

Figure 2 Map of the study site (Source: Atlas map of *P. falciparum* distribution in Ethiopia, 2010).

3.2 Study population and sample size

The study participants were all febrile cases seeking medication in Pawe hospital during the study period, from October 2013 to May-2014. Based on inclusion criteria using a single proportion method and calculated using the following formula developed by Hsieh (1998).

$$n = \frac{n_o}{1 + \frac{n_o}{N}}$$ where,

$$no = \frac{Z^2_{\alpha/2} PQ}{d^2}$$

n= sample size

d= margin of error ()

N= population size

P= proportion of population

α= level of significance

Q=1-P

Where: d=0.05

P=0.5

α=0.05

$$no = \frac{(1.96)^2 x 0.5 x 0.5}{0.05^2} = 384$$

Children <10 years (n=263) and pregnant women (n=121) were enrolled in the study.

3.3 Clinical data collection

Clinical and demographic characteristics of the study participants were documented using pre-designed clinical data record form by health professionals working in the Pawe hospital. Accordingly, body temperature was measured by digital thermometer, severe malaria symptoms such as prostration (inability to sit), hypoglycemia, jaundice, impaired consciousness, splenomegaly, Hepatomegaly, fever, headache, hyperpyrexia, persistent vomiting, respiratory distress, and Hemoglobinuria was assessed for all participants

3.4. Parasitological and hematological tests

About 2ml of blood samples was collected in EDTA coated tubes. Few drops were used for parasite identification and the others used for further analysis. Briefly, a drop of blood sample was collected on clean glass slide for preparation of thin and thick blood smears in duplicate per patient for microscopic examination after patients were confirmed to have malaria infection from finger pricked blood sample. Thick and thin blood smears were stained with 10% Giemsa (pH = 7.2, for 10 minutes), while thin smears fixed in methanol prior to Giemsa staining. Malaria parasite identified under a microscope and parasite load was calculated after counting asexual parasites per 200 white blood cells (WBC), assuming mean WBC count is 8,000/μL.

Small amount of blood samples was used for analysis of hematological parameters such as WBC, RBCs, and platelet, Hb, HCT, and lymphocytes using auto sampler CBC machine. To determine acute and repeated malaria infections among the pregnant women malaria-induced anemia was classified following WHO diagnostic guidelines: mild anemia is defined as hemoglobin concentration < 11 g/dL or hematocirit (HCT) < 33%, severe anemia Hb < 5 g/dL or hematocirit < 15%. Hyperparasitemia (parasite load >100,000 parasite /μL), respiratory distress,

confusion, comma, Hemoglobinuria, prostration, hyperpyrexia (body temp >40^{o}C), mild or severe anemia accompanied by *P. falciparum* infection is designated uncomplicated and severe malarial anemia, respectively (WHO, 2010; MOH, 2012). Furthermore, hematological parameters were classified based on reference ranges such as neutropenia (<50% neutrophil count), Lymphocytopenia (<20% lymphocyte count), Eosinopenia (<1% Eosinophil count) and Monocytopenia (<1% monocyte count).

3.5 Data analysis

Data was analyzed using SPSS software. Descriptive statistical tests were used for analysis of clinical, demographic and parasitological data. Association between variables was evaluated using Pearson correlation test. Median was considered over mean for non-normally described variables. Post hoc Turkey and LSD test was used to identify the real difference after a two-tailed analysis of variance (ANOVA) test. In all analysis significance level ($P<0.05$) was considered at 95%confidence interval.

3.6 Ethical consideration

The study was ethically approved by ethical review committee of Jimma University, College of Natural sciences. Letter of permission was handed to concerned officials in the Pawe hospital. From all study participants consent, oral and written agreement was obtained before data collection.

4. Results

4.1 .Prevalence of malaria in the study area

Malaria was among the top public health concern in the study site. Though the trend of malaria positive cases showing a sort of declining, the five years malaria prevalence data of the area from 2002-2006 in Ethiopian calander showed that the numbers of infected children≤10years are still higher when compared to other parasites (Figure1)

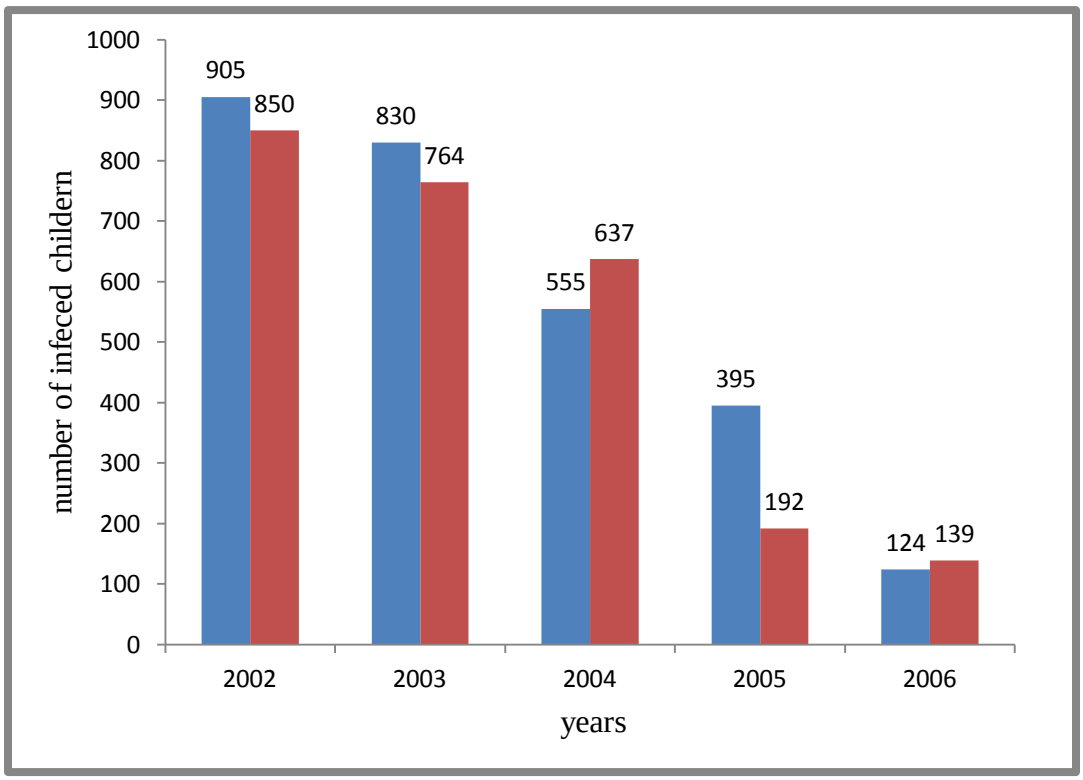

Figure2 Malaria cases among children in the study area

Likewise, proportion of malaria positive pregnant women were showed a decreasing pattern in the study site, but still a major concern (Figure 2).

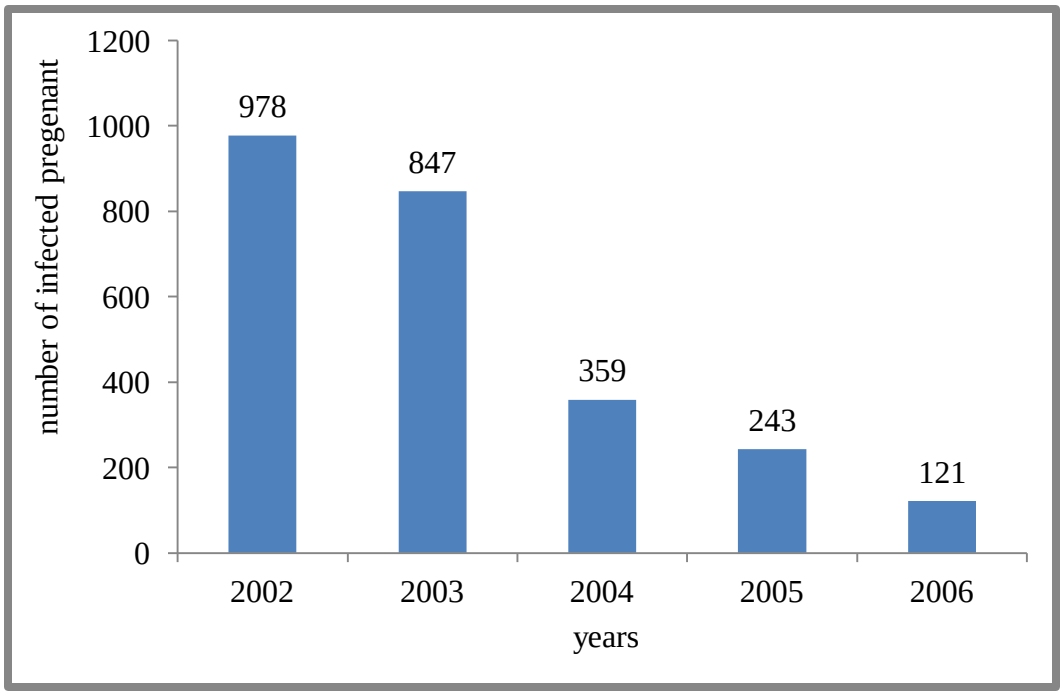

Figure 3 Malaria cases in the study area among infected pregnant women.

4.2 Socio-demographic characteristics of the study participants

During the study period, a total of 1523 blood samples were collected from presumptive malaria cases, children age ≤10 years and pregnant women of all age. About 384 (25.21) were positive for malaria infection. Among these 263 (68.48) were children, among of these 139 (52.85) and 124 (47.14) were males and females respectively. Among these 39 (28.1), 9 (3.4) and 92 (66.2) of male children were infected with mixed and mono *P.vivax* and *P. falciparum* respectively, and 7(2.7), 108 (87.1), and 17 (13.7) of female infected with mixed and mono *P. falciparum* and *P. vivax* respectively.

The rest, 121 (31.5%) were pregnant women. Among this 17(14.0%), 92(76.0%), and 12(9.9%) were infected with mixed, *P. falciparum* and *P.vivax*. The median age of children and pregnant women enrolled in this study was 4.1 and 30 years respectively. Majority of children were males and mean auxiliary body temperature of the children was 38.14^{o}C while pregnant women was

38.03^{o}C. Proportion of children with vomiting and diarrhea symptoms were 162 (61.59) and94 (35.74) respectively, while in pregnant women equal proportion, 84 (69.4) of them had vomiting and diarrhea. Bed net coverage was very limited, 76 (28.89) in children and 20(16.52) in pregnant women. Mean hemoglobin and HCT level were7.8g/dL and 27.5% in children while 8.8g/dl and 26.4% in pregnant women respectively. Geometric mean parasite count of children and pregnant women was 9898 and 8234 parasite/µL respectively table 1 and 2 below.

Table 1 Socio-demographic manifestation of uncomplicated malaria infected children and pregnant women in Pawe hospital.

Characteristics	Children	Pregnant women
Age (median)	4.1(1month-10year)	30 (19-45year)
Sex:Male Female	139 (52.8) 124 (47.14)	-
Mean body temperature(^{o}c)	38.14	38.03
Bed net	76	20
BMI(Body Mass Index)	19.15	22.07

Table 2 Clinical manifestation and laboratory results of uncomplicated malaria infected children and pregnant women in Pawe hospital.

Characteristics	Children	Pregnant women
Vomiting	162 (61.59)	84 (69.4)
Diarrhea	94 (35.74)	84 (69.4)
Mean Hb (g/dl) level	7.78%	8.86%
HCT (hematocirit)	27.52%	26.37%
Geometric mean parasite count	9898±5138	8234±2829
Headache	141 (53.6)	98 (81)
Pre-medication	92 (34.98)	27 (22.3)

4.3 Clinical characteristics of children and pregnant women with malaria parasites

Based on the observed clinical and demographic characteristics, median age of children was 4 years. About, 16.4%, 76.0% and 7.5% of them were infected with mixed and mono infection of *P. falciparum*, and *P. vivax*. Though most children with *P. falciparum*, 119(45.2) and 16(6.1) infected with *P.vivax* had vomiting respectively, 80(30.7) and 16(6.1) of them had diarrhea with *P. falciparum* and *P.vivax*, respectively while 46(17.5) of them had symptom of vomiting and diarrhea with mixed infection (*P.falciparum* and *P.vivax*). Children with *P.falciparum* and *P.vivax* which had bed net were 20(10) and3 (1.1), respectively and 2(0.7) for mixed infection. Mean hematocirit value was 27.52 and 26.17 for *P.falciparum* and *P.vivax and* 28.14 for mixed infection.

Geometric mean parasite count (asexual stage) and average body temperature for *P. falciparum* and *P. vivax* infected children were 10963 and 3902 parasite/µl, 38.63 and 38.62^{o}C respectively. Some participants had symptom of splenomegaly, 6 (2.3) and 4(1.5) for *P. falciparum* and mixed infection, while 36(25.8) and 15(12.4) of the study participant were taken medicine prior to attending the health facility respectively. The study participant had body mass index of 18.44, 19.15, and19.30 for mixed and mono-*P.falciparum* and *P.vivax* infection, respectively. Vomiting, bed net, parasite load, splenomegaly and prior medication were significantly different ($P<0.05$) between *P. falciparum* and *P.vivax* infected children. While, hematocirit level, body temperature, diarrhea, hemoglobin level, body mass index of the study participant didn't show significant difference ($P>0.05$) between the two groups (table 3 and 4) below.

Table 3 Socio-demographic manifestation of malaria infected children with respect to mono and mixed Plasmodium infection in Pawe hospital.

Characteristics	*P. falciparum (n=200)*	Mixed infection(n=34)	*P.vivax* infection (n=29)	P-Value
Temperature (^{0}C)	38.63	38.62	38.62	0.951
Bed net	20(7.6)	3(1.1)	2(0.7)	0.00
Hb (g/dL)	7.87	8.00	8.00	0.84
HCT (hematocirit)	27.52	28.14	26.17	0.67
Geometric mean parasite/µL	10963±3692	7923±2756	3902±1569	0.00
BMI (kg/m^{2})	19.15	18.44	19.20	0.785

Table 4 Clinical manifestation and laboratory results of malaria infected children with respect to mono and mixed Plasmodium infection in Pawe hospital.

Characteristics	*P. falciparum (n=200)*	Mixed infection(n=34)	*P.vivax* infection (n=29)	P-Value
	Proportion (%)	Proportion (%)	Proportion (%)	
Vomiting	119(45.2)	46(17.5)	16(6.1)	0.00
Diarrhea	80(30.4)	46(17.5)	16(6.1)	0.19
Hepatomegaly	0	0	0	-
Splenomegaly	6(2.3)	4(1.5)	0	0.00

The mean body temperature for pregnant women was 38.11, 38.04 and 38.21^{o}C for *P. falciparum*, mixed infection and *P. vivax*, respectively. Significant proportion (P<0.05) of *P. falciparum* infected pregnant women had high prevalence of vomiting 50(35.9), diarrhea 53(38.1), bed net 9(6.4), parasite load 11984parasite/µl, splenomegaly (2.8), chill rigor 42(30.2), nausea 40(28.7), abdominal pain 36(25.9) and self treatment habit 20(14.4) while body temperature, Hb level, parasite load, BMI and abdominal pain, didn't show significant difference (P>0.05). Hb level and BMI 23.05 were higher in mixed infected and *P.vivax* pregnant women respectively (Table5).

Table 5 Clinical manifestation and laboratory results among pregnant women with respect to mono and mixed Plasmodium infection in Pawe hospital.

Characteristics	*P. falciparum* (n=86)	Mixed infection (n=23)	*P. vivax* (n=12)	P. value
	Proportion (%)	Proportion (%)	Proportion (%)	
Temperature (^{0}C)	38.11	38.04	38.21	0.82
Vomiting	50(41.3)	24(19.8)	10(8.2)	0.00
Diarrhea	53(43.8)	25(20.6)	6(4.9)	0.00
Hb (g/dL)	8.9	9.0	8.8	0.84
Geometric mean parasite/µL	11984±4481	7576±5833	3789±3649	0.00
BMI (kg/m^2)	22.07	21.89	23.05	0.37
HCT (%)	26.37	26.70	26.41	0.04
Hepatomegaly	0	0	0	-
Splenomegaly	4 (3.3)	2(1.6)	0	0.00
Chill rigor	42 (34.7)	21(17.5)	10(8.2)	0.02
Nausea	40 (33.1	24 (19.8)	10(8.2)	0.01
Abdominal pain	36 (29.7)	20(16.5)	10(8.2)	0.31
Self medication	20 (16.5)	7(5.8)	0	0.00

4.4. Frequency of severe malaria in the Pawe hospital

Incidence of severe malaria complication among the two biologically risked group, children and pregnant women was comparable. This means proportion of severe anemic children and pregnant women were 15.2 and 14.04% respectively. Also, prevalence of hyperparasitemia and prostration in children and pregnant women were 12.54 and 33.88%, and 19.01 and 24.79%, respectively. Respiratory distress, severe anemia and persistent vomiting were more or less equally appearing in children and pregnant women. Incidence of comma, Hepatomegaly, confusion, and splenomegaly were significantly higher ($P<0.05$) in children than pregnant women, while Hemoglobinuria, hyperpyrexia, prostration and hyperparasitemia were highly observed among mono and mixed Plasmodium infected pregnant women than children (Table 6).

Table 6 Clinical manifestation and laboratory results of complicated severe malaria among children and pregnant women in Pawe hospital.

Characteristics	Children	Pregnant women	P-value
	Proportion (%)	Proportion (%)	
Severe anemia	40 (15.2)	17 (14.04)	0.00
Hyperparasitemia	33 (12.54)	41(33.88)	0.01
Prostration	50 (19.01)	30 (24.79)	0.03
Hyperpyrexia ($\geq 40^{o}C$)	47 (14.8)	8 (33.05)	0.75
Confusion	31(11.78)	4 (3.3)	0.00
Hepatomegaly	3 (1.1)	-	0.000
Splenomegaly	16 (6.08)	6 (5)	0.02
Respiratory distress	19 (7.2)	11 (9.1)	0.01
Hemoglobinuria	15 (5.7)	10 (8.26)	0.85
Persistent vomiting	37 (14.06)	34(28.1)	0.01
Comma	4 (1.52)	-	0.000

4.5 Frequency of severe malaria among *P. falciparum and P. vivax* infected children and pregnant women

Most severe malaria symptoms such as respiratory distress, persistent vomiting, splenomegaly, and confusion were significantly higher ($P<0.05$) among children, while severe anemia, hyperparasitemia, prostration, and Hemoglobinuria were common among malaria infected pregnant women.

Among the 263 children, a total of 166*P.falciparum* infected children were full-filled at least one of the WHO criteria for severe malaria. These were severe anemia 36 (13.68), prostration 41 (20.5), hyperpyrexia ($\geq 40^{o}$C), 16(8), splenomegaly 6(3), respiratory distress 10(5), Hemoglobinuria 14 (7), persistent vomiting 16(6.1), and hyperparasitemia 33 (16.5) but none had pathology of Hepatomegaly and comma. These symptoms of severe malaria complications among the study participants were significantly higher ($p<0.05$) than other Plasmodium infection except symptoms such as confusion and splenomegaly. Most common severe malaria syndromes observed in *P. falciparum* infected pregnant women were hyperparasitemia 41 (46.06), respiratory distress 5 (5.43), Hemoglobinuria 8(8.6), prostration 25 (28.09), severe anemia 15 (16.85), confusion 14 (15.73) and persistent vomiting 20 (22.47%) (Table7).

Table 7 Frequency of severe malaria in the study area among *P. falciparum* infected children and pregnant women.

Characteristics	Children (n=200)	Pregnant women (n=92)
	Proportion (%)	Proportion (%)
Severe anemia	36 (13.68)	15 (16.85)
Hyperparasitemia	33 (16.5)	41 (46.06)
Prostration	41(20.5)	25 (28.09)
Hyperpyrexia (≥40^{o}C)	40 (20)	5 (5.6)
Confusion	3 (1.5)	14 (15.73)
Hepatomegaly	-	1 (1.12)
Splenomegaly	6 (3)	9 (10.1)
Respiratory distress	10(5)	5 (5.43)
Hemoglobinuria	14 (7)	8 (8.6)
Persistent vomiting	16 (8)	20 (22.47)
Comma	-	4 (4.5)

Likewise, among 263 children enrolled in the study, a total of 29 were infected with *P. vivax* malaria. Among these, those who full-filled at least one WHO criteria of sever malaria were 4 (13.8) severe anemia, 9 (31.03) prostration, 7 (24.12) hyperpyrexia, 2 (6.9) splenomegaly, 3 (10.3) respiratory distress, 2 (6.89) Hemoglobinuria, 1 (3.4) confusion, and persistent vomiting, but none of *P. vivax* infected children had symptoms like hyperparasitemia, Hepatomegaly, and comma. Also severe malaria complications associated to a total of n= 12 *P. vivax* in pregnant women were, severe anemia (n=2), prostration (n=5), hyperpyrexia (n=3), confusion (n=5), respiratory distress (n=1), persistent vomiting (n=9), and Hemoglobinuria (n=1). The most prevalent severe malaria symptoms in *P. vivax* infected pregnant women were persistent vomiting, respiratory distress, prostration, and confusion, while respiratory distress, severe anemia, and Hemoglobinuria were highly occur in children (Table 8).

Table 8 Frequency of severe malaria associated to *P. vivax* of the study participants

Characteristics	Children (n=29)	Pregnant women (n=12)
	Proportion (%)	Proportion (%)
Severe anemia	4 (13.8)	2 (16.67)
Hyperparasitemia	0	0
Prostration	9 (31.03)	5 (41.67)
Hyperpyrexia (≥40oC)	7 (24.12)	3 (25)
Confusion	1(3.4)	5 (41.67)
Splenomegaly	2 (6.9)	0
Respiratory distress	3 (10.3)	1 (8.3)
Hemoglobinuria	2 (6.89)	1 (8.3)
Persistent vomiting	1 (3.4)	9 (75)
Comma	0	0

Though there was no significant association (r=0.038) between age and Hb level, as age of children increases Hb level increase. Most children with age <1 years had hemoglobin level <5g/dl (Figure 4).

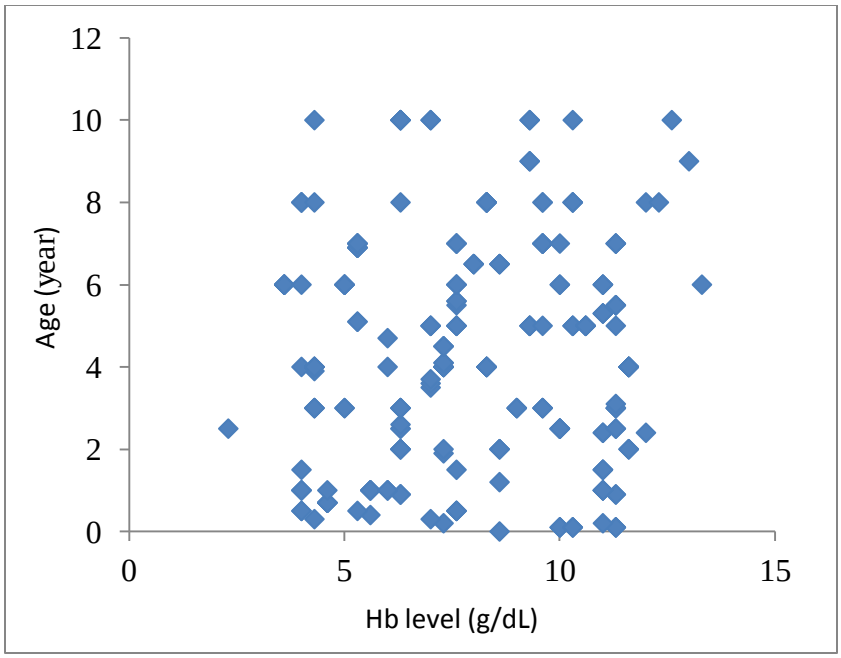

Figure 4 Association between age and hemoglobine level in malaria infected children

BMI had inverse relation with age of children. As age increased BMI value decreased (r= -0.79). Mainly, children with lower age, <1 year had maximum BMI than other. With consistent to this, upto age 5 years, BMI value of children was large, even some of them were in obsity category (Figure 5).

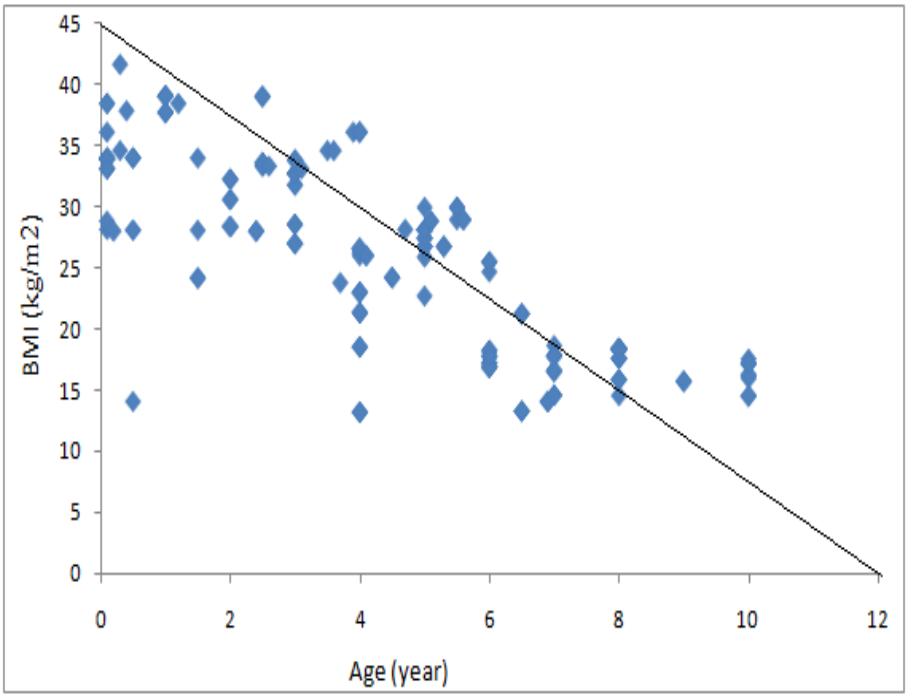

Figure 5 Association between age and BMI in malaria infected children

Though there was no significant differences (P>0.05) in WBC indices between children infected with different Plasmodium species (mixed, *P. falciparum* and *P. vivax*) (Figure 6), significant difference in neutrophil count, <40% or neutropenia condition were observed among children infected with *P. falciparum* (P=0.032) but not among pregnant women. Similarly, lymphocyte count <20% or Lymphocytopenia were significantly higher (P<0.000) among children infected with *P. falciparum* than other. But Eosinophil and monocyte count were not significantly different among children infected with malaria (Table 9).

Table 9 Characteristics of WBC indices in children infected with different Plasmodium species in Pawe hospital, Benishangulgumuz, Northern Ethiopia

WBC indices (Reference range)	Pathologies	Total (%)	P. falciparum (n=200)	P. vivax (n=29)	Mixed (n=34)	P. value
Neutrophil (50-70%)	Neutropenia	84 (31.9)	67 (33.5)	7 (24.13)	10 (29.4)	0.032
Lymphocyte (20-40%)	Lymphocytopenia	49 (18.6)	42 (21)	4 (13.79)	3 (8.82)	0.000
Eosinophil (1-5%)	Eosinopenia	23 (8.7)	16 (8)	3 (10.3)	4 (11.7)	0.437
Monocyte (1-6%)	Monocytopenia	10 (3.8)	7 (3.5)	0	3 (8.82)	0.799

NB: P. value was calculated using one way ANOVA.

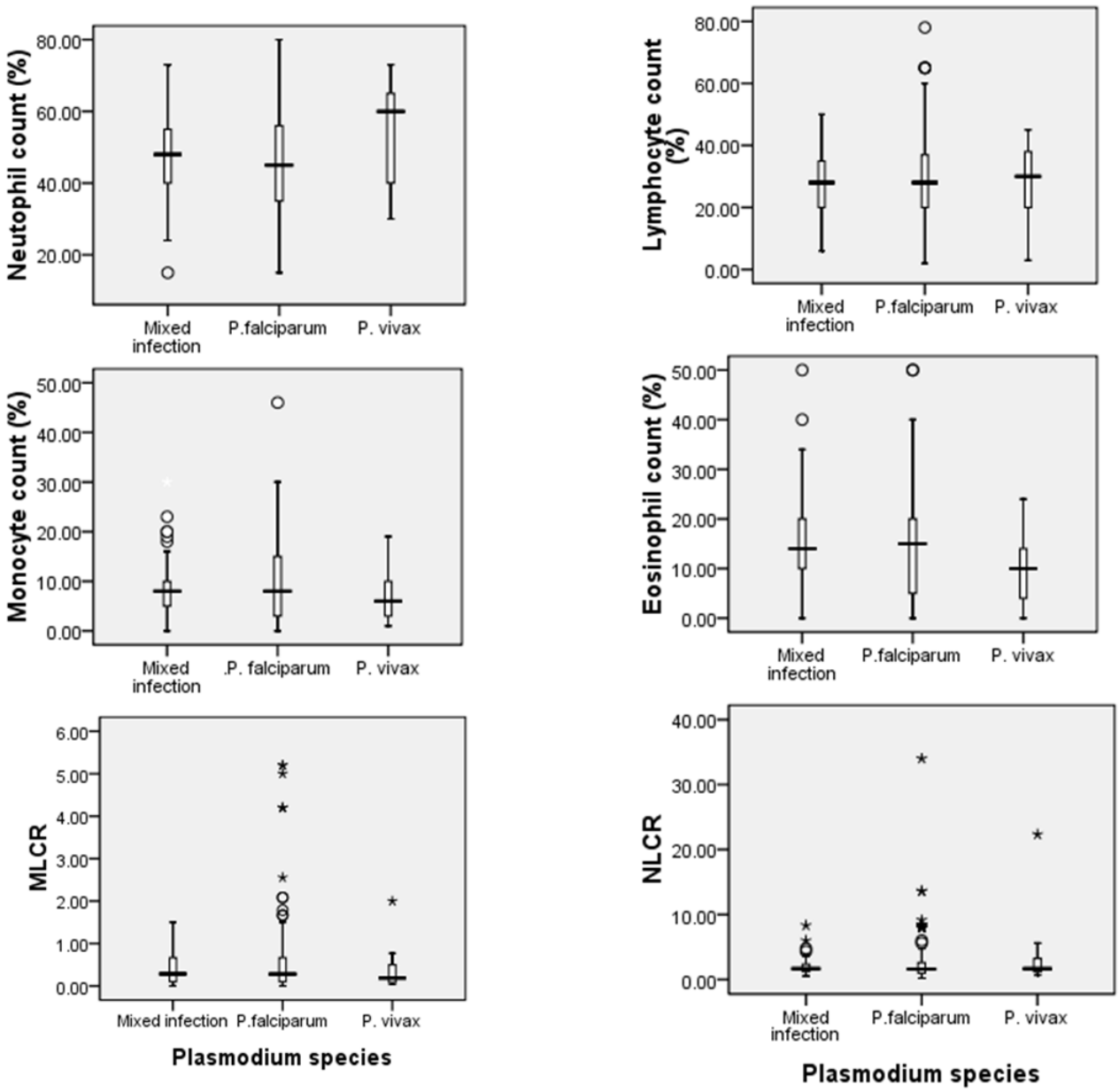

Figure 6 Box-whisker plots showing distribution of WBC indices, Monocyte-Lymphocyte count ratios (MLCR) and Neutrophil-lymphocyte count ratios (NLCR) between children infected with different *Plasmodium* species.

Like in children, WBC indices of pregnant women were not significantly different ($P>0.05$) between different plasmodium species infection (Figure 7).

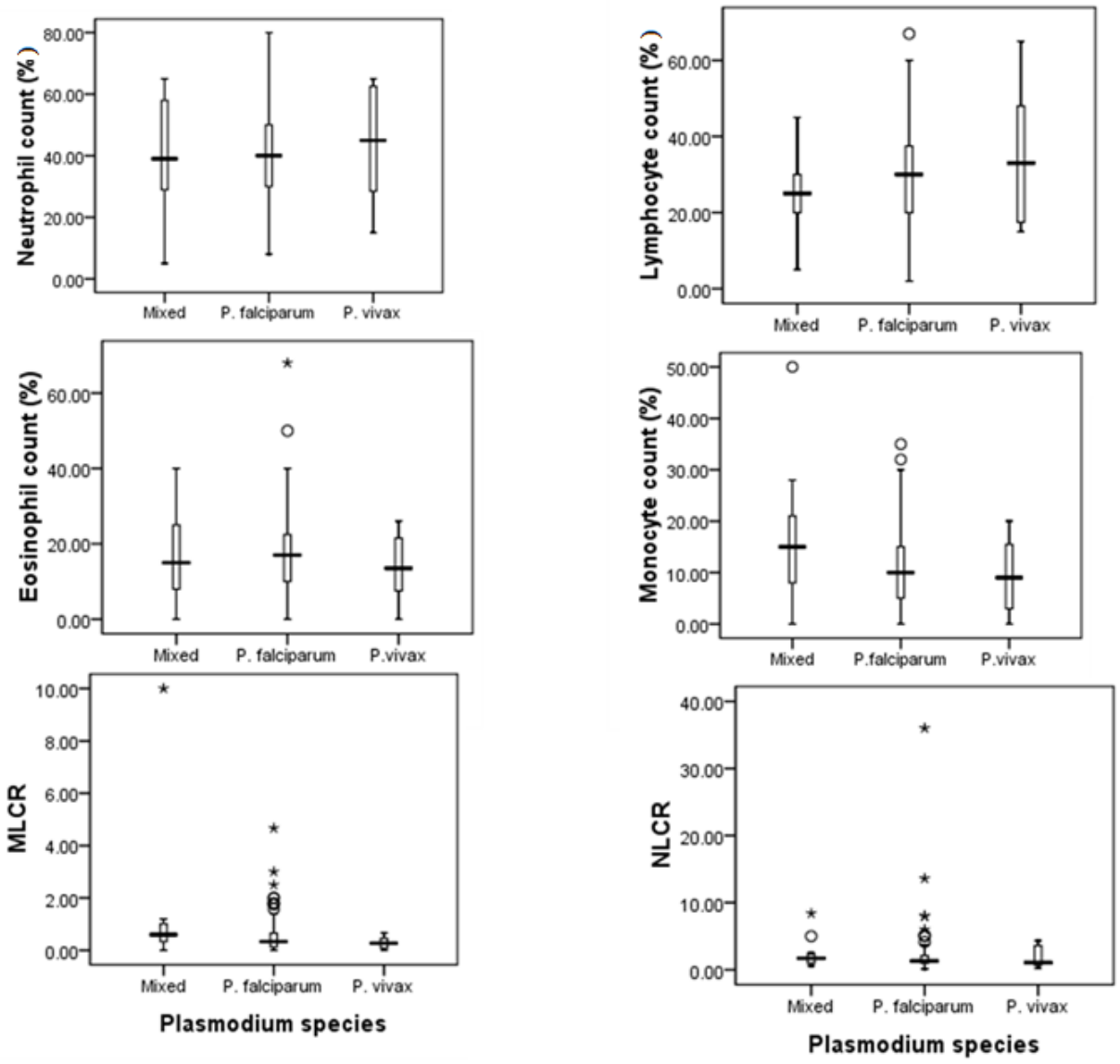

Figure 7 Box-whisker plots showing distribution of WBC indices, Monocyte-Lymphocyte count ratios (MLCR) and Neutrophil-lymphocyte count ratios (NLCR) between pregnant women infected with different plasmodium species.

5. Discussion

Though malaria prevalence in some parts of Ethiopia showing declining pattern, in the study site, still it is the top health concern. According to the five year prevalence report from the hospital, trend of malaria positive cases shows a sort of decreasing, though the numbers of infected children ≤10years and pregnant women were still higher when compared to other parasitic infections. This figure was similar to the recent study conducted in Ethiopia, where due to intensive malaria intervention strategies which involve vector control and symptomatic cases treatment, the morbidity and mortality associated to malaria was decreasing among pregnant women and children respectively (Alemu *et al*., 2012). Though the two Plasmodium species: *P. falciparum* and *P. vivax* are an important parasite in malaria related problems in Ethiopia, *P. falciparum* was the most predominant species that responsible for about 76% of all malaria infections in children and pregnant women assessed in the study.

Malaria is a deadly disease to all human races. But, those at highest risk biologically are infants and young children (from six months to five years) due to their underdeveloped immunity and pregnant women, as their immunity reduce during malaria infection. Thus, in these groups of population besides mortality, mainly in pregnant women it causes increased risk of abortion, stillbirth, premature delivery and low-birth weight infants (Reuben, 1993; Duffy and Fried, 2005). Thus the documented severe malaria among children and pregnant women evidenced the higher risk of these groups to malaria associated morbidity. Furthermore, incidence of severe malaria associated to *P. vivax* in the two study participants strengthen the fact that this parasite is no more benign, rather it is accountable for some life threatening complications among children from endemic regions such as Indonesia, India, Ethiopia and Brazil (Tjitra *et al*., 2008; Kochar *et al*., 2009; Alexandre *et al*., 2010; Tanwar *et al*., 2011; Ketema and Bacha, 2013).

Some of severe malaria complications such as Hemoglobinuria, hyperpyrexia, prostration and hyperparasitemia were higher among malaria infected pregnant women than children (Naha *et al*., 2012). In other report from Ethiopia, cerebral malaria, convulsions, altered mental state and prostration were among the common manifestations of severe malaria symptoms observed in pregnant women (Mengistu *et al*., 2006, Adem *et al*., 2004) was also reported that, severe anemia and jaundice had been observed as the presenting manifestations of severe *P. falciparum* malaria in pregnant women in central and eastern Sudan (Adam *et al*., 2004). In line to the above reports, frequency of malaria associated to *P. falciparum* severe malaria complications documented in pregnant women were severe anemia, hyperparasitemia, prostration, confusion, persistent vomiting, respiratory distress and Hemoglobinuria.

Most of the children enrolled in this study were found in age group ≤ 5 years. In this age category higher load of parasitemia, respiratory distress and incidence of severe anaemia, but lower concentration of haemoglobin and hematocirit level was observed. This strengthens the fact that children in this age group who live in holo or hyper endemic areas are biologically risked group (Le Port *et al*., 2012). This is because of the development of poor immunity against the disease [WHO, 2000], but as they get older and repeatedly exposed to the disease they gradually develop protective immunity to malaria (Dolan *et al*., 2009). Likewise, pregnant women assessed in this study had comparable prevalence of severe anaemia, hyperparasitemia, and respiratory distress to report of Ali *et al* (2011), where hyperparasitemia, and severe anemia were the most common symptoms associated to *P. falciparum* and *P. vivax* malaria observed among pregnant women.

It is well known that *P. falciparum* is generally accepted as a leading cause of anemia in pregnant women (Guyatt and Snow, 2001). It is estimated that anemia causes as many as 10 000 maternal deaths each year (Steketee *et al*., 20014). However, despite the dangerous impact of malaria on pregnant women and their infants, it is estimated that less than 5% of pregnant women have access to effective interventions.

Usually anemia is a hallmark of *P. falciparum* infection due to intense hemolysis (destruction) of infected RBCs due to higher parasitemia caused by *P.falciparum.* Since unlike other plasmodium species, *P. falciparum* infected all types of RBCs found at different stages of development (from immature young to old RBCs) without selection besides hemolysis of non infected RBCs by host immunity, there is no doubt on incidence of severe anemia. However, due to selective preference to only young RBCs by *P.vivax,* it appears that the number of haemolysed RBC during *P.vivax* infection is minimal. Thus, the incidence of anaemia associated to *P. vivax* might occur as a result of rigor inflammatory reactions due to pro-inflammatory response and cytokines activation Andrade *et al*., 2010] and less deformability of RBCs during *P. vivax* infection (Anstey *et al*., 2009;Handayani*et al*., 2009). On the other hand, the rate of non infected RBCs hemolysis for every infected RBC destroyed could contribute to the incidence of anaemia as number of non-parasitized RBCs removed from circulation during *P. vivax* is much higher (~32) than *P.falciparum* (~8) (Colin *et al*., 2003).

Acute respiratory distress syndrome (ARDS), an inflammatory reaction in the lungs that inhibits oxygen exchange, which may occur even after the parasite counts have decreased in response to treatment.

Though bed net is unquestionable tool of malaria vector prevention and widely applicable in most malaria endemic area of the country, in the current study area its distribution and utilization among the biologically risked group supposed to get priority in the family and must be at 100% coverage, it was very limited and only was sleep under bed net. This situation could be among the most important factor responsible for still higher prevalence of malaria in the study area, when the current trend of malaria infection dramatically dropped in most malaria endemic area of Ethiopia.

As Monocyte and lymphocytes play a crucial role in the induction and maintenance of an immune response ratio of the two WBC indices, monocyte-lymphocyte count ratio (MLCR) is recently considered as very important tool in prediction of an individual's protection capacity against clinical manifestation of *P. falciparum* malaria (Warimwe *et al*., 2013). It is considered that increased ratio value of MLCR is associated to severe malaria cases with reduced immunity (Warimwe, *et al*., 2013). MLCR analysis conducted among the study participants didn't show significantly difference between malaria patients infected with different species of plasmodium parasite.

6. Conclusion and Recommendations

Though malaria prevalence is decreasing due to intensive intervention approaches in the country, clinical data showed that, malaria is still a major health concern in the study area. Severe malaria complications associated to *P. falciparum* and *P. vivax* such as respiratory distress, hyperpyrexia, comma, Hepatomegaly, splenomegaly, Hemoglobinuria was observed in a number of children and pregnant women studied. *P.vivax* associated severe malaria complications observed among the study participants strengthen the fact that this parasite will no longer considered mild rather it is virulent. Thus, early detection of infected cases and implementation of effective treatment should be in practice to reduce mortality and morbidity associated to malaria in the study site. Furthermore, awareness creation activities that involve the community on prevention and control measure of the disease, on clinical symptoms of malaria and early seeking medication when they have malaria like symptom must be given.

7. References

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Appendix 1

Clinical record form

Name of Health center_____

- Patient code_____
    - Age_____
    - Sex_____
    - Weight_____
    - Height_____
    - Body temperature_____
    - Bed net_____
- Prior medication_____
- Vomiting_____
- Diarrhea_____
- Prostration (unable to sit) _____
- Jaundice_____
- Headache_____
- Fever_____
- Respiratory distress_____
- Hemoglobinuria (black urine) _____
- Hypoglycemia (<40g/ml) _____
- Hb level _____
- Confusion_____
- Coma_____
- Hepatomegaly_____
- Splenomegaly_____

THANKS!