Jimma University,

College of Public Health and Medical Sciences,

Department of Medical Laboratory Sciences and Pathology

Prevalence of Asymptomatic Plasmodium Infection and Associated Risk Factors among Pregnant Women in Arba Minch Zuria District, Southern Ethiopia: A Community Based Cross-Sectional Survey

By

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A Thesis Submitted to Department of Medical Laboratory Sciences and Pathology, College of Public Health and Medical Sciences, Jimma University;

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Parasitology

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Jimma, Ethiopia

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Abstract

Background*: Pregnant women, after frequent infections with Plasmodium spp., may develop immunity that can result in asymptomatic malaria. Though asymptomatic, the women harbor parasites that can trigger deleterious effects in maternal and child health, and can reserve gametocytes for perpetuation of transmission in an area. Therefore, this study aimed at determining the prevalence of asymptomatic malaria and related risk factors among pregnant women living in the malarious areas of Arba Minch Zuria District, Southern Ethiopia.*

Method: *A community based cross-sectional study comprising multistage sampling was conducted from April to June 2013 on 341 pregnant women in the malarious areas of Arba Minch Zuria District, Southern Ethiopia. Socio-demographic & economic data were collected with questionnaire. Malaria parasites were detected by Giemsa-stained blood smear microscopy and SD BIOLINE Malaria Ag Pf/Pv POCT test. Packed cell volume was determined by microhematocrit centrifugation to define anemia. Data were analyzed by SPSS 16; integrating both descriptive and inferential statistics with 95% confidence interval for odds ratio calculation.*

Results: *Of the total 341 pregnant women participated in this study, 31 women (9.1%) by microscopy with the mean parasite density of 3202.58/µl, and 33 women (9.7%) by the rapid Ag test were positive for malaria. The species diagnosed by microscopy & rapid test, respectively, were P.falciparum (38.71% & 39.4%), P.vivax (48.38% & 48.5%), and mixed Pf+Pv (12.9% & 12.1%). The sensitivity and specificity of the rapid test when compared to microscopy were 100% and 99.35%, respectively. Parasitemia was more likely to occur in primigravidae [Adjusted odds ratio (AOR)= 84.40, 95% CI: 7.30-976.53, P<0.001], secondigravida (AOR=16.34, 95% CI: 2.98-89.53, P=0.001), using insecticide treated net (ITN) sometimes (AOR=10.22, 95% CI: 1.80–57.95, P=0.009), not using ITN at all (AOR=4.61, 95% CI: 1.48-14.41, P=0.009), age group of 31-35 years (AOR=24.74, 95% CI: 2.31-265.42, P=0.008), and age group of >35 years (AOR=69.26, 95% CI: 3.99-1200.86, P=0.00) compared to multigravida, using ITN always & young age(<21 yrs), respectively. Of the total study subjects, 118 (34.6%) were anemic. Anemia was likely higher in Plasmodium infected women (AOR=12.76, 95% CI: 2.40-67.73, P=0.003), using ITN sometimes (AOR=7.33, 95% CI:1.83-29.42, P=0.005), not using ITN at all (AOR=2.06, 95%CI: 1.07-3.99, p-value=0.032) related to malaria negatives & using ITN always, respectively. There was a significant correlation between increasing malaria parasite load and decreasing hematocrit (r = −0.463, P = 0.009).*

Conclusion. *The prevalence of asymptomatic malaria in this study is moderate, however it has shown significant association with anemia in pregnant women. Symptomless malaria may create problems in Ethiopia where malaria control is essentially based on the treatment of symptomatic patients & mosquito control. It is, therefore, critical to design strategies that assist to diagnose pregnant women for asymptomatic malaria through the antenatal care(ANC) service package.*

Key Words*: Asymptomatic Plasmodium spp. Infection, Pregnancy, Anemia, Microscopy, Malaria Rapid tests.*

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Abbreviations

ACT Artemisinin-Based Combination Therapy

Ag Antigen

ANC Antenatal Clinic

CSA Chondriotin sulphate-A

EHNRI Ethiopian Health & Nutrition Research Institute

FMOH Federal Ministry of Health

IRS Indoor Residual Spraying

ITN Insecticide-Treated mosquito Nets

HCT Hematocrit

HIV Human Immunodeficiency Virus

LBW Low Birth Weight

LLIN Long-Lasting Insecticidal Net

MIP Malaria in Pregnancy

MIS Malaria Indicator Survey

PCR Polymerase Chain Reaction

PCV Packed Cell Volume

Pf *falciparum*

***Pf*HRP2** Histidine-Rich Protein 2 of *P.falciparum*

***Psp*LDH** *Plasmodium* Species Lactate Dehydrogenase

Pv *Plasmodium vivax*

POCT Point of care test

RDTs Rapid Diagnostic Tests

SPSS Statistical Package for Social Sciences

WHO World Health Organization

Chapter One

1. Introduction

1. 1 Background

1.1.1 Malaria Etiology

Malaria is an infectious vector-borne disease caused by a single-celled protozoan parasite of the genus *Plasmodium*. Plasmodia are obligate intracellular parasites which are able to infect and replicate within the erythrocytes after a clinically silent replication phase in the liver (1). For the past 80 years, human malaria has traditionally been known to be caused by four *Plasmodium* species (spp.)—*P. falciparum, P. vivax, P. ovale, and P. malaria.* Studies have confirmed the existence of two indigenously sympatric fully distinct *P. ovale* species: *P. ovale curtisi* and *P. ovale wallikeri* in both Africa and Asia (2, 3). These two ovale species have been identified even in a single individual (4, 5). Recently, a fifth species named *P. knowlesi*, which was formerly known to cause malaria only in macaques, has been recognized as a cause of malaria in humans in Southeast Asia (1, 6).

Transmission of malaria among humans is by the bite of female mosquitoes of the genus *Anopheles.* Female mosquitoes take blood from human body which is required for egg production, and hence such blood meals are the link between the human host and the mosquito vectors in the parasite life cycle (1, 7). The successful development of the malaria parasite in the mosquito vector depends on several factors. The most important are ambient temperature and humidity (7-9).

The pathogenesis of malaria is complex and the clinical presentation of the disease is classified in three main clinical groups: asymptomatic (presence of malaria parasite without malaria symptoms), mild, and severe malaria. In malaria-endemic areas, a significant proportion of individuals considered asymptomatic, harbor parasites without presenting signs of clinical malaria. The apparently healthy asymptomatic carriers are a major source of infective gametocytes for the mosquito population and also develop risk of maternal anemia, low-birth-weight baby and still birth (10, 11).

1.1.2 Epidemiology

Malaria is the most prevalent tropical infectious disease in the world. Malaria disease excessively affected the poor, children under the age of 5 years and pregnant women. World Health Organization (WHO) estimated that there were an estimated 216 million episodes of malaria in 2010, of which approximately 81% or 174 million cases were in the African Region(12). There were an estimated 655,000 malaria deaths in 2010, of which 91% were in Africa (12), although a recent systematic analysis indicated that the global death toll exceeded 1.2 million in 2010 (13, 14). Malaria in pregnancy is a major public health problem in endemic countries. There are a plenty of evidences showing that the risk of malaria is higher in pregnant than non-pregnant populations, possibly due to the immunological, hormonal changes or other factors occurring during pregnancy (15).

Malaria is a major public health problem in Ethiopia, where about two-thirds of the population is at risk of infection with an estimated 48 million people—68% of the Population—lives in areas with risk of malaria and three-quarters (75%) of the total land mass is regarded as malarious. *P. falciparum and P. vivax* are the two predominant malaria species, accounting for 60% and 40% of malaria cases, respectively (16, 17). In 2011, approximately 2.6 million cases of malaria were reported in Ethiopia, down from approximately 4 million cases in 2010 (18). In the southern part of the country, malaria remains to be the leading cause of 27.6% outpatient morbidity, 28.7% admission, and 46.4% death in the year 2009/2010 (19).

1.1.3 Vectors and transmission pattern

In Ethiopia, *Anopheles arabiensis* is the major malaria vector followed by *An. pharoensis* and other secondary vectors include *An. funestus* and *An. nili*. Like all other mosquitoes, these breed in water, each species having its preferred breeding grounds, feeding patterns, resting place and variable sensitivity to insecticides **(17, 20)**.

The transmission of malaria in Ethiopia follows a seasonal pattern depending 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. It peaks bi-annually: The major transmission of malaria occurs between September and December following the June to September rainfall, while the minor transmission season occurs between April and May following the February to March rainfall. Some localities also experience perennial malaria, because the environmental and

climatic situations permit the continuous breeding of vectors in permanent breeding sites. Many areas in the south and west of the country have a main rainfall season beginning earlier in March/May or have no clearly defined rainfall season (21, 22).

Epidemics of malaria are relatively frequent involving highland or highland fringe areas of Ethiopia, mainly areas 1,000-2,000 m above sea level. Due to the diversity of topography and variation of climate of the country, the nature of malaria transmission in majority of parts in Ethiopia is unstable (21, 22).

1.1.4 Status of malaria control

Despite the current strong control efforts in reducing the burden of malaria in the areas of stable, seasonal and sporadic malaria transmission, malaria remained an inflexible problem in much of Africa—most deaths by the etiology of *P. falciparum* (23). Given the enormous genetic and genomic diversity of malaria parasites, the emergence of antimalarial drug resistance is unavoidable. Reports of emerging resistance to the newest family of antimalarial drugs—the artemisinins—are particularly disturbing (24), and drug discovery is struggling to keep ahead of the spread of resistance (25).

Moreover, insecticide resistance is again becoming a problem (26), and the parasites showing evasion of immune responses, so resulting in hindrance of vaccine development approaches (27). These problems have led to an acceptance that sustainable reductions in malaria morbidity and mortality will require the development and deployment of drugs and vaccines to prevent the transmission of malaria from human to mosquito, thus interrupting the sexual phase of the parasite life cycle in which new (resistant and multiresistant) genotypes emerge (28).

In Ethiopia, early diagnosis and prompt treatment is one of the key strategies in controlling malaria. Blood smear microscopy and malaria rapid diagnostic tests (RDTs) represent the two diagnostics most likely to have the largest impact on malaria control today. For areas where laboratory facilities are not available, clinical diagnosis is widely used. Microscopical diagnosis is not accessible in most peripheral health facilities and at these sites, RDTs are used (29, 30).

RDT can provide a feasible platform for the diagnosis and treatment of the majority of asymptomatic carriers in rural communities. It has the advantage of detecting circulating antigens, even when the parasites are sequestered in the deep circulation and not visible by

microscopy (31, 32). The mass distribution of insecticide-treated bed nets (ITNs), coupled with increasing use of long-lasting ITNs(LLINs), indoor residual spraying (IRS) and nationwide adoption of artemisinin-based combination therapy (ACT) resulted in substantial declines in malaria-related deaths in Ethiopia (12, 30, 33).

1.2 Statement of the problem

Despite a plenty of studies on the clinical severity of malaria disease, asymptomatic malaria infections are still poorly recognized. In malaria endemic countries, a large proportion of *Plasmodium* infections are asymptomatic or sub-clinical (34, 35). Asymptomatic malaria remains a challenge for malaria control programs as it significantly influences transmission dynamics, and has great health impact on asymptomatic carriers (10, 36).

Asymptomatic infections go undetected and untreated, thus resulting in a major reservoir of gametocytes for transmission by mosquito vector (10, 36, 37) or can be a precursor in progression of symptomatic form of malaria as indicated by other studies that individuals with asymptomatic parasitemia may have a higher risk of developing symptomatic malaria than those without evidence of parasitemia (38). The reason why do asymptomatic individuals eventually develop clinical malaria has also been determined that whenever an "immune" individual becomes exposed to a new *Plasmodium* strain, he or she may develop clinical malaria (39). Gouagna *et al.* have demonstrated that gametocytes from asymptomatic individuals are more infectious to mosquitoes than parasites from symptomatic individuals (38, 40).

Few studies are available on asymptomatic malaria caused by species other than *P.falciparum* (10). Often regarded as "benign," *P.vivax* infections lay in the shadows of the much more virulent *P.falciparum* infections. However, about 1.98 billion people are at risk of both parasites worldwide (41). Although less lethal, *P.vivax* is more difficult to control and eliminate than *P.falciparum* because of its tendency to relapse after resolution of the primary infection (42, 43). *P. vivax's* ability to relapse accelerates the acquisition of clinical immunity more rapid than *falciparum* and thus *vivax* relapse serves as an immunity boosting mechanism (41).

For more than two decades, researchers have investigated the development of two types of immunity, which may result in asymptomatic malaria: 1) an anti-disease immunity that allows one to carry parasite loads without symptoms; and 2) an anti-parasite immunity that may be

responsible for the suppression of parasite loads after a certain age, which is likely a factor of exposure-related clinical immunity. Exposure-related immunity may be achieved much earlier in life for individuals who live in low transmission regions due to predictably low parasite genetic diversity and few overlapping infections (10, 44).

Asymptomatic malaria is commonly known to be occurring in regions of high and moderate transmission including Ghana, Kenya, Senegal, Gabon, Nigeria, Uganda, Thailand, Burma and Yemen where exposure-related immunity is expected to develop, however, currently it has also been reported in low or unstable transmission settings such as hypoendemic Kenya, Amazon region of Brazil and Peru, Colombia, Solomon Islands and Principe (10, 36, 37, 45). Studies have confirmed that asymptomatic parasitaemia occurs in the absence of intense transmission and it persists inter-seasonally in places with seasonal transmission (46-49).

Although transmission intensity is thought to help maintain immunological memory, other factors including parasite and host factors may be associated with the occurrence of asymptomatic malaria within different regions. Studies in the low transmission Peruvian Amazon demonstrated that high parasite exposure is not necessary for the development of B-cell memory or long-lasting antibody titers. As this study indicated, even in conditions where the possibility of re-infection is excluded, *P.falciparum* infection has been shown to persist asymptomatically in semi-immune individuals for more than 18 months (10, 50-52).

Sub-Saharan Africa records each year about thirty-two million pregnant women living in areas of high transmission of malaria (53). Pregnant women have a higher risk of malaria compared to non-pregnant women (15). In sub-Saharan Africa, malaria in pregnancy contributes to 15% of maternal anemia, 14% of low birth weight infants, 70% of intrauterine growth retardation, 36% of premature deliveries, and 8% of infant mortality (54-56).

The susceptibility of pregnant women to malaria parasites has been linked to the level of antibodies to placental sequestrated parasites and depression of the cell-mediated immune response to *Plasmodium* antigens. Subpopulation of *P.falciparum and P.vivax* infected erythrocytes sequester in the placenta by expressing surface antigens, mainly variant surface antigen (VAR2CSA), that adhere to chondroitin sulphate-A (CSA) receptors expressed by syncytiotrophoblasts in the placenta. This is more marked in the first 24 weeks than during the

third trimester. Primigravida and secondigravidae are more susceptible, as anti-adhesion antibodies against CSA-binding parasites associated with protection only develop after successive pregnancies(57, 58).

An asymptomatic infection in pregnant women can cause more severe outcomes than in non-pregnant women because it can result in serious impact on both maternal and infant health that includes maternal anemia, peripheral and placental parasitaemia, intrauterine growth retardation and delivering low birth weight baby(10, 11, 59).

Anaemia is the most common symptom of malaria in pregnancy and usually develops during the first and second trimesters. It is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which varies by age, sex, altitude, smoking, and pregnancy status. It is a reduction in the concentration of hemoglobin (Hgb) level (<11 g/dl at sea level), which can be indicated by packed cell volume (PCV) or red blood cell count when below normal (60, 61). Malaria causes reduction in Hgb concentration by a number of ways, mainly by destruction and removal of parasitized red cells and the shortening of the life span of non-parasitized red cells, and decreasing the rate of erythrocyte production in the bone marrow. Other mechanisms causing reduction of Hgb level during malaria are associated with the acute clinical states (e.g., hemolysis or cytokine disturbances), whereas chronic or repeated infections are more likely to result in dyserythropoiesis (60-62).

The susceptibility of pregnant women to malaria is well established. However, no epidemiological data about pregnant women have been recorded in the study district, where *P. falciparum or P.vivax* malaria is endemic. Therefore, in order to fill these gaps, a community based cross-sectional study was employed to assess asymptomatic *Plasmodium* parasitaemia and its relationship with various sociodemographic and socioeconomic factors among pregnant women in the rural district surrounding Arba Minch, Southern Ethiopia.

1.3. Significance of the study

Malaria control strategy in Ethiopia has more emphasis on vector control and treatment of symptomatic cases, by ignoring asymptomatic malaria carriers possibly due to unstable nature of disease transmission in most parts of the country. However, currently many asymptomatic malaria cases have been reported in both stable and unstable transmission settings. Studies have

confirmed that asymptomatic parasitaemia occurs also in the absence of intense transmission and it persist inter-seasonally in places with seasonal transmission**.**

Asymptomatic carriers do not seek treatment for their infection and, therefore, constitute a reservoir of parasites and thus a real public-health risk may occur. Asymptomatic pregnant women are associated with complications like anemia, intrauterine growth retardation and low-birth-weight baby. Therefore systematic identification and treatment of individuals with asymptomatic *Plasmodium* spp. as part of a surveillance intervention strategy reduces the complications and eliminates the parasite reservoir for disease transmission by mosquito.

Therefore, this study result is useful to determine the existing significant variables related to malaria and anemia to guide the antenatal care service to work towards alleviating the problem with an appropriate care. Developing systematic screening strategies for malaria parasites and anemia at booking, prompt parasite clearance, education on usage of ITN during pregnancy and correction of anemia can reduce the prevalence of malaria and related anemia, and also obstetric complications associated with it. This study finding is also expected to fill gaps in this area of research. By being added to the existing body of knowledge, it will help other researchers as reference for their work.

Chapter Two

2. Literature Review

2.1 Literature review

According to a cross-sectional study, which was conducted at ANC of Saint Camille Medical Centre in Burkina Faso, the prevalence of asymptomatic malaria among pregnant women was found to be 30% and 24% with RDT and microscopy, respectively. The study has shown that the sensitivity and specificity of RDT were 100% and 92% respectively for the detection of asymptomatic malaria as compared to microscopic diagnosis. The mean parasite density among pregnant women as seen from the study was 4058 parasites/μl. The study suggested that anaemia was significantly more common in women infected with malaria (33% prevalence) compared with the uninfected pregnant women (10% prevalence) (11).

Another study which was conducted among asymptomatic pregnant women in Nanoro, Boulkiemde´ Province, Burkina Faso, reported malaria prevalence of 30% by microscopy and 47% by HRP2 RDT. As this study suggests, the HRP2 RDT detects more cases than microscopy. The RDT detect circulating antigen or nucleic acids, which might be adequate for identifying women with placenta malaria (63). Other similar studies conducted among pregnant women in Cote d'Ivoire and Senegal also reported the high sensitivities of rapid malaria detection test relative to blood smear microscopy as 100% and 96%, and specificities of 88% and 87%, respectively (64, 65).

A study carried out in Calabar Nigeria indicated high prevalence of asymptomatic malaria parasitemia (95.4%) by Giemsa stained smear microscopy. In this study it was found that there was a statistically significant association between severity of parasitemia and degree of anemia. All participants who had no parasitemia had normal PCV. 58.4% of participants who had mild parasitemia were mildly or moderately anemic; while the majority (66.7%) of those who had severe parasitemia were severely anemic. Malaria cause anemia mostly in the second trimester of pregnancy when there is accelerated fetal growth and may develop suddenly in severe parasitaemia that may persist into the third trimester (66).

A cross-sectional study conducted in pregnant women of Entebbe, Uganda showed that the prevalence of asymptomatic *P. falciparum* malaria parasitaemia was 10.9%. This study

suggested that, among pregnant women in Entebbe, Uganda, malaria is more important infectious causes of anemia than helminthes. The prevalence of anaemia was 39.7%. Anaemia showed little association with the presence of any helminth, but it showed a strong association with malaria (67).

According to the study conducted in pregnant Nigerian women: almost a decade after Roll Back Malaria, of 125 pregnant women tested, 73 had microscopic *Plasmodium* parasitaemia, giving a prevalence of 58.4%. Asymptomatic malaria parasitaemia was more common in primigravidae, where 32 (65.3%) of 49 women were positive, followed by multigravidae with 39(55.7%) of 70 women. The lowest occurrence was in grandmultigravidae with 2 (33.3%) of 6 women testing positive. These relationships were statistically significant. From this study, Anemia in pregnancy was prevalent (55.2%) and there was no significant difference in the density of parasitaemia in those with mild, moderate, and severe anemia. The prevalence of malaria parasites was higher in younger (65.7%; 46/70) than older pregnant women (49.1%; 27/55). This relationship was also statistically significant (68).

Another cross-sectional study, which was, conducted at ANC of University of Calabar Teaching Hospital, Nigeria with unstable malaria transmission indicated that the prevalence of malaria parasite infection was 70.1% in pregnant women. Prevalence of anaemia and malaria parasite was found to be higher in the primigravidae than in the multigravidae. Primigravidae were found to be susceptible to malaria with a significantly higher parasite density 2112.50±420.90 than the multigravidae 446.70±296.90. Anaemia was also found to be higher (64.4%) in primigravidae than in multigravidae (60.7%) (61).

This study indicated that malaria parasite density increased significantly with gestational age but anaemia was more prevalent in the second trimester than in the other trimesters. Pregnant women appear to be more anaemic in their second trimester (65.8%) than the 3rd trimester (64.5) although those in their 3rd trimester were more infected with malaria parasite (80.2%) than the pregnant women who were in their second trimester (67.6%). The pregnant women who were in their 1st trimester were the least infected with malaria parasite (40.8%). This study indicated that the pregnant women within the age bracket of 25 - 34 years had the highest number of positive sample(45.7%) with the mean parasite density 937±331.5 while those within 35-45 years had the least positive sample (6.8%) with the mean parasite density of 64±135.6 (61).

Similarly, a cross-sectional ANC study in Libreville, the capital city of Gabon indicated 57% women had microscopic parasitaemia by blood films stained with Giemsa. The parasites densities were higher in primigravidae and teenagers. The prevalence of anaemia was 71% and was associated with microscopic *P. falciparum* parasitaemia: women with moderate or severe anaemia had higher parasite prevalences and densities. This study also indicated that parasite densities were higher in primigravidae and teenagers (69).

Study from Ghana also reported the 36% prevalence of *P. falciparum* among the asymptomatic pregnant women and 44% of the participants were anemic (Hgb < 11 g/dL) with the mean hemoglobin level of 10.98±2.32 (38). Findings from studies assured the high prevalence of malaria in asymptomatic pregnants and its impact on Hgb level obviously in lower gravidities. However, there was also a study which have revealed higher rates of anaemia among primiparous women compared to multiparous ones as of Sekyere West District Ghana (70),

According to a study took place on malaria infection among pregnant women attending antenatal clinics in six Rwandan districts, there observed that the overall prevalence of malaria infection was 13.6%. As the study suggests, anaemia prevalence mirrored that of malaria infection. Both malaria and anaemia reflected differences in different districts. The prevalence of infection was significantly higher in primigravidae than in the higher gravidities. Prevalence of anaemia was 11.8% and was higher in primigravidae than in secundigravidae and multigravidae. Parasitaemia, gravidity and district were found to be significant risk factors for anaemia and were kept significant in multivariate analysis. Parasitaemic women had a 75% higher risk of being anaemic than negatives; and this risk inversely related to increasing parity (71).

In a cross-sectional study conducted at ANCs of two district hospitals in Jharkhand State, India, 2,386 pregnant women were enrolled per 12 months period and 1.8% (43/2382) were found to be positive for malaria (53.5% *P. falciparum*, 37.2% *P. vivax*, and 9.3% mixed infections) by Giemsa-stained blood films and/or RDTs using peripheral blood. This study suggested that peripheral parasitaemia was more common in pregnant women in rural settings and in younger age groups below 20 years (72).

Epidemiology of malaria during pregnancy is highly variable in the different transmission settings in sub-Saharan Africa. In a cross-sectional study which took place in Ethiopia, an

asymptomatic peripheral malaria parasitemia was identified in 10.4% of women attending antenatal care clinics at one stable transmission site and in 1.8% of women at three unstable sites; parasitemia was associated with anemia in both stable and unstable sites (56).

A community based study conducted in Gilgel Gibe dam area, Southwest Ethiopia in the year 2011 reported malaria prevalence of 11.59% (45/388) in the pregnant women. This study indicated that malaria had significant association with anaemia. Of these malaria positives, 93.3 % (42/45) were found to be anaemic; while the prevalence of anemia among the whole study subjects was 53.9 % (209/388). Findings from this study indicated that pregnant women from rural residences, not using ITN during the study period, those who were *Plasmodium* infected and those with STH infection were highly likely to be anaemic compared to those from urban residences, using ITN, free of *Plasmodium* and STH infection. There was found a statistically significant correlation between an increasing parasite counts and the decrease of hematocrite values (60). A study from Nigeria (73) also indicated significant correlation between increasing parasite counts and decreasing haemoglobin levels.

The use of ITN has been advocated and has shown potential efficiency in the control of mosquito bites. However, frequently, a pregnant woman might not remain under the net for more than 8 hours a day; hence it does not give complete protection against malaria (73). ITNs only give protection while sleeping inside the net, with increased vulnerability to mosquito bites. The reasons for absence of ITN use among the pregnant women were: psychological effects of suffocation while sleeping under the net, ignorance of the risks of malaria during pregnancy, cost of the net in self-marketing, having only worn-out bed nets (ragged by rats), hanging problem due to dirty bed nets, changes in bed arrangement, unsuitable housing structure and considering alternatives such as insecticide sprays (IRS). Other inconvenient practices are utilization of bed nets as bed sheets to cover the mattress of a bed and using the nets as curtains for doors, windows and traditional pit latrines (74).

Prevalence of anemia among Ethiopian pregnant women was high according to different antenatal care studies. Anaemia prevalence of 41.9% in Jimma health center, Ethiopia (75), 23% in Asendabo health center, Ethiopia (76), and 51.9% in Bushulo health center, Ethiopia (77) were also reported in pregnant women attending ANC. Generally, asymptomatic malaria parasitaemia

is significantly associated with different sociodemographic factors and has great health impact on the level of hemoglobin in pregnant women.

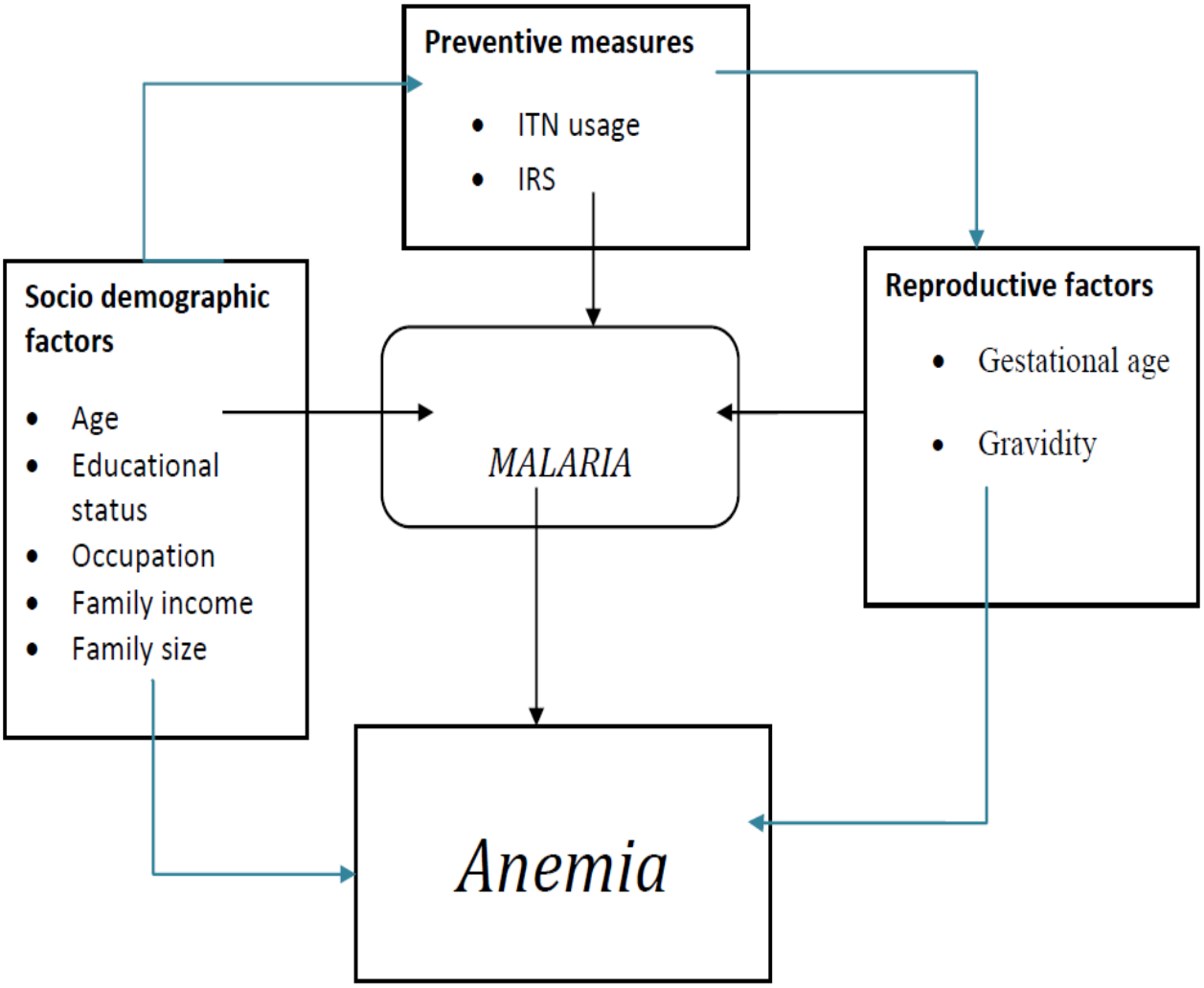

Figure 1. Conceptual framework for factors associated with malaria and anemia among pregnant women in the Arba Minch Zuria District, Southern Ethiopia, from April to June 2013.

Chapter Three

3. Objectives

3.1. General objective

- To determine the prevalence of asymptomatic *Plasmodium* infection and associated risk factors among pregnant women in Arba Minch Zuria District, Southern Ethiopia

3.2. Specific objectives

- To determine the prevalence of asymptomatic *Plasmodium* infection among pregnant women
- To compare asymptomatic malaria prevalence by rapid test with that by light microscopy
- To identify associated risk factors for asymptomatic *Plasmodium* infection
- To assess the effect of asymptomatic *Plasmodium* infection on the hematocrit level of the women

Chapter Four

4. Materials and Methods

4.1 Study setting and period

This study was conducted from April to June 2013; in the malarious villages of the Surrounding District of Arba Minch, Southern Ethiopia. The area locally called as "Arba Minch Zuria Woreda" which is one of the Districts in the Southern Nations, Nationalities, and Peoples' Region of Ethiopia. Arba Minch is the capital of Gamo Gofa Zone which is located 505 km South of Addis Ababa, the capital city of Ethiopia. As part of Gamo Gofa Zone, which is located in the Great Rift Valley, Arba Minch Zuria District included portions of two rift valley lakes, Abaya and Chamo and their islands. Nechisar National Park is located between these lakes. The presence of Lakes, Abaya and Chamo in the surroundings of the district result in intense malaria transmission in the area as the shores of the lakes favor malaria vector breeding. The distance of the household from the shores of the lakes was found to be one of the significant predictors of malaria infection in the area (78).

The Arba Minch town lies in tropical climatic zone locally known as *"Kolla"* (1,200-1,300 m above sea level) with an average annual temperature of 29.7^0C and rainfall of 700 mm. There are 11 highly malarious villages out of 30 villages in the Arba Minch Zuria District. The villages are found at an average altitude of 1,206 m above sea level with a rainfall of 950 mm and with the same average annual temperature to the town. The rainfall pattern of the area is almost similar to other parts of Ethiopia with the long rainy season starting in June and extending up to September, while the short rainy season begins in February/March and extends to April/May or irregular, the whole year round rainfall pattern occurs (79, 80).

Based on the 2007 Census conducted by the Cental Statistical Agency, Arba Minch Zuria Woreda has a total population of 164,529, of whom 82,199 are men and 82,330 women. The five largest ethnic groups reported in Arba Minch Zuria were Gamo (69.53%), Amhara (7.94%), Wolayta (6.75%), Zayse (6.02%), and Oromo (3.64%); all other ethnic groups made up 2.28% of the population (81, 82).

The dominant income generation source is agriculture for this rural population. The main socio-economic activities of the local communities are mixed farming involving the cultivation of staple crops combined with cattle and small stock raising. Mango, banana, and maize are the major cash crops in the area. Almost all households in the District have mango and banana plantation within their compound.

4.2 Study design

A community based cross-sectional survey

4.3 Population

4.3.1 Source population

All pregnant women in the study community

4.3.2 Study population

Apparently healthy pregnant women in the study community

4.3.3 Study participant

Apparently healthy pregnant women who meet the inclusion criteria

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- Absence of disease symptom/sign within the past 48 hours
- Axillary temperature <37.5º
- Permanently resident in the study area

4.4.2 Exclusion criteria

- Individuals having taken anti-malarial drugs in the past six weeks prior to sampling.
- Those who are undergoing any kind of long term medical treatments.

4.5 Sample size determination and sampling technique

4.5.1 Sample size calculation

The required sample size for this study was calculated using a formula for a single population proportion. The peripheral malaria prevalence of 11.59% from a community based study conducted among pregnant women in Gilgel Dam Area, Southwest Ethiopia (60) was used for

estimation of the sample. Taking 95% confidence interval and ±5% marginal error, sample size (n) is determined using the following statistical formula.

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

$$= (1.96)^2 (0.1159) (1-0.1159) / (0.05)^2 = 157.45 \sim 157$$

Where, P= Prevalence rate of 11.59%,

n = Sample size,

Z = 95% confident interval

d= Bond on sampling error tolerated between the sample and population: ±5%

α = Critical value at 95% confidence interval of certainty (1.96)

By taking a design effect of 2 and non-response rate of 10%, the total sample size became 346.

4.5.2 Sampling technique

Multistage sampling technique was employed in selection of the study subjects. Arba Minch Zuria District consists of 30 villages, of which 11 have been known to be malarious villages with intense transmission pattern (79). Eight Study villages, from the known 11 malarious villages in the District, were selected by simple random sampling using lottery method. The sample size was then distributed proportionally to the villages based on the size of their pregnant women population (Figure 3). The selected study villages were chano Mile, Chano Dorga, Chamo Shele, Chano Chalba, Aelgo Gonto, Genta Kenchama, Chano Lante, and Kolla Shara.

At the village level, the households where pregnant women live were selected by simple random sampling by using the sampling frame which was prepared after having identified the pregnant women in the households by the preliminary assessement through active house-to-house visits. Computer generated random numbers were used for the random selection of the study households or study participants. In the cases where more than one eligible women were encountered in a single household, a lottery method was used to select a woman to be recruited to the study. Pregnant women were identified with the support of rural health extension workers, who are working in each of the villages. The health extension workers guided us in the preliminary assessement by indicating the houses where pregnant women were living.

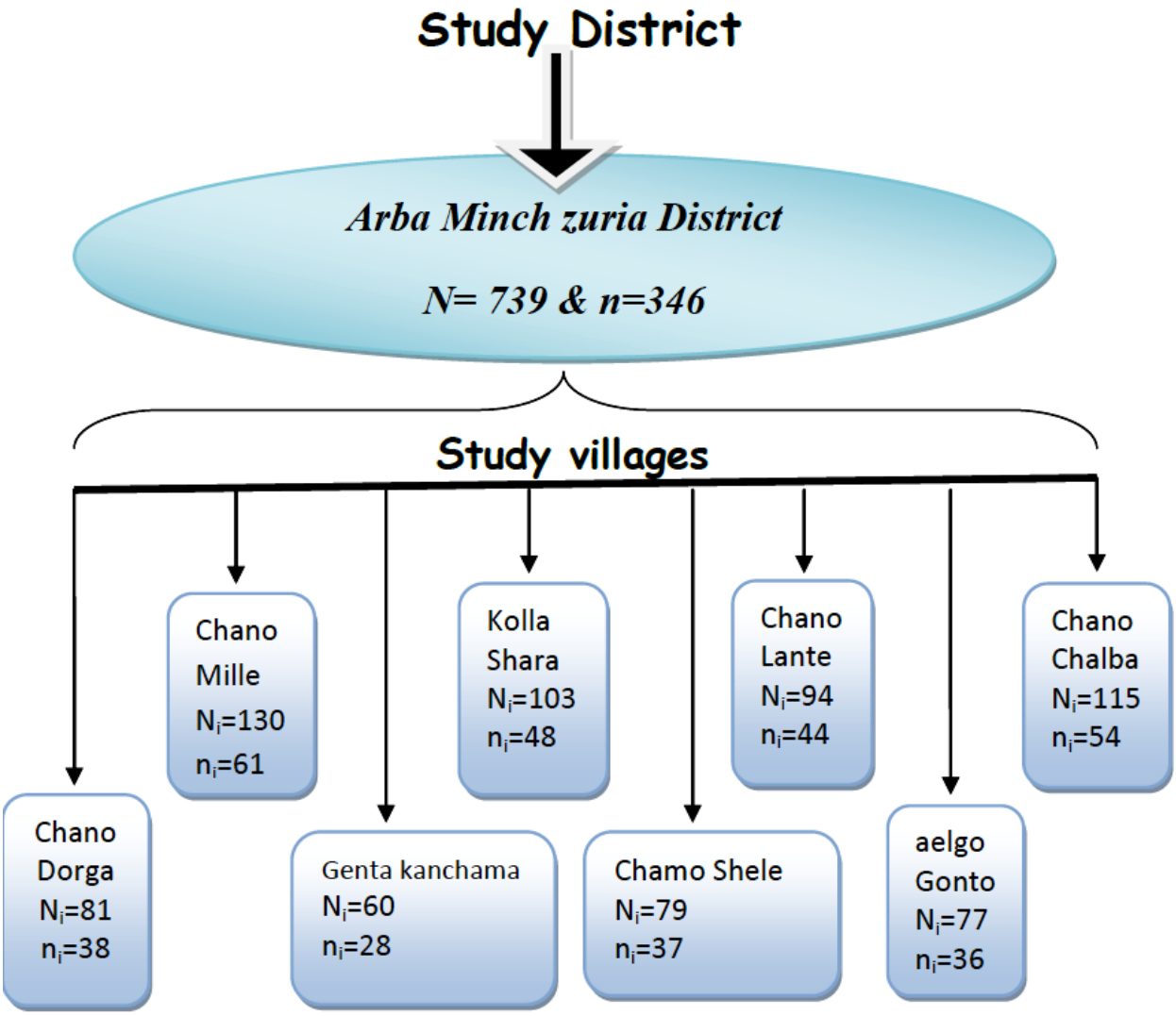

Figure 2. Flow chart indicating the sampling procedure for a study conducted on asymptomatic malaria among pregnant women in the Arba Minch Zuria District, Southern Ethiopia, from April to June 2013.

Where; **N**=the whole number of pregnant women in all study villages, **n**=the total study subjects/sample, $\mathbf{N_i}$=the whole number of pregnant women in each study village, $\mathbf{n_i}$=the number of pregnant women proportionally sampled from each study village

Proportional allocation of the sample from each study village was performed as :

$$ni = \frac{n}{N} X \, Ni$$

4.6 Measurement

4.6.1 Study variables

4.6.1.1 Dependent variable

- *Plasmodium* infection
- Hematocrit level

4.6.1.2 Independent variable

- Age
- Gestational period
- Gravidity
- Educational status
- Occupation
- Family income
- Family size
- ITN use
- IRS of insecticides with in past 12 months

4.6.2 Data collection instrument

4.6.2.1 Questionnaire & Laboratory request

A semi-structured questionnaire having both closed and open ended questions was prepared. The questionnaire was initially prepared in English and then translated to Amharic by two independent translators, and the consistency was checked. Reverse translation of the questionnaire ensured the accuracy and compatibility of the first translation. The questionnaire incorporated socio-demographic and socio-economic informations, reproduciove histories of mothers such as parity, gravidity, and antenatal clinic visits, and disease prevention methods including IRS and ITN usage. The Laboratory format was also used to record the laboratory test results. Prior to data collection, the questionnaire was pre-tested on 5% of the total sample size in similar setup outside the study area and appropriate changes were made according to the feedback received. The questions were checked for clarity, completeness, consistency, sensitiveness and setting of time required to conduct interviews and the questions which posed

difficulty or unclear were rephrased and corrected. Unnecessary questions were excluded and missed questions were incorporated where necessary.

4.7 Data collection

4.7.1 Socio-demographic and socio-economic survey

The survey was conducted using semi-structured questionnaire having both closed and open ended questions. Data collectors who could speak both Amharic and the local language were hired for this particular study to attain standardization and maximize the interviewer reliability. Two laboratory technicians from Arba Minch hospital and the District Health Center, one having a work experience of 5 years and the second having 6 years; one supervisor who was also a laboratory technician, field technical assistants and guide persons were involved for interviewing the pregnant women after obtaining their verbal and written consent. Each pregnant woman resident in the study villages was assigned a household number and name identifying the household number. A face-to-face interview was held to collect relevant data from each pregnant woman. Interviews were conducted privately to maintain confidentiality and avoid family and peer pressure.

4.7.2 Parasitological and hematological Survey

Together with the socio-demographic and socioeconomic information, peripheral blood samples were collected from a finger prick using disposable lancet by the laboratory technicians following standard WHO protocol (83). The specimen processing was organized in such a way that all three tests: malaria RDTs, both thick and thin blood films for microscopic examination of malaria parasites and hematocrit determination were performed simultaneously from each surveyed individual‘s single finger prick blood as per WHO guidelines (83).

4.7.2.1 Blood film preparation and examination of malaria parasites

Before taking the blood sample, new frosted glass slides were extracted from the slide packets and labeled in the field in such a way that the slide code could match with the file of the particular pregnant woman. Then, the finger was cleansed with a swab moistened with 70% isopropyl alcohol, dried with a piece of dry cotton, punctured with a disposable blood lancet. Through wiping off the first drop of blood, thick and thin films were made on the same slide by the laboratory technitians. After being air-dried in a horizontal position, the slides were placed in

a slide box and transported daily to Arba Minch University Hospital for staining and microscopic examination. During the collection period, samples were dried at room temperature before storage and were shipped at 4°C for examination.

Soon after the completion of each day field work, the thin blood films were fixed on the day of collection in absolute (100%) methanol for 30 seconds at the Hospital Laboratory. Then staining and examination of the films was conducted according to WHO protocol. With in 24-48 hrs of collection, the films were stained with 10% Giemsa working solution for 10 minutes and examined by light microscopy. Malaria parasites were detected by observing the morphological appearance of parasites in the infected red blood cells by microscopy of blood films under 100x oil immersion objective. Thick films were used to search/detect malaria parasites and quantify the parasity density, while thin films were used to confirm/identify the parasite species.

Two laboratory technologists from the Hospital, each having a work experience of above 5 years, were hired to examine the blood films. Initial microscopic examination of thick films using high power magnification for the presence of parasites and parasite species identification using thin films under 100x oil immersion objective was carried out. Parasite density was determined on the basis of the number of parasites per 200 white blood cells (WBC) on a thick blood film assuming a total WBC count of 8000/μl. The degree of parasite density was graded as mild or +, moderate or ++, and severe or +++ when the counts were between 1–999 parasite/µl, 1000-9999/µl, >10,000/µl, respectively, following the method described by Cheesbrough (84) and based on WHO guide (83). The principal investigator in collaboration with the other data collectors performed the field data collection and the initial microscopic examination.

The slides were again read by the next microscopist who was blinded to the other's readings. Criteria for a second microscopy reading were: slides positive for *Plasmodium* spp. at first microscopy reading; individuals with discrepant microscopy and RDT results; severely anaemic individuals (HCT <21%); and a randomly selected 10% of negative slides. The crosscheck or second round confirmatory reading was performed at the same hospital laboratory by the next microscopist who was blinded to the findings of the first microscopic reading and the RDT result (83). Discrepancy between the first and second readings was settled by a third senior microscopist, whose readings were considered final. The discrepant microscopy result for parasite counts was resolved by calculating the percentage discrepancy. The total leukocyte

count determination for each participant were done using the improved Neubauer chamber as described by Baker and Silverton (85). [Details on Annex 8.1]

4.7.2.2 SD BIOLINE Malaria Ag **P.f/P.v** *POCT.*

Results of malaria RDTs were recorded onsite at the field. The rapid diagnostic test used in this study is SD BIOLINE Malaria Ag P.f/P.v POCT (point of care test), Korea. This test is one step, rapid, qualitative and differential test for the detection of HRP-II (Histidine-rich protein II) specific to *P.falciparum* and *p*LDH (*Plasmodium* lactate dehydrogenase) specific to *P.vivax* in a human blood sample. Same finger prick blood samples, used for blood films, were also used to perform RDTs onsite at the field based on the manufacturer instructions of the kits and the results were recorded there. All RDT devices were labeled with similar patient ID numbers to that of the blood films. The manufacture and expiry date of RDT were checked and handled at the temperature recommended by the manufacturer suggesting that they were in a supposedly good condition during the study period (31, 32). [Details on annex 8.2].

4.7.2.3 HCT determination

Samples were estimated for PCV using the gold standard microhaematocrit centrifugation method. The PCV was used as a simple screening test for anemia, as a reference method for calibrating automated blood count systems and as a rough guide to the accuracy of hemoglobin measurements (86). Capillary blood samples, from same finger prick used for other preparations, were collected from all the pregnant women following aseptic technique. The blood filled hematocrit tubes having been sealed with a sealing-wax were placed in a labeled larger tube container and then transported for estimation in Arba Minch University Hospiatal. The PCV were determined using capillary tubes and read from a Hawksleys microhematocrit reader (66).

According to WHO guidelines, pregnant women are normal with hematocrit concentration of 33% and above. The pregnant women with HCT values less than 33% were categorized as anemic. Anemic women were further categorized as women with mild anemia (HCT $\geq$ 30% & < 33%), moderate anemia (HCT $\geq$ 21% & < 30%) and severe anemia (HCT below 21%) (87). [Detail on annex 8.3].

4.8 Data quality assurance

To assure the quality of data reliable: data collection, application of standard operating procedure (SOP), accuracy of test results were strictly & closely followed up by the principal investigator. All data collection reagents and equipments, sufficient enough per the need, were gathered before starting the collection. Properly designed and pretested data collection instrument was developed. Training was given to all data collectors on the objective of the study, on the instrument to be used, on how to interview mothers, and on how to collect and examine blood specimens. Every day the collected data were reviewed and checked for completeness and consistency by the supervisor and principal investigator. Problems encountered were reported to the supervisor and then to the principal investigator for immediate action. Discussions were made with the data collectors at the end of each day to minimize errors committed during the fieldwork and to take corrective actions timely. Repeated visits were made when study households were found closed or when respondents were unavailable. The non-response rate was very small and negligible for this study. [Details in annex 9].

4.9 Data processing and analysis

Data from laboratory and field survey were checked for completeness and consistency, and then entered into and analyzed by Statistical Package for Social Sciences (SPSS) version 16 for windows**.** The data were then cleaned by checking for error, impossible or implausible values, and inconsistencies that might occur due to coding or data entry errors. In the analysis process, descriptive statistics were used to scan the data, assess normality, and identify missing values and outliers.

Both descriptive and inferential statistics were integrated in the analysis of study variables. Descriptive statistics were done to determine the proportions of the variables. Chi-square test was used to check the statistical assumption fulfillment of the data as well as to measure the strength of association and statistical significance between the outcome and independent variables. Binary logistic regression model was employed to analyze the adjusted effect of each independent variable on the outcome variables. Based on purposeful selection of variables in logistic regression as proposed by Hosmer and Lemeshow (88, 89), Preliminary Univariate analysis for each independent variable was performed to start with; and those variables

significant at P-value of 25% at the Univariate regression were then selected to Multivariate analysis model.

Multivariate analysis was done to identify the independent predictors of the outcome variable. Odds ratio were calculated with a 95% confidence interval to determine the strength of association and statistical significance at two sides' P-value of ≤ 5%. Pearson and Spearman rank correlation procedures were used to evaluate the relationship between parasite counts and hematocrit values. Malaria rapid test performance and acceptability evaluation indices: sensitivity, specificity, positive & negative predictive values, accuracy and overall reliability of a test were calculated by using blood smear microscopy as gold standard. Finally, the results were presented using tables, charts, and graphs.

4.10 Ethical consideration

The research proposal was first approved by the Department of Medical Laboratory Science and Pathology, and ethically cleared by the Research Ethics Review Board of College of Public Health and Medical Sciences, Jimma University. Communication with Gamo Gofa Zonal and Arba Minch Zuria District health departments was done through official letters from Jimma University. Following a discussion with the head of Arba Minch Zuria District Administrative Health Office and coordinator of disease prevention and control unit, a brief cooperation and agreement letter was written to each study village council. Correspondingly, community agreement and local oral consent was gained from village leaders through contact in village administrative offices and meetings with villagers. Informed verbal and written consent was taken from each pregnant woman after the objectives and the nature of the study were clarified to the participants so as to get their voluntary consent.

Data collected from each study participant and results of laboratory tests were kept confidential and used only for the research purpose. Results of participants with parasitic infection and low HCT level were addressed to the study participants. The pregnant women who were found to be infected with *Plasmodium* parasite and anemic were referred for treatment and medical consultation in the ANCs of nearby health facilities. All anemic women requiring treatment (whether or not tested parasitemic) were referred to a health facility. Since there are many causes

of anemia, further assessment by medical professionals is necessary so that the women could receive the appropriate treatment.

4.11 Operational definitions

Asymptomatic *Plasmodium* parasitaemia: No history of fever within the past 48 hours, or temperature ≤37.5°C but associated with the presence of asexual forms of *Plasmodium* spp. on blood smear or a positive rapid malaria antigen detection test.

Peripheral parasitaemia: Presence of asexual *P.falciparum* or *P.vivax* parasitic forms on blood smears and/or positive RDT from the peripheral blood.

Parasite density: The number of parasites/µl of blood which is determined by counting the number of parasites on thick blood film against 200 or 500 WBCs in relation to the standard number of WBCs/µl (8000).

Apparently healthy pregnant women: Pregnant women who look healthy because they are without disease symptom, but suspected or diagnosed for asymptomatic carriage of *Plasmodium* spp.

Trimester: A period of 3 months, or one of the three three-month periods into which human pregnancy is divided for medical purposes.

Chapter Five

5. Results

5.1 Socio-demographic and socio-economic survey

From the total 346 intended sample size, data were collected from 341 pregnant women with response rate of 98.6%. The mean age of the study participants was 26.36 (95% CI: 25.83-26.89) with a standard deviation (STD) of ± 4.985 years. The highest proportion of pregnant women (41.1%; 140/341) were in the age range of 21-25 years and the least in >35 years. The majority of the women (80.35%; 274/341) were young (age ≤ 30 years); while 67 (19.65%) were over the age of 30 years. Regarding marital status, 95% (327/341) of the women were married, and almost three-fourth (66.6%; 227/341) of the study participants were protestants with fewer orthodox followers. Three-fourth of the women were housewives and farmers (like their husband). About 70% of the women completed their primary and secondary school education (Table. 1).

We categorized monthly income of the respondents by putting US$ 1.25 income per day as a cut off point for poverty line as indicated by World Bank 2010; and the respondents monthly income was calculated using a dollar exchange of 1USD as 18.82 ETB according to the average exchange rate for Ethiopian Birr which was last updated on August 12, 2013 from Yahoo Finance (90). The mean monthly income of the study participants was 718.48(95% CI: 680.22-756.73). Two-third of the study subjects (59.8%; 204/341) had a monthly income of less than 650 Br. The mean family size in this study is 4.55(95% CI: 4.34 - 4.77) with a STD of ±1.987. Half of the study houses had a family size in the group of 4-7 (Table. 1).

The gestational age of the women was assessed from the last normal menstrual period. It was defined as first trimester (<14 weeks), second trimester (14-27 weeks) and third trimester (>27 weeks). Majority of the women were at their second trimester (41.1%; 140/341) and were multigravidae (> 2 births) (44.3%; 151/341) (Table. 1). Most of the women began their prenatal checks late at the second trimester as observed in our study that 33.3% at first, 71.2% at second and 95.5% at third trimester were attending their ANC follow up. There was also statistically significant difference among these three gestational trimesters in terms of ANC attendance (chi-square =84.606[a], p <0.001). More than half proportion of the study subjects (56.3%; 192/341) were using ITN always, while others using sometimes and/or not at all (Table. 1).

Table 1. Sociodemographic and economic characteristics of the pregnant women in Arba Minch Zuria District, South Ethiopia, April to June, 2013.

Variables	No (%)	Variables	No (%)
Age groups		Marital status	
≤20	32(9.4)	married	327(95.9)
21-25	140(41.1)	single	4(1.2)
26-30	102(29.9)	divorced	6(1.8)
31-35	47(13.8)	widowed	4(1.2)
>35	20(5.9)		
Parity		Gestational age	
Primigravidae	91(26.7)	1st trimester	83(24.3)
Secondgravida	99(29.0)	2nd trimester	140(41.1)
Multigravida	151(44.3)	3rd trimester	118(34.6)
Occupation		Education	
Farmer	98(28.7)	Illiterate	59(17.3)
Daily laborer	40(11.7)	Read/write	15(4.4)
Merchant	57(16.7)	Primary	134(39.3)
House wife	134(39.3)	Secondary	95(27.9)
Civil servant	12(3.5)	College/above	38(11.1)
Family size		ANC Attendance	
1-3	116(34.0)	Yes	
4-7	168(49.3)	No	275(80.6)
>7	57(16.7)		66(19.4)
Income		ITN use	
<650 ETB	204(59.8)	Use always	187(54.8)
650-1300 ETB	102(29.9)	Use rarely	19(5.6)
>1300 ETB	35(10.3)	Do not use	135(39.6)
Religion		IRS(past 12 months)	
protestant	227(66.6%)	Yes	289(84.8)
orthodox	114(33.4%)	No	52(15.2)
Roof material		Floor material	
Thatched roof	155(45.5)	earth	316(92.7)
Corrugated iron	186(54.5)	local dung plastic	11(3.2)
		cement	14(4.1)
Room's wall material			
mud blocks	323(94.7%)		
cement blocks	8(2.3%)	/**.....**……../	
sticks	10(2.9%)		

5.2 Parasitological and hematological survey

5.2.1 Parasite prevalence by microscopy and SD BIOLINE Malaria Ag *P.f/P.v* POCT

Of the total 341(98.6%) pregnant women who responded in the study, 31 women (9.1%) had a positive diagnostic result for malaria by blood smear examination. Thirty-three women (9.7%) were positive by SD BIOLINE Malaria Ag *P.f/P.v* POCT (point of care test), of which two cases__one *P.falciparum* and one *P.vivax* were later examined to be negative by blood smear microscopy. The species diagnosed positive by blood smear microscopy were 12(38.71%) *P.falciparum*, 15(48.38%) *P.vivax*, and 4(12.9%) mixed *P.f/P.v* infections. The species diagnosed positive by RDT were 13(39.4%) *P.falciparum*, 16(48.5%) *P.vivax*, and 4(12.1%) mixed *P.f/P.v* infections. The mean parasite density observed among the 31 pregnant women was 3202.58(95% CI: 2225.83 - 4174.17) parasites/μl. Parasitaemia was classified as 5(16.1%) of the *Plasmodium* infected had mild, 23(74.2%) had moderate, and 3(9.7%) had severe parasitaemia.

5.2.2 Measures of diagnostic performance of RDT against microscopy as gold standard

To calculate malaria rapid test performance and acceptability evaluation indices; microscopy was used as the gold standard. Variables measured included the number of true positive (TP), true negative (TN), false positive (FP), and false negative (FN) results. Results were considered false positive for if microscopy failed to detect parasites which had been detected by rapid test, and false negative for if microscope detected parasites which had not been detected by RDTs. The true values were the same values by the two methods (91-93).

The table below shows the results of diagnostic tests presented in a 2x2 table: columns summarize the gold standard test result or blood smear result and the rows summarize RDT test results. The labels positive and negative refer to the presence or absence, respectively, of the malaria infection. The numbers of subjects with malaria by microscopy, and testing positive and negative by RDT are denoted by “a and c”, respectively. The numbers of subjects without malaria by microscopy, and testing positive and negative by RDT are denoted by b and d, respectively. The total number of study subjects is a + b + c + d.

Table 2. Diagnostic performance of the SD BIOLINE Malaria Ag *P.f/P.v* POCT test, Arbaminch Zuria District, April-June, 2013.

Result of diagnostic test (SD BIOLINE Malaria Ag *P.f/P.v* POCT test)	**Results of Gold Standard Test (Blood smear microscopy)**		Total
	Positive	Negative	
Test positive	a→31	b→2	33
Test negative	c→0	d→308	308
Total	31	310	341

*Note: True positive (**a**), False positive (**b**), False negative **(c)** and True negative (**d**).*

The indices were calculated as follows based on the method evaluation guides **(94, 95)**.

$$\text{Sensitivity of RDT} = \frac{\text{True Positive(a)}}{\text{True Positive(a)} + \text{False Negative(c)}} \text{X100\%}$$

$$= 31/31\text{x}100\% = 100\%$$

$$\text{Specificity of RDT} = \frac{\text{True Negative(d)}}{\text{True Negative(d)} + \text{False Positive(b)}} \text{X100\%}$$

$$= 308/310 \text{ x}100\% = 99.35\%$$

Positive predictive value (PPV) = TP/ (TP+FP) =31/33 = 93.94%

Negative predictive value (NPV)= TN/ (TN+FN) =308/308 = 100%

Accuracy of test = (TP + TN)/number of all tests=(31+308)/341 = 99.41%

Reliability of test = [(TP × TN) - (FP × FN)]/[(TP + FN)(TN+FP)]

= [(31x308)-(2x0)]/[(31+0)(308+2)]=308/310=99.35%

5.2.3 Assessement of possible risk factors associated with *Plasmodium* infection

Preliminary univariate analysis on binary logistic regression model was performed individually between each individual independent and the dependent variable. The outcome variable in our study is dichotomous, having two response categories__being positive or negative. Thus binary logistic regression model was chosen. Crude univariate analysis of the related risk factors with *Plasmodium* infection was placed below (Table. 3).

Table.3: Univariate regression analysis of predictors for *Plasmodium* spp. infection among asymptomatic pregnant woman in Arba Minch Zuria District, Southern Ethiopia, April to June 2013.

Variables	No(%)	Malaria status		Crude OR (95%CI)	P-value
		Negative (310=90.9%)	Positive (31=9.1%)		
Age groups					
≤20	32(9.4)	24(75.0)	8(25.0)	1.00	
21-25	140(41.1)	135(96.4)	5(3.6)	0.11(.03-0.37)	0.000*
26-30	102(29.9)	97(95.1)	5(4.9)	0.16(.05-0.52)	0.002*
31-35	47(13.8)	38(80.9)	9(19.1)	0.71(.24-2.10)	0.535
≥36	20(5.9)	16(80.0)	4(20.0)	0.75(.19-2.91)	0.678
Occupation					
Farmer	98(28.7)	89(90.8)	9(9.2)	0.30(0.07-1.33)	0.113*
Daily laborer	40(11.7)	30(75.0)	10(25.0)	1.00(0.23-4.44)	1.000
Merchant	57(16.7)	54(94.7)	3(5.3)	0.17(0.03-0.96)	0.045*
House wife	134(39.3)	128(95.5)	6(4.5)	0.14(0.03-0.66)	0.013*
Civil servant	12(3.5)	9(75.0)	3(25.0)	1.00	
Education					
Illiterate	59(17.3)	49(83.1)	10(16.9)	1.35(0.42-4.30)	0.615
Read/write	15(4.4)	12(80.0)	3(20.0)	1.65(0.34-7.98)	0.534
Primary	134(39.3)	125(93.3)	9(6.7)	0.48(0.15-1.51)	0.208*
Secondary	95(27.9)	91(95.8)	4(4.2)	0.29(0.07-1.15)	0.077*
College/above	38(11.1)	33(86.8)	5(13.2)	1.00	
Income					
<650 ETB	204(59.8)	182(89.2)	22(10.4)	1.63(0.51-5.17)	0.409
650-1300 ETB	102(29.9)	95(93.1)	7(6.9)	0.87(0.27-2.84)	0.822
>1300 ETB	35(10.3)	33(94.3)	2(5.7)	1.00	
Family size					
1-3	116(34.0)	102(87.9)	14(12.1)	1.00	
4-7	168(49.3)	157(93.5)	11(6.5)	0.51(0.22-1.17)	0.111*
>7	57(16.7)	51(89.5)	6(10.5)	0.86(0.31-2.36)	0.766

- Note: Variables with asterisk(*) are significant at P-value of 0.25 and thus are candidate for multivariate binary logistic regression model!

Continued from Table 3…

Variables	No (%)	Malaria status		Crude OR (95%CI)	P-value
		Negative (310=90.9%)	Positive (31=9.1%)		
Parity					
Primigravidae	91(26.7)	77(84.6)	14(15.4)	3.25(1.30-8.09)	0.011*
Secondgravida	99(29.0)	90(90.9)	9(9.1)	1.79(0.67-4.80)	0.249*
Multigravida	151(44.3)	143(94.7)	8(5.3)	1.00	
Gestational age					
1st trimester	83(24.3)	77(92.8)	6(7.2)	0.84(0.29-2.41)	0.748
2nd trimester	140(41.1)	125(89.3)	15(10.7)	1.30(0.56-3.01)	0.545
3rd trimester	118(34.6)	108(91.5)	10(8.5)	1.00	
ITN use					
Use always	187(54.8)	181(96.8)	6(3.2)	1.00	
Use sometimes	19(5.6)	14(73.7)	5(26.3)	10.77(2.92-39.75)	0.000*
Do not use	135(39.6)	115(85.2)	20(14.8)	5.25(2.05 -13.46)	0.001*
IRS(past 12 months)					
Yes	289(84.8)	267(92.4)	22(7.6)	1.00	
No	52(15.2)	43(82.7)	9(17.3)	2.54(1.10-5.88)	0.03*

- Note: variables with asterisk (*) are significant at P-value of 0.25 and thus are candidate for multivariate binary logistic regression model!

Based on the purposeful selection of variables in logistic regression as proposed by "Hosmer and Lemeshow(88, 89)", preliminary univariate analysis results with a P-value of less than 25% were selected as a candidate variable for the multivariate analysis. This rule designed with the aim of maintaining numerical stability of the parameter estimates and generalizability of the results by decreasing the standard error. Accordingly, variables having a P-value of less than 25% in the univariate analysis (Table 3), were selected and entered in to multivariate analysis on Binary Logistic Regression Model (Table 4).

Table. 4: Multivariate regression analysis of predictors for *Plasmodium* spp. infection among asymptomatic pregnant woman in Arba Minch Zuria District, Southern Ethiopia, April to June 2013.

Variables	No (%)	Malaria status		AOR(95%CI)	P-value
		Negative 310=90.9%	Positive 31=9.1%		
Parity					
Primigravidae	91(26.7)	77(84.6)	14(15.4)	84.40(7.30-976.53)	0.000*
Secondgravida	99(29.0)	90(90.9)	9(9.1)	16.34(2.98-89.53)	0.001*
Multigravida	151(44.0)	143(94.7)	8(5.3)	1.00	
Age					
≤20	32(9.4)	24(75.0)	8(25.0)	1.00	
21-25	140(41.1)	135(96.4)	5(3.6)	0.29(0.07-1.21)	0.089
26-30	102(29.9)	97(95.1)	5(4.9)	3.21(0.45-22.78)	0.244
31-35	47(13.8)	38(80.9)	9(19.1)	24.74(2.31-265.42)	0.008*
≥36	20(5.9)	16(80.0)	4(20.0)	69.26(3.99-1200.86)	0.004*
Occupation					
Farmer	98(28.7)	89(90.8)	9(9.2)	0.55(0.06-4.75)	0.586
Daily laborer	40(11.7)	30(75.0)	10(25.0)	2.94(0.29-29.67)	0.360
Merchant	57(16.7)	54(94.7)	3(5.3)	0.69(0.07-7.11)	0.756
House wife	134(39.3)	128(95.5)	6(4.5)	0.29(0.04-2.22)	0.231
Civil servant	12(3.5)	9(75.0)	3(25.0)	1.00	
ITN use					
Use always	187(54.8)	181(96.8)	6(3.2)	1.00	
Use sometimes	19(5.6)	14(73.7)	5(26.3)	10.22(1.80 –57.95)	0.009*
Do not use at all	135(39.6)	115(85.2)	20(14.8)	4.61(1.48-14.41)	0.009*
IRS(past 12 months)					
Yes	289(84.8)	267(92.4)	22(7.6)	1.00	
No	52(15.2)	43(82.7)	9(17.3)	2.19(0.62-7.76)	0.225
Family size					
1-3	116(34.0)	102(87.9)	14(12.1)	1.00	
4-7	168(49.3)	157(93.5)	11(6.5)	1.92(0.40-9.26)	0.419
>7	57(16.7)	51(89.5)	6(10.5)	2.16(0.31-14.88)	0.435
Education					
Illiterate	59(17.3)	49(83.1)	10(16.9)	0.47(0.06-3.59)	0.463
Read/write	15(4.4)	12(80.0)	3(20.0)	0.76(0.06-9.17)	0.829
Primary	134(39.3)	125(93.3)	9(6.7)	0.44(0.08-2.51)	0.351
Secondary	95(27.9)	91(95.8)	4(4.2)	0.23(0.03-1.62)	0.142
College/above	38(11.1)	33(86.8)	5(13.2)	1.00	

- Note: Asterisk (*) stands for significant association at 5% P-value in multivariate analysis.

From the variables that were included in the multivariate analysis, being primigravidae, secondigravidae, rare and/or total absence of ITN use, and age group showed significant association with malaria infection at P-value of ≤ 5%. The odds of being infected with *Plasmodium* was 84.40[(95%CI: 7.30-976.53), P-value<0.001] times higher among primigravidae, 16.34[(95%CI: 2.98-89.53), P-value = 0.001] times higher among secondigravida, 10.22[(95% CI: 1.80 –57.95), P-value=0.009] times higher among women who were using ITN sometimes, 4.61[(95% CI: 1.48-14.41), P-value=0.009] times higher among those who did not use ITN at all compared to multigravida and those who were using ITN always, respectively. Pregnant women in the age group of 31-35 years were 24.74[(95% CI: 2.31-265.42), P=0.008] times, and those in ≥36 years were 69.26[(95% CI: 3.99-1200.86), P-value=0.004] times, more likely to have asymptomatic malaria when compared to pregnant women of young age (Table 4).

5.2.4 Hematocrit determination

Of the total 341 study participants, 118 (34.6%) were anemic. The minimum and maximum HCT values were 18% and 46%, respectively, while the mean was 35.2% (95% CI:34.6%–35.8%) with STD of ±*5.55%*. Of those 118 anemic women; 73(61.9%) were mildly anemic, 38(32.2%) were moderately anemic, and 7(5.9%) were found to be severely anemic. Prevalences of anemia in different sociodemographic and economic factors were placed in the table (Table 5).

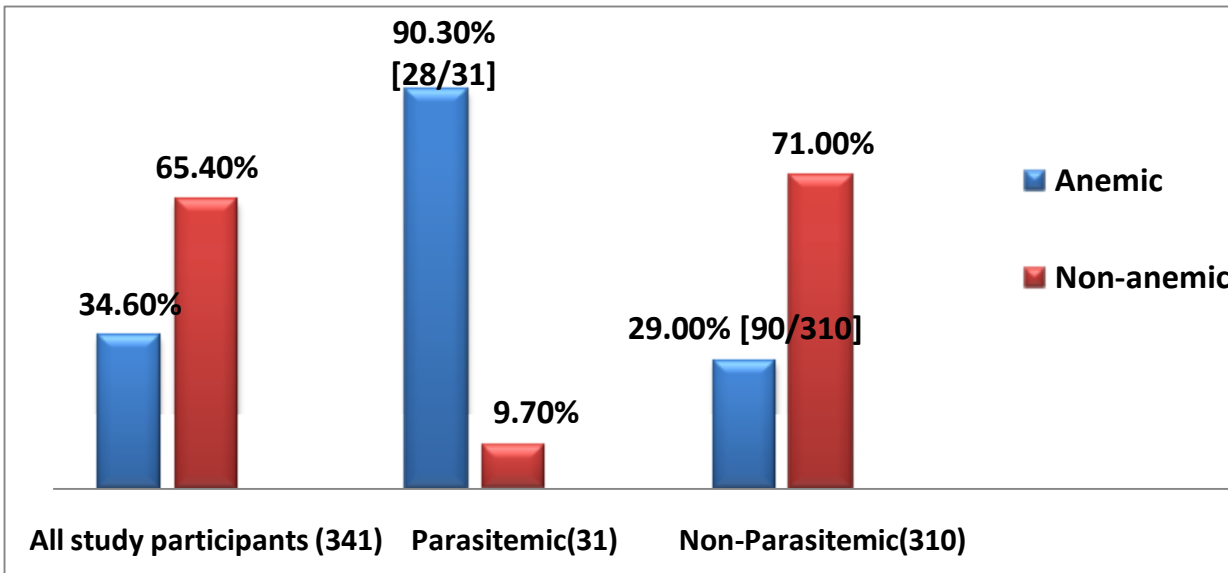

Figure 3. Anemia prevalence among pregnant women in the Arba Minch Zuria District, Southern Ethiopia, from April to June 2013.

5.2.5 Asssessment of possible risk factors to anemia

As used above for assessement of risk factors of *Plasmodium* infection, the same steps and rules for variable inclusion and analysis were used here for assessing risk factors of anemia (Table 5).

Table 5: Univariate regression analysis of predictors for anemia among pregnant woman in Arbaminch Zuria District, Southern Ethiopia, April to June 2013

Variables	No(%)	Anemia status		COR (95%CI)	P-value
		Non-Anemic (223=65.4%)	Anemic (118=34.6%)		
Age					
≤20	32(9.4)	21(65.6)	11(34.4)	0.35(.11-1.11)	.074*
21-25	140(41.1)	99(70.7)	41(29.3)	0.28(.11-.73)	.009*
26-30	102(29.9)	71(69.6)	31(30.4)	0.29(.11-.78)	.014*
31-35	47(13.8)	24(51.1)	23(48.9)	0.639(.22-1.85)	.408
>35	20(5.9)	8(40.0)	12(60.0)	1.00	
Occupation					
Farmer	98(28.7)	61(62.2)	37(37.8)	1.21(.34-4.31)	.765
Daily laborer	40(11.7)	20(50.0)	20(50.0)	2.00(.52-7.72)	.315
Merchant	57(16.7)	40(70.2)	17(29.8)	.850(.23-3.21)	.810
House wife	134(39.3)	94(70.1)	40(29.9)	.851(.24-2.99)	.801
Civil servant	12(3.5)	8(66.7)	4(33.3)	1.00	
Education					
Illiterate	59(17.3)	29(49.2)	30(50.8)	3.88(1.53-9.85)	.004*
Read/write	15(4.4)	8(53.3)	7(46.7)	3.28(.91-11.80)	.069*
Primary	134(39.3)	85(63.4)	49(36.6)	2.16(.92-5.10)	.077*
Secondary	95(27.9)	71(74.7)	24(25.3)	1.27(.51-3.14)	.608
College/above	38(11.1)	30(78.9)	8(21.1)	1.00	
Income					
<650 ETB	204(59.8)	122(59.8)	82(40.2)	3.01(1.38-6.58)	.003*
650-1300 ETB	102(29.9)	70(68.6)	32(31.4)	1.97(.92-4.22)	.027*
>1300 ETB	35(10.3)	31(88.6)	4(11.4)	1.00	
Family size					
1-3	116(34.0)	76(65.5)	40(34.5)	0.90(.55-1.49)	0.680
4-7	168(49.3)	114(67.9)	54(32.1)	1.38(.72-2.65)	0.330
>7	57(16.7)	33(57.9)	24(42.1)	1.00	
Malaria					
Negative	310(90.9)	220(71.0)	90(29.0)	1.00	
Positive	31(9.1)	3(9.7)	28(90.3)	22.82(6.77-76.94)	0.000*

Note: variables with asterisk (*) were candidate for multivariate analysis (p<0.25).

Continued from Table 5…

Variables	No (%)	Anemia status		COR (95%CI)	P-value
		Non-Anemic (223=65.4%)	Anemic (118=34.6%)		
Parity					
Primigravidae	91(26.7)	60(65.9)	31(34.1)	.93(.54-1.60)	.789
Secondgravida	99(29.0)	66(66.7)	33(33.3)	.90(.53-1.53)	.693
Multigravida	151(44.3)	97(64.2)	54(35.8)	1.00	
Gestational age					
1st trimester	83(24.3)	55(66.3)	28(33.7)	1.26(.69-2.30)	.457
2nd trimester	140(41.1)	84(60.0)	56(40.0)	1.647(.98-2.78)	.061*
3rd trimester	118(34.6)	84(71.2)	34(28.8)	1.00	
ITN use					
Use always	187(54.8)	149(79.7)	38(20.3)	1.00	
Use sometimes	19(5.6)	7(36.8)	12(63.2)	6.72(2.48-18.23)	0.000*
Do not use at all	135(39.6)	67(49.6)	68(50.4)	3.98(2.44-6.50)	0.000*
IRS(past 12 months)					
Yes	289(84.8)	195(67.5)	94(32.5)	1.00	
No	52(15.2)	28(53.8)	24(46.2)	1.78(0.98-3.23)	0.059*

Note: variables with asterisk (*) were candidate for multivariate analysis ($p<0.25$).

As used above for "malaria infection", the variable inclusion rule for multivariate analysis by Hosmer and Lemeshow (89) was again applied here for the second dichotomous dependant variable__ "anemia". Variables with P-value of less than 25% in the bi-variable analysis(Table. 5), were selected as a candidate variable and entered in multivariate analysis model(Table. 6).

From multivariate analysis, the independent predictors of anemia were malaria positivity, and rare use and/or complete non-use of ITN (Table. 6). The odds of being anemic were 19.59 [(95% CI: 5.06-75.81), P-value≤0.001] times more likely to occur in malaria positives, 5.96 [(95% CI: 1.82-19.47), P-value=0.003] times more likely to occur in rare ITN users, 2.99 [(95% CI: 1.72-5.22), P-value<0.001] times more likely to occur in those who did not use ITN completely relative to malaria negatives and those who were using ITN always, respectively.

Table 6: Multivariate regression analysis of predictors for anemia among pregnant woman in Arbaminch Zuria District, Southern Ethiopia, April to June 2013.

Variables	No(%)	Anemia status		AOR (95%CI)	p-value
		Non-Anemic (223=65.4%)	Anemic (118=34.6%)		
Age					
≤20	32(9.4)	21(65.6)	11(34.4)	.17(.01-5.94)	0.327
21-25	140(41.1)	99(70.7)	41(29.3)	.70(.09-5.42)	0.734
26-30	102(29.9)	71(69.6)	31(30.4)	.61(.08-4.60)	0.632
31-35	47(13.8)	24(51.1)	23(48.9)	.83(.23-3.02)	0.774
>35	20(5.9)	8(40.0)	12(60.0)	1.00	
Education					
Illiterate	59(17.3)	29(49.2)	30(50.8)	2.45(.43-14.04)	0.316
Read/write	15(4.4)	8(53.3)	7(46.7)	2.14(.27-17.19)	0.475
Primary	134(39.3)	85(63.4)	49(36.6)	1.52(.30-7.78)	0.618
Secondary	95(27.9)	71(74.7)	24(25.3)	.71(.13-3.90)	0.692
College/above	38(11.1)	30(78.9)	8(21.1)	1.00	
Income					
<650 ETB	204(59.8)	122(59.8)	82(40.2)	2.15(.65-7.05)	0.207
650-1300 BR	102(29.9)	70(68.6)	32(31.4)	1.51(.49-4.68)	0.476
>1300 ETB	35(10.3)	31(88.6)	4(11.4)	1.00	
Malaria					
Negative	310(90.9)	220(71.0)	90(29.0)	1.00	
Positive	31(9.1)	3(9.7)	28(90.3)	19.59(5.06-75.81)	0.000*
Gestational age					
1st trimester	83(24.3)	55(66.3)	28(33.7)	0.56(0.21-1.51)	0.249
2nd trimester	140(41.1)	84(60.0)	56(40.0)	1.59(0.78-3.22)	0.199
3rd trimester	118(34.6)	84(71.2)	34(28.8)	1.00	
ITN use					
Use always	187(54.8)	149(79.7)	38(20.3)	1.00	
Use sometimes	19(5.6)	7(36.8)	12(63.2)	5.96(1.82-19.47)	0.003*
Do not use at all	135(39.6)	67(49.6)	68(50.4)	2.99(1.72-5.22)	0.000*
IRS(In past 12 months)					
Yes	289(84.8)	195(67.5)	94(32.5)	1.00	
No	52(15.2)	28(53.8)	24(46.2)	0.80(0.32-2.03)	0.644

- Note: variables with asterisk (*) are significant in multivariable model (P<0.05).

5.2.6 Correlation between malaria parasite counts and hematocrit values

We assessed Pearson correlation between the continous variables malaria parasite counts and hematocrit values. This was carried out to determine the effect of malaria parasite load on the value of HCT. There was found statistically significant correlation between increasing malaria parasite load and decreasing hematocrit values ($r = -0.463$, $P = 0.009$) (Table 7).

Table 7. Correlation between HCT value and parasite density

		HCT value	Parasite density
HCT value	Pearson correlation	1	-.463**
	Sig.(2-tailed)		0.009
	No	341	31
Parasite density	Pearson correlation	-.463**	1
	Sig. (2-tailed)	0.009	
	No	31	31

**. Correlation is significant at the 0.01 level (2-tailed).

Chapter Six

6.1 Discussion

This work aimed at studying asymptomatic *P.falciparum or P.vivax* infection among pregnant women living in the areas of stable malaria transmission in Arba Minch Zuria District, Southern Ethiopia. This study has revealed malaria prevalence among the women as 9.1% and 9.7% by using blood smear microscopy and the SD BIOLINE Malaria Ag *P.f/P.v* POCT test, respectively. This prevalence has a little bit the characteristic of malaria-endemic areas, where malaria prevalence among pregnant women is from 10% to 65% (54).

Malaria prevalence obtained in this study matches with some of previous works done in Ethiopia. Almost similar figures have been reported by similar studies in Ethiopia which reported asymptomatic malaria prevalence of 10.4 % among pregnant women living in stable malaria transmission areas (56) and prevalence of 11.59 % among pregnant women living in Gilgel Gibe Dam area, Ethiopia (60). This is also confirmed that asymptomatic malaria prevalence is high in pregnant women in Ethiopia as indicated in the study conducted in Fincha Sugar Factory area where the majority (70.3%) of malaria cases found were asymptomatic with only small proportion (29.7%) being febrile. Among the infected individuals, 8.1% were pregnant women (96).

There are almost matching asymptomatic malaria prevalences that have been reported from similar studies carried out in Africa such as 10.9% prevalence from Uganda (67), and 13.6% prevalence from Rwanda (71). However, this study finding is contrary to findings from most studies from Africa such as 57% of asymptomatic malaria prevalence from Gabon (69), 58.4% from Enugu, Nigeria (68), 95.4% from Calabar, Nigeria (66), 36% from Ghana (38). This variation might be mainly due to a very higher malaria transmission intensity in these African countries than Ethiopia and less disease transmission in all areas currently (12). This might also be due to the differences in the diagnostic methods used by these different studies.

The difference between the two diagnostic methods in this study was minor that it may perhaps be explained by the persistence of *Plasmodium* antigens after the clearance of parasites from past malaria exposures (97). In this study, the SD BIOLINE Malaria Ag P.f/P.v POCT test had a sensitivity of 100% and a specificity of 99.35% for the detection of asymptomatic malaria

compared to microscopy. This study results agree with findings from similar studies conducted among pregnant women in Burkina Faso, Cote d'Ivoire and Senegal with sensitivities of 100%, 100%, and 96%, and specificities of 92%, 88% , and 87%, respectively (11, 64, 65).

The overall prevalence of infection was significantly higher in primigravidae, where 14 (15.4%) of 91 women were positive, followed by secondigravida with 9 (9.1%) of 99 women tested positive. The lowest occurrence was in multigravida with 8 (5.3%) of 151 women tested positive. There was a strong association between increasing gravidity and decreasing rates of peripheral malaria infection. Primigravidae and secundigravidae had nearly 84 times (P-value < 0.001)) and 16 times (P-value = 0.001) higher risk of being infected with *Plasmodium* than multigravidae, respectively. This agrees with findings of several similar studies from sub-Saharan African countries (61, 68, 71, 98) where the prevalence of asymptomatic *Plasmodium* infection was significantly higher in the lower gravidity than the higher gravidity.

This can be attributed to a low recovery rate of infections during early pregnancy which might be linked to infection-specific immunological factors. Some *Plasmodium* infected erythrocytes sequester in the maternal placenta by producing surface antigens, mainly variant surface antigen (VAR2CSA), that adhere to chondroitin sulphate-A (CSA) receptors expressed by syncytiotrophoblasts in the placenta. Primigravidae and secondigravida are more susceptible to infection, as they lack these anti-adhesion antibodies against CSA-binding parasites, which develop only after successive pregnancies (57).

The prevalence of asymptomatic malaria infection in pregnant women who did not use ITN totally was 14.8 % (20/135), in those who used ITN rarely was 26.3 % (5/19) and in those who used always was 3.2% (6/187). This was statistically significant that the pregnant women who used ITN sometimes were 10.22 times (P-value=0.009)) and those who did not use ITN totally were 4.61 times (P-value=0.009) more likely to be infected with *Plasmodium* than those who were using ITN always. This finding matches findings from other similar studies conducted from Nigeria (68, 73), from Rwanda (71), from Gilgel Gibe Dam area, Ethiopia (60) and from Burkina Faso (11).

A prospective cohort study in one of our study villages in the District also indicated the low utilization of ITN in the area and its effect as risk factor for malaria which was possibly

attributed to psychological effects of suffocation by pregnant women while sleeping under the net, ignorance of the risks of malaria during pregnancy, cost of the net in self-marketing, having only worn-out bed nets (ragged by rats), hanging problem due to dirty bed nets, changes in bed arrangement, unsuitable housing structure and considering alternatives such as insecticide sprays (IRS), utilization of bed nets as bed sheets to cover the mattress of a bed, using the nets as curtains for doors, windows and traditional pit latrines (74).

Malaria was more common in the 2nd trimester, where 15(10.7%) of 140 women were positive, followed by those in 3rd trimester with 10(8.5%) of 118 women positive, and the lowest rate was in the 1st trimester with 6(7.2%) of 83 women were found positive. However, these relationships were not statistically significant as in 1st trimester (P-value=0.748), 2nd trimester (P-value=0.545). This is similar to findings from study in central Nigeria (68).

Pregnant women in old age (≥30 years) were more likely to have asymptomatic malaria infection than women of young age (<30 years). The prevalence of asymptomatic malaria parasitemia among young women was 6.57% (18/274) while among old women was 19.4% (13/67). This difference was statistically significant that pregnant women in the age group of 31-35 years were 24.74 times (P=0.008), and those in ≥ 36 years were 69.26 times (P-value=0.004) more likely to have asymptomatic malaria when compared to pregnant women of young ages. This finding meets the truth that asymptomatic malaria prevalence is higher in older age groups and in those living for longer periods in malaria endemic region (10, 39). However, our finding is contrary to findings of some similar studies from Nigeria (68), Gabon (69) and India (72) which suggested that peripheral parasitaemia was more common in pregnant women of younger age groups than old ages. This contrast might be due to differences in the size of cases, sampling technique, physiologic and biochemical factors of pregnant women and the study setting such as geography, altitude and temperature (22).

In the current study, the prevalence of anaemia in the pregnant women was 34.6% (118/341). Anaemia prevalence in this study almost agrees with those of previous works from Jimma health center, Ethiopia 41.9% (75) and Asendabo health center, Ethiopia 23% (76), Uganda 39.7% (67), and from Ghana 44% (38) but lower than the anaemia prevalence of 53.9% in Gilgel Gibe Dam area, Ethiopia (60) and 51.9% from Bushulo health center, Ethiopia (77), 61% from Burkina

Faso (11), 55.2% from Nigeria (68), 71% from Gabon (69) and higher than that from Rwanda 11.8% (71).

Of those 118 anemic pregnant women; 73 (61.9%) were mildly anemic, 38 (32.2%) were moderately anemic, and 7(5.9%) were severely anemic. This finding is almost akin to findings from Gilgel Gibe Dam area Study, Ethiopia (60), which reported; 55%, 42.1%, and 2.9% as mild, moderate and severe anaemia, respectively. Nevertheless, current study finding is higher than the result of comparable study in Malaysia, particularly in the cases of moderate anemia, which reported 45%, 9.8%, and 1.85% with mild, moderate, and severe anaemia, respectively (99).

Anaemia was significantly associated with the occurrence of *Plasmodium* spp. infection. In this study, the independent predictors of anemia were malaria positivity, rare and complete non-use of ITN. This study findings indicated that pregnant women who were *Plasmodium* infected, and those using ITN sometimes and/or did not use ITN at all during the study period were more likely to be anaemic relative to those not infected of *Plasmodium* and using ITN always, respectively. In fact, 90.3%(28/31) of *Plasmodium* infected pregnant women were anaemic compared with 29.0% (90/310) were anaemic among non-parasitemic women. This difference was statistically significant that pregnant women infected with *Plasmodium* had 19.59 times (P-value $\leq$ 0.001) higher risk of developing anaemia, relative to malaria negatives. The odds of being anemic were 5.96 times (P-value=0.003) more likely to occur in rare ITN users, 2.99 times (P-value < 0.001) more likely to occur in those who did not use ITN totally relative to those who were using ITN always.

This result matchs with the findings from Gilgel Gibe Dam area study, Ethiopia (60), where 93.3% of *Plasmodium* infected pregnant women were anemic, as the independent predictors of anaemia in the study were non-ITN use and *Plasmodium* positivity. Other related findings were from Burkina Faso where anaemia was significantly more common in women infected with *Plasmodium* (33% anemia prevalence) compared with the uninfected pregnant women (10% prevalence of anemia) (11), from Uganda where anemia showed a strong association with malaria (67), and from Rwanda where parasitaemia, gravidity, and district were found to be significant risk factors for anaemia (71).

The prevalence of anemia in primigravidae was 34.1 % (31/91), in secondigravida was 33.3 % (33/99), in multigravida was 35.8% (54/151). We did not find a statistically significant relationship between anaemia and gravidity. The likelihood of being anemic in primigravida (P-value=0.789), secondigravida (P-value=0.693) comparative to multigravida. This is in accordance with a previous report in Nigerian pregnant women (61, 66) and Burkinafaso (11). However, there are other studies which have revealed higher rates of anaemia among primiparous women compared to multiparous ones as of Sekyere West District Ghana (70) and Rwanda (71). This difference could be explained by physiological changes and other factors associated with pregnancy.

In the current study, anemia prevalence in 1st trimester was 33.7 %(28/83), in 2nd trimester was 40 %(56/140) and 3rd trimester was 28.8 %(34/118). There was no significant difference seen among gestational age groups in terms of anemia status as in 1st trimester (P-value=0.249), 2nd trimester (P-value=0.199) when related to those in 3rd trimester. This finding is akin to that of analogous study from calabar Nigeria (66).

The prevalence of anemia reflects that of malaria. It was lower in younger (30.29%; 83/274) than older pregnant women (52.24%; 35/67). However, this relationship was not statistically significant. This is contrary to findings from Nigeria (61). The variation might be due to geography, life style, physiology and other factors of the study subjects. Degree of anemia did not differ significantly by parasite density. There was no significant difference (P-value=0.470) in the density of parasitaemia in those with mild, moderate, and severe anemia. However, Pearson correlation carried out to determine the effect of malaria parasite load on the value of HCT indicated that there was a significant correlation between increasing malaria parasite load and decreasing hematocrit values ($r = -0.463$, $P = 0.009$). This result was comparable to findings from Gilgel Gibe Dam area study, Ethiopia (60), Nigeria(73) where there was found a significant correlation between increasing parasite counts and decreasing haemoglobin levels.

Chapter Seven

7. Conclusion, Recommendation, Strength and Limitation of the Study

7.1 Conclusion

The prevalence of asymptomatic *Plasmodium* infection among the pregnan women in the current study is moderate. However, it showed significant association with the high prevalence of anemia in the women. Given the high risk of the pregnant women and their infants, asymptomatic malaria can result in different adverse health effects during pregnancy if it is left undetected & untreated. Symptomless malaria may pose new problems for the currently adopted strategy for the control of malaria in Ethiopia, which is essentially based on the treatment of symptomatic patients. Thus to better manage the disease, it is worthwhile to screen both sick and 'healthy' people and treat them promptly. Malaria had shown significant association with anemia and it may account in mother to child transmission. There was a significant correlation between increasing parasite load and decreasing hematocrit values, particularly amongst the pregnant women of first and second pregnancy. The independent predictors of malaria infection in our study were first and second pregnancy, total absence or rare ITN use and maternal age. Where as, the rare use and/or total absence of ITN utilization and *Plasmodium* infection were found to be independent predictors of anaemia. As part of ANC service package, education on how to use the bed nets and encouraging early ANC attendance among pregnant women could rapidly enhance benefits for the women's health. The SD BIOLINE Malaria Ag *P.f/P.v* POCT rapid test had shown a high sensitivity and specificity against blood smear microscopy in diagnosis of malaria in the current study. The RDTs have the advantage of detecting circulating antigens, even when the parasites are sequestered in the deep circulation and not visible by microscopy**.** Therefore, the RDTs can provide a feasible platform for diagnosis of the asymptomatic malaria in rural or peripheral communities.

7.2 Recommendation

For areas in which malaria transmission is stable but of relatively low intensity, mass distribution of free ITNs, coupled with increasing use of long-lasting ITNs, at first ANC visits as part of health education on malaria during pregnancy, spraying of houses with insecticides(IRS) atleast twice and more times per a year, nationwide adoption of artemisinin-based combination therapy

(ACT) and possibly vector control measures are an attractive alternative. Finally, based on the findings of this study, the following specific recommendations were forwarded:

1. The Health office of the Arba Minch Zuria District should again design comprehensive preventive strategy including scheduled spray of houses with insecticides and education on effective use of ITN for these low-income rural pregnant women living in malaria endemic area. Given the challenges of re-impregnating bednets, the use of long-lasting ITNs would be preferable.

2. ANC clinic of the District Health Post should further promote its service by providing health education to mothers regarding ANC follow up and ITN utilization

3. As indicated in the current study, majority of the ANC non-attendee pregnant women were at their first trimester. So, the women should be thought & encouraged strongly to attend antenatal care early in their gestation; this could rapidly increase benefits for their health.

4. National malaria control programmes should design strategies that would help the systematic diagnosis of malaria among pregnant women (living in endemic region) during their ANC visits in addition to those already in practice: Hgb level, syphilis and blood group.

5. The District Health Bureau must hold conversations with the District Kebele Leaders and the community on the efforts that should be made to incorporate mosquito prevention measures such as reduction of breeding sites like bushes near living homes.

6. Finally, we recommend further investigation related to asymptomatic malaria parasitemia and anemia among pregnant women should be given due attention in order to determine true effect of asymptomatic malaria on birth outcomes and to identify the most predictor variables to enhance compliance.

7.3 Strength of the study

This study was successfully accomplished in a rural community with a difficult socio-topographic environment, irrespective of the usual difficulties encountered during community assessment. In view of the fact that this study is community based and built-in multistage probability sampling techniques, it increases the chance of generalizability to the whole pregnant population in the district. Additionally, incorporation of the two diagnostic methods in detection

of asymptomatic malaria in the major risk groups (pregnant women) is also one of the strong approachs in malaria control.

7.4 Limitation of the study

Since this is cross-sectional study which measures both the exposure and outcome simultaneously, it does not allow for an understanding of the natural history of asymptomatic parasitaemia during the course of gestation. Unluckily, we did not also evaluate some confounding factors to anemia such as helminthic infections and chronic medical conditions including human immunodeficiency virus sero status. We acknowledge that the lack of follow-ups to determine the true impact of asymptomatic *Plasmodium* parasitemia is a limitation of this study, attributed to the unsuitable conditions of pregnancy related risks.

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Annexes

Annex 1: Information sheet for study subjects

Principal investigator: Desalegn Nega

Organization: Jimma University, College of Public Health and Medical Sciences, Department of Medical Laboratory Sciences, and Pathology

Sponsor: Jimma University, College of Public Health and Medical Sciences

We would like to conduct a medical research entitled "Asymptomatic *Plasmodium* infection among pregnant women in Arba Minch Zuria District, southern Ethiopia". We are requesting you to give your consent to participate in this study voluntarily. We are going to inform you about the purpose, responsibility of investigators or data collectors to keep confidentiality and how we are going to use the data.

Description and purpose of the study

Malaria is a major public health problem in Ethiopia, where about two-thirds of the population is at risk of infection**.** Pregnant women are highly susceptible to malaria than other human population. The symptom of malaria are fever, sweating, malaise, head ache, joint and back pain. Asymptomatic carriers of malaria parasite constitute a reservoir for disease transmission and asymptomatic malaria in pregnant women can cause maternal anemia and risks to new baby. Therefore this study aimed at diagnosing and treating asymptomatic pregnant women to reduce health risks of the mother and her baby, and to halt down parasite reservoirs for further disease transmission.

Procedures

If you agree to participate in this study and sign the consent form, then the following will be done:

- Your past and present medical histories, and socio-demographic factors will be assessed
- You will provide us a 10 minutes' interview
- We will take a little blood sample (3-4 drops) from your finger for malaria diagnosis and hematocrit determination.
- The results of laboratory will be given to you for treatment if positive.

Risks and discomforts

During all sample collection, we will follow standard operational procedures. You may experience some minor pain during the collection of blood by finger prick. Appropriate medical care will be provided to you.

Benefits

This study will benefit you directly. If your result from the diagnosis is found positive, immediately you will be referred for treatment in your nearby health facilities.

Compensation

You will not be offered any payment for participating in this study.

Costs

There are no costs associated with the study

Confidentiality

We respect your privacy and confidentiality. Your records in the study will be identified by a subject identification number/code and not by your name. Any information that identifies you will not be shared with anyone else without your written permission. If a research article is to be published from this study, you will not be identified by any name. The information we collect from you as part of the study will be kept in a locked file cabinet in a locked building. We will be entering some of your information into a computer file. This will be protected by a password only accessible to personnel involved in the study.

Voluntary Participation and Withdrawal from the Study

Your participation in this study is completely voluntarily, and you have a full right to accept or refuse it. You can stop your participation in the study at any time after giving your consent. This decision will not affect in any way your current or future medical care or any other benefits to which you are otherwise entitled. The professional/investigator may stop you from taking part in this study at any time if they decide it is in your best interest.

Annex 2: Amharic version of information sheet

ቅጥያ 7: የጥናቱ ተካፋዎች የመረጃ ቅጽ

የዋና ተመራማሪ ስም፡ ደሳለኝ ነጋ

የድርጅቱ ስም፡ በጅማ ዩኒቨርሲቲ የህብረተሰብ ጤና እና የህክምና ሰይንስ ኮሌጅ፤ሜድካል ላቦራቶሪ ሰይንስ እና ፓቶሎጂ ትምህርት ክፍል

ድጋፍ አድራጊ (ስፖንሰር) ፡ ጅማ ዩኒቨርሲቲ የህብረተሰብ ጤና እና የህክምና ሰይንስ ኮሌጅ፣ የድህረ ምረቃ ትምህርት ቤት

ምንም ዐይነት የበሽታ ምልክት በማይታባቸዉ ነፍሰ ጡር እናቶች ላይ የወባ በሽታን የሚያስከትሉ ተህዋሲያን በደማቸዉ ዉስጥ መኖር አለመኖራቸዉንና ከዚህ ሁኔታ ጋር ተጓዳኝ ሆነዉ የሚከሰተዉ የደም ማነስ በሽታ በእነሱ ምን እን□ሚመስል መፈተሽ በሚለው ጥናት ውስጥ ተሳታፊ ለሚሆኑ ነፍሰ ጡር እናቶች የተዘጋጀ የማብራሪያ ጽሁፍ፡፡

ከላይ በተጠቀሰው ርዕስ ላይ **በአርባ ምንጭ ዙሪያ ገጠር ወረዳ ዉስጥ** ጥናት ለማካሄድ አስበናል፡፡ በከፍተኛ አክብሮት እና ትህትና እርስዎ በዚህ ጥናት ውስጥ እንዲሳተፉ ይጠየቃሉ፡፡ ይህ ፎርም በዚህ ጥናት ውስጥ እርስዎ ለመሳተፍ ወይም ላለመሳተፍ ከመዎሰንዎ በፊት ሊያውቋቸው የሚገቡ ጠቃሚ መረጃዎችን በሙሉ አካቶ የያዘ በመሆኑ በጥሞና እንድያነቡ እና እንዲመረምሩ እንምክራለን፡፡

ስለ ጥናቱ በጥቂቱ

የወባ በሽታ ፕላስሙድዬም በሚባል ረቂቅ ተህዋሲያን የሚመጣ በሽታ ነው፡፡ ይህ በሽታ ሲመጣ የሚያሳያቸዉ ምልክቶች፡ ትኩሳትን መጨመር፤ ራስ ምታት፤ የማቅለሽለሽ ስሜት፤ የመገጣጠሚያና

የሰዉነት ጡንቻዎች ህመም፤ የድካም ስሜት ያስከትላል፡፡ ይህም የሚሆነው የበሽታዉ ተህዋሲያን እንግዳ አካል የሰዉነታችንን ደም ሴሎች ስለሚመገብ ነው፡፡ የዚህ ጥናት አላማም በአርባምንጭ ዙሪያ ወረዳ ዉስጥ በወባማ አከባቢ ባሉ **ነፍሰ ጡር** እናቶች ላይ የዚህ ረቂቅ ተህዋሲያን መኖር ወይም አለመኖሩን ለመፈተሽ ነው፡፡ በዚህ ጥናት ውስጥ ይህ ረቂቅ ተህዋሲያን ከተገኘ የማስተካከያ እርምጃዎች እንዲዎሰዱ ይደረጋል፡፡

የጥናቱ ሂደት ዝርዝር

በዚህ ጥናት ውስጥ እርስዎ ለመሳተፍ ከተስማሙ የሚከተሉትን ነገሮች እናደርጋለን፡፡

1. ከእርስዎ አንደበት በአጭር ቃለ መጠይቅ የእርስዎ የህክምና መረጃ ይሰበሰባል አካላዊ ምርመራም ይደረጋል፡፡
2. ከእጅዎ ጣት እጅግ በጣም አነስተኛ በሆነ ንጽህናው በተረጋገጠ መርፌ ከሦስት እስከ አራት ጠብታ የሚያህል የደም ናሙና ይወሰዳል፡፡
3. ከእርስዎ በተወሰደው ናሙና ላይ የወባ በሽታን የሚያስከትሉ ረቂቅ ተህዋሲያን ከተገኙና የሌሎች ተጓዳኝ ምርመራዎች ዉጤት በአቅራቢዎ ባሉ የህክምና ተቋሞች ተገቢዉን ህክምና እንዲያገኙ እንሰጦታለን፡፡

ስጋትና ጉዳት

በአጠቃላይ ከላይ የተጠቀሰዉ ናሙና ሲወሰድ ህክምናው የሚያስገድደውን የአሰራር ሂደት ስለምንከተል ሊያጋጥሙ የሚችሉ ጉዳቶች በጣም አነስተኛ ናቸው፡፡ ቢሆንም በጣት የደም ስር ስወጋ እጅግ በጣም አነስተኛ የሆነ የሕመም ስሜት ሊሠማ ይችላል፡፡ ይህ የሕመም ስሜት በመርፌ ስወጋ ብቻ ነዉ የሚሰማዉ፤ ከተወጋ በኋላ ወዲያሁኑ ይተዋል፡፡

ሊያገኟቸው የሚችሉ ጥቅሞች

የወባ በሽታን የሚያስከትሉ ረቂቅ ተህዋሲያን ከተገኙና የሌሎች ተጓዳኝ ምርመራዎች ዉጤት ነፍሰ ጡር እናቶች በአቅራቢያቸዉ ባለዉ የህክምና ተቋም ተገቢዉን ህክምና እንዲያገኙ ይሰጣቸዋል፡፡ በተጨማሪም የዚህ ጥናት ውጤት ለወደፊት ነፍሰ ጡር እናቶች ለክትትል ወደ ክሊንክ ስመጡ እንደሌሎች የተለሙዱ ምርመራዎች የተሻለ የወባ ምርመራ እንዲደርግላቸዉ ይጠቅማል፡፡ በዞኑ ወይም በክልል/በፈደራል ደረጃ የሚደረገዉ የወባ መከላከያ እና መቆጣጠሪያ ስትራተጂ የዚህን ጥናት ዉጤት እንዳስፈላግነቱ ግንዛበ ዉስጥ እንዲያስገባ ይጠቅማል፡፡

የካሳ ክፍያ

እርስዎ በዚህ ጥናት ውስጥ በመሳተፎ ከላይ ከተጠቀሰው ጥቅም በስትቀር በጥሬ ገንዘብ የሚደረግ የካሳ ክፍያ አይኖርም፡፡ ይህንን ጥናት በገንዘብ የሚደጉሙት ድርጅቶች ከዋና በሽታው ጋር የተያያዙ የህክምና ችግሮች ክፍያ ወይንም የተለየ የህክምና እርዳታ አያደርጉም፡፡

በጥናቱ ምክንያት የሚያዎጡት ትርፍ ወጪ

እርስዎ በዚህ ጥናት ሲካተቱ ለጥናቱ ተብሎ ቀጠሮ ስለማይያዝ ተጨማሪ ወጭ ያስከትልብዎታል ተብሎ አይታመንም፡፡

የጥናቱ ሚስጥራዊነት

ይህ ጥናት የህክምናና የሳይንሳዊ ጥናቶች ስነምግባርን በመከተሉ ሰብአዊ እንዲሁም የእርስዎን ግለሰባዊ መብትን በማክበር ሁሉንም እርስዎን የሚመለከቱ የጤና መረጃዎችን በሚስጥር ይጠብቃል፡፡ የእርስዎን

ማንነት በማያጋልጥ መልኩ የተዘጋጀዉን መረጃ በፊርማዎ የተረጋገጠ ፈቃድዎን ሳናገኝ ይፋ አናደርግም፡፡የጥናቱ መረጃዎች በሙሉ የሚቀመጡት ከእርስዎ ስም ጋር ሳይሆን ለጥናቱ ተብሎ በሚሰጣቸው ስውር ቁጥር ሲሆን ጥናቱን ከሚያካሂዱት ባለሞያዎች በስተቀር ማንም ሊያውቅ አይችልም፡፡ ይህ ጥናት ሳይንሳዊ መረጃ እንደመሆኑ መጠን በወረቀት ታትሞ ቢዎጣ ወይንም በሚዲያ ቢነገር የእርስዎ ስም በምንም መልኩ አይጠቀስም፡፡ ስለ እርስዎ የምንሰበስበው ማንኛውም መረጃ በተቆለፈ ቢሮ እና በተቆለፈ ቁምሳጥን ውስጥ ይቀመጣል፡፡ከተሰበሰቡት መረጃዎች የተወሰኑትን ወደ ኮምፒውተር በማስገባት የምንጠቀምባቸው ሲሆን መረጃው የተቀመጠበት መዝገብ በስውር ኮድ ይታሰራል፡፡ይህን ስውር ኮድ ከአጥኝዎች በስተቀር ማንም እንዳያውቅ ይደረጋል፡፡

ያለመቀበል ወይንም ጥሎ የመውጣት መብት

እርስዎ በዚህ ጥናት ውስጥ የሚኖሮት ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ይሆናል፡፡ እርስዎ በዚህ ጥናት ውስጥ የመሳተፍ መብትዎ ሙሉ በሙሉ የተጠበቀ ነው፡፡ እርስዎ በዚህ ጥናት እንዲሳተፉ ፈቃድ ቢሰጡም በማንኛውም ጊዜ ከጥናቱ የመዉጣት አማራጭ መውሰድ ይችላሉ፡፡ በጥናቱ ባለመሳተፎ ወይም ከጥናቱ በመገለሎ ምክንያት በአሁኑ ወይም የወደፊት የህክምና እርዳታ ላይ ተጽዕኖ አይፈጥርም፡፡ ከዚህ በፊት ሲያገኙ ከነበሩት ጥቅሞች አንድ እንኳን አይጎድልቦትም፡፡ ጥናቱን የሚያከናውነው አካል ወይም ድጋፍ አድራጊ አካል ለእርስዎ ጥቅም ሲባል በጥናቱ እንዳይሳተፉ ሊከለከሉ ይችላሉ፡፡

ጥያቄ አለዎት?

- ✓ ስለዚህ ጥናት ወይም እርስዎ በዚህ ጥናት ውስጥ ስለሚኖርዎ ድርሻ
- ✓ በዚህ ጥናት አማካይነት በእርስዎ ላይ ደረሰብኝ ስለሚሉት ጉዳት
- ✓ ስለ ጥናቱ ማንኛውንም ጥያቄ፣ አሳሳቢ ጉዳት፣ ወይንም ቅሬታ ካለዎት የሚከተሉትን ስልኮችና ኢ-ሜይሎች በመጠቀም የጥናቱን ባለቤቶች ማነጋገር ይችላሉ፡፡

Annex 3: Consent form for study subjects

Participant Code Number_____Date_____

Participant Full Name_____

I confirm that I have understood the purpose of the study entitled "Asymptomatic *Plasmodium* infection among pregnant women in Arba Minch Zuria District, Southern Ethiopia."

I have been informed that information about malaria, and little blood sample will be taken from my fingertip and that there will be minimal risk during sample collection. In addition, I have been informed that all data collected from me will be kept confidential. I have understood that my current and future medical services will not be affected even if I refuse to participate or withdraw from the study. I have also been informed that results from the laboratory diagnosis will be given to me; for treatment if it is found positive for malaria parasite and anemia.

Agree ☐ Do not agree ☐

Therefore, I give my consent freely to participate in this study.

Participant Name_____Signature _____Date____

Investigator's Name_____ Signature _____Date_____

Witness;

1. Name _____Signature_____Date_____
2. Name_____Signature_____Date_____

Signature of Principal investigator _____ Date _____

Annex 4: Amharic version of consent form

ቅጥያ 8: የጥናቱ ተሳትፍዎች የፈቃደኝነት መግለጫ ቅጽ

የተሳታፊ ልዩ መለያ ቁጥር_____
የተሳታፊ ሙሉ ስም_____

እኔ_____ስሜ ከለይ የተጠቀሰው የጥናቱ ተሳተፊ መሆኔን አያረጋገጥኩ የወባ በሽታ እና የሌሎች ህመሞች መንስኤ የሆነውን ፕላስሙድዬም በመባል የሚታወቀውን ፓራሳይት ላይ ሊደረግ ስለታሰበው ጥናት መረጃ አግኝቻለሁ፡፡ ለዚህም ይረዳ ዘንድ ከእኔ እጅ ጣት የደም ናሙና በመርፌ ለመውሰድ እንደሚፈለግ ተረድቻለሁ፡፡ ስለ ጥናቱ አላማ እንዲሁም ናሙናዉ በሚወሰድበት ወቅት በመርፌ ስወጋ በእኔ ላይ ምናልባትም መጠነኛ የሆነ የህመም ስሜት ሊሰማኝ እንደሚችልም ተገንዝቤያለሁ፡፡
በተጨማሪም በመጠይቁ ውስጥ በተካተቱት ጥያቄዎች መሰረት የምሰጣቸው መረጃዎች በጠቅላላ በሚስጥር እንደሚያዙ ተገልጾልኛል፡፡እንዲሁም የምጠየቀውን መረጃ ያለመስጠት እና በጥናቱ ያለመተባበር እነዲሁም በማንኛውም ወቅት የማግለል መብቴ የተጠበቀ መሆኑ የተገለጸልኝ ሲሆን ይህንንም ማድረጌ በእኔ ላይ ምንም አይነት እክል የማይፈጠርብኝ መሆኑን በሚገባ ተረድቻለሁ፡፡ከዚህ በላይ በእኔ ላይ ናሙናዉ በሚወሰድበት ወቅት ብቻ በጣም መጠነኛ የህመም ስሜት እንደሚሰማኝና ያ ስሜት ወዲያኑ በራሱ ጊዜ እንደሚተወኝ ተገንዝቤያለሁ፡፡

ለተፈለገዉ ጥናት ይውል ዘንድ የደም ናሙናና ለሎች መረጃዎችን ለመስጠት ተስማምቻለሁ፡፡

ተስማምቻለሁ ☐ አልተስማማሁም ☐

ስለዚህ ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳት በፍጹም ፈቃደኝነት ነው፡፡
*የጥናቱ ተሳታፊ ስም*_____ *ፊርማ*_____*ቀን*_____
*የዋናው ተመራማሪ ስም*_____ *ፊርማ*_____*ቀን*____
ምስክሮች፤

1. *ስም*_____ *ፊርማ*_____*ቀን*____
2. *ስም*_____*ፊርማ*_____*ቀን*____

Annex 5: Questionnaire

Organization: Jimma University, College of Public Health and Medical Sciences, Department of Medical Laboratory Sciences, and Pathology

Asymptomatic *Plasmodium* infection among pregnant women in Arba Minch Zuria District, Southern Ethiopia.

Name of kebele……..….Code ……House No…….

Socio-demographic and other associated factors to malaria

1. How old are you? …….
2. What is your marital status?
   A. Married B. Single
   C. Divorced D. Widowed
   E. Other……...
3. What is your occupation?
   A. Farmer B. Housewife
   C. Merchant D. Daily laborer
   E. Civil servant
   F. Other……..
4. What is your educational status?
   A. Illiterate B. Read and write
   C. 1 – $6^{th\ grade}$ D. 7 – $12^{th\ grade}$
   E. College graduate and above
5. What is your religion?
   A. Orthodox Christian B. protestant Christian
   C. Catholic Christian D. Muslim
   E. Other……

6. What is your gestational age?
   A. First trimester(< 14 weeks),
   B. Second trimester (14–28 weeks)
   C. Third trimester (> 28 weeks).
7. What is your gravidity?
   A. Primigravida B. Secondigravidae
   C. Multigravidae(greater than 2 births)
8. What is the number of your family? ……

9. What is your estimated monthly income? .....

10. Did you follow antenatal care in your previous pregnancy?

A. Yes B. No

11. Do you follow antenatal care for the current pregnancy?

A. Yes B. No

12. Do you sleep under impregnated anti- malaria bed net (ITN)?

A. Yes B. No

13. If your answer to question #13 is yes, how often do you use it?

A. always B. sometimes

14. Main material of the Room's Walls

A. Mud Blocks B. Cement Blocks

C. Sticks D. Wood Planks

E. Corrugated Metal F. Other (*please specify*)_____

15. Main material of the Room's Roof

A. Thatch B. Sticks and mud

C. Corrugate D. Other (*please specify*) _____

16. Main material of the Room's Floor

A. Earth B. Local dung plaster

C. Cement D. Wood

E. Other (*please specify*)_____

17. At any time in the past 12 months, have the interior walls of this room been sprayed against mosquitoes?

A. Yes B. No C. Don't Know

18. If No, how many months ago was the room sprayed? __ ___ Months Ago

Annex 6: Amharic version of questionnaire

ቅጥያ 9: የጥናቱ ዋና መጠይቅ

በጅማ ዩኒቨርሲቲ የህክምና እና የሕብረተሰብ ጤና ሰይንስ ካሊቲ፣ ህክምና ላቦራቶሪ ሰይንስ እና ፓቶሎጂ የትምህርት ክፍል::

ሙሉ ቃኛ በሆኑ እና ምንም ኢይነት የበሽታ ምልክት በማይታይባቸው ነፍሰጡር እናቶች ላይ በደም ስኮ ሚኙ ኮኛ የወባ ተህዋሲያንና ሌሎች የተገናኙ መንስኤዎች ለወባ በሽታ ክትውን እገዛ ለማጥናት የተዘጋጀ መጠይቅ::

የጥናቱ ተካፋይ መለያ ቁጥር.....

አድራሻ ወይም ቀበሌ.....የበት ቁጥር.....

ሰነ ማህበራዊ እና ህ□ባዊ መ□□ቅ

1. ክ□ሜ- ት ስንት ነው?.....

2. የጋብቻ ሁኔታ?□□

ሀ. ያገባች **ለ. ያላገባች**

ሐ. አግብ □□ታች **መ. ባል የሞተባት**

3. ሥራዎት ምንድ ነው?

ሀ. አርሶ አደር **ለ. የቀን ሰራተኛ**

ሐ. ነጋዴ መ. የቤት እመቤት

ሰ. ሌላ (□□ቀስ).....

4. የትምህርት ደረጃ- ት ምን ያህል ነወ?ሩ

ሀ. ማንበብና መፃፍ አልችልም ለ. ማንበብና መፃፍ □እችላለሁ

ሐ. ከ1-6 ክፍል መ. ከ7-12 □□ል

ሠ. ኮሌጅና በላይ

5. ሀ□ማኖትዎት ምንድ ነው?

ሀ. ፕሮተስተንት ክርስቲያን ለ. ኦርቶዶክስ ተ□ህ□

ሐ. ካቶሊክ መ. ሙስሊም

ሠ. ሌላ.....

6. ካረገዙ ስንተኛ ወር- ት ነው?

ሀ. ከ 14 ሳምንት በታች

ለ. ከ 14-28 ሳምንት

ሐ. ከ 28 ሳምንት በላይ

7. ለስንት ጊዜ አርግዘዋል?

ሀ. ለመ□ሪ□ □□ ለ. ሁለተኛ □□

ሐ. ከ 3ና በላ□

8. የበተሰብዎ ቁጥር ስንት ነዉ?.....

9. የወር ገቢዎት ስንት ነዉ?.....

10. ከዚህ በፊት በነበር- ት እርግዝና ቅድመ ወሊድ ክትትል ያደርጉ ነበር?

ሀ. አ-

ለ. አላደረግኩም

11. በአሁኑ የእርግዝና ወቅት የቅድመ ወሊድ ክትትል እያደረጉ ነዎን?

ሀ. አዎን

ለ. አላደረግኩም

12. በ□ረ-ወባ ኬሚክል የተነከረዉን የአልጋ አጎበር ይጠቀማሉ?

ሀ. አ-

ለ. አልጠቀምም

13. ለ□□ቱ 13 መልስ- አ- ከሆነ፡ መች መች ይጠቀማሉ?

ሀ. ሁል □□

ለ. አልፎ አልፎ

14. የዚህ ማደሪያ ክፍል ግድግዳ የተሰራው ከምንድነው?

ሀ. ጭቃ ጡብ ለ. ከብሎኬት ሐ. ከኦንጨት

መ. ከጣው ሠ. ከቆርቆሮ ረ. ሌላ(□□ሰ□) ----------

15. የዚህ ማደሪያ ክፍል ጣርያ የተሰራው ከምንድነው፡

ሀ. ሣር ክዳን ለ. ኦንጨትና ጭቃ ሐ. ቆርቆሮነ

መ. ሌላ (□□ሰ□) ----------

16. የዚህ ማደሪያ ክፍል ወለል የተሰራው በምንድነው?

ሀ. አፈር ለ. በአበት የተለቀለቀ

ሐ. ከስሚንቶ መ. ከጣውላ

ሠ. ሌላ (□□ሰ□) ---------

17. ባለፉት 12 ወራት ውስጥ የዚህ ማደሪያ ክፍል የውስጥ ግድግዳዎች በፀረ-ትንኝ መድሀኒትተረጭተው ነበር?

ሀ. አዎን ለ. አልተረጨም

ሐ. አላውቅም

18. ከተረጨ ስንት ወር ይሆነዋል?----------ወራት

Annex 7: Laboratory requesting and recording format

Jimma University, College Of Public Health And Medical Science, Department Of Microbiology And Parasitology

Parasitological investigation and hematocrit determination for pregnant women in Arba Minch Zuria District, Southern Ethiopia.

1. Personal data

1.1 Code no……..…….. ……..…….

1.2 Age …….

1.3 Address/Kebele ……..……...

1.3 Date of sample collection ……

2. Laboratory data

2.1 SD BIOLINE Malaria Ag Pf/Pv POCT Test….……..……...……

2.2 Blood smear microscopy…….……..…...……

A. Types of haemoparasite seen……...……

B. Parasite density ……..……...……

C. No haemoparasite seen……...……..……

2.3 Hematocrit determination…….……...……

Name of investigator _____

Signature _____ Date _____

Annex 8: Specimen Collection and Processing

8.1 Malaria blood film preparation and its microscopic examination

Kinds of blood film: In malaria microscopy, two kinds of blood film are used: thick and thin.

The thick film: A thick film is always used to search for or detect malaria parasites. The film consists of many layers of red and white blood cells. During staining, the haemoglobin in the red cells dissolves (dehaemoglobinization), so that large amounts of blood can be examined quickly

and easily. Malaria parasites, when present, are more concentrated than in a thin film and are easier to see and identify.

The thin film: The thin film is used to confirm the malaria parasite species, when this cannot be done in the thick film. It is used to search for parasites only in exceptional situations. A well-prepared thin film consists of a single layer of red and white blood cells spread over less than half the slide. The frosted end of the slide is used for labelling.

Required equipments

- ***Sterile lancet***—Retractable type is preferred; tip less than 2.4 mm.
- ***Alcohol wipes***—Wipes containing 70% isopropyl alcohol was used.
- ***Sterile gauze pads***—for removal of first free-flowing drop of blood and for pressure application after collection.
- ***Gloves***—made of latex, rubber, vinyl, etc.; worn to protect the patient and the collector
- ***Sharps disposal unit***—Lancets were placed in a proper disposal unit after use.
- ***Frosted end slides***—Cleaned, wrapped frosted end slides; frosted ends used for labelling
- ***Absorbent cotton wool***—to clean the finger & wipe off the blood
- ***A slide box or tray*** for drying slides horizontally and protecting them from flies and dust;
- ***Record forms or a register***; to record the field & laboratory data
- ***Ballpoint ink-pen*** for the record forms or register; and
- ***A lead pencil*** to give code or write on the frosted end of slides film and small sharpener.
- ***Absolute Methanol***—to fix the thin blood films
- ***Giemsa stain***—to stain the blood fims
- ***Slide rack***—to put the slides on for staining
- ***Light microscope and immersion oil***—to examine the blood films

1.1 Procedure for finger pricking and blood film preparation

1. The retractable lancet is used most often for safety reasons. The retractable lancet is spring-loaded and the lancet retracts into the body of the device after skin puncture.
2. The recommended depth of puncture is 2.5 mm for adults, 2.0 mm for children, and 1.5 mm for infants less than 6 months of age. All lancets were sterile and for one-time use only.
3. The pregnant women positioned in a chair, lie down, or sit up in bed. The patient's arm hyperextended.
4. The finger was massaged to increase the blood flow. This may be done by ***gently*** squeezing the finger from hand to fingertip 5 or 6 times. This maneuver is not overused as it may cause erroneous results due to concentration of tissue fluids.
5. Fingertip cleansed with 70% isopropyl alcohol. It was then wiped, dried with a clean, dry piece of gauze or cotton. The finger should be thoroughly dry, as blood will not well up and form a drop at the puncture site of a moist finger.

6. The lancets were removed from its package and grasped between the thumb and forefinger. The finger puncture devices were used referring to the instructions for the device we were using.
7. Using a sterile lancet, a skin puncture was made just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges. The first drop of blood, which tends to contain excess tissue fluid, was wiped away.
8. The drops of blood were collected into the collection device by gently massaging the finger. Excessive pressure was avoided because it may squeeze tissue fluid into the drop of blood.
9. Through wiping off the first drop of blood, thick and thin films were made on the same slide from the next blood drops. For thick films, ~12 µl of blood was spread over a diameter of 15 mm, while ~2 µl of blood was used for thin films.

1.2 Staining of malaria blood smears

1) The staining technique and blood film examination was conducted employing WHO guidelines.
2) We used 10% giemsa working solution at PH of 7.2 to stain for 10 minutes. One hand of commercially available Giemsa liquid stock solution and 9 hand distilled water were diluted, checked for PH and used immediately for staining. Every time of staining, new working solutions were used for a new batch, discarding the remaining solution.
3) After the thin slides had been fixed with 100% methanol for 30 sec, the stain was gently poured on to the slides (or a pipette was used to drop the stain on to the slide).
4) After 10 minutes of staining, the stain gently flushed off the slide by adding drops of clean water. The stain never poured off the slides, otherwise the surface scum will stick to the film and spoil it for microscopic examination.
5) The slide was placed in the drying rack, film side downwards, to drain and dry. The thick film placed carefully as not to touch the edge of the rack.

1.3 Microscopic examination of the films

1. Microscopic examination of thick films, using high power magnification for the presence of parasites and parasite species identification using thin films under a 100× Oil immersion objective was carried out by an experienced laboratory technicians/technologists. Parasitic load was also counted following WHO guidelines**.**
2. Parasite density was determined from thick smears at 100x oil immersion objective by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes when the number of parasites was below 10) and expressed as parasites/µl, assuming a standard white blood cell count of 8,000leukocytes/µl.

$$\text{Parasites}/\mu\text{l} = \frac{\text{no. of asexual parasites} \times 8000 \text{ leukocytes}}{200 \text{ leukocytes}}$$

3. The degree of parasitaemia was graded as (1-999/µL) as mild or +, (1000-9999 /µL) as moderate or ++, and (>10000/µl) as severe or +++ based on WHO guide.
4. Each slide was read by two microscopists blinded to the other's readings. Slides with discrepant results between the first and second readings were settled by a third microscopist. Criteria for a second microscopy reading were: slides positive for Plasmodium spp. at first microscopy reading; individuals with discrepant microscopy and RDT results; severely anaemic individuals (HCT <21%); and a randomly selected 10 % of negative slides.
5. The discrepant microscopy result was resolved by calculating the percentage discrepancy.

$$\%\ Discrepancy = \frac{\text{Count 1} - \text{Count 2}}{\text{Mean Of Counts 1 \& 2}} \text{x} 100\%$$

6. For parasite counts with percentage discrepancy less than 20%, the count was accepted and the mean parasite count was taken as the parasite density.
7. For parasite counts with % discrepancy ≥ 20%, the films were examined by Reader 3.
8. The count by Reader 3 and the closest from either Reader 1 or Reader 2 were used to calculate the % discrepancy, and their mean count was taken as parasite density providing the % discrepancy is < 20%.
9. Alternatively, the slides were re-read by Reader 1 and Reader 2. The total leukocyte count determination for each participant were done using the improved Neubauer chamber as described by Baker and Silverton.
10. A definitive diagnosis of malaria were made when a reddish chromatin dot with a purple or blue cytoplasm of the malaria parasites were seen together.
11. The microscopist spent an average of 15 min to 1 hr on each thick and thin film, respectively. A slide was reported negative after 100 high power fields have been examined using ×100 oil immersion objective lens and no parasite seen.

1.4 Preparation of Giemsa Working Solution from Giemsa Stock

Principle: Light microscopy, usually applying the Giemsa staining technique, is the established method for the laboratory diagnosis of malaria. Giemsa is a Romanowsky stain used for staining blood films. Romanowsky stains contain Eosin Y, an anionic acidic dye, and Azure B, a cationic basic thiazine dye obtained by oxidation of methylene blue. When the dyes are diluted in a buffer, the anionic dye stains the acidic components (nucleus) of cells red, and the cationic dye stains the basic components (cytoplasm) of cells blue.

Materials and Reagents

- Giemsa stock solution, Buffered Distilled water, Measuring cylinder 10 and 100 ml capacity, Filter paper and Funnel

Procedure to prepare 10% Giemsa working solution

1. Pour 90 ml of buffered water (pH 7.0 – 7.2) into the measuring cylinder.
2. Add 10 ml of filtered Giemsa stock into the measuring cylinder
3. Mix well before using.

1.5 Preparation of Buffered Water

Principle: The importance of buffering the Giemsa stain solution resides in creating the optimal PH environment for staining. As a usual, distilled water was used for Giemsa dilution purpose because of its purity. Since distilled water is slightly acidic, it safely buffers Giemsa to obtain a PH of 7.0 to 7.4, with the optimum pH being 7.2. This "distilled water-Giemsa Stain Solution" is excellent for staining of blood parasites, particularly for use in cases of malaria, where Maurer's clefts, characteristic of *P. falciparum*, or schuffners dots of *P.vivax* are readily evident in blood smears**.**

Materials and Reagents

- Beaker, 250ml capacity
- Graduated cylinder, 1000ml capacity
- Buffer tablet(Glaxo make) or powder pack (Ranbaxy make) of 7 pH in 100 ml dist. H2o
- Distilled water

pH of Giemsa working solution was checked by "Deluxe pH meter, model SE-963"

Digital pH meter; "Deluxe pH meter, model SE-963". To measure hydrogen ion concentration $pH = \log10\ [1/H^+]$

Procedure

1. connect the instrument A.C supply
2. put the selector switch to stand by. The instrument should read 000±1
3. instrument is now ready for use

Procedure for pH buffer dilution

1. Add 150 ml of distilled water to beaker.
2. Add one buffer tablet, either acidic or basic based on the pH. Now pH is 7.0
3. Shake the water until the tablets dissolve.
4. When dissolved, add the fluid from the beaker to the measuring cylinder.
5. Fill the fluid in the measuring cylinder with distilled water until it is made up to 1L mark.

Quality control: Check expiry date of buffer tablet

1.6 Preparation of control slides

1. To control the quality of Giemsa stain for proper staining results, a known positive smear was used with each new batch of working Giemsa stain.
2. Control slides were prepared from a patient's blood and stored for future use. From a patient known to have a malaria infection, blood sample was collected in an EDTA (ethylene diamine tetra acetic acid) or citrated blood tube. [If we can't get known patient having malaria infection we can also prepare panel slides from negative patient].
3. An ideal blood sample has at least one parasite in every 2–3 fields on a thin blood smear. As many thin smears as possible were made, preferably within one hour of drawing the blood from the patient.
4. We allowed the smears to dry quickly, using a fan at room temperature.
5. The smears were fixed in absolute (100%) methanol and allowed to dry.
6. They were placed, touching back to back, in a box with separating grooves.
7. The outside of the box was labeled with the species, date and 'Giemsa control slides.' The slides were stored at room temperature or stored at -20°C or -70°C for longer staying.
8. Just before use, the slides were removed from the box and allowed to evaporate the condensation; the slides were labeled with the date and '+ control.'
9. The smears were then stained and examined to check that the working solution of Giemsa stain is of good quality.

8.2 SD BIOLINE Malaria Antigen(Ag) P.f/P.v POCT.

This kit was manufactured by STANDARD DIAGNOSTICS, INC. 156-68 Hagal-dong, Giheung-gu, Yongin-si, Gyeongggi-do, Korea. Authorized Representative___MT Promedt Consulting GmbH, Altenhofstrasse 80 D-66386 ST. Inbert Germany. SD BIOLINE Malaria Ag P.f/P.v Point Of Care Test is a *one step, rapid, qualitative and differential test* for the detection of HRP-II(Histidine-rich protein II) specific to *P.falciparum* and pLDH (Plasmodium lactate dehydrogenase) specific to *P.vivax* in human blood sample. This is accurate or differential diagnosis between *P.f* HRP-II and pLDH**.**

Precaution

These malaria rapid tests were performed according to the manufacturer's instruction on the kit. The storage and stability of the kit were maintained as recommended on the kit. The warnings taken into consideration. The expiry and manufacture dates were checked before use.

Procedure

1. All kit components were allowed to room temperature prior to testing.
2. Test devices were opened from the foil pouch and placed on a flat and dry surface.
3. The test cassettes were labeled with similar patient ID numbers to blood film.
4. Finger tips to be pricked were firstly cleansed with alcohol swab in the kit.
5. The end of fingertip was squeezed and pierced with a sterile lancet provided in the kit.
6. First drop of blood was discarded with a dry cotton to protect interference of tissue fluids in the test result
7. The second blood drop was taken with a capillary pipette(5µl) provided in the kit.

8. The blood sample were transferred directly to the sample pad/well and the assay diluent in the kit were opened and dispended to diluents pad.
9. Finally, the results were read for waiting up to 30 min.

Note: Presence of red band on both the control and test lines indicated a positive result. The formation of only one band on control line indicated a negative result. For no band formation on the control line, the test were read invalid**.**

8.3 Hematocrit determination

Hematocrit level was estimated using manual hematocrit centrifuge. The micro hematocrit method was carried out on blood contained in capillary tubes of 75mm in length and about 1mm in an internal diameter. The tubes were plain for use with anticoagulated blood samples. The micro hematocrit method has an adequate level of accuracy and precision for clinical utility.

1. Capillary tubes per individual were filled with capillary blood from finger prick
2. After capillary bloods were filled in the tubes, the capillary tubes were sealed with sealing clay; giving care to avoid breakage of the tube when placing it in sealing clay.
3. Capillary tubes for the determination of packed cell volume were sealed with the tube perpendicular to the sealing clay. This ensures a flat seal.
4. The specimen was labeled immediately following collection and mixing and before leaving the patient's side. The label stated the patient's first and last names, identification number, date and time of specimen collection, and initials of the person collecting the specimen.
5. Each micro collection device was labeled individually, or placed in a larger tube or waterproof plastic bag that was labeled for transport to the laboratory. Alternatively, the label was placed around capillary tubes, like a flag, and the labeled group then placed into a large test tube.
6. Microhematocrit centrifugation took place in the laboratory. The microhematocrit centrifuge was used to centrifuge blood filled and sealed capillary tubes at a centrifugal force of 12,000g-15,000g, for 5 minutes; resulting in a constant PCV.
7. The PCV was determined by reading the centrifuged capillary tubes from a Hawks leys microhematocrit reader

Annex 9: Data quality management

To assure the quality of data reliable: The data collection, application of standard operating procedure by health professionals, accuracy of test results were supervised by principal investigator. Close follow ups by the investigator during the whole data collection process were done.

- Questionnaire was evaluated by pretesting on about 5% of the total sample size.
- Laboratory request was prepared clearly in precise way and patient/specimen identification was coded orderly.
- Training on data collection procedures, focusing on issues related to the way of respondents' approach, how to fill questionnaires, and how to record data, were given for interviewers.

- Training was also given for laboratory technicians on examination of malaria blood films to apply standard operational diagnostic procedures.
- Disinfection procedures during finger prick sampling were applied cautiously.
- Freshly reconstituted and filtered Giemsa stains, standard positive and negative films as well as standard operational procedures were considered.
- The quality of Giemsa working solution was checked using known quality control slides for every batch of new stain solution.
- The manufacture and expiry date of RDTS were checked before use and the kits were handled at the temperature recommended by the manufacturer suggesting that they are in a supposedly good condition during the study period.
- All RDT devices were labeled with similar patient ID numbers to that of the blood film.
- Data collectors were regularly overseen by the principal investigator for proper data collection
- The staining technique and blood film examination were conducted employing WHO guidelines and/or laboratory SOPs.
- Experienced laboratory technicians and technologists having experience on detection of malaria blood films were assigned to examine both thick & thin films
- In addition to the qualitative examination, parasitic load was determined by following WHO guidelines/SOPs.
- Each slide was read by two microscopists blinded to one's readings. A third microscopist settled discrepancies between readings 1 & 2.
- The discrepant microscopy result was resolved by calculating the percentage discrepancy
- Comparison was made with both known positive and negative blood films.
- Malaria rapid diagnostic tests were performed according to the manufacturer's instruction.
- The hematocrit centrifugation was adjusted to a centrifugal force of 12,000g, and time of 5 minutes that results in a constant PCV.
- The capillary tubes were filled with blood carefully to secure the sudden breakage of tube.
- Filled questionnaires and lab requests were collected on a daily base after checking for consistency and completeness by the supervisor and the PI.
- Reporting/addressing the laboratory result to the subjects were done immediately after its completion.
- Immediate reaction to laboratory reporting, checking correct interpretation, appropriate and adequate follow-ups, and ordering appropriate consultation on whole data collection process were applied by the principal investigator

Annex 10: Declaration

I, the undersigned, hereby declare that this thesis finding is my original work and has never been presented for any degree in Jimma University or any other institutions of higher learning in Ethiopia. I also declare the duly acknowledgement of all material sources used for this thesis work.

Name of the student: Desalegn Nega (BSc, MSc Candidate)

Signature: _____

Place :_____

Date of submission :_____/_____/_____

This thesis has been approved by the supervision of university advisors and examiners:

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   Place: _____
   Date of submission : _____/_____/_____
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   Place: _____
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3. Examiner: Dr. Delenasaw Yewhalaw (PhD, Associate Professor)
   Signature :_____
   Place: _____
   Date of submission : _____/_____/_____

Name of Department head: _____

Signature : _____

Place: _____

Date of submission : _____/_____/_____