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Full Length Research Paper

Prevalence of Malaria and associated factors among pregnant women attending antenatal care at Angar Gute health centers, Western Ethiopia

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Malaria in pregnancy is a major public health problem in Sub-Sahara African countries, including Ethiopia. The unwanted outcomes of malaria in pregnancy are morbidity and mortality of the pregnant women and their newborns. **In moderate and high malaria transmission sub-Saharan Africancountries about 11 million pregnant women would have been exposed to malaria infection in 2018.** ***To determine prevalence of malaria and associated factors among pregnant women attending antenatal care at AngarGute Health Center, Western Ethiopia. A health facility-based cross-sectional study comprising a convenient sampling technique including 239 pregnant women was conducted from September to November 2020. Blood specimen were examined to detect plasmodium species using microscopy and rapid diagnostic test. Hemoglobin concentration was performed. Bivariate and multivariate logistic regression were employed to assess factors associated with malaria. Of the total 239 pregnant women who participated in the study the pooled prevalence of malaria was 5.65. of the positive cases 13(5.4%)*** **, and 14 (5.9%)** ***were detected by microscopy and rapid diagnostic tests, respectively. Of 13 malaria positive, 11 (84.6 %),*** **95% CI (***57.8-95.6) and* **2(15.4%), 95% CI (4.3-42.3)** ***were infected with P. falciparum and P.vivax, respectively.*** **Residence (AOR = 0.16, 95% CI: 0.015 -0.89, p <0.038), gravidity (AOR:9.62 95% CI: 1.2-77.8 , p <0.034), ITNs ownship (AOR: 5.4, 95% CI: 1.2-25.2, p <0.032) and ITNs utilization sometimes (AOR:0.114, 95% CI: 0.02-0.70, p <.019) and never use (AOR= 3.4, 95% CI (0.63-12.3), p <0.001) were significantly associated with malaria. Anemia was associated with malaria at chi-squere (X^2=13.15, *P*=0.01). The prevalence of malaria among pregnant women in our study was high and the predominant species was *P. falciparum*.**

Keywords: Malaria, Antenatal care, Plasmodium,Pregnant women, AngarGute

INTRODUCTION

Background

Malaria is one of the top fatal diseases caused by parasites belong to the genus *plasmodium*. There are five common species of *plasmodium* which can infect humans. These are *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi*. Globally, malaria infection was decreased from 238 million across 108 malaria-endemic countries to 229 million malaria cases in 87 malaria prevalent countries in 2000 to 2019. In 2019 the World Health Organization (WHO) African County estimated 215 million malaria cases and 384 000 malaria deaths which accounted for 94% of cases and deaths globally. Malaria mortality rate reduced by 44% while morbidity rate decreased by 67%in 2019(1). Malaria in pregnancy has significant threats for pregnant mothers and the newborn. For the pregnant woman, malaria infection can lead to complicated disease and death, maternal anaemia; mortality before and after childbirth. For the newborn stillbirth, preterm birth, poor fetal growth and low birth weight due to Placental infection which in turn can lead to child growth retardation and poor cognitive outcomes, also being a major risk factor for perinatal, neonatal and infant mortality.According to the 2020 WHO malaria report in 33 moderate to high malaria transmission countries in the WHO African Region, among 33.2 million pregnancies 11.6 million were exposed to malaria. By WHO sub-region, central Africa had the highest prevalence 40% followed by West

Africa 39% and East and Southern Africa 24%(1).
In moderate and high malaria transmission sub-Saharan African (SSA) countries about 11 million pregnant women would have been exposed to malaria infection in 2018. Prevalence of exposure to malaria infection in pregnancy is highest in the West and Central African (35%) followed by East and Southern Africa (20%). About 39% of these were in the Democratic Republic Congo and Nigeria (2).
The clinical manifestation of Plasmodium infection varies from asymptomatic to fatal symptomatic in malaria-endemic areas. Asymptomatic *plasmodium* infections can be associated with high levels of gametocytes which serve as parasite reservoir for malaria transmission. Frequently exposure to *Plasmodium* parasites produce partial immunity in high and moderate malaria transmission. Malaria infection is an obstacle in the concept of a malaria elimination program. It also creates an asymptomatic carrier for the persistence of malaria transmission (3).
Innovative and cost-effective malaria control strategies that clear malaria infections are required to reach malaria elimination goals. As the world move towards malaria elimination, the focus of interventions has to shift to include a group of people such as pregnant women with malaria infection. 'Asymptomatic carriers' (subclinical) pregnant women defined as malaria-infected but healthy individuals who may be the source of infection for malaria vectors, a silent reservoir of gametocytes, thereby maintaining transmission (4, 5). In clinical malaria studies, 'apparently healthy' usually means the absence of fever, the most symptom of malaria (6). Research shows that low-density infections, including sub-microscopic infections, could be important factors for malaria transmission in areas with very low transmission intensity (7).
The possibility of continuous transmission maintained due to malaria infection shows the 'human reservoir of infection'. Therefore, this study helps to determine the prevalence and factors associated with malaria infection among pregnant women in the study area which indirectly strengths the previous intervention in malaria elimination and control programme at 2030 and great attention should be given to the malaria infection to eliminate and control malaria in Ethiopia.
Malaria in pregnancy (MIP) is a major public health problem in Sub-Saharan African countries, including Ethiopia. Morbidities and mortalities of pregnant mothers and their newborn were the most significant problem associated with MIP. Some of the major outcomes due to malaria in pregnany are maternal anemia, low birth weight, premature births and still birth. The sequestration of *Plasmodium* species in the placenta was the main factor resulted with MIP.In Africa about 30 million pregnant women live in malaria-endemic regions become exposed each year. Malaria infection affects both the mothers and their newborns, with up to 200,000 new born deaths occurring each year and 25% of maternal death was due to malaria in pregnancy(1).
Malaria in pregnancy is the main community health problem in both tropic and sub-tropical regions of the world(8, 9) and *P. falciparum* is the prevalent species in the region. The presence of undetected and untreated parasites in the placenta responsible for anemia and parasitemia in pregnant women which causes adverse effects to the fetus and newborn (8).
Pregnant women have an increased sensitivity to infection with *Plasmodium* parasites due to decreased immunity during gestation period(10). Pregnant women are three times at risk than non -pregnant women in malaria-endemic areas. It is now known that pregnant women are more susceptible to malaria than their non-pregnant peers and that the severity of the infection depends on pre-pregnancy and immunity status (11).
Compared to men with naive immune systems and non-pregnant women, pregnant women with malaria infection do not have symptoms due to acquired immunity and considered to be the highest risk populations for malaria-related deaths. The study has indicated that asymptomatic infections can contribute to anemia and impairment of cognitive development in children (12). It can be expected that 1-50% of pregnant women may carry malaria parasitemia without notice depending on the endemicity of malaria (13, 14). The sequestration of Plasmodium species in the placenta is believed to be associated with LBW, preterm delivery, miscarriage, and stillbirth (15). The epidemiology of malaria in different transmission settings increased attention due to asymptomatic individuals are still able to produce gametocytes and onward malaria transmission (4, 16). In malaria-endemic regions, pregnant women are constantly exposed to malaria parasites which lead to effective immunity to malaria(17).

MATERIALS AND METHODS

Study Area

The study was conducted on pregnant women in Anger Gute town (Anger Gute 01 and 02) and from all kebeles and the surrounding pregnant women follow-up ANC at AngarGute and Warabo health centers . The study was undertaken from September to November 2020. Anger Gute area is found in GidaAyana district in the East Wollega Zone of Oromia Regional State, western Ethiopia (Fig. 2). The study area is located about 360 km west of Addis Ababa along the main road connecting Jimma to Bahir Dar via Nekemte and set in the Anger River Valley (upper Blue Nile Valley) in western Ethiopia. The population of this sub-district is 59,445The altitude of the area is between 1200m to 1500m above sea level and on global positioning, the study area is located at latitude of N90 33'57" and longitude of E360 37'57".The daily mean temperature is 28ċ and the area is affected by

seasonal/unstable malaria transmission. In the study area malaria is characterized by its high transmission seasonality where the peak transmission season is from October to December with a second peak in June. *Plasmodium falciparum* and *P. vivax* are the predominant species in the area (60).

Study Design and Study Period

A facility-based cross-sectional study was conducted from September to November 2020.

Source Population

All pregnant women attending ANC at AngarGute health centers during the study period.

Study Population

The study population was all pregnant women attending ANC in the current pregnancy at AngarGute health centers.

Study Participants

All pregnant women who fulfil the inclusion criteria during the study period.

Sample Size Determination and Sampling Technique

The required sample size was calculated using a formula for a single population proportion. Taking the previous prevalence of malaria infection reported from Northwest Ethiopia 11.2% (53); 95% confidence interval and 4% margin of error the calculated sample size was $n=Z^2 p (1-p)/d^2=1.96x 1.96(0.112) (1-0.112)/.04x.04=239$
Since the source population of our study was <10,000 desired sample size was corrected using the finite population correction formula.
Ncorrected= n/1+n-1/N
239/1+(239-1/4500)
Ncorrected= 228
By taking a non-response rate of 5%, the total sample size was 239 pregnant women fulfilling inclusion criteria were included.
Where
nf= desired sample size
N = The estimate population, 4500 pregnant women in the study area.
z= confidence interval (95%)
d=margin of error 4%.
P=previous Prevalence of malaria infection among pregnant women 11.2%.
A convenient sampling technique was applied due to the lack of sampling frame and pregnant women not follow-up ANC regularly.
Eligibility Criteria

Inclusion Criteria

All pregnant women living in AngarGute more than six months and presenting to the health center for ANC were enrolled in the study.
Pregnant women who volunteered to sign informed consent were included.

Exclusion Criteria

Pregnant women who had taken anti-malaria four weeks before study period was excluded.
Pregnant women who were severily ill and cannot interviewed was also excluded.

Study Variables

Dependent Variable

Prevalence of *Plasmodium* parasites

Independent variables

- ✓ Socio-demographic characteristics: age, family size, education, occupation and marital, residence
- ✓ Housing condition: the presence of eaves and hole in the wall, doors, windows
- ✓ LLINs coverage: availability, the ratio of ITN per family size and utilization of ITN
- ✓ IRS spray last 12 months
- ✓ Distance of house to water bodies
- ✓ Maternal hemoglobin
- ✓ Pervious infection with *plasmodium*
- ✓ Number of pregnancy(gravidity)
- ✓ weeks of pregnancy (trimester)

Data Collection

A pre-tested semi-structured questionnaire was used to gather information on socio-demographic characteristics and factors associated with malaria infection. These factors include age, occupation, and educational level, regular use of an LLIN, gestational age, and gravidity, history of malaria symptoms, socio-economic status, marital status, maternal hemoglobin and housing condition. The questionnaire was prepared in the English using questions adopted from literature and questions based on knowledge of the subjects by the investigator. The questionnaire was translated to the local language Afaan Oromo. It was back-translated to English to ensure correct consistency. Interviews were conducted by trained midwives after the pregnant women have attended the ANC room. Malaria was examined by using light microscopy and RDT and hemoglobin level determination was done by portable Hemocue 301 analyzer.

Blood Sample Collection and Processing

For each participant two blood films (thick and thin) were prepared from sample collected by finger-prickand labelled with pencil on frosted slide. The thin blood film were fixed with methanol for 30 seconds. Then, it was stained with 10% Giemsa solution for 10-15 minutes after being air-dried. Following SOP, the stained smears were investigated with a light microscope high power magnification (100x) objective to detect the presence of malaria parasites. Thick film preparations were examined first by 100x objective for the presence of *Plasmodium* parasites. Finally, when the slide is found to be positive, *Plasmodium* species were identified by examining the thin blood smears under 100x objective. Species identification was done based on the shape of ring stage, infected red blood cell size, gametocyte shape, presence of chromatin dots and merozoites numberin schizont. The results were classified qualitatively as either negative or positive for specific *Plasmodium* species or mixed infection. At least 100 high power fields (100x objective) were examined before reporting a negative result as WHO recommended.

Parasitemia was estimated in the thick film by counting the number of asexual parasites along with 200 white blood cells (WBC) or 500 WBC if the parasite count is less than 10 parasites per 200 WBC. A total of 8,000/µl white blood cells count was considered for the determination of parasitemia.

The degree of parasite density was graded as mild, moderate and severe when the counts were between 1–999 parasites/µl, 1000–9999/µl and>10,000/µl, respectively, following the formula described below.

$$\text{Parasite /ul} = \frac{\text{No. asexual stages counted x 8000 WBC}}{\text{200 WBC}}$$

Gametocyte density was quantified against 500 WBC. This was converted to the number of gametocytes per microliter of blood, assuming a standard approximation of leukocyte count of 8,000/ µl(61).

Rapid Diagnostic Test

Malaria RDT (CareStartTM Malaria HRP2/pLDH (Pf/Pv) Combo) is a qualitative immune chromatographic test that contains a membrane strip, which is pre-coated with two monoclonal antibodies as two separate lines across a test strip to detect malaria antigen in peripheral blood.

One monoclonal antibody (test line 'P. v') is specific to *Plasmodium* lactate dehydrogenase
(pLDH) of the *P. vivax*and the other line (test line 'Pf') consists of a monoclonal antibody specific to histidine-rich protein (HRP2) of the *P. falciparum.*

The test was performed according to the manufacturer's instruction (Access Bio, Inc., Addis Ababa, Ethiopia). The kit was labelled with respective sample code and 5 µl of blood specimen was added into the sample well of the test device. Two drops of lysis buffer are added into the buffer well to lyse the cells, release the antigen and facilitate antibody recognition. The RDT test results are read after 20 min and interpreted as follows:

Two bands: one band in the control area and another band in the *P vivax* area indicates a positive result for *P. vivax*;

Two bands: one band in the control area and another band in the *Pf* area (test line Pf) indicating infections due to *P. falciparum*;

Three bands: bands in the control area, *Pv* area and *Pf* area indicates a mixed infection;

Only one band in the control area in the result window indicates a negative result and In case when the control line did not appear, the result is interpreted as invalid.

Determination of Hemoglobin Level

Hemocue Hb 301 works by the absorbance of whole blood is measured at Hb/HbO2 isobestic point. It is read by spectrophotometry at 506 and 880 nm to compensate for turbidity. Hemoglobin level was determined using a portable HemocueHb 301 analyzer (Hemocue, Angelholm, Sweden). The status of anemia was reported based on WHO hemoglobin concentration cut-off level. Anemia was reported based on WHO hemoglobin concentration cut-point as mild (10 g/dL-10.9 g/Dl), moderate(7 g/dL- 9.9 g/dL) and severe anaemia (below 7 g/dL)(62).

Quality Control

Questionnaire quality was assessed by conducting a pre-tested before data collection. Training was given for the data collectors before data collection started. Before data entry, the completeness and consistency of the questionnaires were checked. All the test procedures and the interpretation of results was accomplished according to SOP. The expiry date of reagents, materials and RDTwere checked daily before data collection takes place. The quality of Giemsa staining was assessed by using known positive and negative control blood films. All positive and 10% negative microscope slides were reexamined by a blinded senior medical laboratory technologist. If there was no discordant result between the first and the second examiner it will be reported.

Data Analysis

The data were coded, entered using EPI data version 3.1, checked, cleaned and exported to SPSS version 20 for analysis. Descriptive statistics were employed to explain the study participant's relation to socio-demographic and other important variables.

Bivariate and multivariate logistic regressions were used to assess the association between dependent and independent variables. Variables with a p-value ≤ 0.25 from the binary logistic regression were entered to

multivariate logistic regression model. Variables having a p-value ≤ 0.05 from multivariate logistic regression were considered as statistically association with malaria infection. Adjusted Odds Ratio with 95% CI was used to measure strength association between malaria and associated factors. A chi-square test was used to determine the association between malaria and anemia among pregnant women.

Ethical Considerations

Ethical clearance was obtained from the Institutional Ethical Review Board of Jimma University. Consultation and permission to conduct this study were also obtained from East Wollega zonal Health Offices. Written informed consent was obtained from every study participant after explain the purpose and objective of the study.Results of participants with malaria infection were addressed to the study participants and got prompt treatment according to WHO guideline.

RESULTS

Prevalence of Malaria Infection among Pregnant Women In The Study Area

The prevalence of malaria among pregnant women in the study area was 13 (5.4%), 95% CI: (3.1- 9.4) and 14 (5.9%), 95%CI: (3.5-9.8)by microscopy and RDTs respectively.The percentage of *P. falciparum and P. vivax* among positive confirmed case were 11(84.6%) (95% CI, *57.8-95.6) and* 2(15.4%) (95% CI, 4.3-42.3) as detected by microscopy.

The Proportion of *P. falciparum* and *P.vivax*among positive malaria parasites byRDT was11 (78.6%), 3(24.4%) respectively.Mixed *Plasmodium* infection was not detected both by microscopy and RDTs tests.

The proportion of anemia among pregnant women was normal 133(56.65), mild anemic 103 (43.1%)and 3(1.2%) were severe anemic.

There was statistically significant association between malaria infection and anemia among pregnant women (X^2=13.15, *P*=0.01).The proportion of anemia among malaria infected and non-malaria infected pregnant women was 11(84.3%) and 2(15.7%) respectively.

Table 1: Prevalence of anemia among malaria infected and non-infected pregnant women of the13 pregnant women infected with malaria; the calculated density of plasmodium parasites are 120-720 para/µ mild,1200-1320 para/µ moderate and 10400para/µ had severe parasitemia as shown in Table 5**.**

Hemoglobin level	Malaria infection			
	Positive (%)	Negative (%)	*Chi square*	*p-value*
>10.9g/dl	2(1.5%)	133(98.5%)	*13.15*	*0.01*
7-10.9g/dl	11(10.8%)	91 (89.2%)		
< 7g/dl	0	3 (100%)		

Table 2: *Plasmodium* parasites density among the pregnant women in the study area

Parasitemia	Parasite Density (%)	Total n (%)
Mild	120-720 para/µ	9(69.2%)
Moderate	1200-1320 para/µ	3(23.1%)
High	10400para/µ	1(7.7%)

Socio-demographic Characteristics and Associated Factors of the Pregnant Women in the Study Area

A total of 239 pregnant women participated in this study during their ANC follows up with a 100% response rate. The age of pregnant women were range from 15-42with the mean age of 24.6. The majority, 150 (62.8%) of them were in the age of 20–29 years. Concerning the educational status, more than half, 127 (53.1%), of the mothers had primary education. The majority of pregnant women's trimester of pregnancy during ANC follow up were 1st trimester 119(49.8%). Among participated pregnant women 87(36.4%) were secundigravidae and 81(33.9%) were multigravidae. More than half 130(54.4%) of the pregnant women came from urban area.Of the participants, 188 (78.7%) owned at least one LLINs. In terms of bed net usage in the current pregnancy, 93 (38.8%) reported having slept under a bed net every night and 88(36.9%) use some times and 58 (24.3%) never use bed nets. More than half of the participant house had not been sprayed within the last 12 months 131 (54.8) as presented below in Table 3.

Associated factors with respect to the distribution of malaria infection

The current study reports that high proportion of malaria infections among pregnant women who were in1st trimester9 (8.1%), and unable to read and write 7 (7.3%). In this study, we reported the highest proportion of

Table 3: Socio-demographic Characteristics and Associated Factors of the Pregnant Women Attending ANC at Angar Gute Health centers from September-November 2020, (N = 239).

Variables	Category	Number (%)
Education level	Unable to read and write	63(26.4)
	Primary school	127(53.1)
	secondery school	37(15.5)
	High school	5(2.1)
	>College	7(2.9)
Age in years	15-19	34 (14.2)
	20-29	150 (62.8)
	30-39	53 (22.2)
	40-49	2 (0.8)
Marital status	Single	10 (4.2)
	Married	211 (88.3)
	Divorced	18 (7.5)
Occupation	Daily laborer	57 (23.8)
	government worker	11 (4.6)
	private work	69 (28.9)
	House wife	87 (36.4)
	others	15 (6.3)
Residency	Urban	130 (54.4)
	Rural	109 (45.6)
Gravidity	Primigravidae	71 (29.7)
	Secundigravidae	87 (36.4)
	Multigravida	81 (33.9)
Trimester	1st trimester	119 (49.8)
	2nd trimester	98 (41.0)
	3rd trimester	22 (9.2)
LLINs own	No	51 (21.3)
	Yes	188 (78.7)
LLINs use /week	Every night	93 (38.8%)
	Sometimes	88 (36.9%)
	Never	58 (24.3%)
IRS sprayed < 12 month	No	131 (54.8)
	Yes	108 (45.2)

Plasmodium infections among age 20-298 (8.8%).The distribution of malaria infection was higher among house wife pregnant women 6 (6.9%). The pregnant women whose house were not sprayed were more infected for malaria parasites 9 (9.7%).

Factors Affecting Malaria Infection among Pregnant Women

Socio-demographic and associated factors were analyzed in relation to malaria infection using binary and multivariate logistic regression model. In the binary variable logistic regression analysis, malaria infection was associated with residence, gravidity, ITNs own,, LLINs utilization, IRS spray,holes and eaves in the house (Table 2). Predictors p < 0.25 in bivariate analysis were entered to multivariate logistic regression model analysis.

Residence (AOR = 0.16, 95% CI:0.015 -0.89, p<0.038), Gravidity (AOR: 9.62 95% CI: 1.2-77.8, p <0.034), ITNs own (AOR: 5.4, 95% CI: 1.2-25.2, p <0.032), ITNs utilization(AOR: 0.114, 95% CI:0.02-0.70, p <.019) were found to be significantly associated with malaria among the pregnant women on multivariate analysis (Table 3). The pregnant women who came from urban area were 84% (AOR = 0.16, 95% CI: 0.015 -0.89, p <.038) less likely to be infected with malaria than those came from rular area. Primigravidae pregnant women were 9.6 times (AOR: 9.62 95% CI: 1.2-77.8, p <0.034) more likely to be infected than secundigravidae and 1.3 times multigravida.Pregnant women who had no ITNs were 5.4 times(AOR: 5.4, 95% CI: 1.2-25.2, p <0.032) more likely to be at risk than those who had ITNs.Those pregnant women who utilize ITNs sometimes were 81% (AOR: 0.114, 95% CI: 0.02-0.70, p <0.02) more protected and those who use never ITNs were 3.4(AOR:3.4 95% CI:(0.63-12.3) times more infected with malaria than those pregnant women who utilize ITNs always.Although IRS sprayed within last 12 months were not significantly associated with malaria infection, the participants whose house were not sprayed within 12 last months were 4.2 times (AOR:4.2, 95% CI: 0.0.80-53.2) more likely infected with malaria than those house sprayed last 12 months.

Table 4:Bivariate analysis of Associated Factors for Malaria Infection among Pregnant Women Attend ANC at Angar Gute Health Centers from September to November 2020N= 239

Variables	Category	Positive (%)	Negetive (%)	p-value	COR
Residency	Urban	5 (3.8)	125 (96.2)		1
	Rural	8 (7.3)	101 (92.7)	0.091*	0.34(0.11-1.2)
Gravidity	Primigravidae	8(10.4)	69(89.6)	0.064*	0.22(0.046-1.09)
	Secundigravidae	3 (3.6)	80 (96.4)	0.692	0.69(0.11-4.26)
	Multigravidae	2 (2.5)	77 (97.5)		1
ITNs own	No	6(11.8)	45(81.81)	0.001*	0.12(0.034-0.40)
	Yes	7(3.7)	181(96.3)		1
ITNs use	Always	2 (2.1)	93 (97.9)		1
	Sometimes	5 (5.6)	85 (94.4)	0.003*	3.8(0.12-1.06)
	Never	6 (11.1)	48 (88.9)	0.03*	10.1 (2.14-47.3)
IRS sprayed	No	9 (9.7)	84 (90.3)	0.05	4.2(0.80-53.2)
	Yes	4 (2.7)	142 (97.3)		1
Eaves	No	7 (5.0)	140 (58.6)	0.23	9.1(1.98-42.2)
	Yes	6 (7.0)	86 (36.0)		1
Holes	No	2 (2.2)	92 (38.5)	0.043	8.1(1.07-65.6)
	Yes	11 (8.2)	134 (56.0)		1

COD: Crude odd ratio; AOR: Adjusted Odd Ratio, CI: Confidence Interval; Neg: Negative, Pos: Positive, ITNs: Insecticide Treated Nets, IRS: Indoor Residual Spray,
***: Significant at p-value < 0.25**

Table 5: Multivariate Analysis Results of Associated Factors for Malaria Infection among Pregnant Women Attend ANC at Angar Gute Health Centers N= 239 from September to November 2020.

Variables	Category	Pos (%)	Neg (%)	p-value	AOR (95%CI)
Residency	Urban	5 (3.8)	125 (96.2)		1
	Rural	8 (7.3)	101 (92.7)	**0.038****	0.16(0.15-8.9)
Gravidity	Primigravidae	8(10.4)	69(89.6)	**0.034****	9.62(1.2-77.8)
	Secundigravidae	3 (3.6)	80 (96.4)	0.787	1.34(0.16-10.8)
	Multigravidae	2 (2.5)	77 (97.5)		1
ITNs own	No	6(11.8)	45(81.81)	**0.03****	5.4(1.2-25.2)
	Yes	7(3.7)	181(96.3)		1
ITNs use	Always	2 (2.1)	93 (97.9)		1
	Sometimes	5 (5.6)	85 (94.4)	0.**02****	0.114(0.02-0.70)
	Never	6 (11.1)	48 (88.9)	**0.001****	3.4(0.63-12.3)

AOR: Adjusted Odd Ratio, CI: Confidence Interval; Neg: Negative, Pos: Positive, ITNs: Insecticide Treated Nets, ** Significant at p-value < 0.05.

The diagnostic performance of malaria RDT (Care Start TM Malaria HRP2/pLDH (Pf/Pv) Combo) was determined by taking blood smear microscopy as a gold standard.There was good measure of agreement (Kappa = 0.92) between RDTs and light microscopy.
The Sensitivity and specificity of RDTs were 100%, 99.1% respectively and positive predictive value and negative predictive value of the RDTs were 93.7%, 100%, respectively.

DISCUSSION

This study helps to determine the prevalence of malaria infection and associated factors among pregnant women in Angar Gute, Gida Ayana district, Oromia region, West Ethiopia.The prevalence of malaria among pregnant women in the study area was 5.4% and 5.9% by microscopy and RDTs respectively.The percentage of *P. falciparum and P. vivax* detected by microscopy were 84.6%and15.4% respectively. The proportion of *P. falciparum and P. vivax*d etected by RDTs were78.6%*P. falciparum* and 24.4%*P.vivax*. This result was similar to studies conducted in North-Shoa Ethiopia 5.7% (52).Our finding was higher than the study conducted in Haiti 1.2%(40), Temotu province 2.7%(63), Nigeria 2.1% (42).These difference may be due to methodology, utilization of intervention measures, geographical location and malaria transmission season.

However, the prevalence of our finding was lower than studies conductedin Arbaminch 9.1% (51), Benishangul Gumuz 10.2% (55), Bangladesh 10.2%(21), Nigeria 38.2% (10), Southern Ghana 10.1%(39), Kenya 12.9%(41). The difference might be due study design(community based), sample size, sampling technique, geographical location and Laboratory personnel skill and Laboratory quality.

Rapid diagnostic tests (RDTs) detected 5.9% of the malaria infection among pregnant women. This result is in line with the study done in, North Ethiopia 5.7% (52), northwest Ethiopia 5.1%(53). But, it is lower than reports from Arbaminch 9.7%(51). This difference might be due to sample size, storage condition of RDTs, geographical location, type of RDT and density of parasitemia in blood circulation.

Urban pregnant women were 84% (AOR = 0.16) less likely to be infected with malaria than rural pregnant women .This finding was comparable with reports from the North-west Ethiopia which reveals that pregnant women from urban were more knowledgeable on malaria infection than from rural area(52).

Primigravidae pregnant women were 9.6 times more likely to be infected with malaria than secundigravidae and 1.3 times multigravidea. This agrees with findings from Ghana which indicates strong association between increasing gravidity and decreasing rates of malaria infection (39).These result also agree with previous studies conducted in Nigeria which found *Plasmodium* infections are more common in primigravidae compared to multigravida (30).

Because of adults who live in malaria-endemic regions have some acquired immunity to malaria infection (7).The decreased in the occurrence of malaria infection with increased gravidity related to exposure-acquired immunity. This could be explained by asymptomatic malaria infection of *P*. *falciparum* results in sequestration and adherence of infected red blood cells with CSA on the surface of the placenta(29).

The current study reports that lower proportion of malariainfections among pregnant women who were in second 3.1% and third trimesters 5% and a higher proportion in the first trimester18.1%which is similar to the study reported from Ghana(39, 53). Studies in Nigeria have also reported high malaria prevalence in pregnant women who were in their second trimester(42). These result was disagree with study conducted among pregnant women on which reveals malaria infection was higher among pregnant women in the second trimester 50.9% followed by the third trimester 42.9% and the first trimester 6.2% (30).

This difference might be due to our study had a higher number of the first trimester compared with 2nd and 3rd trimesters.

The reason for the present result of gravidae-associated factors was*P*. *falciparum* infections may be due to adults who live in malaria-endemic regions generally have some acquired immunity to malaria infection. This acquired immunity diminishes significantly in pregnancy. It has also been suggested by different authors that the early onset of antibody response in multigravida and the delayed antibody production in primigravidae may be responsible for the gravidity-dependent and differential prevalence of *falciparum* malaria among pregnant women (7).

Pregnant women who had no ITNs were 5.4 times more likely to be at risk than those who had ITNs. Those pregnant women who use ITNs were 81% more protected from malaria infection than those pregnant women use ITNs always. ITNs ownership and usage have been reported in several studies to be protective against malaria infections (39, 41, 64). It was also reported from Arbaminch ,Southern Ethiopia- to be protective effects for *Plasmodium* infections(51).

Our study assessed through interviewing study participants on bed net ownership and usage. The majority of our respondents owned 78.6% and 38.8% of participants reported they always sleep under a bed net and 36.9 % sleep under bed nets sometimes. The Ethiopia malaria indicator survey 2015, reported similarly high rates of ITNs ownership 83.7% and usage 44.5% of pregnant women reported that they slept under ITNs the previous night (47).

Although age of pregnant women is not significant, mothers with increased age were found to have a lower risk of developing malaria infection and partial immunity developed though long time exposure to malaria in malaria endemic area. MIP among young women 15-29 years of age were greater 8(5.2%) than of MIP among >30years women. This may reflect continuous exposure to malaria infection developed immunity(65). this result in line with a study done in Ghana(39, 52).

Our study shows that malaria infection can cause maternal anemia. The proportion of anemia among pregnant women in the study area was 43.1%. There were statistically significant association between malaria infection and anemia among pregnant women (X^2=13.15, P=0.01).Malariainfection is one of the major causes of anemia in malaria-endemic region. The most significant malaria in pregnancy outcome ismaternal anemia. Of all severe anemia among pregnant women in Africa, it is estimated that 25 % is caused by malaria(31, 32).Similar to our findings, study conducted in the Arbaminch, reported 57%(51), southern Ethiopia 58.2% (54). Another prevalence of anemia in pregnancy was also reported from Kenya 62.7% and lower than our finding reported from North-west Ethiopia 16.5% (41, 52).Although,the etiology of anemia is variable and potentially multi-factorial, malaria may contribute tomaternal anemia.

The percentage of *P. falciparum* and *P. vivax* among positive blood film was 84.6% and15.4% as detected by microscopy respectively. The *Plasmodium* species identified as positive by RDTs were 78.6% *P. falciparum*

and 24.4% *P. vivax*. This result agrees with study conducted at Angar Gute Among malaria positive case 64.75 % and 32 % of malaria infection was caused by *P. falciparum* and *P.vivax* respectively. Also agrees with Ethiopia PMI report that malaria transmission is seasonal and unstable with major parasites species, *P. falciparum* 70% and *P. vivax*30% (47). The result also agree with study conducted in Ghana which identified species of parasites *P. falciparum* accounted for 95.5% while *P.vivax* malaria accounted for 4.5% (29). In moderate and high malaria transmission, a significant proportion of malaria infections are caused by *P*. *falciparum*, while few infections are caused by other species of *Plasmodium*(51).

This high percentage *P.falciparuim* in our study need for aggressive prevention and control of the diseases, especially among pregnant women.Because *P. falciparum* causes the most severe form morbidlyand mortality to the mother and the fetus and the most outcomes maternal anemia.

Our findings provide the prevalence of malaria infection and associated factors among pregnant women attending ANC at AngarGute health centers during the major malaria transmission season.

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However,there are some limitations need to be considered.We detected *Plasmodium* species of malaria parasites using a malaria RDT and microscopy, which could lead to underestimation of true prevalence. False negatives from the malaria RDT and microscopy may be due to the detection threshold of the test. The routine examination of blood films by microscopy reliably detects parasite densities >10 parasites per µl of blood(66).

CONCLUSION

The prevalence of malaria among pregnant women in our study was relatively high (5.65%) and the predominant species was *P. falciparum*. In our study area, Residence, gravidity, ITNs ownership, ITNs utilization and anemia were the main predictors of malaria infection among pregnant women in the study area.

Therefore, existing malaria prevention and controlling strategies should be reviewed and focus on associated risk factors among pregnant women. So, the responsible body at different level particularly health workers and health extension need to strengthen pregnant women atrular area to protect them selves by utilizing ITNs in safe way.

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