



St. Paul's Hospital Millennium Medical College

School of Public Health

Graduate Study Program

The assessment of Asymptomatic Malaria Infection and Its Associated Factors Among Pregnant Women in Gambella Town, Southwest Ethiopia.

Investigator: Ashenafi Kure (BSc in Medical Laboratory Science)

Research thesis submitted to the Department of Epidemiology, School of Public Health, St. Paul's Hospital Millennium Medical College in the Partial Fulfillment of the Requirements for the Degree of Master of Public Health in Epidemiology

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Gambella, Ethiopia

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List of Acronyms and Abbreviations

ACT: Artemisinin-based combination therapy

ANC: Ante-Natal clinic

EPHI: Ethiopian Public Health Institute

HCT: Hematocrit

HIV: Human immunodeficiency virus

IRGR: Intrauterine Growth Retardation

IRS: Indoor residual spraying

ITN: Insecticide-treated mosquito nets

LBW: Low birth weight

LLIN: Long-lasting insecticidal net

MIP: Malaria in pregnancy

MIS: Malaria Indicator Survey

MOH: Ministry of Health

PCR: Polymerase chain reaction

PCV: Packed Cell Volume

Pf: *Plasmodium falciparum*

PfHRP2: Histidine-rich protein 2 of *P. falciparum*

PPS: Probability proportional to size

PspLDH: *Plasmodium* species lactate dehydrogenase

Pv: *Plasmodium vivax*

RDTs: Rapid diagnostic tests

RHB: Regional Health Bureau

WHO: World health organization

Abstract

Background: Malaria during pregnancy is a major cause of fetal and maternal morbidity and mortality. In malaria endemic areas, Plasmodium infections tend to remain asymptomatic yet causing significant problems like maternal anemia, low birth weight, premature births, and still birth. Asymptomatic carriers also provide a fundamental reservoir of parasites, contributing to the persistence of malaria transmission.

Objective: To assess the prevalence of asymptomatic malaria and its associated factors among pregnant women in Gambella town, southwest Ethiopia.

Methods: A hospital -based cross-sectional study was conducted from May to July 2022 among 269 randomly selected pregnant women attending antenatal clinics at Gambella town. Socio demographic data were obtained using structured questionnaire and about 3ml of peripheral blood samples were collected for malaria parasite and hemoglobin estimation. Microscopy and rapid diagnostic test (RDT) were used to identify malaria parasites. The filled data were analyzed using SPSS version 20 for Windows. The estimates were presented as odds ratio (OR) with a 95% confidence interval (95% CI). P-values less than 0.05 were considered statistically significant.

Results: The prevalence of asymptomatic malaria infection was 23 (8.6%) and 17 (6.3%) by microscopy and RDTs, respectively. All of them were found infected with *P. falciparum*. Prevalence of anemia among pregnant women were 58 (21.6%) and 12 (20.7%) of them were infected with asymptomatic malaria. Being anemic (AOR = 0.23, 95% CI = 0.068-0.791); not using of Insecticide-treated mosquito nets (ITN) regularly (AOR = 0.07, 95% CI = 0.010-0.546), not spraying of indoor residual spray (IRS) for the last 12 months (AOR = 0.11, 95% CI = 0.031-0.354), being primigravidae (AOR = 15.22, 95% CI = 1.473-157.35), and lower monthly household income (AOR = 0.12, 95% CI = 0.018-0.801) were independent predictors of asymptomatic malaria among pregnant women at p-value of < 0.05.

Conclusion: The present study showed asymptomatic malaria is prevalent in pregnant women and it has statistically significantly associated with the haemoglobin level, being primigravidae, not using of ITN and spraying of IRS. This indicates pregnant women have to be screened for asymptomatic malaria, anemia and educate regular utilization of ITNs and IRS to avoid health consequences of malaria infection and anemia during pregnancy for the mother and fetus.

Keywords: Asymptomatic malaria, Anaemia, Pregnant women, Prevalence, Plasmodium species, Gambella, southwest Ethiopia.

CHAPTER ONE: INTRODUCTION

1.1. Background

Malaria is a life-threatening disease caused by plasmodium species that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. It is preventable and curable (1,2). There are five major *Plasmodium* species which can infect human beings, including *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*. Two of these species; *P. falciparum* and *P. vivax* pose the greatest threat. *P. falciparum* is the deadliest malaria parasite and the most prevalent on the African continent. *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan African (SSA) countries (3,4).

The transmission of malaria in humans occurs through the bite of a female *Anopheles* mosquito. In addition, blood transfusion and maternal transmission have been demonstrated as other routes of malaria infection with less frequent occurrences (2,3). The typical symptoms; fever, headache and chills usually appear 10 to 15 days after the infective mosquito bite and may be mild and difficult to recognize as malaria. Left untreated, *P. falciparum* malaria can progress to severe illness and death within a period of 24 hours. In some cases, symptoms may not develop for several months or dormant. The pathogenesis of malaria is complex and the clinical presentation of the disease is classified in three main clinical groups: asymptomatic, mild, and severe malaria (4).

Malaria is one of the most severe public health problems worldwide. It is a leading cause of death and disease in many developing countries, where young children and pregnant women are the groups most affected. According to the 2022 World Malaria Report, nearly half the world's population lives in areas at risk of malaria transmission. In 2021, there were an estimated 241 million malaria cases, and 627,000 deaths. Africa, continues to carry a huge share of the global malaria burden. In 2021 the it was home to 95% of all malaria cases and 96% of deaths (3).

Malaria is one of the leading public health problems in Ethiopia. Nearly 75% of the landmass of Ethiopia is estimated to be “malarious, with 52% people lives in areas at risk of malaria infections, and transmission is highly seasonal and varies geographically across the country. *P. falciparum* and *P. vivax* are the two main causative agents of malaria accounting for 60% and 40% cases respectively. *Anopheles arabiansis* is the major vector for the transmission of malaria in Ethiopia

(5). Gambella is one of the areas of Ethiopia with high malaria endemicity due to its suitable climate and high rainfall (6).

Malaria in pregnancy (MIP), remains a major public health threat in tropical and subtropical region where about 125 million pregnancies are at risk of malaria, and up to 200,000 babies die as a result. It affects an estimated 24 million pregnant women in SSA annually (7) . In malaria-endemic areas, malaria is the cause for almost 25% of maternal deaths each year, with the greatest risk of infection and morbidity occurring in primiparous women, adolescents, and those co-infected with human immunodeficiency virus (HIV) (8).

Malaria during pregnancy may cause a variety of adverse consequences including maternal anaemia, placental accumulation of parasites, low birth weight (LBW) from prematurity and intrauterine growth retardation (IUGR), congenital infection and infant mortality (9). Malaria in pregnancy is caused mainly by the specie *P. falciparum*, which is the most common species in Africa including Ethiopia. Asymptomatic infection with *Plasmodium* species is common in malaria endemic areas (10).

Protection of pregnant women living in malaria endemic countries has been of particular interest to many malaria control programmes because of this group's higher susceptibility and reduced immunity. Measures recommended by the WHO to reduce morbidity and mortality among pregnant women include use of insecticide-treated bed nets (ITNs), intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP), and prompt diagnosis and treatment of infection. Such measures have led to preventive efforts, though with continued rise in the cost of malarial treatment in pregnancy to the individual and the nation(11,12). IPT in pregnancy (IPTp) reduces maternal malarial episodes, maternal and fetal anemia, placental parasitemia, LBW, and neonatal mortality (13).

In Ethiopia, early diagnosis and prompt treatment is considered as key strategy for malaria control. Currently, special attention is given to the reduction of maternal mortality and reduction of malaria cases.(14).

1.2. Statement of the problem

Malaria is one of the most severe public health problems worldwide, and being one of the greatest causes of mortality in tropical regions. Asymptomatic malaria remains a challenge for malaria control programs as it significantly influences transmission dynamics, and has great health impact on asymptomatic carriers. Asymptomatic infections remain undetected and untreated, result in a major reservoir of gametocytes for transmission by mosquito vectors or can be a precursor in the progression of symptomatic form of malaria (15–18).

Depending on the endemicity of malaria in an area, it can be expected that 1-50% of pregnant women may carry malaria parasitemia, especially in the placenta, without noticing it (19). Pregnant women are three times more likely to suffer from severe diseases as a result of malarial infection compared with their non-pregnant counterparts, and have a mortality rate that approaches 50% (20). The principal impact of malaria infection is due to the presence of parasites in the placenta, which causes maternal anaemia and low birth weight (LBW) (21). Beyond the post-partum period, the long term consequences of malaria during pregnancy on the infant include poor development, behavioral problems, short stature and neurological deficits (22).

Different studies have shown individual with an asymptomatic malaria, can be a reservoir for transmission by *Anopheles* mosquitoes and may progress to symptomatic disease (23,24) among whom pregnant women are at higher risk (25). The sequestration of *Plasmodium* species in placenta is also believed to be associated with LBW, preterm delivery, miscarriage, and still birth (26,27). Besides, asymptomatic carriers serve as silent reservoir of gametocytes for transmission by mosquito vectors (28,29).

Pregnant women are particularly vulnerable to malarial infection as the infection poses a lot of threats to them and the fetus. Malaria in pregnancy is one of the major causes of mortality and morbidity in tropical regions, causing maternal anemia, intrauterine growth retardation (IUGR), preterm birth, and LBW (30,31). Nearly 32 million pregnant women living in areas of high transmission of malaria in SSA every year (32). In SSA, MIP contributes to 15% of maternal anemia, 14% of LBW infants, 30% of preventable LBW, 70% of IUGR, 36% of premature deliveries, and 8% of infant mortality (33).

Studies have shown the level of endemicity of malaria transmission, the level of immunity acquired prior to pregnancy, gravidity, gestational age anemia, ITNs utilizations and maternal age are some

of the primary risk factors that contribute to the epidemiology of malaria during pregnancy in various regions (34,35).

An asymptomatic infection in pregnant women can cause more severe outcomes than in non-pregnant women because it remains undetected and continues to have serious impact on both maternal and infant health that include maternal anemia, peripheral and placental parasitaemia, IUGR and delivering LBW baby (15,34–36).

Anaemia is the most common symptom of malaria in pregnancy and usually develops during the first and second trimesters. Malaria causes reduction in hemoglobin 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(37).

To this end, malaria causes much damage to the health and socioeconomic development of the in Ethiopia (38). Pregnant women have a higher risk of malaria compared to non-pregnant women(34). Currently, protection of pregnant women living in malaria endemic countries has been of particular interest to many malaria control programmes because of this group's higher susceptibility and reduced immunity. Many studies have been published on the prevalence of clinically symptomatic malaria in Ethiopia but little information is available on the prevalence of asymptomatic malaria among pregnant women (39,40). Despite the high risk of malaria transmission in the study area, there is limited evidence about the magnitude and risk factors of asymptomatic malaria among pregnant women, additional knowledge of malaria and adequate preventive practices among women most likely will aid in the control of Malaria in Pregnancy.

Therefore, the aim of this study, was to assess the prevalence of asymptomatic malaria and its associated factors among pregnant women in Gambella town, Southwest Ethiopia.

1.3. Significance of the study

Malaria control strategies in many developing countries including Ethiopia have emphasized on symptomatic cases, by ignoring asymptomatic carriers possibly due to unstable nature of disease transmission in the country. However, currently many asymptomatic malaria cases have been reported in low or unstable transmission settings and studies have confirmed that asymptomatic parasitaemia occurs also in the absence of intense transmission and it persist inter-seasonally in places with seasonal transmission.

The prevalence and associated factors of asymptomatic malaria among pregnant women have not been studied in the study area. This study was attempted to assess prevalence of asymptomatic malaria and its associated factors among pregnant women in the study area. Information on this regard is useful to determine the existing significant variables related to asymptomatic malaria to work towards alleviating the problem with an appropriate care. Thus, this proposed study is expected to fill gaps and may be important to (1) healthcare providers and patients, to prevent and treat asymptomatic malaria early (2) for policymakers to have the evidence they need to make informed decisions about how to allocate resources to best improve the health of pregnant women at risk of asymptomatic malaria and (3) for researchers as a baseline data for further research agendas.

CHAPTER TWO: LITERATURE REVIEW

2.1. Overview of asymptomatic Malaria

Malaria is a preventable and treatable mosquito-borne illness and one of the world's most common and serious tropical diseases. Malaria during pregnancy is a major cause of fetal and maternal morbidity and mortality. Asymptomatic malaria is referred to as a positive result from malaria diagnostic tests in the absence of clinical symptoms of malaria. In malaria endemic areas, Plasmodium infections tend to remain asymptomatic yet causing significant problems like maternal anemia, LBW, premature births, and still birth. Asymptomatic carriers also provide a fundamental reservoir of parasites, contributing to the persistence of malaria transmission (30).

2.2. Prevalence of asymptomatic malaria among pregnant women

Many antenatal clinic studies carried out in different SSA countries including Ethiopia reported high prevalence of asymptomatic *P. falciparum* parasitaemia and related anemia(A) among pregnant women with greater complications in primigravida: Burkina Faso (*Pf*=24%, *A*=33%)(36), central Nigeria (*Pf* =95.4%, *A*=66.7%)(41), Southeast Nigeria (*Pf* =58.4%, *A*=55.2%)(42) and Ethiopia (*Pf/Pv*= 9.7%)(43).

In a cross-sectional study conducted at ANCs of two district hospitals in India, 1.8% of pregnant women were found to be positive for malaria (53.5% *P. falciparum*, 37.2% *P. vivax*, and 9.3% mixed infections) by Giemsa-stained blood smear and/or RDT using peripheral blood (44). Another study conducted in Bangladesh, the prevalence of asymptomatic malaria among pregnant women were 2.3% using RDT(25).

According to a study conducted at Burkina Faso the prevalence of *P. falciparum* asymptomatic malaria among pregnant women was found to be 30% and 24% with RDT and microscopy, respectively. The study suggests that anaemia was significantly more common in women infected with *P. falciparum* with 33% prevalence compared with the uninfected pregnant women (10% prevalence) (36). Another study conducted among asymptomatic pregnant women in Burkina Faso also indicated, malaria prevalence of 30% (95% CI: 25–34%) by microscopy, and 47% (95% CI = 42–52%) by RDT (36).

A study done in Nigeria among pregnant women, Plasmodium parasitemia was found in 58.4% of the study participants (42). Another study done in Nigeria with unstable malaria transmission indicated that the prevalence of malaria parasite infection was 70.1% among pregnant women (37).

Study from Ghana also reported the 36% prevalence of *P. falciparum* among the asymptomatic pregnant women and 44% of the participants were anemic (hemoglobin < 11 g/dL) with the mean hemoglobin level of 10.98 ± 2.32 (45).

According to a study took place on malaria infection among pregnant women attending ANCs in six Rwandan districts, indicated that the overall prevalence of malaria infection was 13.6% (46).

According to a study done to investigate the prevalence and predisposing risk factors among pregnant women in Greater Khartoum area, Sudan malaria was prevalent among 26.2% of pregnant women (47).

Based on a cross-sectional study done among pregnant women in Arbaminch southern Ethiopia, 9.1% and 9.7% of the respondents were confirmed to be infected with Plasmodium species by microscopy and RDTs, respectively (43). Another study conducted in malaria endemic parts of north shoa, Ethiopia revealed that, asymptomatic malaria was found 5.7% and 3.4% of the study participants using microscopy and RDT respectively (48).

2.3. Factors associated with asymptomatic malaria among pregnant women

Different risk factors for malaria among pregnant women were identified by previous studies. These include residency, educational status, age, ANC visit, gestational age, parity, gravidity, anemia, ITN utilization and IRS practices.

A study conducted in Nigeria showed that, there was a statistically significant association between severity of parasitemia and degree of anemia ($\chi^2 = 441.1$, $P: 0.001$). 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(41).

In a cross-sectional study conducted at ANCs of two district hospitals in India, suggested that peripheral parasitaemia was more common in pregnant women in rural settings (aRR:4.31, 95%CI 1.84-10.11) and in younger age groups below 20 years (aRR: 2.68, 95%CI: 1.03-6.98)(47).

A study conducted in Nigeria among pregnant women showed that asymptomatic malaria parasitaemia was more common in primigravida, where 32 (65.3%) of 49 women were positive, followed by multigravida 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 ($\chi^2 = 31.753$; $P: 0.001$). 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%) than older pregnant women (49.1%). This relationship was also statistically significant ($X^2 = 4.945$; $P = 0.026$)(42).

Another study done in Nigeria with unstable malaria transmission area indicated that primigravida were found to be susceptible to malaria with a significantly higher parasite density 2112.50 ± 420.90 than the multigravida 446.70 ± 296.90 ($P < 0.05$). Anaemia was also found to be higher (64.4%) in primigravida than in multigravida (60.7%) ($P < 0.05$). This study indicated that malaria parasite density increased significantly with gestational age but anaemia was more prevalent in the 2nd trimester than in the other trimesters. Pregnant women appear to be more anaemic in their 2nd 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 2nd 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 (37).

Study from Ghana also assured the high prevalence of malaria in asymptomatic pregnant and its impact on hemoglobin level obviously(45).

According to a study undertaken on malaria infection among pregnant women attending ANC in six Rwandan districts, indicated that, anaemia prevalence mirrored that of malaria infection. The prevalence of infection was significantly higher in primigravida than in the higher gravidities ($P: 0.01$). Prevalence of anaemia was 11.8% and was higher in primigravida than in secondigravidae and multigravida ($P: 0.035$). Parasitaemic women had a 75% higher risk of being anaemic than negatives; and this risk inversely related to increasing parity(46).

According to a study done among pregnant women in Greater Khartoum area, Sudan indicate that, Low parity and young-aged pregnant women were significantly associated with parasitemia ($p=0.001, 0.004$). Second trimester pregnancy [adjusted odd ratio (aOR), 3.2 (95% CI, 2.9-5.8,)] and season of antenatal visit [(aOR), 1.7 (95% CI, (0.9-1.1))] constituted a significant risk factor for contracting malaria. In a multivariate analysis, mothers <20 years [(aOR), 2.4 (95% CI, 1.3-3.0)], first and second pregnancies [(aOR), 2.0 (95% CI, 1.1-1.9)], poverty related factors (low-income, illiteracy) were independent predictors for *P. falciparum* infection. Pregnancy-associated *P. falciparum* malaria is prevalent among Sudanese pregnant women with evidence of close correlation to low parity, young age and socioeconomic status(47).

A study done in Arbaminch town southern Ethiopia, indicated that, parasitemia was more likely to occur in primigravida (AOR: 9.4, 95%; CI: 4.3–60.5), secondigravidae (AOR: 6.3, 95% CI: 2.9–27.3), using ITN sometimes (AOR: 3.2, 95% CI: 1.8- 57.9), not using ITN at all (AOR: 4.6, 95% CI: 1.4–14.4) compared to multigravida and using ITN always, respectively (43). Another study conducted in malaria endemic parts of north shoa, Ethiopia also revealed that, malaria parasitemia was significantly associated with not using ITN (AOR: 18.16; 95% CI: 1.84-179.07) and Anemia ((AOR: 3.78; 95% CI: 0.98-14.58)(48).

In general, asymptomatic malaria parasitaemia is significantly associated with different sociodemographic factors and has great health impact on the level of hemoglobin in pregnant women. This study was evaluated the prevalence of asymptomatic malaria and associated risk factors among pregnant women attending antenatal clinics in Gambella town, Southwest Ethiopia.

2.4. Conceptual frameworks

The independent risk factors causing increased asymptomatic malaria prevalence among pregnant women include maternal risk factors such as younger maternal age, low gestational age, low gravidity, and other associated risk factors such as low economy, no/low frequency of ITN usage, no/low frequency of IRS, rural than urban residence, and larger family size. Parasite density is greater in primigravida, teenagers, and first and second trimesters than in higher maternal risk factors. Anemia directly reflects the prevalence of malaria among the women. It has higher prevalence in all higher malaria cases (43,48).

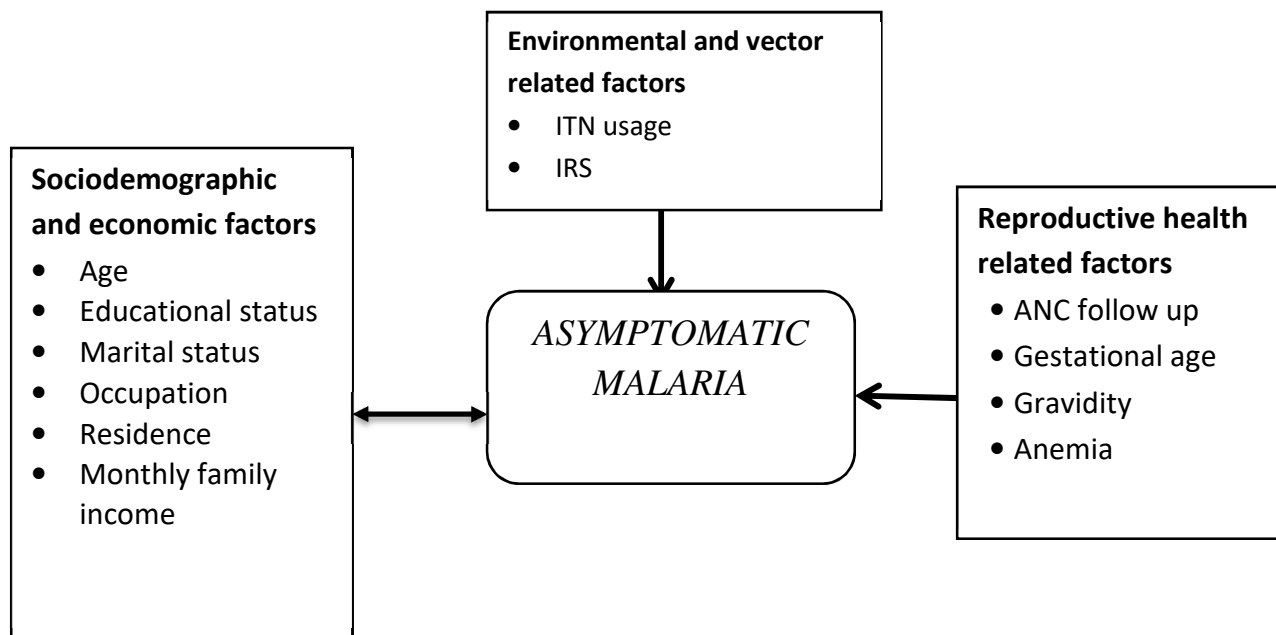


Figure 1: Conceptual framework for factors associated with asymptomatic malaria, Gambella southwest, Ethiopia, July 2022.

CHAPTER THREE: OBJECTIVES OF THE STUDY

3.1. General objective

The aim of this study was to assess the prevalence of asymptomatic malaria infection and its associated risk factors among pregnant women in Gambella town, Southwest Ethiopia, July 2022.

3.2. Specific objectives

1. To determine the prevalence of asymptomatic *Plasmodium* species infection among pregnant women living in Gambella town.
2. To evaluate the impact of asymptomatic malaria on the level of hemoglobin.
3. To identify the associated factors with asymptomatic malaria among pregnant women.

CHAPTER FOUR: METHODS AND MATERIALS

4.1. Study settings and period

The study was conducted in Gambella town, Southwest Ethiopia. Gambella town is the capital city of Gambella region, located at 766 km away from Addis Ababa (the capital city of Ethiopia). The town is known by its flat land scape and hot and humid climate. The averages annual rainfall of the town is 600 mm and the average temperatures ranges between 21.10 °C and 41.90 °C. There is a diversity of ethnic group found in the town. Gambella town has five malarias Kebeles served by one general hospital, one primary hospital and one health center. Under Gambella primary hospital there is one health post that provide primary health care services to the community including malaria, vaccination and malnutrition screening activities. The total population of the town is estimated to be 65,395,000 in 2021 (18). During this study there were, 1962 pregnant women who are obtaining antenatal care (ANC) service in Gambella town of whom, 840 (43%) had been obtaining from Gambella town primary hospital and the rest 1122 (57%) of them from Gambella general hospital. The study was conducted at ANC clinics of Gambella general hospital and Gambella town primary hospital in Gambella town, from May to July, 2022. The study hospitals were selected purposely for this particular study because they were located at the areas of high malaria transmission, possess most human population in the region and providing ANC services in the town.

4.2. Study design

A hospital -based cross-sectional study design was used to conduct the study.

4.2.1. Population

4.2.2. Source population

All women attending ANC clinics at Gambella general hospital and Gambella town primary hospital was the source population.

4.2.3. Study population

Selected pregnant women attending ANC follow up at Gambella general hospital and Gambella town primary hospital was the study population.

4.2.4. Study units

Apparently healthy pregnant women attending ANC during the study period who meet the inclusion criteria.

4.3. Eligibility Criteria

4.4.1. Inclusion criteria

Pregnant women with the absence of malaria symptoms/signs since past forty-eight hours including (fever, nausea, headache, body ache, weakness, anorexia and joint/muscle pains), having an Axillary temperature ≤ 37.5 °C, permanent residents in the town, and those willing and who consented to participate in the study and signed the informed consent were included in this study.

4.4.2. Exclusion criteria

Pregnant women who had taking anti-malarial drugs in the past four weeks prior to the study and those undergoing any kind of long term medical treatments were excluded from this study.

4.4. Sample size determination and sampling technique

4.4.1. Sample size calculation

Sample size was calculated using asymptomatic malaria prevalence of 9.7% reported from a study conducted in the rural surroundings of Arbaminch Town, Southern Ethiopia (43), and 10% for non-response rate. The random error of 5% and confidence level of 95% were assumed in the sample size determination. The calculated sample size was 269.

Formula for single population proportion:

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

Z = level of confidence (1.96)

P = Malaria prevalence among pregnant women living in rural surrounding of Arbaminch town, Southern Ethiopia (9.7%)

d = margin of error (5%)

Calculation

$$n = (1.96)^2 * 0.097 (1 - 0.097) / (0.05)^2 = 135$$

Non-response rate= 10% of n = 14

Total sample size = 135 +14= 149 pregnant women

For the second objective, sample size was calculated using open Epi version 3.03 calculations for unmatched cross sectional study as stated in **table 1** where, Anemia gives the maximum sample size among associated predictors of asymptomatic malaria among pregnant women in some related literature which was **269**.

Table 1: Sample size determination by using factors associated with asymptomatic malaria among pregnant women in Gambella town, southwest Ethiopia, July, 2022.

Variables	CI (%)	Power	% unexposed	AOR	Sample size	n+10%NR	References
Young maternal age (<20yrs)	95	80%	14.5%	3.62	124	137	(49)
Primigravida	95	80%	44.3%	9.4	44	49	(43)
Not using ITN at all	95	80%	31.3%	2.41	84	84	
Anemia	95	80%	82.5	3.78	244	269	(48)

4.4.2. Sampling technique and procedures

The two hospitals were selected by purposive sampling while the study participants were selected by using simple random sampling. Apparently healthy pregnant women who attend the antenatal clinics during the study period was assessed and, those who fulfill the inclusion criteria were counseled, signed the informed consent were obtained and recruited to this study by *simple random sampling using a “lucky dip of Yes or No”*.

Thus a total of **269** pregnant women were selected by using simple random sampling technique. First the two hospitals were listed since there were no other health facilities providing ANC service in Gambella town. The two hospitals were different in population size of pregnant women; thus the study participants were allocated by probability proportional to size (PPS) for each selected study unit as indicated the **figure 2** below.

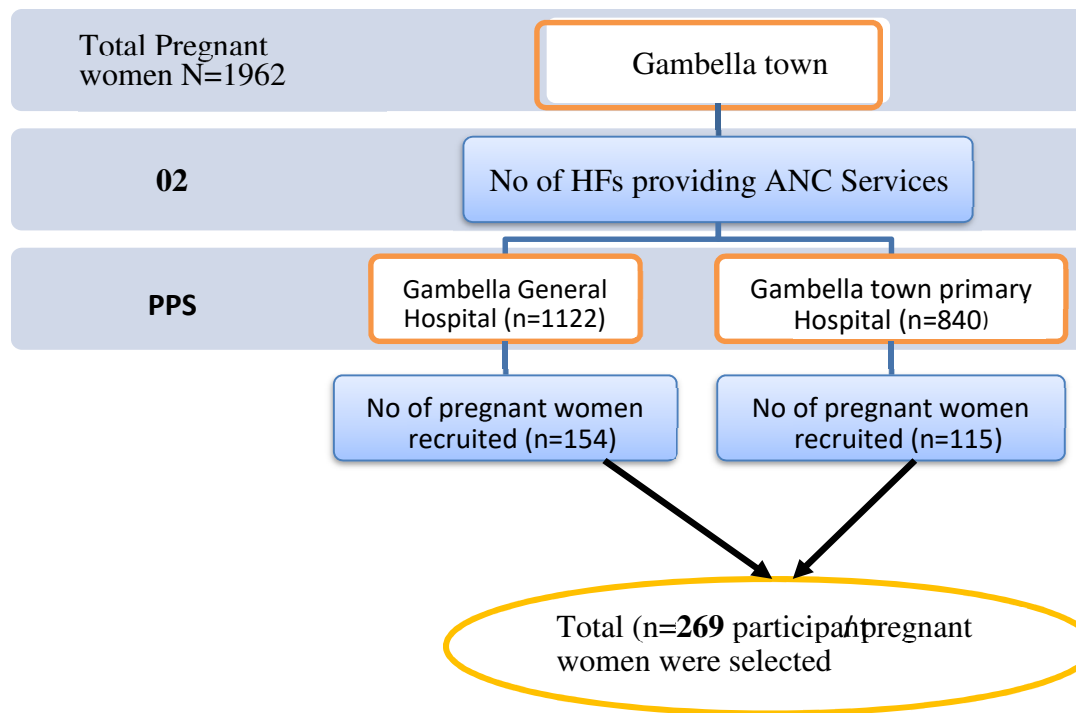


Figure 2: Schematic presentation of sampling procedure for the study Gambella southwest, Ethiopia, July 2022

4.5. Study variable

4.5.1. Dependent variable

- Prevalence of asymptomatic malaria among pregnant women

4.5.2. Independent variable

- Age, Gestational period, Gravidity, Educational status, Occupation, Bed net usage, IRS, Residence, monthly family income and Anemia.

4.6. Operational definitions

Asymptomatic malaria parasitaemia: is defined as the presence of *Plasmodium* by microscopy and RDTs and with an axillary temperature of $< 37.5^{\circ}\text{C}$ and no record of fever within the past 2 weeks (50).

Anaemia: Anaemia in pregnancy is defined as $\text{Hb} < 11.0 \text{ g/dL}$ and further categorized as severe ($\text{Hb} < 7.0 \text{ g/dL}$), moderate (Hb between 7.0 and 10.0 g/dL), and mild (Hb between 10.1 and $< 11 \text{ g/dL}$)(50)

Insecticide-treated mosquito net (ITN): Mosquito nets treated with an effective insecticide to repel or kill mosquitoes. ITN use is defined as a pregnant woman having slept under an ITN the night before the survey/interview (51).

Low birth weight: Infants weighing less than 2,500 grams at birth (52).

Peripheral parasitaemia: Presence of asexual parasitic forms on blood smears or positive RDT.

4.7. Data collection methods, tools, and procedures

The semi-structured questionnaire both with open and close-ended questions were developed and used from previous related studies to obtain sociodemographic and economic information (age, resident, and monthly family income), medical histories (parity, gravidity, antenatal clinic visit), and the use of ITNs and IRS history.

Gestational age was assessed from the last normal menstrual period or gestational calendar. Trimester was defined as first trimester (<14 weeks), second trimester (14-27 weeks) and third trimester (>27 weeks). Data collectors were regularly supervised by the principal investigator for proper data collection. Medical and obstetric examinations were done. Axillary temperature was taken to exclude fever and temperature of less than 37.5°C was considered as normal.

Two clinical nurses or midwives who can speak both Amharic and the local language (Agnua or Nuer) obtained training on data collection procedure for this particular study to attain standardization and maximize interviewer reliability.

About 3ml of blood samples were collected for malaria parasite and hemoglobin estimation from vein by experienced and trained medical laboratory personnel's. Malaria parasites were identified by thick and thin blood films, and malaria RDTs. Three readers were involved to read the result, and discrepancy were resolved by the third reader in case happen.

4.7.1. Data quality assurance

Questionnaire were prepared first in English, translated into the local language Amharic, and then retranslated back to English by language expert to maintain its consistency. Training was given for data collectors and supervisor. Pre-testing of the questionnaires was made on 5% pregnant women's attending ANC clinics at Bonga HC, a week prior to the study and appropriate changes were made according to the feedback received. The questionnaire also checked for its clarity,

completeness, consistency, sensitiveness and setting of time required to conduct interviews and the questions which posed difficulty or unclear were rephrased and corrected. To ensure the quality of the data reliability: The data collection, application of standard operating procedure by professionals, accuracy of test results was supervised by principal investigator. Close follow up by the investigator during the whole data collection process were done.

All the necessary reagents and equipment's were checked by known positive and negative samples before sample preparation and examination using control samples. The blood smears were examined independently by two experienced trained laboratory professionals for microscopy and RDT techniques blindly to avoid observer bias and finally the results were checked by the principal investigator in case where the results were discordant, the results of the third expert reader were considered as the final results.

Furthermore, data collection process was strictly followed on daily basis by the principal investigator. Standard operating procedures were adhered during sample collection, processing, transportation, and detection of malaria and anemia.

4.8. Data processing and analysis

Data were coded, entered and cleaned before and during data processing using statistical software Epidata version 3.1 and was exported to SPSS for windows version 26 (IBM SPSS Statistics, IBM Corp., New York) statistical software package for analysis. Dependent variable frequencies, percentage, mean, range, and proportion were calculated.

Descriptive statistics such as frequency, proportion, mean and standard deviation ($\pm$ SD) were used to summarize the characteristics of the study participants and to determine the prevalence of asymptomatic malaria among pregnant women. Chi-square test and bivariate logistic regression analyses were performed to identify the predictors of asymptomatic *Plasmodium* infection. A multivariable logistic regression model was used to identify risk factors associated with malaria adjusting for potential confounders. The estimates were presented as odds ratio (OR) with a 95% confidence interval (95% CI). P-value less than 0.05 were considered statistically significant in all comparisons. Results were presented using tables, charts, and graphs in addition to text description.

4.9. Ethical considerations

Ethical clearance was obtained from the research and ethics committee of SPHMMC. Letter of permission was obtained from administrative body of the respective hospitals. The study participants were informed about the purpose and procedures of the study. Written consent was obtained from each study participants. Only those (i) who were willing to participate and (ii) signed the written informed consent form were included in the study. All information collected from study participants were kept anonymous and confidential.

Study participants who were found with undesired result were linked to the concerned department for further consideration such as treatment and health education relevant to prevention and control. Results of participants with parasitic infection and low HCT level were given to the study participants, as soon as possible, for treatment and medical consultation in their corresponding ANC.

Furthermore, WHO recommended aseptic technique were applied to avoid contamination while taking samples. The disposal of biological wastes produced during data collection and laboratory investigation were done in an environmentally friendly manner, following WHO recommended standard procedure. Biological samples were only stored for the intended duration and purpose stated in this study with the consent of the study participant.

4.10. Dissemination and communication of findings

The finding or the result of this study were submitted to SPHMMC and School of Public Health. It was also presented to SPHMMC scientific community through thesis defense. Then the final report was disseminated to the concerned bodies so that the outcomes of this study was used by healthcare policy makers, budget planners, researchers and other health professionals for the prevention and control of asymptomatic malaria and its associated factors among pregnant women. Furthermore, great endeavors were made to publish on peer-reviewed reputable journal and present the findings during different meeting /conferences.

CHAPTER FIVE: RESULTS, DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

RESULTS

5. Results

5.1. Sociodemographic and economic characteristics of the study participants

A total of 269 pregnant women were participated in this study giving a response rated of 100%. The age of the study participants ranged from 18 to 38 years with the mean age of 24.25 years (SD = $\pm$ 4.22). The vast majority of the study participants 263 (97.8%) were married. Regarding educational and occupational status, 165 (61.3%) were completed secondary school and about 135 (50.2%) were daily laborer, respectively. The vast majority of the study participants 259 (96.3%) earn more than one thousand Ethiopia Birr (>1000 ETB) per month and 260 (96.7%) of the study participants resides in Urban setting. The mean family size in the present study was 3.01 (SD $\pm$ 1.16). About 104 (38.7%) the pregnant women were Primigravida, 218 (81%) of them were in their second trimester of pregnancy and majority 245 (91.1%) of them were following ANC in the respective hospitals. Regarding vector prevention measures, the majority 244 (90.7%) of the study participants were utilizing ITNs regularly and 216 (80.3%) them had history of IRS for the last 12 months (Table 2).

Table 2: Socio-demographic and economic characteristics by asymptomatic malaria infection among pregnant women in Gambella town, Southwest Ethiopia, July 2022 (n=269).

Variable	All cases		Asymptomatic malaria				P-value)
			Yes		No		
	n	%	n	%	n	%	
All patients	269	100	23	8.6	246	91.4	-
Age (Years)							0.153
<20	72	26.8	10	13.9	62	86.1	
20-30	174	64.7	12	6.9	162	93.1	
>30	23	8.5	1	4.3	22	95.7	
Marital status							0.228*
Married	263	97.8	21	8	242	92	
Unmarried	6	2.2	2	33.3	4	66.7	
Educational status							0.179
No formal education	5	1.9	1	20	40	80	
Primary School	87	32.3	3	3.4	84	96.6	
Secondary School	165	61.3	18	10.9	147	89.1	
Tertiary School	12	4.5	1	8.3	11	91.7	
Occupational status							0.001*
Merchant	20	7.4	6	30	14	70	
Daily laborer	135	50.2	7	5.2	128	94.8	

House wife	84	31.2	5	1.9	79	29.4	
Government employee	30	11.2	23	8.6	246	91.4	
Monthly income (ETB)							0.001*
<1000	10	3.7	5	50	5	50	
≥1000	259	96.3	18	6.9	241	93.1	
Place of residence							0.001*
Urban	260	96.7	19	7.3	241	92.7	
Rural	9	3.3	4	44.4	5	55.6	
Anemia							0.001*
Anemic (<11 g/dl)	58	21.6	12	20.7	46	79.3	
Normal (≥11 g/dl)	211	78.4	11	5.2	200	94.8	
Gestational age							0.929
1 st trimester	32	11.9	3	9.4	29	90.6	
2 nd trimester	218	81	18	8.3	200	91.7	
3 rd trimester	19	7.1	2	10.5	17	89.5	
Parity							0.028*
Primigravida	104	38.7	10	9.6	94	90.4	
Secondigravida	99	36.8	8	8.1	91	91.9	
Multigravida	66	24.5	5	7.6	61	92.4	
Family size							0.225
≤3	212	78.8	21	9.9	191	90.1	
>4	57	21.2	2	3.5	55	96.5	
ANC follow up							0.024*
Yes	245	91.1	18	7.3	227	92.7	
No	24	8.9	5	20.8	19	79.2	
ITNs use							0.001*
Always	244	90.7	14	5.7	230	94.3	
Rarely	17	6.3	6	35.3	11	64.7	
Don't use	8	3	3	37.5	5	62.5	
IRS (Last 12 months)							0.001*
Yes	216	80.3	8	3.7	208	96.3	
No	53	19.7	15	28.3	38	71.7	

ANC: Antenatal Care, ITNs: Insecticide-treated net; IRS: Indoor residual spraying; OR: odds ratio; 95%CI: 95% confidence interval. *Statistically significant association (p < 0.05).

5.2. Prevalence of Asymptomatic malaria infection and anemia among pregnant women in Gambella town, southwest Ethiopia, July 2022

The prevalence of asymptomatic malaria infection among pregnant women were 23 (8.6) % and 17 (6.3%) by microscopy and RDTs, respectively. All of them were found infected with *P. falciparum* as *P. falciparum* was being a dominant *Plasmodium* infection in general population in the region.

In this study, the mean haemoglobin was estimated at 11.9 (SD = 1.15) g/dL and the overall prevalence of anemia (haemoglobin level of <11 g/dl) among pregnant women were 58 (21.6%) and 12 (20.7%) of them were infected with asymptomatic malaria.

5.3. Factors associated with asymptomatic malaria infection among pregnant women in Gambella town, southwest Ethiopia, July 2022

Socio-demographic and economic characteristics and other health related factors were computed using Bivariate and Multivariable logistic regression analyses. In the bivariate analysis, occupational status, monthly income, place of residence, gravidity, haemoglobin level, ANC follow up, ITN usage and IRS history were found to be significantly associated with asymptomatic malaria among pregnant women at p-value of < 0.05 levels (see **Table 3**).

However, in multivariable logistic regression analyses (i.e. after adjusting for possible confounder), lower monthly household income, being anemic, being primigravidae, not using of ITN, and not spraying of IRS for the last 12 months were the independent variables statistically significantly associated with the outcome variable at p-value <0.05.

In this study asymptomatic malaria infected pregnant women were more likely to be anemic (AOR: 0.23, 95% CI 0.068-0.791) compared to non-infected pregnant women. Asymptomatic malaria was more likely to occur in primigravidae (AOR: 15.22, 95%CI= 1.473-157.35) compared to multigravida. ITN usage was another factor assessed for possible association with asymptomatic Plasmodium infection among the pregnant women. It was found that asymptomatic malaria was higher among those who did not use ITN (AOR= 0.07, 95%CI=0.010-0.546) compared to those who were using ITN always (Table 3). Not spraying of IRS was significantly associated with higher rates of asymptomatic malaria infection (AOR= 0.11, 95%CI=0.031-0.354) compared to those who did IRS for the last 12 months (see **Table 3**).

Table 3: Bivariate and multivariable analysis of association between socio-demographic and Asymptomatic malaria infection among pregnant women in Gambella town, Southwest Ethiopia, July 2022.

Variable	All cases		Asymptomatic malaria				COR (95% CI)	P-Value	AOR (95% CI)	P-Value
	n	%	Yes		No					
			n	%	n	%				
All patients	269	100	23	8.6	246	91.4	-	-	-	-
Occupational status										
Merchant	20	7.4	6	30	14	70	0.467(0.120-1.810)	0.270	0.948(0.144-6.241)	0.956
Daily laborer	135	50.2	7	5.2	128	94.8	3.657(1.074-12.450)	0.038*	4.771(0.959-23.745)	0.056
House wife	84	31.2	5	1.9	79	29.4	3.160(0.845-11.813)	0.087	2.920(0.551-15.468)	0.208
Government employee	30	11.2	23	8.6	246	91.4	1		1	
Monthly income (ETB)										
<1000	10	3.7	5	50	5	50	0.075(0.020-0.282)	0.001*	0.118(0.018-0.801)	0.029*
≥1000	259	96.3	18	6.9	241	93.1	1		1	
Place of residence										
Urban	260	96.7	19	7.3	241	92.7	1		1	
Rural	9	3.3	4	44.4	5	55.6	0.099 (0.024-0.398)	0.001*	0.512 (0.059-4.440)	0.543
Anemia										
Anemic (<11g/dl)	58	21.6	12	20.7	46	79.3	0.211(0.088-0.508)	0.001*	0.232 (0.068-0.791)	0.020*
Normal (≥11g/dl)	211	78.4	11	5.2	200	94.8	1		1	
Parity										
Primigravida	104	38.7	10	9.6	94	90.4	0.5.762 (0.996-33.322)	0.050	15.223 (1.473-157.35)	0.022*
Secondigravida	99	36.8	8	8.1	91	91.9	0.467(0.120-1.810)	0.270	0.948(0.144-6.241)	0.956
Multigravida	66	24.5	5	7.6	61	92.4	1		1	
ANC follow up										
Yes	245	91.1	18	7.3	227	92.7	1		1	
No	24	8.9	5	20.8	19	79.2	0.301 (0.101-0.901)	0.032*	0.481(0.104-2.216)	0.348
ITN use										
Always	244	90.7	14	5.7	230	94.3	1		1	
Rarely	17	6.3	6	35.3	11	64.7	0.112 (0.036-0.346)	0.001*	0.188 (0.031-1.156)	0.071
Don't use	8	3	3	37.5	5	62.5	0.101(0.022-0.468)	0.003*	0.074 (0.010-0.546)	0.011*
IRS (Last 12 months)										
Yes	216	80.3	8	3.7	208	96.3	1		1	
No	53	19.7	15	28.3	38	71.7	0.097(0.039-0.246)	0.001*	0.105(0.031-0.354)	0.001*

ANC: Antenatal Care, ITN: Insecticide-treated net; IRS: Indoor residual spraying; COR: crude odds ratio; AOR: adjusted odds ratio; 95%CI: 95% confidence interval. *Statistically significant association (p < 0.05).

6. DISCUSSION

In the present study, the prevalence of asymptomatic *malaria* infection among pregnant women in Gambella town was found to be 23 (8.6%) and 17 (6.3%) by using Giemsa-stained blood smear microscopy and ONE STEP Malaria HRP-II (P.f) and PLDH (P.v) Antigen Rapid Test, respectively. This prevalence was lower than the finding reported in Burkina Faso where the prevalence of 24% and 30% among pregnant women using microscopy and RDT respectively(36). In contrast, the prevalence of asymptomatic malaria in the present study was higher than the finding reported in Nigeria where the prevalence of 3.1% and 4.8% by microscopy and RDTs respectively(54), in Bangladesh with the prevalence of 2.3% using RDT, in North Shoa, Ethiopia, with prevalence of 5.7% and 3.4%% using microscopy and RDTs respectively(48) and in Merti Ethiopia, with the prevalence of 3.6% using microscopy(55). However, these findings corroborated with the results in Lagos, where a prevalence rate of 7.7% among pregnant women attending ANCs(49) and in Arbaminch, Ethiopia, where the prevalence of 9.1% and 9.7% by microscopy and RDT, respectively among pregnant women attending ANCs(43). This substantial rate of asymptomatic malaria among pregnant women in the area calls for the need to review the control measures available, with a view to possibly redesigning the control programmes.

This observed difference in prevalence of asymptomatic malaria among pregnant women might be partly explained by the difference in the size of the study participants, sampling techniques, the study settings, altitudes, study season, weather conditions and socio demographic and economic variability of the study participants. Besides, in this study, strict inclusion criteria were used to recruit pregnant women who were asymptomatic with a random lucky dip of yes or no while the other employed by the systematic sampling method. Moreover, many reason also could be explained for the wide variations in the reported prevalence among pregnant women due to the area of the present study is among the high malaria transmission areas in Ethiopia. In areas with high malaria transmission, individuals frequently exposed to malaria infection which leads to development of protective immunity that makes plasmodium infection to be asymptomatic(43) .

In this study the prevalence of asymptomatic *Plasmodium* infection was higher using microscopy than RDT which disagrees with a report from Arbaminch, Ethiopia and Nigeria(43,54). However, the finding was consistent with the study findings reported from north shoa, Ethiopia(48). This is

may be due to the fact that the most recent report of parasites with deleted *pfhrp2/3* genes remain undetected and untreated due to ‘false-negative’ RDT results(56).

In this study, the variables identified as a risk factor for asymptomatic malaria among pregnant women were lower household income, anemia, gravidity (primigravidae), ITNs use practices, and history of IRS (for the last 12 months).

The presence of malaria and anaemia in pregnancy, regardless of the gestational stage, are potentially harmful to both the fetus and mother as well as to the family and community(57,58). This study provides insights into the extent of asymptomatic MiP and its effect, anaemia, in Gambella town.

This study showed a high prevalence of anemia among pregnant women were 21.6% of them were anemic and of which 20.7% of them were infected with asymptomatic malaria, though this prevalence seemed to be much lower compared with the prevalence reported in Burkina Faso where 59.4% and 61% among pregnant women attending health facilities ANC(36,57). According to the literature, the nutritional deficiencies, particularly deficiency in iron and folic acid constitute the main causes of anaemia in pregnancy(36,57).

The present study indicated that the malaria risks could also be linked with low household income(47). This may be indicating the role socio economic impact on the overall success of malaria control programmes in the study area. Government policies should be focused towards improving citizens’ socio economic status in order to reduce the burden of the disease in the country, especially among the most vulnerable population.

In this study, it has been demonstrated that infection rates are higher in women in their first pregnancies and significantly associated with asymptomatic malaria. This was in agreement with the finding reported from Ethiopia and Nigeria(43,59). This is clear as pregnancy is naturally accompanied by general immune suppression that may cause loss of acquired immunity to malaria especially among primigravidae. This is because they lack the specific immunity to placental malaria that is acquired from exposure to malaria parasites during pregnancy. This immunity accumulates with successive pregnancies, provided there is exposure to malaria infection(59)

The use of ITNs decreases both the number of malaria cases and malaria deaths in pregnant women(3). In our study, the use of ITNs was found to be associated with malaria infection;

pregnant women who did not use ITNs regularly were more affected by malaria as compared to those who did. A previous study conducted in Burkina Faso and Ethiopia also indicated that the rate of malaria infection proportionately decrease with the use of ITNs(48,57). This finding is in line with the fact that effectiveness of ITNs usage is considered as one of the most important prevention and control tools(3). There was also statistically significant association between IRS and plasmodium infection in pregnant women. This was in agreement with the finding reported from Arbaminch Ethiopia(43).

In the present study there was no significant difference in the rate of asymptomatic malaria parasitemia with respect to age, marital status, parity and ANC follow-up of the pregnant women. However, the findings of some similar studies(43,48,50), suggested that asymptomatic malaria was higher in pregnant women of younger age, with second trimester, parity and ANC follow up. This contrast might be due to differences in the sample size, sampling technique, physiologic and biochemical factors of pregnant women and the study setting such as geographical differences, altitude, temperature, and age categorization scheme.

6.1. Strengths and limitations of the study

As part of strengths, (1)our study demonstrated the finding of other previously studies (43,48,50) that asymptomatic malaria, particularly *P. falciparum* was prevalent and also responsible for anaemia in pregnant women. (2) our study also adhered all the possible research protocols thus increases the chance of generalization to the whole pregnant population in the study areas. And (3) our study incorporated the two diagnostic methods in detection of asymptomatic malaria among pregnant women increased the accuracy of the diagnosis, were the strong side of this study.

As parts of limitations, (1) our study were based on a cross-sectional design that did not allow us to determine cause and effect. (2) data on living, and environmental conditions factors, were not collected despite they are important risk factors for malaria infection. (3) our study also identified asymptomatic malaria among pregnant using RDT and light microscopy, where the sensitivity of these tests is low for the detection of very low parasitaemia comparing with PCR which underestimate the prevalence rate of asymptomatic malaria (60). And (4) to define anaemia we only measured haemoglobin levels among pregnant women though the impact of asymptomatic malaria on hematological markers like blood count, ferritin, and transferrin levels are necessary (60). Therefore, further research with larger sample size and detailed information regarding additional risk factors for asymptomatic malaria and anemia among pregnant women is necessary.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

Even though prevalence of malaria is decreasing in the country because of scale-up of intervention and prevention measures, this study showed that, malaria is still the major public health problem among pregnant women. Being married, low house hold income, anemia, not sleeping under long lasting insecticide treated nets and not spraying IRS were factors that increased the prevalence of malaria in malaria symptomatic pregnant women.

The present study also showed anemia highly prevalent among pregnant women infected with asymptomatic malaria

7.2. Recommendation

This study makes recommendations based on the findings,

- This study indicated that the prevalence of asymptomatic malaria among pregnant women were considerably high, thus, it is recommended to screened for asymptomatic malaria to avoid health consequences of malaria infection during pregnancy for the mother and fetus and, special attention should be given to pregnant women prone to those identified factors.
- The findings of the present study also suggested the need of further malaria control strategies to screen the pregnant women for asymptomatic plasmodium infection followed by prompt malaria treatment in the ANC service package. Such initiatives not only safeguard the pregnant women from malaria associated morbidities and mortalities but also the fetus and the newborns.
- As part of ANC service package, educating on the appropriate usage and benefits of the bed nets, IRS and encouraging early ANC attendance among pregnant women could enhance benefits for the women's health.
- Therefore, screening of pregnant women, increasing awareness and enhancing the prevention and control of malaria and anemia should be strengthened.
- Further research is also highly recommended along with other factors using large sample size and covering wide area.

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Annexes

Annex I: Data Collection Procedure

1. Parasitological examination

Blood specimens were collected for preparation of blood smears and malaria rapid test (RDT) from finger prick using sterile lancet by experienced medical laboratory technician.

1.1 Blood film examination

Semi-structured questionnaire was administered to each participant and two thin and thick blood films were used to identify malaria parasites and estimate density. The finger was cleansed with an alcohol-moistened swab, dried with a piece of dry cotton, and pricked with a disposable blood lancet. Through wiping off the first drop of blood, thick and thin films were made on the same slide. For thick films, 6µl of blood were spread over a diameter of 15 mm, while 2µl of blood were used for thin films. Before staining the air-dried blood films, the thin blood films were fixed in methanol for 30 sec. Then blood films were stained with 10% Giemsa stain solution at pH 7.2 for 10 min. The staining technique and blood film examination were conducted employing WHO guidelines. 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 were carried out by experienced laboratory personnel's. In addition to the qualitative examination, parasitic load were determined following WHO guidelines (61).

Each slide was read by two microscopists blinded to the other's readings. A third microscopist will settle discrepancies between readings. Parasitemia were determined by counting the number of asexual forms of *P. falciparum* parasites against 200 leukocytes. Enumeration was done on the thick smear under oil immersion and viewed using 100X objective lens. The number of asexual parasites were calculated using the following formula(62):

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

A definitive diagnosis of malaria was made when a reddish chromatin dot with a purple or blue cytoplasm of the malaria parasites are seen together. Quality control were ensured by using freshly reconstituted and filtered Giemsa stains and using standard positive and negative films as well as standard operation procedures. Where there were doubts, films were cross-examined by a senior microscopist. The microscopist was spend an average of 5 min to 10 min 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.

1.2 RDT (SD BIOLINE Malaria Ag Pf/Pv POCT, standard diagnostics, inc., Korea).

Malaria (ONE STEP Malaria HRP-II (P.f) and PLDH (P.v) Antigen Rapid Test). RDT is currently being used in Ethiopia. Histidine-rich protein 2 of *P. falciparum* (PfHRP2) is a water-soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum*, expressed on the red cell membrane surface, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy. All the antigens targeted by the RDTs are expressed by the asexual as well as the sexual forms of the parasites and persistent antigenemia can cause positive tests on RDTs up to one month. PfHRP2 test kit has generally shown higher sensitivity for *P. falciparum* and can be less costly than the plasmodium spp. Lactate dehydrogenase (*PspLDH*) alternative, though it contributes to false-positive results and limiting specificity due to the prolonged persistence of PfHRP2 antigen(63). These malaria rapid tests were performed according to the manufacturer's instruction. All RDT devices were labeled with similar patient ID numbers to that of the blood film. Then the collected blood sample were transferred directly to the sample pad and 4 drops of clearing buffer were added. Finally, the results were read after 15 minutes. Presence of band on both the control and test lines indicated a positive result for *P. falciparum*/*P. vivax* infection. The formation of only one band on control line indicated a negative result. If no band formation on the control line, the test was read invalid.

The manufacture and expiry date of RDT were checked and handled at the temperature recommended by the manufacturer suggesting that they are in a supposedly good condition during the study period. Quality controls and discrepancies were resolved in same way as for blood film examination(63).

The sensitivity, specificity, predictive positive and negative values of the kits were calculated using microscopy as the gold standard(61–64).

2. Hematocrit determination

Hemoglobin level were estimated using manual hematocrit centrifuge. According to WHO guidelines, pregnant women are normal with hemoglobin concentration of 11 g/dl or 33% hematocrit value and above. The average of two packed cell volumes at booking were determined using two capillary tubes and read from a Hawks leys micro hematocrit reader. The packed cell volume (PCV) were used as a simple screening test for anaemia, as a reference method for calibrating automated blood count systems and as a rough guide to the accuracy of hemoglobin measurements. The microheamatocrit method is carried out on blood contained in capillary tubes 75mm in length and having an internal diameter of about 1mm. The tubes may be plain for use with anticoagulated blood samples. The centrifuge were used for capillary tubes provides a centrifugal force of 12,000g, and 5 minutes' centrifugation result in a constant PCV. The microheamatocrit method has an adequate level of accuracy and precision for clinical utility. Then pregnant women with HCT values less than 33% were categorized as anaemic. Anaemic women will further be categorized as women with mild anaemia (Hct 30%-33%), moderate anaemia (Hct 21%-30%) and severe anaemia (Hct below 21%)(65).

Annex II: Laboratory Requesting and Recording Format

St Paul Hospital Millennium Medical College, Department of Public Health

Parasitological investigation and hemoglobin determination at Health Facilities in Gambella town

1. Personal data

1.1 Code no.....

1.2 Age

1.3 Address

1.4 Occupation

1.5 Date of sample collection

2. Laboratory data

2.1 Malaria RDT examination.....

2.2 Blood film examination.....

A. Types of haemoparasite seen.....

B. Density of *P. falciparum*.....

c. No haemoparasite seen.....

2.3 Hemoglobin determination.....

Name of investigator _____

Signature _____ Date _____

Annex III: Consent Form

Code number_____

Date.....

Consent form

St Paul Hospital Millennium Medical College

This is an agreement request to assess the prevalence of Asymptomatic Malaria in pregnant women. With this request, those pregnant women attending antenatal clinic at Gambella General Hospital, and Gambella town Primary hospital of Gambella town who fulfill the study criteria and willing to participate in the study will be invited to assess the prevalence of asymptomatic malaria. Based on the laboratory investigation, those who become positive for *plasmodium* parasite and anemia will get treatment from their clinics.

Objective of the study.

Questionnaires will be requested on factors associated with asymptomatic malaria to assess its prevalence and impact on the level of hemoglobin. On the request paper, your name or your identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose. You have all the right to accept or refuse participation of this study. You will be requested to provide 3ml of blood from your vein and there will be some pain during injecting your vein but not harmful to your health. If you agree to give samples, you will be requested to answer for questionnaire. Are you willing to participate with this study by giving blood sample and answer to the requests?

A. Yes B. No

Thank you

Address of participant _____ House number.....

Participant name Signature.....Date.....

Name of data collector.....Signature..... Date.....

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Annex V: Questionnaire

Assessment of associated risk factors for asymptomatic malaria among pregnant women in Gambella town, Southwest Ethiopia.

Socio-demographic factors, past and present histories of pregnancy and other medical conditions.

(I) A verbal questionnaire regarding the symptoms of malaria for inclusion of the study participants:

1. Are you suffering from fever, headache, body ache, nausea since last fortnight?
2. Did you suffer from malaria within the last one month?
3. Are you undergoing any kind of medical treatment?
4. This is a study regarding asymptomatic malaria cases among pregnant women attending ANC.

Do you mind giving your blood sample for this study?

When we find the answer “No” for all the four questions, we will select the person for blood sampling.

(II) Assessment of associated risk factors for asymptomatic malaria among pregnant women in Gambella town, Southwest Ethiopia.

Annex V.I: Questionnaires (English)

Questionnaire Code _____

Instructions to the interviewer's/data collectors

This questionnaire contains two parts; Read each part carefully and encircle appropriate number of response. If more than one response is needed, it's possible to encircle more than one response only in response column.

Part I: Socio-demographic factors			
No.	Questions	Response	Remark
	ID number of the patient	_____	
1	Age of the Patients in Year	_____ Years	
2	Marital status	1.Married 2.Unmarried	
3	Educational status	1.No formal education 2.Primary school 3.Secondary school 4.Tertiary school	

4	Occupational status	1.Merchants 2.Day laborer 3.House wife 4.Jobless 5.Government employee	
5	Monthly household income	1 < 1000 ETB 2 > 1000 ETB	
6	Place of residence	1.Urban 2.Rural	
Part II: Clinical and medication characteristics			
7	Laboratory data	1. Blood film result: _____ 2. RDT result: _____ 3. Hemoglobin result:_____	
8	What is your gestational age?	1. First trimester (< 14 weeks), 2. Second trimester (14–28 weeks) 3. Third trimester (> 28 weeks).	
9	What is your gravidity?	1. Primigravida 2. Secondigravidae 3. Multigravidae(3-5 births) 4. Grandgravidae(>5 births)	
10	How many children do you have?	----- childrens	
11	Did you follow antenatal care in your previous pregnancy?	1. Yes 2. No	
12	Do you follow antenatal care for the current pregnancy?	1. Yes 2. No	
13	Do you sleep under impregnated anti- malaria bed net (ITN)?	1. Yes 2. No	
14	If your answer to question #14 is yes, how often do you use it?	1. Frequently 2. Rarely	
15	Did you spray Insecticide spray (past 12 months)	1. Frequently 2. Rarely	

[illegible]

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Annex VI: Declaration

I, Ashenafi Kure, hereby declared that this proposal is my original work and has never been presented for any degree in SPHMMC or any other institutions of higher learning in Ethiopia. I also declare that all material sources used for this proposal have been duly acknowledged.

Signature _____

Place: SPHMMC

Date of submission 18/11/2022

This proposal has been submitted for approval with my supervision as university advisor.

Name of Primary advisor: **Mr. Kifle Haile (MPH, Lecturer)**

Signature _____

Place: SPHMMC

Date: **18/11/2022**

Name of secondary Advisor: **Mr. Takele Gezahegn (MPH, Assistant Professor)**

Signature: _____

Place: SPHMMC

Date: **18/11/2022**

Approval of the Examiners

External Examiner: Dr Lemessa Oljira Signature _____ Date _____

Internal Examiner: Mrs Hanna Feleke Signature _____ Date _____