JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

SEVERE MALARIA SYMPTOMS ASSOCIATED WITH *PLASMODIUM VIVAX* AMONG PATIENTS VISITING HEALTH FACILITIES IN MENDI TOWN, OROMIYA REGIONAL STATE, WESTERN ETHIOPIA

By: Yohannes Demissie

October, 2015

Jimma, Ethiopia

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By: Yohannes Demissie

A Thesis submitted to Department of Biology, College of Natural Sciences, Jimma University, in Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology (General Biology)

Advisor: Tsige Ketema (Associate professor)

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Title: Severe malaria symptoms associated with *Plasmodium vivax* among patients visiting health facilities in Mendi town, Oromiya Regional state, western Ethiopia.

By: Yohannes Demissie

The thesis entitled "Severe malaria associated with *Plasmodium vivax* among patients visiting Mendi Health Facilities in Mendi town, Oromiya Regional state, western Ethiopia" has been approved by the Department of Biology for partial fulfillment of the Degree of Master of Science in Biology.

Approved by the examining board

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Declaration

I, the under signed, declare that this is my bona fida original work, has never been presented in this or other University, and that all the resources and materials used for the thesis have been dully acknowledged.

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This thesis has been submitted for examination with my approval as candidate' advisor

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List of Abbreviations /Acronyms

ARDS	acute respiratory distress syndrome;
ARF	acute renal failure
G6PD	glucose-6-phosphate dehydrogenase
CDC	Centers for Disease Control and Prevention
FMOH	Federal Ministry of Health
Hb	hemoglobin
PMI	President's Malaria Initiative
PCR	Polymerase chain reaction
RBC	Red Blood Cell
RBM	Roll Back Malaria
SMA	Severe malaria anemia
SPSS	Statistical Package for Social Science
SSA	sub-Saharan Africa
UNICEF	United Nation Children's Fund
WBC	White Blood Cell
WHO	World Health Organization

Abstract

Malaria is a serious global health problem, a vector-borne infectious disease of humans and other animals caused by unicellular obligate intracellular protozoan parasites of the genus Plasmodium. Five primary species of malaria parasites infect humans. P. vivax malaria is usually an uncomplicated disease, which runs a benign course, changing trends with severe and life-threatening complications has been reported from different endemic regions. Thus, this study was designed to assess the occurrence of severe malaria complications (one or more complications that causes multi-organ dysfunction occurring in the absence of an identified alternative causes and in the presence of Plasmodium asexual parasite.) associated with P. vivax in Western parts of Ethiopia. Presumptive malaria patients of all age groups seeking medication at the selected health facilities in Mendi town were recruited for the study. Socio-demographic, clinical and parasitological characteristics of uncomplicated and complicated malaria were assessed following standard procedures. Data were analyzed using IBM SPSS statistical software version 20. Median age of the participants was 14 years (from one month to 85 years). Geometric mean parasite count and mean hemoglobin level were 3745 parasite/µL and 12.4 g/dL, respectively. Among the 384 patients enrolled in the study based on the inclusion criteria for P. vivax mono-infection, 55 of them were fulfilled at least one of the WHO criteria for severe malaria indicators. Those fulfilled at least one of the WHO criteria for severe malaria syndromes were, 36 (65.5%), while 19 (34.5%) had two or more overlapping severity indicators. Some of these syndromes were: prostration 14 (25.45%), persistent vomiting 9 (16.36%), respiratory distress 6 (10.9%), hypoglycemia 5 (9.1%), hyperpyrexia 8 (14.5%), and severe anemia 13 (23.63%). In none of the cases symptoms such as epistaxis, confusion, coma, hemoglobinuria and hypotension were observed. Differences in parasite load did not affect the frequency of respiratory distress, hyperpyrexia, and hypoglycemia. However, severe anemia, prostration, and persistent vomiting were significantly affected ($P<0.05$) by relatively higher load of parasitemia (>10,000 parasite/µL), (OR=3.8, 95% CI, 1.1-13.7; OR=4.4, 95% CI, 1.4-13.9; and OR=7, 95% CI, 1.8-27.4) respectively. Similarly, these three severe malaria symptoms were significantly higher ($P<0.01$) in children <5 years. P. vivax malaria cases seems decreasing but from the total malaria burden its prevalent rate was high for instance the prevalence rate of P. vivax was 36.4%, 38% and 37.1% in the year 2010, 2012 and 2014 respectively. P.vivax associated severe malaria complications observed in this study participants strengthen the fact that this parasite will no longer be considered mild rather it is virulent. Thus, to meet international and regional targets for reduction of malarial mortality and morbidity, responsible bodies should no longer neglect the impact of P. vivax.

Keywords*: Hypoglycemia, P. vivax, Prostration, Respiratory distress, Severe anemia.*

1. Introduction

1.1. Background of the study

Malaria is a serious global health problem, a vector-borne infectious disease of humans and other animals caused by unicellular obligate intracellular protozoan parasites of the genus *Plasmodium* (Rodriguez-Morales *et al*., 2015). Malaria is currently endemic in the tropical and sub-tropical zones of Asia, Africa, South and Central America, but also constitutes a serious problem for travelers as well as for people working in endemic regions. Nearly, half of the world's population (3.3 billion people) is at risk in more than 100 countries (WHO, 2008; Angel *et al*., 2013) A recent study has estimated that 1,238,000 people died from malaria in 2010 (Angel *et al*., 2013). Five primary species of malaria parasites infect humans: *P. falciparum*, *P. ovale*, *P. malariae, P. vivax* and *P. knowlesii* (Douglas *et al*., 2011; Angel *et al*., 2013; Rodriguez-Morales *et al*., 2015).

Malaria is one of the major public health problem facing the poorest countries in the world, not only causing sickness and death, but also hinder economic development; as a result of lost working hours and the high costs of treating those affected, and of vector control (Bekele *et al*., 2013). Among the most important public health problems, the World Health Organization (WHO) reported that 300-500 million clinical cases per year, with at least one million of these cases resulting in death are caused by malaria (WHO, 2012). *P. vivax* malaria is usually an uncomplicated disease, which runs a benign course. In the last few years, changing trends with severe and life-threatening complications due to *P. vivax* has been reported from different endemic regions such as India, Indonesia, and Brazil (Tijitra *et al*., 2008; Alexandre *et al*., 2010).

Although, it is a rare parasite in most parts of Africa, it has significant public health importance in Ethiopia. In some areas of the country the prevalence rate exceeds even 70% of total malaria infections (Ketema and Bacha, 2013). This was previously reported to be accounted to high Duffy blood group positivity of most population of the country (Miller *et al.*, 1976). But recently, contradictory reports are coming in that *P. vivax* malaria is highly prevalent in some parts of Ethiopia and its risk to drug resistance is increasing (Ketema *et al*., 2009). The paradigm of *P. vivax* red blood cell (RBC) invasion only in Duffy-positive individuals has been recently challenged, as it was shown that Duffy negative individuals can also be infected by *P. vivax* in

Brazil (Cavasini *et al*., 2007). It may evolve and may use alternative receptors other than Duffy (DARC) for erythrocyte invasion (Poespoprodjo *et al*., 2009).

Recently severe life threatening malaria syndromes, frequently associated with *P. falciparum* have been reported from *P. vivax* mono-infections in Ethiopia (Ketema and Bacha, 2013). These manifestations may be due to the change in the clinical spectrum of disease, increase in resistance, indiscriminate use of anti-malarial drugs, delayed treatment, or missing the primaquine therapy (Alexandre *et al.*, 2010).

1.2. Statement of the problem

P. vivax threatens almost 40% of the world's population, causing an estimated 72 to 390 million clinical infections worldwide each year (Price *et al*., 2007; Battle *et al*., 2014). And 2.85 billion people living at risk of infection. The challenges in controlling and eliminating *P. vivax* are far greater than those for *P. falciparum*. In areas where intensive control measures have been implemented, the relative proportion of malaria due to *P. vivax* usually increases when compared with *P. falciparum* (Price *et al.*, 2009).

Several important biological differences account for these observations, in particular, the development of dormant liver stages causing recurrent blood stage infections (relapses), the early appearance of gametocytes and the greater transmission potential of *P. vivax* at low parasite densities. Although often regarded as causing a benign infection, there is recent increasing evidence that the overall burden, economic impact, and severity of *P. vivax* have been underestimated, in part due to a bias in the scientific literature, policy makers and malaria researchers have generally focused on *P falciparum*, which is the main cause of malaria mortality (Anstey *et al*., 2009).

Even in places where *P. vivax* represents the major local problem to be tackled, clinical research is still focused on *P. falciparum* (Lacerda *et al*., 2012) There is robust evidence in the past decade from hospital-based studies in India and Indonesia that *P. vivax* is able to cause severe disease (Lacerda *et al*., 2012). In summary, *P. vivax* which has long been neglected and mistakenly considered 'benign' (Mueller *et al*., 2009). It is receiving an increasing amount of importance in the debates taking place on malaria epidemiology, control, drug resistance, pathogenesis and vaccines (Anstey *et al*., 2009).

There is a consensus among malaria experts that eliminating *P. vivax* will prove more technically challenging than eliminating *P. falciparum* and that there exist fewer tools and a weaker knowledge base from which to start an effective global elimination program (Mueller *et al*., 2009). As Ketema and Bacha (2013) argued although *P. vivax* malaria is highly prevalent in some parts of Ethiopia and its risk to drug resistance is increasing there are only few studies conducted on *P. vivax* associated severe malaria complication in order to estimate the associated burden among biologically risked groups (children and pregnant women), mainly children in the endemic areas (Ketema and Bacha, 2013).

The vast majority of these reports on severe *P. vivax* malaria are from south East Asia and India, Few studies were conducted about the prevalence of severe malaria associated with *P. vivax* on children and pregnant women in another region of Ethiopia (ketema and Bacha, 2013). Thus, this particular study was conducted to investigate the occurrence of severe malaria associated with *P. vivax* in all age groups that visit Mendi health facilities to fill this broad gap and try to answer the following questions.

- What is the current prevalence of *P. vivax* in the study area?
- Is *P. vivax* cause severe or complicated malaria?
- What is the prevalence of sever malaria associated with *P. vivax*?
- What are the demographic and clinical characteristics of the study populations in relation to severe malaria associated to *P. vivax*?

1.3. Objective of the study

1.3.1 General objective

- To assess the occurrence of severe malaria complications associated with *P. vivax* in patients visiting a health center and clinics in Mendi town, Oromiya regional state, Western Ethiopia.

1.3.2. Specific objectives

- To assess the prevalence of vivax malaria in the study area,
- To estimate prevalence of severe malaria complications associated with *P. vivax* among all age groups in the study area,
- To explore demographic and clinical characteristics of the study populations in relation to severe malaria associated to *P. vivax.*

1.4. Significance of the study

This study will provide information on the current prevalence of malaria in Mendi district, especially on severe malaria complications associated with *P. vivax*. Likewise; it will provide evidences for prevalence of severe malaria associated to P. vivax in Ethiopia. In addition, the result of this study will enrich the current available literature on prevalence of severe malaria complications associated with *P. vivax.*

2. Literature Review

2.1 *P. vivax* malaria as a Public Health Concern

Malaria is considered the most important parasitic disease in tropical areas, being responsible for a considerable amount of morbidity and mortality, especially in sub-Saharan Africa, where *P. falciparum* predominates, primarily affecting children and pregnant women (WHO, 2012). It has been a leading public health problem despite several malaria control strategies. According to World Health Organization (2013) approximately 207 million cases of malaria and 627,000 deaths were reported in 2012 (Dasgupta *et al*., 2015).

Currently *P. vivax* is the most geographically distributed cause of malaria worldwide (Battle *et al*., 2014), with annual infections range between 132–391 million (O'Brien *et al.,* 2014). In the Americas, about 5.5% of people are at-risk and *P. vivax* accounts for more than 70% of cases (Mueller *et al*., 2009). Furthermore, in the Americas about 30% of the population of the 21 countries with active transmission are at some degree of risk; Brazil and Colombia accounted for 68% of the cases in 2011 (O'Brien *et al.,* 2014). In Colombia, approximately 27 million individuals live in areas suitable for transmission and, although the mortality of the disease has diminished, the associated morbidity has remained relatively constant (WHO, 2012).

Risk among the billions living in areas of endemicity in Asia and the Americas should be considered a major problem involving both species as essentially equal agents of severe morbidity and mortality, focusing therapy and other interventions solely on *P. falciparum* malaria, with exclusion of a strategy to address the impact upon *P. vivax* malaria, is irrational and irresponsible (Anstey *et al*., 2009).

Furthermore, control measures limited to mosquito nets and diagnosis and treatment of the acute attack, even with successful diagnosis of *P. vivax* malaria, have a relatively limited impact on endemic *P. vivax* malaria (Baird, 2013). *P. vivax* malaria is typically carried with lower levels of parasitaemia, making it relatively difficult to diagnose (Mendis *et al*., 2001). However, there is evidence that, despite lower blood parasite loads, *P. vivax* immunity is acquired more rapidly than *P. falciparum* and may result in an earlier age-prevalence peak in

areas of high transmission (Mueller *et al*., 2009). The perception of *P. vivax* malaria as benign, however, has thwarted development of effective prevention, control, diagnosis, and treatment tools for this infection. The current frontline therapies for *P. vivax* malaria, chloroquine and primaquine, have been in continuous use since 1952. This treatment was never suitable for malaria as it occurs in settings of endemicity due to concerns of toxicity, and its efficacy has been known to be eroding for over 2 decades (Baird, 2013). Chloroquine (CQ) remains the first-line treatment for *P. vivax* asexual stages in most areas where this parasite is endemic, despite increasing reports of CQ resistance from many regions, especially from Southeast Asia and elsewhere (Baird 2009; Ketema *et al*., 2009).

The infection is common in tropical and sub-tropical areas and more than 50% of malaria cases are caused by *P. vivax*, especially in South East Asia and India (Dasgupta e*t al.*, 2015). Once considered benign, the number of complicated *P. vivax* malaria is increasing over the past few years. Usual complications reported from various parts of the world include common gastrointestinal symptoms, severe anemia, thrombocytopenia, pulmonary complications, and renal failure (Dasgupta *et al*., 2015). How *P. vivax* does this is a very important question, but the lack of understanding of pathogenesis should not diminish the importance of the core implication found in the sum of these many findings, *P. vivax* regularly kills people, and probably in very substantial numbers (Gething e*t al.*, 2011).

P. vivax gametocytes are present earlier in the progression of a primary or recrudescent infection than *P. falciparum* (Mendis *et al.*, 2001), such that the majority of patients have sufficient gametocytaemia to allow transmission before the infection is diagnosed or treated (Rat cliff *et al*., 2007; Douglas *et al.*, 2010). *P. vivax* gametocytes are transmitted more efficiently to *Anopheles* mosquito vectors than those of *P. falciparum* and are transmissible at lower parasite densities (Collins *et al*., 2002). Within the mosquito, *P. vivax* sporozoites develop faster than *P. falciparum* and with slightly wider viable temperature ranges, allowing for a greater geographical distribution (Gething e*t al.*, 2011).

2.2 Epidemiology of P. vivax

P. vivax is responsible for more than 50% of malaria episodes, in areas outside the African continent, is endemic in the Middle East, Asia and Pacific, and in many places, especially where elimination of transmission is within close sight, it is the predominant or even sole

species causing malaria, as is the case in large regions of the Americas, the Indian subcontinent and Western Pacific Region (Mendis *et al*., 2001; WHO, 2012). *P. vivax* is the most geographically widespread species that infects humans and the perception and wide-spread acceptance that *P. vivax* presents additional challenges for its elimination (Feachem *et al*.,2010) and that it can cause severe clinical manifestations and fatalities (Price *et al*., 2007, Lacerda *et al*., 2012; Baird., 2013). This has led to increased interest in this parasite (Mueller *et al*., 2009; Carlton *et al*., 2011).

P. vivax can account for up to a quarter of all severe cases detected (Price *et al*., 2009). A broad range of severe manifestations has been reported, some of which have been associated with subsequent death (Tjitra *et al*., 2008). Infants are particularly vulnerable to infection with *P. vivax.* Indeed in southern Papua, where *P. falciparum* is the most prevalent species, more infants are hospitalized with *P. vivax* malaria than with *P. falciparum* malaria (Poespoprodjo *et al*., 2009).

Most West Africans are negative for the Duffy blood-type, which is shown to be associated with receptor sites for *P. vivax* merozoites on the RBC. Therefore, many West Africans are not susceptible to infection with *P. vivax* (Cavasini, *et al*., 2007). However, as population of East Africa, including Ethiopians are duffy blood positive, they are susceptible to malaria infection (Ric *et al*., 2014). In some parts of the country, prevalence of *P. vivax* is more than 70% (Ketema *et al*., 2011). The epidemiological pattern of malaria transmission in Ethiopia is generally seasonal and highly unstable due to variations in topography and rainfall patterns (Adhanom *et al*., 2006).

Marked variations in the level of transmission from place to place or seasonal fluctuations in the number of cases are the main features of malaria transmission in Ethiopia, it is estimated that three-fourths of the land below 2000 meters is malarious with two thirds of the country's population at risk (Gebreyesus, *et al*., 2006). Malaria in Ethiopia often occurs below 2000 meters, with short-lived transmission following the rains. However, malaria epidemics have been recorded up to 2400 meters during periods when increased temperature and adequate precipitation are conducive for both vector survival and parasite development within the vector (Gebreyesus, *et al* ., 2006).

2.3 Life cycle of malaria Parasite

Malaria infection may be acquired congenitally from mother to baby across the placenta, from platelet or blood transfusions and from the use of shared needles; however it is most frequently initiated with the bite of an infected, female *Anopheles* mosquito, which injects the sporozoite stage of the parasite with its bite (Moriya *et al*., 2009). The malaria parasite life cycle involves two hosts (Figure 1). During a blood meal, a malaria infected female Anopheles mosquito inoculates sporozoites into the human host (Cox, 2010). After sporozoites or the infective stages, are injected they make quick work of invading liver cells (hepatocytes) using the apical organelles (Garnham, 1988).

In most cases, relatively few sporozoites are injected (approximately 8-15), but up to 100 may be introduced in some instances, after injection, they enter the circulation, either directly or via lymph channels (approximately 20%), and rapidly target the hepatic parenchyma cells (Garnham, 1988). Within 45 min of the bite, all sporozoites have either entered the hepatocytes or have been cleared. Each sporozoite bores into the hepatocyte and there begins a phase of asexual reproduction. This stage lasts on average between 5.5 for *P. falciparum* and 15 days for *P. malariae* before the hepatic schizont ruptures to release merozoites into the blood stream (Garnham, 1988; Moriya *et al*., 2009).

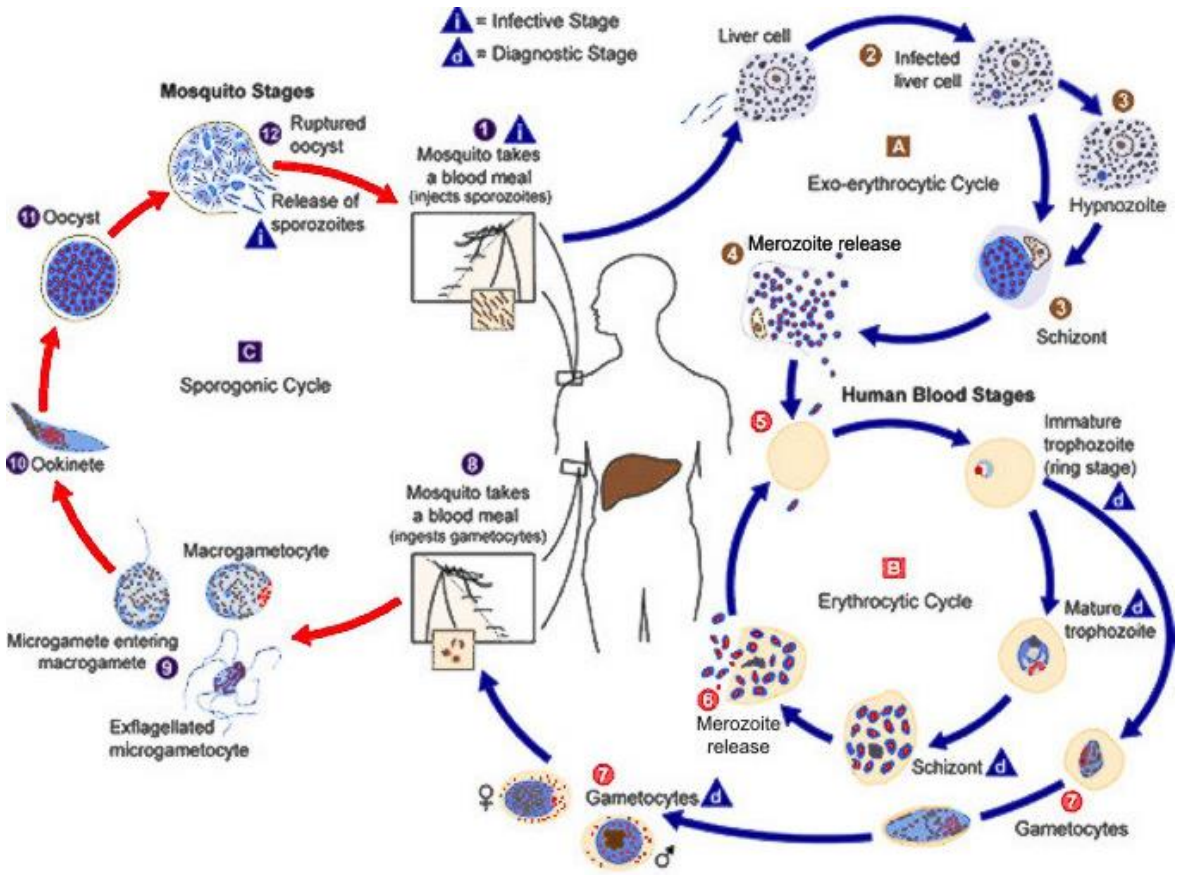

Fig. 1 Life cycle of Plasmodium species.

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

The infective stages, which are injected by a mosquito are carried around the body until they invade liver hepatocytes where they undergo a phase of asexual multiplication (exoerythrocytic schizogony) resulting in the production of many uninucleate merozoites (Bannister *et al*., 2001). At the end of the hepatic stage of development, a single sporozoite can develop into a schizont that contains thousands of daughter parasites that fill the hepatocyte. Infected hepatocytes burst and release numerous merozoites into the bloodstream. *P. falciparum* can complete this liver stage within 7 days and each of its sporozoites produces about 40,000 daughter parasites (Moriya *et al*., 2009).

For *P. vivax*, these values are 6-8 days and 10,000 merozoites; for *P. malariae*, 12-16 days and 2000 merozoites; and for *P. ovale*, 9 days and 15,000 merozoites. In the case of *P. vivax* and *P. ovale*, some sporozoites transform to the dormant hypnozoite, remaining viable for up to 50 years (Bannister *et al*., 2001). This stage is responsible for relapses when it re-enters its

developmental cycle. Inside the host's liver cell it undergoes asexual replication. Next stage of development, called the erythrocytic or blood stage, is initiated when exo-erythrocytic merozoites from the liver invade red blood cells (RBCs) (James *et al*., 2004).

Merozoites of *P. falciparum* can infect RBCs of all ages, whereas those of *P. vivax* and *P. ovale* infect reticulocytes and those of *P. malariae* invade only older RBCs (James *et al*., 2004; Moriya *et al*., 2009). Shortly after merozoites are released from hepatocytes, they invade RBCs and over a period of 2 or 3 days, develop asexually. The time from invasion to exit varies with species, 48 hrs for *P. falciparum* and *P. vivax* and 72 h or *P.malariae* and *P. ovale*, the synchronous release of merozoites coinciding with fever peaks (Bannister *et al*., 2009). The stages of asexual development include the ring (early trophozoite), trophozoite and schizont stages (James *et al*., 2004; Moriya *et al*., 2009).

At maturation, the schizont bursts and releases merozoites into the blood circulation. Most of the released merozoites re-invade a new erythrocyte, thereby repeating their asexual life cycle (blood stage cycle). In some instances, however, invasion of an erythrocyte by a merozoite initiates sexual development instead of asexual development (James *et al*., 2004; Moriya *et al*., 2009). Thus, merozoites may develop into male gametocytes (microgametocytes) or female gametocytes (macrogametocytes).

These gametocytes can develop further only when they are taken up by an appropriate species of *Anopheles* mosquito during a blood meal. They subsequently mate within the gut of the mosquito, the definitive host (Cox, 2010). The parasites eventually become sporozoites, which reach the salivary gland of the mosquito. With the next bite, the infected mosquito releases sporozoites into the host, thereby completing the life cycle (Bannister *et al*., 2001).

The life cycle's continuation now depends on gametocytes being taken into the gut of a feeding female mosquito where both types of gametocyte escape from their host cells. Male gametocytes divide rapidly into a number of motile flagellated micro-gametes each of which can fertilize a female macrogamete to form a zygote (Bannister *et al*., 2009). The parasite then becomes a motile ookinete, penetrating the mosquito gut wall and encysting as a rounded oocyst. The parasite multiplies asexually within this to form many hundreds of motile sporozoites (sporogony). Mature sporozoites escape through the oocytst wall into the insect's blood cavity (haemocoel) and thence to the salivary glands, penetrating their walls to reach the mosquito's

stored saliva in readiness for transmission to a vertebrate at another blood meal (Bannister *et al*., 2009).

2.4 Pathologies and pathogenesis

Severe and life-threatening complications due to *P. vivax* may be due to the change in the clinical spectrum of disease, increase in resistance, indiscriminate use of anti-malarial drugs, delayed treatment, or missing the primaquine therapy (Alexandre *et al*., 2010; Kute *et al*., 2011; Kumar and Ghildiyal, 2014).

All age groups suffer *P. vivax* infections and endure repeated, incapacitating febrile attacks, and develop clinical complications very similar to those observed in *P. falciparum* severe disease. such as jaundice, acute renal failure, shock and coma, severe anemia, and respiratory distress, cerebral malaria, dysfunction of different organs, hypoglycemia, jaundice, thrombocytopenia, renal impairment, hepatic dysfunction, acute kidney injury and hypotension with poor outcomes in pregnancy and learning impairment in children (Alexandre *et al*., 2010; Lacerda *et al*., 2012; Ric *et al*., 2014; Dasgupta *et al*., 2015). How *P. vivax* does this is a very important question, but the lack of understanding of pathogenesis should not diminish the importance of the core implication found in the sum of these many findings: *P. vivax* regularly kills people, and probably in very substantial numbers (Gething *et al.*, 2011).

Malaria in pregnancy is associated with increased risks of maternal anemia, hypoglycaemia, ARDS, spontaneous abortion, premature delivery and other adverse effects on health, placental parasitaemia is associated with low birth-weight infants, the risk of severe disease extends into the immediate postpartum period (WHO, 2000).

Key features of malaria are the adherence of infected red blood cells to the endothelium of small blood vessels compromising blood flow through tissues, and the production of pro-inflammatory cytokines (Parise and Lewis, 2005). Factors that determine whether a patient develops mild or severe disease are complex and multi-factorial and are related to both the parasite and the host. Parasites causing severe malaria have a greater multiplication potential than those causing uncomplicated infections (Parise and Lewis, 2005).

Compared with *P. falciparum*, *P. vivax* has a slightly longer incubation period and produces fewer merozoites per schizont (Ric *et al*., 2014). It is generally believed that *P. vivax* merozoites

require a single cell receptor, the Duffy antigen, to invade host erythrocytes (Ketema and Bacha, 2013). Humans lacking this antigen are not susceptible to infection, explaining why *P. vivax* is largely absent from West Africa, a highly malarious region where the Duffy negative blood group is ubiquitous (Ric *et al*., 2014).

With *P. vivax* malaria, the pyrogenic threshold, the level of parasitemia at which *P. vivax* causes fever is known to be lower than that of *P. falciparum* malaria because of this, the inflammatory response to *P. vivax* has been hypothesized to be of greater magnitude than that to *P. falciparum*, with plasma levels of the fever-inducing cytokine tumor necrosis factor (TNF)-α being higher in *P. vivax* than in *P. falciparum* malaria (Nicholas *et al*., 2009; Ric *et al*., 2014).

P. vivax preferentially infects young RBCs, parasitemia rarely exceed 2% of circulating RBCs, and high parasite burdens are not a feature of severe disease (WHO, 2000; WHO, 2014; Picot, 2006). *P. vivax* can cause both sequestration-related complications such as cerebral malaria, renal dysfunction, hepatic dysfunction, and ARDS and non sequestration-related complications such as anemia and thrombocytopenia (Kumar and Ghildiyal, 2014).

In contrast *P. vivax*-infected red blood cells become increasingly more deformable as they mature and are usually considered not to cytoadhere or sequester in the microvasculature (Ric *et al*., 2014). These characteristics underlie the reason why severe pathology in vivax infections is much less common than with *P. falciparum* infection. Interestingly recent studies have challenged this paradigm by suggesting that *P. vivax*-infected red cells may sequester in organs such as the lung (Nicholas *et al*., 2009; Ric *et al*., 2014).

As Price *et al*. (2007) due to *P. vivax* infection severe malaria results from a combination of parasite-specific factors, such as adhesion and sequestration in the vasculature and the release of bio-active molecules, together with host inflammatory responses (Price *et al*., 2007). Some cytokines and *vivax*-specific "malaria toxins" are believed to cause greater organ-specific inflammation, increased alveolar-capillary membrane permeability, capillary leakage, and leukocyte aggregation (Anesty *et al*., 2007; Andrade *et al*., 2010).

2.5 Severe malaria and associated morbidity and mortality Due to P. vivax

Severe malaria by definition is associated with a high mortality, from a clinical perspective, there is a continuum from asymptomatic malaria to uncomplicated illness through to severe and lethal

malaria (Murray *et al.,* 2012). Almost all severe forms and deaths from malaria are caused by *P. falciparum*. With an implementation of molecular diagnosis, it has become evident that *P. vivax* mono infection could also result in multiorgan dysfunction as well as severe life threatening and fatal disease both in adults and in children as seen in *P. falciparum* infection (Anstey *et al*. 2012; Patil *et al*., 2015).

It was previously presumed that the severe disease with vivax infection is actually caused by co infection of vivax and falciparum species (Picot, 2006; Murray *et al.,* 2012). However with application of the recently developed tests of malarial antigen and the nucleic acid amplification technique it has become evident that vivax mono infection can be a cause of severe malaria and death. PCR test which is used mainly for the academic purpose can also differentiate between vivax mono-infection and falciparum infection (Picot, 2006; Murray *et al.,* 2012).

In 1990, the World Health Organization (WHO) established criteria for severe malaria in order to assist future clinical and epidemiological studies (WHO, 1990). In 2000, the WHO revised these criteria to include other clinical manifestations and laboratory values that portend a poor prognosis based on clinical experience in semi-immune patients (WHO, 2000). The major complications of severe malaria can develop rapidly and progress to death within hours or days (WHO, 2000).

There are many risk factors that are related to severe malaria and death include age greater than 65 years, female sex (especially when associated with pregnancy), paediatric patients especially children under 5 years, non-immune status, coexisting medical conditions, no anti-malarial prophylaxis, delay in treatment, and severity of the illness at admission (Rodriguez-Morales *et al.,* 2015).

2.6. Challenge towards elimination of P. vivax Malaria

As Carlton *et al.* (2011) noted *P. vivax* malaria control program require early diagnosis combined with highly efficacious treatment to cure dormant liver and blood-stage parasites. In addition, disruption of mosquito bite transmission is key, preferably through sustainable community-based vector control programs and routine use of drugs that target the mosquito transmission (‘‘gametocyte’’) stages found in human blood. However, case detection is inherently more challenging for *P. vivax* malaria due to two of its biological properties (Carlton *et al.*, 2011). First, the parasite’s strong preference to infect the minor population of reticulocytes (immature

red blood cells) in the bloodstream results in significantly lower parasitemia (WHO, 2014). In many endemic regions *P. vivax* is often overlooked in co-infections with *P. falciparum*, and newly available rapid diagnostic tests have sub-optimal sensitivity for *P. vivax* (Carlton *et al.*, 2011). Carlton *et al.* (2011) explained the second and more significant problem stems from invisible dormant liver stages that can give rise to multiple periodic ‘‘relapse infections’’ up to several years after an infectious mosquito bite (Carlton *et al.*, 2011).

The unpredictable nature of relapse infections, which can vary from as short as three weeks for tropical strains to five years for strains circulating in temperate climates, further complicates elimination programs since gametocytes typically appear at the earliest onset of clinical symptoms, allowing transmission of *P. vivax* before treatment can be initiated (Battle *et al*., 2014, White, 2008). *P. vivax* control may become even more difficult in coming years as there is increasing prevalence of clinically defined chloroquine-resistant *P. vivax*, for which little monitoring is possible without in vitro culture or a genetic marker for resistance (Price *et al.*, 2009; Carlton *et al.*, 2011)

3. Materials and Methods

3.1 Description of the study area

The study was conducted in Mendi Town, Oromiyia Regional State, Western Ethiopia, found at 567 km from Addis Ababa. Geographically the study site is located at a latitude and longitude of 9°48′N35°6′E9.800°N 35.100°E coordinates: 9°48′N35°6′E9.800°N 35.100°E and the mean elevation of 1538 ranged from 1500 - 1730 meter above sea level and annual rainfall ranged 900-1500 mm. The main rainy season is from May to October. The mean annual maximum temperature of the area is about 32^{o}C ranged 25-32^{o}C(unpublished data of district Agriculure) (Figure 2).

Mendi is one of the districts where altitude and climatic factors such as, rainfall that provide suitable moisture medium for aquatic stage of mosquito life cycle and the mean annual temperature which affects directly the developmental period in the life cycle of mosquitoes. Thus malaria outbreaks are some of the most serious major public health emergencies of the study site (unpublished data) and the two most important cause of malaria in the study area during the study period were *P. falciparum* and *P. vivax* accounted for 59.3% and 37.2% respectively. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia, this town has a total population of 45700 of women 24400 are males and 21300 females (Mendi district Unpublished data).

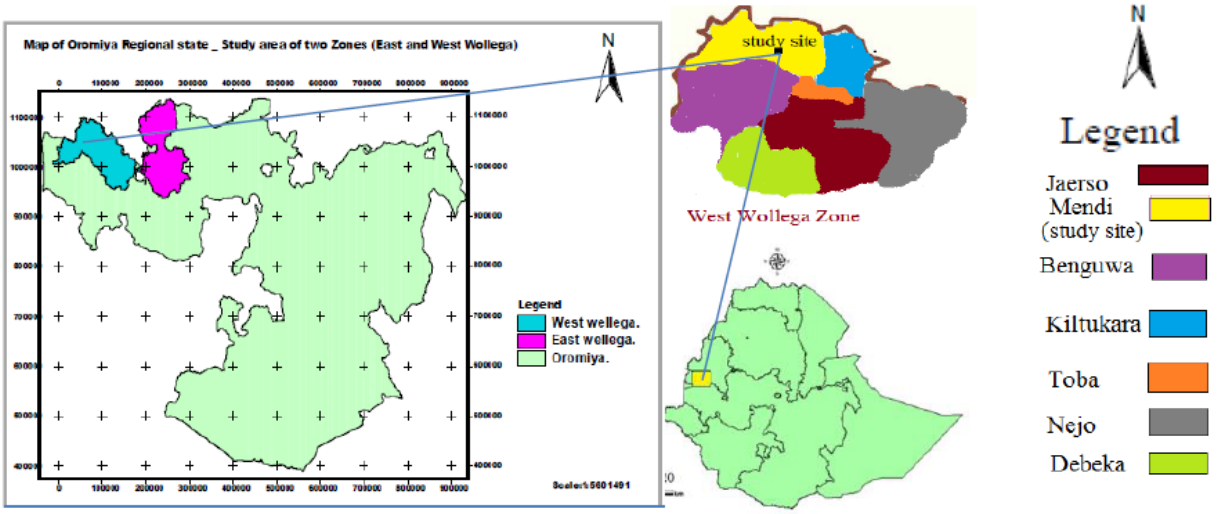

Figure.2. Map of Study site (Fita, 2014).

3.2 Study population

The study participants were all patients seeking medication at the health facilities in Mendi town, during the study period, from September, 2014 to June, 2015 and those who had symptoms of malaria infection.

The inclusion criteria: were any patient with *P. vivax* mono-infection, without any chronic illness or not admitted to Tb or ART clinic, without prior medication, having clinical symptoms like fever, chills, malaise, headache, vomiting, and history of fever for about 48 hours before admission, and volunteer to participate in the study (agreed to sign assent and consent) .

The exclusion criteria: were any patient with mixed infection, with any chronic illness or admitted to Tb or ART clinic, who took prior medication, not volunteer to participate in the study (disagreed to sign assent and consent) .

3.3 Sample size determination

Sample sizes was estimated by 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, $no = \underline{Z^2\alpha/2\ PQ}$

d^2

n= sample size

d= margin of error (0.05)

N= population size = 45700

P= proportion of population P=0.5

α= level of significance

Q=1-P Where: d=0.05, estimated prevalence P=0.5, α=0.05

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

3.4 Data collection instrument

Clinical and demographic data of the study participants were recorded on pre-designed case record form by trained health professionals working at the two health facilities.

3.4.1 Clinical assessment

Body temperature of each patient was measured using digital thermometer; clinical symptoms such as fever, headache, diarrhea, hyperpyrexia, hemoglobunria, persistent vomiting, impaired consciousness, convulsion, respiratory distress, persistent vomiting, hypotension, anorexia, nausea, rigor and others were assessed (WHO, 2010).]Patients with at least one or more symptoms of severe malaria complications set by WHO were classified as severe *P. vivax* cases..

3.4.2 Laboratory diagnosis of malaria

3.4.2.1 Parasite Detection and Quantification

Data from laboratory tests were collected by experienced laboratory technicians as follows:

Thick and thin film preparation : Pre-cleaned slides were labeled, finger selected to be punctured, the area to be punctured were cleaned with 70% alcohol, the ball of the finger were punctured, the first drop of blood were wiped away with clean gauze, Next drop of blood were placed on a clean glass slide, smear were allowed to air dry and stained with 10% Giemsa (pH= 7.2, for 10 minutes), only thin smear was fixed with absolute methanol prior to gimsa stain

immersion oil Applied and were examined using 100x oil immersion objective microscope after allowed to air dry.

Presence of plasmodium parasite were checked using thick film but identification of plasmodium species were done using thin blood film. Malaria parasites were identified by observation of the smears and the morphological appearance of the parasite in the infected RBC under oil immersion objective. Parasite load was calculated using thick film after counting asexual parasites per 200 white blood cells (WBC), assuming mean WBC count is 8,000/µL, using the following formula: (Cheesbrough, 2005).

$$\text{Parasite load } /\mu L = \frac{\text{Number of observed asexual parasites x 8000 WBC count}/\mu L}{200 \text{WBCs}}$$

The degree of parasitaemia was graded as mild, moderate, and severe, when a count is between 1–999 parasite/µl, 1000-9999/µL, >10000/µL, respectively, following method described by Cheesbrough (2005).

3.4.2.2 Blood glucose concentration measurement

From the same pricked finger, few drops of blood samples was taken for measurement of blood glucose concentration (Glu) using handheld portable blood glucose analyzer Senso Card Hungary, accordingly blood sample were taken in the kit reagent zone and inserted in the glucose analyzer and the output was taken from the screen.

Hypoglycemia was considered when blood glucose concentration is < 40 mg/dL (WHO, 2014).

3.4.2.3 Hemoglobin level measurement

Hemoglobin (Hb) concentration were measured using hemoglobin analyzer Hemocue™ (haemoglobinometer, Angelholm, Sweden, Hb 301).

From the same pricked finger drop of blood was taken using microcuvete and feed in the analyzer and the output was read from the screen.

Degree of anemia in children were considered as severe, moderate and mild, when Hb concentration <5 g/dL, between 5 and 8 g/dL, and between 8 and 11 g/dL respectively Adult anemic cases were considered severe anemia <7g/dl, moderate anemia between 7 and 9 g/dl but

mild anemia were considered for male Hb level <13g/dL and for female <12g/dL following the WHO (2010) anemia classification for severe *P. falciparum* malaria.

A trained physician on the study research staff completed a standard clinical evaluation of all malaria symptomatic participants. The local health provider treated all individuals as soon as the blood sample was drawn following national guideline for malaria diagnosis and treatment. Patients infected with *P. vivax* were treated orally with curative doses of chloroquine (FMoH, 2012).

3.5 Data analysis

Data was analyzed using SPSS statistical software (IBM SPSS version 20.0). Descriptive statistical tests were used for analysis of some clinical, demographic and parasitological data. Associations between variables were evaluated using Pearson correlation test and presented using scatter dot. Responses were compared using chi-square test, and odds ratio to describe strength of associations between variables in groups. In all data analysis, significance level 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 study area, Mendi Health center and Clinic prior to data collection. From all study participants, oral and written consent or assent for children <18 years were obtained prior to data collection and for positive cases treatment was given according to malaria diagnosis and treatment guidelines of Ethiopia.

4. Result

4.1 Socio-demographic and clinical characteristics of the study participants

In the current study, a total of 533 patients were clinically diagnosed and admitted as cases of *P. vivax* malaria during the study period but 384 patients who fulfilled the inclusion criteria were selected as a study participants. Prevalence of malaria among males and females patients were 202 (52.6%) and 182 (47.4%) respectively. Among the three age groups, distribution of malaria infection with respect to sex was almost similar, except in those found in age group <5years, where *P. vivax* positive males children were higher than females of the same age (Figure 5). From a total of 101 children, under 5 years, 58 (57.4%) and 43 (42.6%) were males and females respectively. While, among 97 children in age group 5-14 years, 51 (52.6%) were males and 46 (47.4%) were females. But patients in the age group above 14 years were accounted for (n= 186), where males and females show the same frequency.

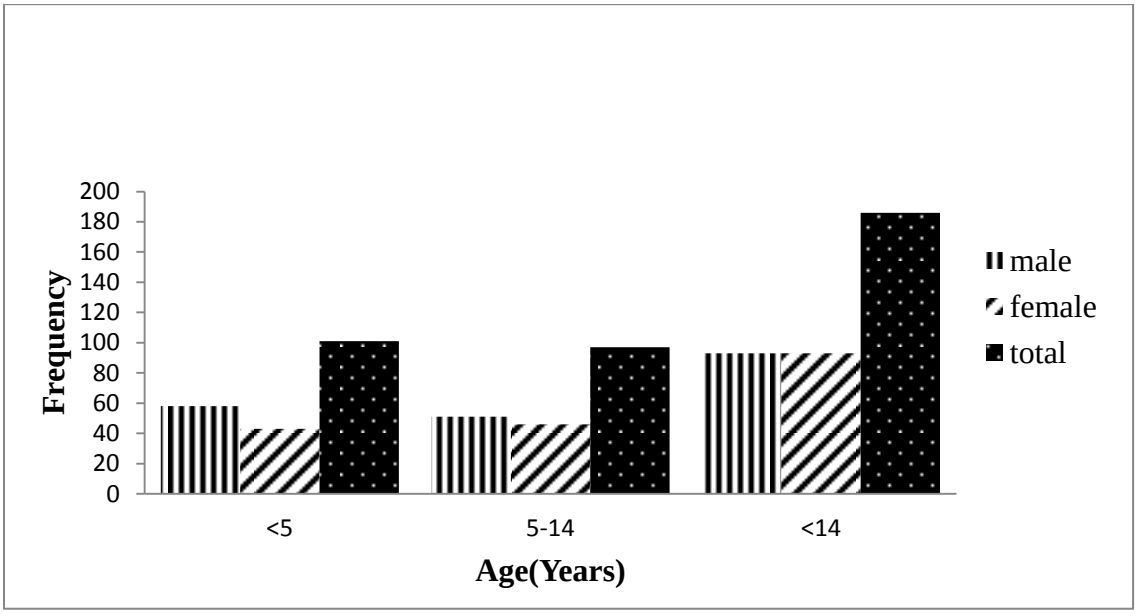

Figure 3 Sex distributions of patients infected with P. vivax in the study site, 2014/15.

4.2. The Current Survey

During the present study period, a total of 14844 (8751 females and 6093 males) patients seeking medication visited both health facilities. Among the 4813 presumptive malaria cases from which blood samples were collected, 1434 (818 males and 616 females) were positive for malaria parasites. A total of 533 (37.2%) and 851 (59.3%) were infected with *P. vivax* and *P. falciparum*,

respectively while, the rest, 50 (3.5%) were due to mixed infection (*P. vivax* and *P. falciparum*). The highest peak season of general malaria cases as well as for *P. falcipharum* was observed in November followed by October but highest peak for *P. vivax* were December (Figure 4).

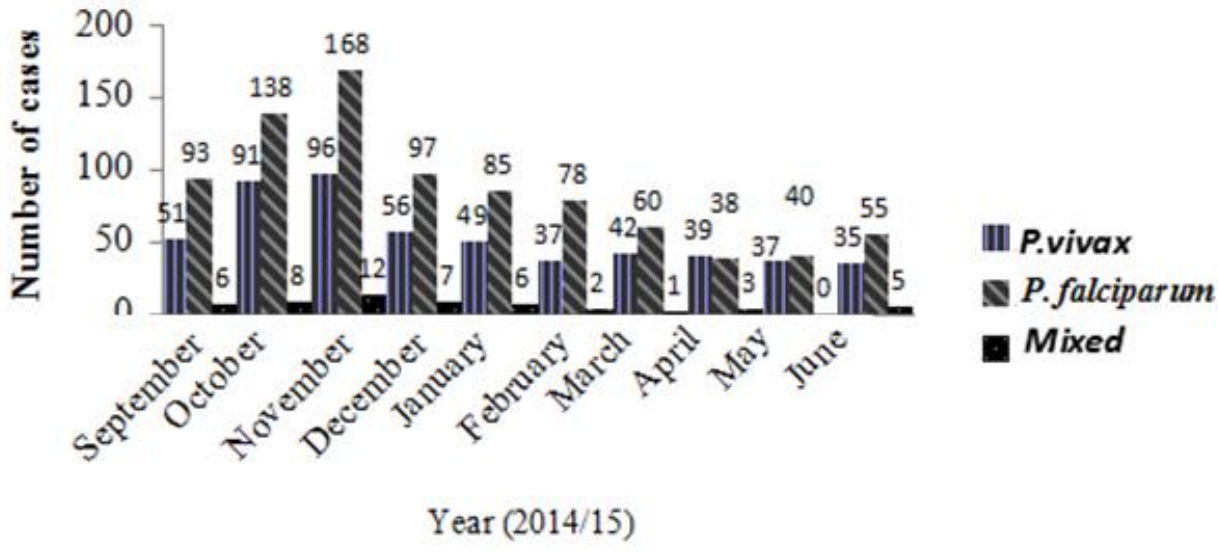

Figure 4 Slide Confirmed Malaria Cases observed during the study period, Mendi town, Oromia, Ethiopia, 2014/15.

4.3. Prevalence of malaria in the study area (Retrospective Data)

Even though the trend of malaria cases showed an irregular declining pattern, the five years prevalence report (2010-2014) from the health facilities of the study sites showed that the number of malaria infected patients were still higher. To mention, in contrast to the study period (2014/15), in the year (2012) among a total of 6028 malaria suspected patients, 2161 (35.8%) slide positive for plasmodium parasite, of these, 1267 (58.6%) were due to *P. falciparum,* and 822 (38.1%) were due to *P. vivax* and the rest were accounted for mixed infection, in addition *P. vivax* malaria cases seems decreasing but from the total malaria burden its prevalent rate was high for instance the prevalence rate of *P. vivax* was 36.4%, 38% and 37.1% in the year 2010, 2012 and 2014 respectively. (Figure 3).

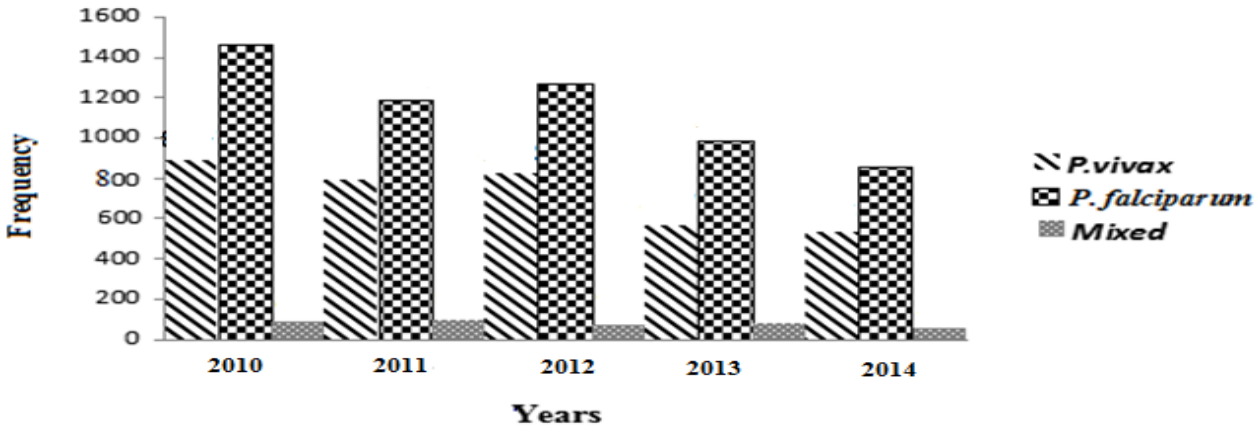

Figure 5 Malaria positive cases documented in the two health facilities during a period from 2010 to 2014

This study showed that the most common age category who were positive for *P. vivax* mono infection were adults >14 years (48.4%, n=186) followed by children <5 years old (29.4%, n=101 and the median age of the study participants was 14 years. Mean body temperature of the study participants was 37.89 ^{o}C (35.5- 40.8). Also levels of Hb and geometric mean parasite count were 12.4 g/dL and 3745 (280-31600) respectively. Anemic cases observed in this study were 115 (29.9 %) (Table 1).

Table 1 Clinical and demographic characteristic of *P. vivax* malaria infected patients, at Mendi town, Ethiopia, 2014/15.

Clinical and demographic features	Frequency (%)
Age (years) Median age • <5 • 5-14 • >14	 14 101 (26.3) 97 (25.26) 186 (48.4)
Sex ▪ Male ▪ Female	 202 (52.6) 182 (47.4)
Mean body Temperature	37.89 (^{o}C)
Mean Hemoglobin level	12.4 (g/dL)
Anemic cases	115 (29.9)
Geometric mean parasite (Parasite count/µL) range	3745 (280-31,600)

In this study, age of the study participants and parasite load showed inverse association. As age of the patients increased the parasite load was significantly reduced ($r = -0.217$, $p < 0.01$) (Figure 6). In the contrary, age and hemoglobin level of patients showed statistically significant positive association ($r=0.536$, $p<0.01$). This was as age increase hemoglobin level was increasing (Figure 7). Unlike, parasite load and hemoglobin, blood glucose level of the participants did not show significant association with age of the study participants ($r = .030$, $p >0.05$) (Figure 8).

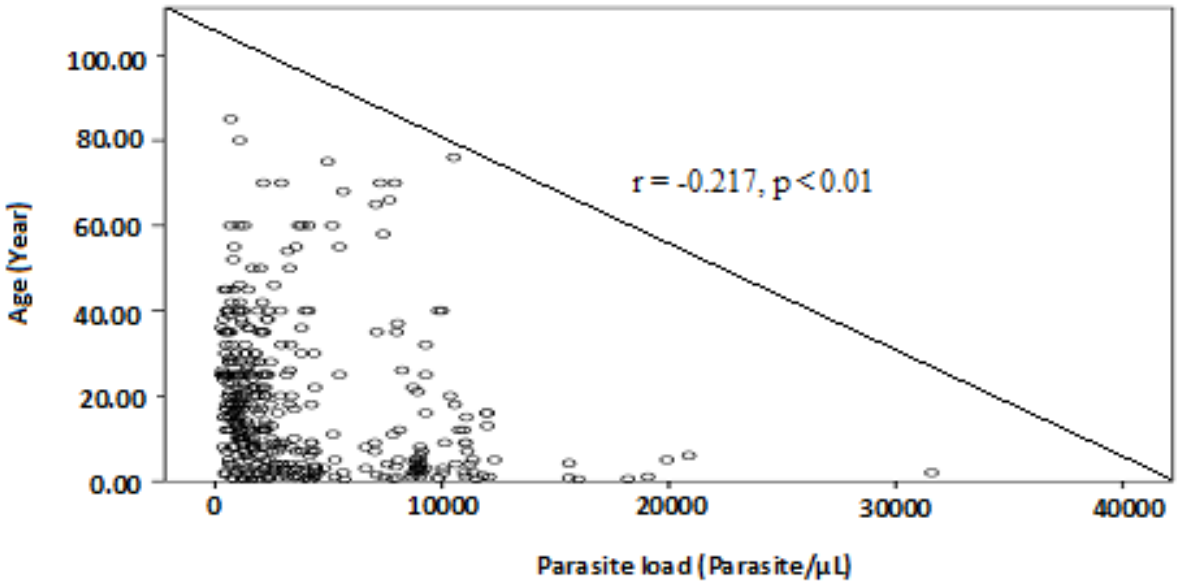

Figure 6 Association between age and parasite counts among patients infected with *P. vivax* at Mendi town, Ethiopia, 2014/15.

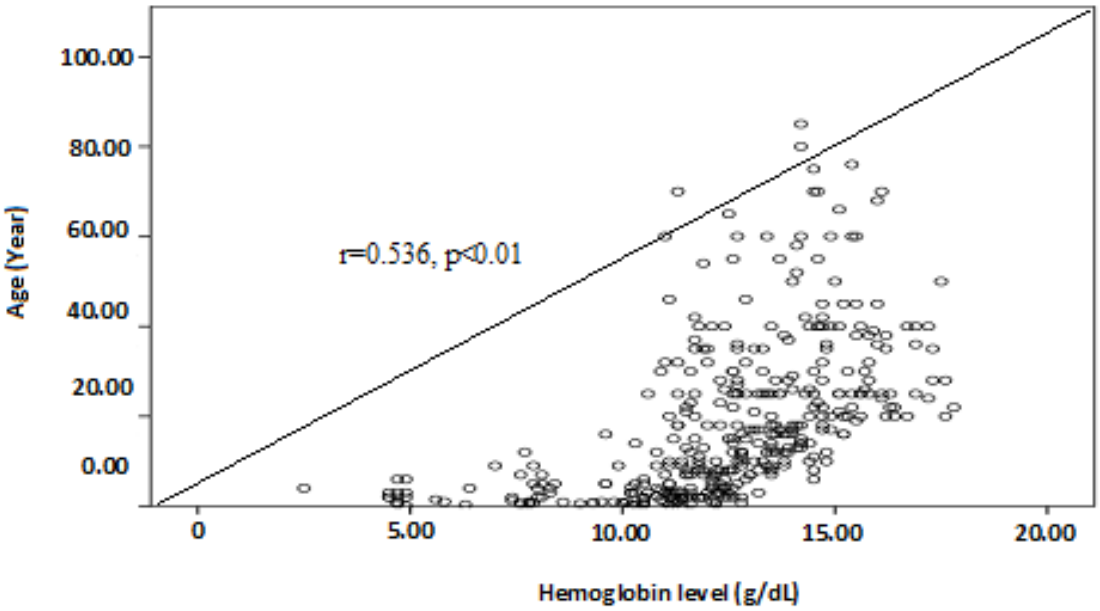

Figure 7 Association between age and hemoglobin level among patients infected with *P. vivax* at Mendi town, Ethiopia, 2014/15.

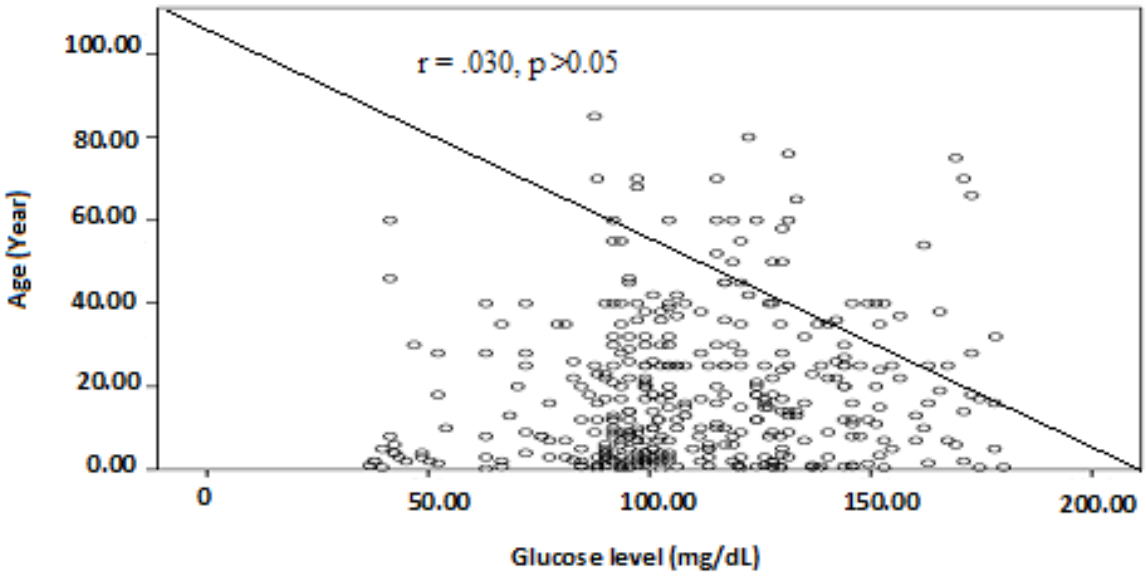

Figure 8 Association between age and glucose concentration among patients infected with *P. vivax*, Mendi town, Ethiopia, 2014/15.

Higher Hb level was observed among adults, while the lowest was recorded in children <5 years. Hemoglobin level <5g/dL, indicator of severe anemia, was observed in none of participants with age > 14 years, but in 2 participants among those in age range from 5 to 14 was detected. In contrary, lowest Hb level, which is <5 and 5-7.9 g/dL were frequently observed among children in age group < 5 years (Figure 9).

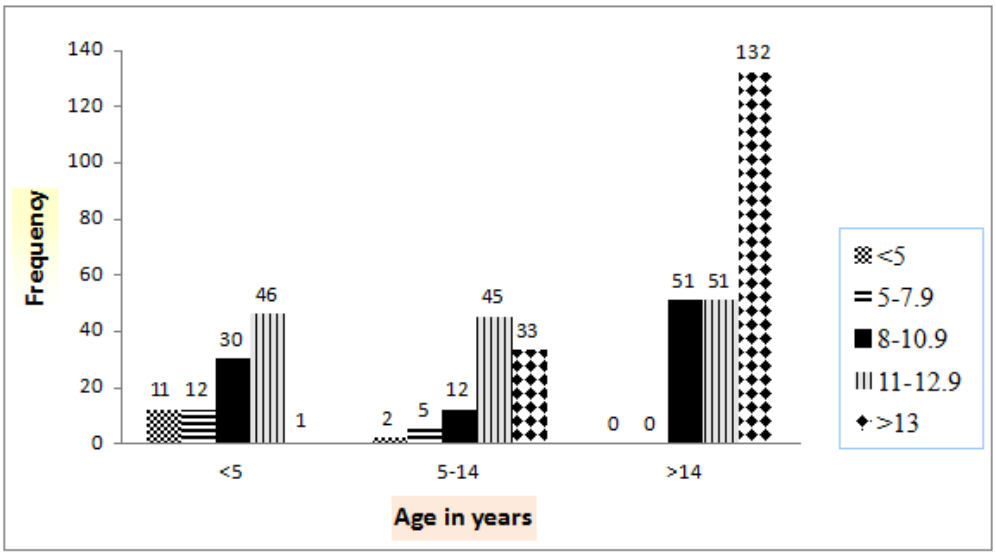

Figure 9. Hb level among different age groups of *P. vivax*, Mendi town, Ethiopia, 2014/15.

Frequencies of uncomplicated malaria symptoms observed were headache 255 (66.4%), chills 225 (58.6%), aching 231 (60.2%), vomiting 179 (46.6%), diarrhea 22 (5.72%), anorexia 61 (15.9%), nausea 147 (38.3%), rigor 152 (39.6%), shivering 151 (39.3%), and cramp 150 (39.1%). At the time of admission, about 179 (46.6%) and 22 (5.7%) of the patients had vomiting, and diarrhea, respectively. Although most patients, 360 (93.8%) had a history of fever for the past 48 hours, only 227 (59.1%) of patients were febrile or had axillary temperature ≥37.5°C (Table 2).

Table 2 Frequency of symptoms of uncomplicated *P. vivax* patients, at Mendi town, Ethiopia, 2014/15.

No	Symptoms of uncomplicated malaria	Frequency (%)
1	Headache	255 (66.4)
3	Chills	225 (58.6)
4	Aching	231 (60.2)
5	Vomiting	179 (46.6)
6	Anorexia	61 (15.9)
7	Nausea	147 (38.3)
8	Rigor	152 (39.6)
9	Shivering	151 (39.3)
10	Cramp	150 (39.1)
11	History of fever for about 48 hours	360 (93.8)
12	Fever at time of enrollment (Febrile) body temperature ≥ 37.5°C	227 (59.1)

4.4 Frequency of severe malaria among *P. vivax* infected patients

Severe malaria symptoms were observed in 55 (14.32) patients infected with *P. vivax* mono-infection. Those fulfilled at least one of the WHO criteria for severe malaria were 65.5% (n= 36), while 34.5% (n=19) had two or more overlapping severity indicators. Of which 63.2% (n=12/19) had two combined symptoms, 26.3% (n=5/19) had three symptoms and the rest 10.5 % (n=2/19) showed four multiple WHO defined sever malaria symptoms.

Some of these syndromes were: prostration n=14 (25.45%), persistent vomiting n= 9 (16.36%), respiratory distress n= 6 (10.9%), hypoglycemia or glucose concentration <40 mg/dL, n= 5 (9.1%), hyperpyrexia or temperature ≥40 °C, n= 8 (14.5%) and severe anemia n= 13 (23.63%). Major severe malaria symptoms observed were prostration, and severe anemia, in combination with other syndromes including persistent vomiting, hyperpyrexia, and hypoglycemia. However, none had signs of confusion, coma, hemoglobinuria or discoloration of urine and hypotension or algid malaria (Table 3).

Table 3 Clinical manifestation and laboratory results of complicated severe malaria of patients (n=55) infected with *P. vivax* at Mandi town, Ethiopia, 2014/15.

No	Clinical symptoms	Frequency (%)
1	Severe anemia	13 (23.64)
2	Respiratory distress	6 (10.91)
3	Prostration	14 (25.45)
4	Hyperpyrexia	8 (14.55)
5	Persistent vomiting	9 (16.36)
6	Hypoglycemia	5 (9.1)
6	Comma	0(0)
7	Convulsion	0(0)
8	Hypotension	0(0)
9	Hemoglobunuria	0(0)

Most severe malaria manifestations were presented in children less than five years. To mention, severe anemia was observed in n=11 patients in under five years, which were significantly higher (P<0.001) in this age groups, while n=2 in those in age range 5-14, but none among adults. The Chi-square test result showed that association between severe anemia, prostration, persistent vomiting and age were significantly different (P<0.05) in children under age 5. These three severe symptoms were higher in children under age 5 years than other age groups (Table 4).

Table 4 Frequency of severe malaria symptoms among different age groups of patients infected with *P. vivax* malaria, at Mandi town, Ethiopia, 2014/15.

Clinical features	Age (year)			P. value
	<5	5-14	>14	
Respiratory distress	3 (5.5%)	2 (3.6%)	(1.8%)	0.287
Hyperpyrexia	5 (9.1%)	1 (1.8%)	2 (3.6%)	0.116
Persistent vomiting	5 (9.1%)	3 (5.5%)	(1.8%)	0.027
Severe anemia	11 (20%)	2 (3.6%)	0 (0)	<0.001
Prostration	8 (14.5%)	4 (7.3%)	2 (3.6%)	0.022
hypoglycemia	3 (5.5%)	2 (3.6%)	0(0)	0.09

From the analysis made on association between parasite load and severe malaria syndromes, it was observed that differences in parasite load did not affect the incidence of respiratory distress, hyperpyrexia, and hypoglycemia. Since significant differences were not observed (P>0.05) with respect to relative risk of *P. vivax* infected patients of different parasite load (between 1000 and 9999, and ≥10,000). However, severe anemia, prostration, and persistent vomiting were significantly associated (P<0.05) with severe parasitemia (>10,000 parasite/µL), (OR=3.8, 95% CI, 1.1-13.7; OR=4.4, 95% CI, 1.4-13.9; and OR=7. 95% CI, 1.8-27.4) respectively (Table 5).

Table 5 Measure of association between parasitemia and severe malaria indicators in patients infected with *P. vivax* malaria, at Mandi town, Ethiopia, 2014/15.

Parasite load	Sever malaria indicators (%)											
	Severe anemia		Respiratory Distress		Prostration		Persistent vomiting		Hyperpyrexia		Hypoglycemia	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
1000-9999	9 (3.4)	258 (96.6)	4 (1.5)	263 (98.5)	10 (3.7)	257 (96.3)	5 (1.9)	262 (98.1)	5 (1.9)	262 (98.1)	4 (1.5)	263 (98.5)
≥10,000	4 (11.8)	30 (88.2)	2 (5.9)	32 (94.1)	4 (12.1)	30 (85.3)	4(11.8)	30 (88.2)	3(8.57)	32 (94.1)	1(2.9)	33(97.1)
Odd Ratio (OR)	OR=3.8 (95% CI, 1.1- 13.2), P=0.034		OR=4.1 (95% CI, 0.72 -23.3),P= 0.11		OR=3.4 (95%CI, 1.4-13.9), P = 0.048)		OR =6.98 (95% CI, 1.8-27.4), P= 0.005		OR =3.3 (95% CI,0.61- 17.6), P=0.17		OR=1.9(95% CI, 0.2 -18.4), P=0.54	

Note: yes = presence of the symptom, No = absence of the symptom

5. DISCUSSION

During the study period, from September, 2014 to June, 2015, the overall prevalence of malaria documented was 29.8%, which was declined by 3.3% from 2012 (Unpublished data, Annual Report on Malaria Prevalence of Mendi district, 2007). The observed prevalence of malaria in this study was comparable to reports of different researchers, but slightly lower than the recent report from southern part of Ethiopia (31.9%) (Ketema and Bacha, 2013), and much lower than Nigeria (81.9%) (Oladeinde *et al*., 2012).

The possible justification for this discrepancy might be associated with the intense and diverse malaria control strategies undertaken in most parts of Ethiopia, such as vector control using utilization of insecticide treated bed net to prevent contact of mosquito with human body, covering exposed skin surfaces and remaining indoors or under a net at peak biting times will obviously reduce exposure (Lindsay, 1988) which are the principal strategy for malaria prevention in areas where sustained vector control is required (WHO, 2002; WHO, 2005).

In the current study the most common age category confirmed to be positive for *P. vivax* mono infection were adults >14 years (48.4%, n=186) followed by children <5 years old (29.4, n=101). This finding was supported by report from Papua, Indonesia where *P. vivax* infection were more pronounced in adults compared to other age groups (Tijitra *et al*., 2008).

In this study the most common uncomplicated non-specific malarial symptoms presented were characterized by fever, chills, headache, aching and chills with other accompanied symptoms such as vomiting, rigor, shivering, cramp, nausea and anorexia with a large number of febrile cases. Compared to the study by Anstey *et al*. (2012) regardless of age and gender in the non-specific symptoms like headache, abdominal pain, fatigue and a non-fever phase (chills) occurs, followed by intermittent fever phase, The periodic fever phase is also known as "paroxysm" that occurs after the rupture of schizont-infected red cells (Anstey *et al*., 2012).

In this study, a total of 384 patients infected with *P. vivax* were recruited based on the set inclusion criteria. Among these severe malaria symptoms were observed in 55 (14.3%) patients, which was almost similar to report from southern Ethiopia (13.67%) and Indian States among hospitalized patients (17.2%) with *P. vivax* malaria (Ketema and Bacha, 2013; Verma *et al*., 2014). Other report of severity observed was in Papua, Indonesia with the risk of severe malaria showed greater among *P.v*ivax (23%) infections (Tjitra *et al*., 2008). Also very recently study conducted in India on *P.*

vivax infected adult patients, it was reported that out of 460 *P. vivax* positive patients, 102 (22.1%) were having severe malaria as per criteria with more cases on male than female 62.75% 37.25% respectively (Patil *et al*., 2015). *P. vivax* mono infection has also been associated with severe and fatal disease in endemic areas, (Tjitra *et al.* 2008; Nurleila *et al.* 2012; Yadav *et al.* 2012). Severe malaria is a potentially fatal condition encountered in all age groups and if not treated timely can cause mortality (Verma *et al*., 2014).

The most common documented severe malaria manifestations of *P.vivax* mono infection in this study were, prostration, followed by severe anemia, persistent vomiting, hyperpyrexia, respiratory distress, and hypoglycemia. Some of these complications such as severe anemia, hypoglycemia, and persistent vomiting among children were reported from the same country (Ketema and Bacha, 2013).

In this study prostration or unable to walk, unable to stand and sit (unable to feed, drink in infants) have been the most common severe complication observed (25.45 %, n=14). This number was much higher than reported in a study conducted in Children (5.8%) admitted in a Tertiary Care Centre of Central India (Verma *et al*., 2014).

Frequency of respiratory distress observed in this study was 10.9 % (n=6). In line to report from India (6.8%), this figure was relatively higher, but comparable with other study conducted among adults living in malaria-endemic areas in Bikaner, Northwestern India, which is 10% of the patients had respiratory distress (Kochar *et al*., 2009; Verma *et al*., 2014). In contrast to this, higher frequency of respiratory distress (74%) was observed in southern Papua, Indonesia in *P. vivax* infected patients from which 83% were children while 58% were adults (Duglas *et al.*, 2014). The wide variations in values might be due to frequent drug resistance by *P. vivax* and endemicity of the parasite in the locality (Tjitra *et al*. 2008).

The mechanisms underlying respiratory distress caused by Plasmodia are not well understood. Red blood cells parasitized by *P. vivax* do not cytoadhere to endothelial cell. Thus, the occurrence of ARDS in benign malaria suggests that lung injury in malaria cases is also determined by causes other than microvascular sequestration of parasitized red blood cells (Kasliwal *et al*., 2009). Since *P. vivax*-infected red cells sequester in organs such as the lung (Nicholas *et al*., 2009; Ric *et al*., 2014). Small airway obstruction, gas exchange alteration, increased phagocytic activity, and

accumulation of pulmonary monocytes is suggested mechanisms for respiratory complications (Anstey *et al.*, 2007).

According to the current study, frequency of hypoglycemia or glucose concentration <40 mg/dL was 9.1% (n=5) much lower than reports from similar study in Colombia (41%) (Echeverri *et al.*, 2003), but comparable with report from South west Ethiopia (10.5%) and Brazil (10%) (Ketema and Bacha, 2013; Siqueira e*t al*. 2015), but, relatively higher than the findings of the three municipalities from Colombia (6.25%) (O'Brien *et al*., 2014). This pathology was believed to occur as a result of parasites derives energy from anaerobic glycolysis of glucose which will yield lactic acid. Increased peripheral requirement of glucose consequent upon anaerobic glycolysis, increased metabolic demands of febrile illness, obligatory demand of parasites, failure of hepatic gluconeogenesis and glycogenolysis (parasites consume about 70 times as much glucose as uninfected cells) (WHO, 2000).

In this particular study the prevalence of persistent vomiting and hyperpyrexia were observed in 2.3% and 2.1% of patients respectively with a slight difference from other similar study reported from south west Ethiopia in which persistent vomiting 1.4% (n = 2), and hyperpyrexia 2.9% (n = 4) of patients were recorded Most of the hyperpyrexia and its associated signs such as vomiting have been largely attributed to production of various cytokines such as TNF-α produced in response to the parasite and toxin products released during rupture of infected RBCs (Clark *et al*., 2006).

Also, hemozoin released from infected RBCs (iRBCs) leading to the release of pro-inflammatory cytokines that in turn induce COX-2 (cyclooxygenase-2) up-regulating prostaglandins leading to the induction of fever (Parroche *et al*., 2007; Schumann, 2007). As there is evidence that there is *rigor* inflammatory reactions due to pro-inflammatory response and cytokines activation during *P. vivax* infection (Andrade *et al*., 2010), the hyperpyrexia and persistent vomiting observed in this study could be accounted to the intense inflammation reaction caused by the parasite and its components (Parroche *et al*., 2007).

The mean Hb concentration measured in this study was 12.6 (2.5-17.8 g/dL). This was relatively higher than reports from Central India 7.5 (2.1-11) in *P. vivax* infected patients (Jain *et al*., 2013). However, about 29.9% of patients were found to be anemic which was higher than the very recent report from Colombia (19.7%) (Arévalo *et al*., 2015), but lower than from southern Ethiopia (43%)

(Ketema and Bacha, 2013), India in children (34%) (Singh e*t al*., 2012), and among adults (30.5% and 32%) (Naha *et al*., 2012; Jain *et al*., 2013).

The impact of *P. vivax* infection on Hb concentration varies from negligible to dramatic (Genton *et al*., 2008; Kochar *et al*., 2009). That leads to severe anemia, one of the most important manifestations of severe malaria in areas of high stable transmission and occurs predominantly in children. Severe anemia is the major complication associated with *P. vivax* infection, accounting for 87% of severe disease compared to 73% of severe manifestations with *P. falciparum* (Tjitra *et al*., 2008). Similarly, significant number of severe anemia patients were observed among children in age group <5 years (Ketema and Bacha, 2013).

In agreement to our findings, a study by Andrade *et al.* (2010) in Brazil, patients with severe anemia due to *P. vivax* malaria were younger, had lived in the endemic area for shorter time and had less previous episodes of malaria. In comparison of the report from Venezuela (58.8 %) and India (31.8%) in children (Rodriguez-Morales *et al*., 2006; Jain *et al*., 2013), severe anemia observed in the current study was much lower, but higher than report from Eastern Sudan (7.2%) (Abdallah *et al.,* 2013). In Latin America, anemia generates an important burden, being the most frequent complication of severe malaria in Brazil, causing a significant increase in morbidity and mortality especially in children and in pregnant women (Costa *et al.*, 2012).

Although there is no exact information on mechanism of severe anemia associated with *P. vivax* malaria, its pathogenesis is multi factorial. As this parasite has selective preference to some red blood cells type, hemolysis it will cause cannot be significant to cause anemia. On the other hand, the low parasite biomass of *P. vivax* indicates that severe anemia is not due to destruction of infected RBCs alone (WHO, 2014). Studies have shown that, for every infected RBC destroyed during *P. vivax* infection, 32 non-infected RBCs are removed from the circulation, compared to the loss of 8 RBCs for every infected erythrocyte in *falciparum* malaria (Anesty *et al*., 2009).

Rather, anemia might occur as a result of rigor inflammatory reactions due to pro-inflammatory response and cytokines activation (Andrade *et al*., 2010) and phagocytosis of non-parasitized red blood cells, increased splenic clearance, and dyserythropoiesis in bone marrow (Jain *et al*., 2013). In addition, *P. vivax* infected RBCs show greater deformability while passing the endothelial slits of the splenic sinus and other reticulo-endothelial organs and the mature RBCs infected with *P.*

vivax avoid splenic clearance. Hence uninfected RBCs which show increased fragility are removed (Siqueira *et al*., 2015).

Naturally *P. vivax* causes an acute febrile illness with no complications or death, however, in recent years complications due to *P. vivax* are being increasingly reported from different parts of the world (Lacerda *et al*., 2012) or many cases of severe *P. vivax* malaria were seen and some cases resulted in death (Tjitra *et al*. 2008). However, its exact causes of changes in the clinical profile are uncertain. There may be due to genetic alterations of the parasite or change in vector and its biting habits, indiscriminate use of anti-malarial drugs, delayed treatment, or missing the primaquine therapy (Alexandre *et al*., 2010; Kute *et al*., 2011), or due to declining efficacy of chloroquine or a high prevalence of chloroquine- resistant *P. vivax* (Ratcliff *et al*., 2007).

A study from Venezuela assessing morbidity and mortality from malaria reported that among a total of 407 deaths, the most affected age group (28.3%), was patients under 10 years old, followed by 15.7% in the group of 20–29 years (Rodriguez-Morales *et al.* 2008). Likewise, the highest prevalence of severe malaria complications (63.6%, n=35/55) observed in the current study was in children age < 5 years (White *et al*., 2014).

In this study differences in parasite load did not affect some of the incidence of severe malaria symptoms among patients assessed. This was consistent to report of Price *et al*. (2009), which explained *P. vivax* is capable of inducing fever at levels of parasitemia lower than those causing fever in *P. falciparum* infection (Price *et al*., 2009). Also WHO reported in western Thailand, a region of low endemicity, the pyrogenic density for *P. vivax* was 180 parasites/μL compared to 1000 parasites/μL observed in *P. falciparum* infection.

In addition, patients with *P. vivax* infections also tend to present with all parasite stages visible on the peripheral blood film (WHO, 2000; WHO, 2014). However, although difference in age did not affect the occurrence of severe malaria infection, severe anemia was prevalent in children <5 years. Different studies also concluded that *P. vivax* has immense potential to cause life threatening complications (Rizvi *et al*., 2013). Especially countries endemic for both major *Plasmodium* species, *P. vivax* infection can account for up to 38% of patients hospitalized with malaria (Carrara *et al*. 2006; Tjitra *et al*. 2008). In Indonesia, Papua, *P. vivax* accounted for 24% of malaria admissions in all age groups, and 47% of infants, the need for hospitalization indicates significant morbidity and at least moderately severe disease (Tjitra *et al.* 2008; Anstey *et al*. 2012).

6. Conclusions and Recommendations

6.1. Conclusions

Although prevalence of malaria seems decreasing due to intensive intervention and combined strategies approaches in the country, clinical data showed that, malaria is still a major health problem in the study area.

Severe malaria complications associated with *P. vivax* such as prostration, severe anemia, respiratory distress, hyperpyrexia and persistent vomiting were observed in some of the study participants. *P.vivax* associated severe malaria complications observed among the study participants, could strengthen the fact that this parasite will no longer considered mild rather it is virulent.

6.2. Recommendations

- Further large scale studies are required to know the exact pathogenesis of complications of *P. vivax* malaria. Also there should be an urgent need of public health measures to estimate the burden of *P. vivax* malaria so that adequate planning and control measures can be taken against this emerging problem. 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.
- Every effort to reduce or eliminate the malaria burden must also target *P. vivax* along with *P. falciparum* in regions where both species coexist. In addition, to meet international targets for reduction of malarial illness and death as well as regional elimination, the malariologists and control communities should no longer afford to neglect the impact of *P. vivax*.

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8. Annex

8.1. Case record form

Case Record for Clinical and demographic data on sever Malaria related to *P. vivax*

CODE	AGE	SEX	Pf/pv	Pre -	Persiste	tempera	par.	Hyper	diarrhe	prostrat	Convuls	comma	hemoglo	Respir.	hypoten	Hb.	Hypogly	black	Epistaxi	cramp	shiverin g	rigor	nausea	anorexi	Aching	fever	Vomitin	Chill	headach

Data collected by Name _____sign_____ Date_____

8.2 written consent form

Code Number ----------

Name of study participant ----------Age---------- Sex----------

Physician Name----------Site /Health center----------

I was informed about a study that plans to investigate the malaria transmission in Mendi town entitled " severe malaria symptoms associated to *plasmodium vivax* among patients visiting health facilities in mendi town" which is believed to provide substantial information on the level of current situation of malaria in Mendi ,western Ethiopia.

For the study I was requested to give a drop of blood from finger prick or intravenously. by experienced health professional according to the established aseptic procedure of the blood collection, the investigator informed me that if I want all the laboratory results will be kept confidential, in addition to this, they gave me enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation I agreed to give enough blood for the investigation

Name (participant) ----------Signature ----------Date ----------

Name (investigator) ----------Signature ----------Date ----------

8.3. Assent Form

Code number ----------

Name of study participant child ----------Age---------- Sex----------

Parent Name ----------Age ----------Sex ----------

Physician Name---------- Site /Health center----------

I was informed about a study that plans to investigate the malaria transmission in Mendi town entitled " severe malaria symptoms associated to *plasmodium vivax* among patients visiting health facilities in mendi town" which is believed to provide substantial information on the level of current situation of malaria in Mendi ,western Ethiopia.

The study was involved in children 1-18 years old, For the study I was requested to give a drop of blood from my child finger prick or intravenously by experienced health professional according to the established aseptic procedure of the blood collection and the investigator also informed me that if I want all the laboratory results will be kept confidential, in addition to this, they gave me enough time to think over before I signed this informed Assent. It is therefore, with full understanding of the situation I agreed to give enough blood for the investigation

Parent Name (participant child parent) ----------Signature ----------Date -------

Name (investigator) ----------Signature ----------Date ----------

8. 4. Waadaa waliigaltee (Consent)

Koodii _____

Maqaahirmaataaqorannichaa_____Umurii_____ Saala_____

Maqaa ogeessa fayyaa _____ Maqaa Buufata Fayyaa _____

Severe malaria symptoms associated to *plasmodium vivax* among patients visiting health facilities in mendi town," mataduree jedhu irratti yeroo ammaa Godina Wallaggaa Lixaa, Magaalaa mandiitti babal'ina dhukkuba busaa jiru baruuf qorannoo adeemsifamuuf gahee koo ba'uuf, odeeffannoo gahaan naaf kenname irratti hundaa'uun, qorannoo kana milkeessuuf gahee narraa eegamu ba'uuf akkaataa mootummaa Rippaapilikaa Dimookiraatawaa Federaalawwaa Itoophiyaatti, Ministeerri Eegumsa Fayyaa, kenniinsa dhiigaa ilaalchiseeseera (danbii) baasee jiruun nama muuxannoo ogummaa fayyaa qabuun dhiiga koo kennuuf yeroon gaafatamutti qorannichaaf hangan gaafatametti dhiiga koo kennuuf waadaa galeera.

Sakatta'a booda firiin dhiiga kootii argames iccitiin akkanaaf eegamuuf waadaa akka naaf galtan ta'uufi qorannichaaf dhiiga koo kennuun dura, akkanitti yaaduuf yeroo dheeraa akkanaaf kennitan gaafachaa, irratti hirmaachuuf yaada koo guutuun waadaa galeera.

Maqaa Hirmaataa_____ Mallattoo_____ Guyyaa_____

8.5. Waadaa waliigaltee (Assent)

Koodii _____

Maqaa mucaa hirmaataa qorannichaa_____Umurii_____ Saala_____

Maqaa maatii_____ Umurii_____ Saala_____

Maqaa ogeessa fayyaa _____ Maqaa Buufata Fayyaa _____

severe malaria symptoms associated to *plasmodium vivax* among patients visiting health facilities in mendi town,," mataduree jedhu irratti yeroo ammaa GodinaWallaggaa Lixaa, Magaalaa mandiitti babal'ina dhukkuba busaa jiru baruuf qorannoo adeemsifamuuf gahee koo ba'uuf, odeeffannoo gahaan naaf kenname irratti hundaa'uun, qorannoo kana milkeessuuf gahee narraa eegamu ba'uuf akkaataa mootummaa Rippaabilikaa Dimookiraatawaa Federaalawwaa Itoophiyaatti, Ministeerri Eegumsa Fayyaa, kenniinsa dhiigaa ilaalchisee seera (danbii) baasee jiruun nama muuxannoo ogummaa fayyaa qabuun dhiigni mucaa kootii akka fuudhamuuf yeroon gaafatamutti qorannichaaf hanga barbaachisetti dhiiga dhiigni mucaa kootii akka fuudhamuuf waadaa galeera.

Qorannoo kana keessatti kan hirmaatan ijoollota umuriin isaanii waggaa 18n gadii akka ta'e hubadhee fedha kootiin dhiiga mucaa kootii yeroon kennuuf waadaa galu, sakatta'a kana booda firiin dhiiga mucaa kootii argamees iccitiin akka naaf eegamuuf waadaa kannaaf galtan ta'uufi qorannichaaf dhiiga mucaa kootii kennuun dura, akkanitti yaaduuf yeroo dheeraa akkanaaf kennitan gaafacha, mucaa koo hirmaachuuf yaada koo guutuun waadaa galeera.

Maqaa maatii mucaa/guddisaa hirmaachisee_____Mallattoo_____Guyyaa_____

Mallattoo_____Guyyaa_____