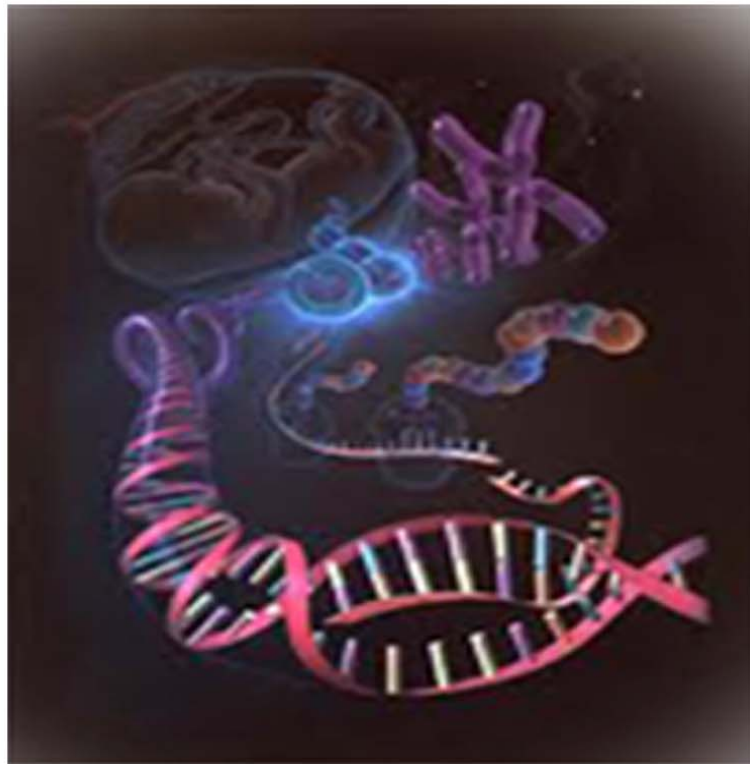




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## Research Paper

# PREVALENCE OF MALARIA AND HIV AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS AT FELEGE HIWOT REFERRAL HOSPITAL AND ADDIS ZEMEN HEALTH CENTER IN NORTHWEST OF ETHIOPIA

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Pregnant women are more susceptible to malaria, which is associated with serious adverse effects on pregnancy. The largest burden of malaria and HIV infections lies in Africa and sub-Saharan Africa. In view of the rapidly increasing of malaria and HIV overlapping in the country Ethiopia, there is a pressing need to improve our understanding of the epidemiology of malaria and HIV. Therefore, this study was initiated as to assess prevalence of malaria and HIV among pregnant women. A cross-sectional study was conducted from February 2011 to May 2012. Socio-demography data and risk factors were collected using questionnaires from a total of 212 pregnant women. Capillary blood were collected and screened for HIV by rapid test and microscopically examined *plasmodium* species detection. All the data were entered and analyzed by using SPSS-15. From a total of 212 pregnant women screened for malaria only 6 (2.83%) individuals were positive for *P. falciparum*, 23 (10.85%) were positive for HIV and only 2 individuals were co infected. Most of malaria positive patients (4.9%) were in the age range <20 years and a higher number (17.9%) of HIV sero-positive clients were in the age range between 30-40 years. Majority (83.3%) of malaria positive pregnant mothers had fever and vomiting during clinical examination but headache is the most common (73.9%) symptom among HIV positive pregnant mothers.

**Keywords:** Prevalence, Malaria, HIV, Pregnant women, Ethiopia

## INTRODUCTION

Malaria is a protozoan disease caused by parasites called genus *Plasmodium*. It is one of the leading causes of illness and death in the world. It is the leading cause of death in children under the age of 5 years and pregnant women in developing countries (Martens and Hall, 2000; Lagerberg, 2008). In 2010 there were an

estimated 216 million cases of malaria worldwide, of which 91% were due to *Plasmodium falciparum* (World Health Organization, 2011). Also, there are approximately 39.5 million people living with HIV/AIDS, including an estimated 17.7 million women and 2.3 million children under the age of 15 in worldwide. 25 million pregnant women are exposed to malaria yearly and HIV prevalence

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among pregnant women is 9%. Women currently represent the population with the fastest increase in HIV infection rates; in the hardest hit countries of Sub-Saharan Africa, more than 60% of all new HIV infections are occurring in women, infants, and young children (Conclusions of a Technical Consultation Convened by WHO, 2004; Elizabeth *et al.*, 2002).

The largest burden of co-infection lies in Africa and sub-Saharan Africa, the continent with the greatest burden of malaria, and where more than three quarters of all HIV-infected women live. Most affected by HIV/malaria co-infection are the Central African Republic, Malawi, Mozambique, Zambia, Zimbabwe and Ethiopia, where some 90% of adults are exposed to malaria and average adult HIV-prevalence reaches 10% (Brahmbhatt *et al.*, 2003). The most common species of malaria are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* and the effects of the disease may vary by the degree of immunity a women has achieved by the time she becomes pregnant (Ticconi *et al.*, 2003).

HIV in pregnancy combined with malaria increases the risk of severe anemia and reduces any acquired immunity that women living in areas of stable malaria transmission may have developed effectively meaning that HIV-positive women in their second, third and fourth pregnancies have the same low immunity to malaria as women in their first pregnancy. Pregnant women infected with HIV become twice as susceptible to clinical malaria, regardless of gravidity (Steketee *et al.*, 1996; Ayisi *et al.*, 2003). In these women, malaria can restrict fetal growth, cause preterm delivery and low birth weight in newborns (Mockenhaupt *et al.*, 2002). Recent evidence suggests that HIV-positive mothers with malaria are more likely to have low-birth weight infants; in turn, low-birth

weight infants were shown to have significantly higher risks of mother-to-child transmission of HIV compared with infants of normal birth weight (Leke *et al.*, 1999). The effects of malaria on HIV are less clear, though episodes of acute malaria can increase viral load and hasten disease progression (WHO, 2007).

The Ministry of Health in Ethiopia has done a great job bringing the high rates of Malaria down but the biggest problem by now is to infection of malaria and HIV among pregnant women living in endemic areas. Pregnant women suffer particularly serious consequences when infected with both HIV/AIDS and malaria. Women living with HIV/AIDS face increased risk of becoming ill with malaria and of developing severe malarial illness due to a weakened immune system. Despite this reality, few studies were conducted regarding prevalence of these diseases in pregnant mother in Ethiopia. Additionally, in view of the rapidly increasing of malaria and HIV overlapping in the country Ethiopia, there is a pressing need to improve our understanding of the epidemiology of malaria and HIV. Therefore, this study was initiated as to assess prevalence of malaria and HIV among pregnant women attending Antenatal Clinics at Felege Hiwot Referral Hospital and Addis Zemen Health Center, Northwest of Ethiopia. The study also envisage that it might strengthen the information so far for scaling up and to design effective communication strategy to combat malaria and HIV in the study area.

## METHODS

### Study Area

The study was conducted in two areas, namely Addis Zemen Health Center in Libo Kemkem

Woreda and Felege Hiwot Referral Hospital in Bahir Dar. Libo Kemkem Woreda is found in the South Gondar Zone of the Amhara Regional State. It extends from a latitude of 37°15'36" E to 38°06'36" E and from a longitude of 11°54'36" N to 12°22'48" N. The mean annual temperature in the area is 19.7°C. According to the 2007 census report of the Ethiopian Central Statistical Agency (ECSA), its total estimated population was 198,374 of which 88.9% live in rural areas. Felege Hiwot Referral Hospital is located at Bahir Dar which is the capital city of Amhara regional state. Bahir Dar city is located approximately 578 km North-west of Addis Ababa, having a latitude and longitude of 11°36'N 37°23'E / 11.6°N 37.383°E / 11.6; 37.383 and an elevation of 1840 m above sea level. Based on figures from the Central Statistical Agency in 2005 Bahir Dar has an estimated total population of 167,261, of whom 86,355 (52%) were males and 80,906 (48%) were females. According to lab reports malaria is common health problems in these areas.

### Study Design and Sample Size

A cross-sectional study was conducted from February 2011 to May 2012 among pregnant mothers attending antenatal care at Felege Hiwot Referral Hospital and Addis Zemen Health Center. All Pregnant women who were suspected of malaria and visiting Felege Hiwot Referral Hospital and Addis Zemen Health Center and provided written consent were included in this study. Pregnant women who were taking anti-malaria treatment and/or seriously sick were excluded from the study. During study period a total of 225 pregnant women had requested malaria microscopic examination and included in our study.

### Microscopic Examination of Malaria Parasites and Hemoglobin Determination

Socio-demographic survey and other necessary data were collected by trained data collectors. The staining techniques and blood film examination for malaria parasite detection was conducted according to a Standard Operating Procedure (SOP) in study site laboratory. In brief, peripheral blood was collect from finger by disposable blood lancet and thick and thin films were made on the same slide. After being air-dried in a horizontal position, the thin blood films were fixed in methanol for 30 s. Then smears were stained with 10% Giemsa solution for 20 min. Each slide was examined under oil immersion microscopic objective by experienced laboratory technicians who were certified on malaria diagnosis and species identification from Ethiopia Ministry of Health. 100 fields were examined before negative result was reported. The thin smear was used to identify the type of *Plasmodium* species. The second round confirmatory microscopic examination done by experienced laboratory technician who was blind for the first result.

Haemoglobin concentration was determined using a portable haemoglobin spectrophotometer, Hemocue Hb 201 analyzer (HemoCue, Angelholm, Sweden) and specially designed microcuvette (the Hemocue Hb 201 Microcuvette, Hemocue, Angelholm, Sweden).

### HIV SEROLOGY

The presence of HIV-1/2 antibodies in the serum was determined using rapid HIV-1/2 diagnostic test kits following the manufacturers' instructions. The results were then interpreted following the current national algorithm for screening of sera for HIV-1/2 infection that was adopted from WHO.

In brief, capillary whole blood was first tested with KHB HIV-1/2. If the result was found negative, it was taken as negative. If not, it was further tested with STATPACK HIV-1/2. If the result of STAT PACK was found to be positive, then the sample was considered as positive for HIV-1/2 antibodies.

## DATA ANALYSIS

Data were checked for its completeness before entering for analysis. The laboratory investigation result recording format for each participant were carefully filled and attached with the respective questionnaire. Data were double entered and analyzed by using SPSS-15.0 statistical software (SPSS Inc. Chicago, 2007). Descriptive analysis was computed for both dependent and independent variables. The frequency distribution of both dependent and independent variables were worked out and the association between the independent and dependent variables were measured and tested using OR and 95% CI.

## ETHICAL CLEARANCE

Ethical clearance was obtained from the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar Ethical Committee. Additionally, after explaining the importance, purpose and procedure of the study briefly an informed written consent was obtained from study participants. Anyone not willing to take part in the study had full right to do so and confidentiality of the results was also maintained. Positive cases were instantaneously linked for treatment and follow up propose.

## RESULTS

Sociodemography characteristics of study participants. Out of 225 pregnant women approached during the study period, 13 (5.5%)

pregnant women were excluded due to refusal to participate. Thus, 212 pregnant women were included in the study. The majority of study participants 130 (61.3%) reported that they were residence of urban dwellers and 188(88.68%) were married. More than 83% (178) participants were orthodox religion followers and 203(95.8%) were Amhara by Ethnicity. The mean age and income was 25 Years and 1384.82 Ethiopian birr, respectively (Table 1).

**Table 1: Socio-Demographic Characteristics of Study Participants At Felege Hiwot Referral Hospital and Addis Zemen Health Center, Northwest Ethiopia, 2012**

Variables	Frequency	Percentage
Age group		
<20 years	41	19.3
20-30 years	143	67.5
30-40 years	28	13.2
Residence		
Urban	130	61.3
Rural	82	38.7
Marital status		
Single	22	10.4
Married	188	88.7
Separated	2	0.9
Educational status		
Illiterate	72	34
Read and write	53	25
High school and above	87	41
Ethnicity		
Amhara	203	95.8
Tigre	4	1.9
Others	5	2.3
Religion		
Orthodox	178	84
Muslim	21	9.9
Others	13	6.1
Note: * ETB = Ethiopian birr		

**Table 2: Prevalence of malaria and HIV among Women's attending ANC at Felege Hiwot Referral hospital and Addis Zemen Health center, Northwest Ethiopia, 2012**

Variables	Total Sample Tested No (%)	Malaria Infection No (%)	COR/95% CI	P. Value	HIV Infection No (%)	COR/95% CI	P. Value
<b>Age group</b>							
<20 years	41(19.3)	2(4.9)	1*	0.384	3(7.3)	1*	0.014
20-30 years	143(67.5)	4(2.8)	.56(.013-1.2)		15(10.5)	.36(.08-1.7)	
30-40 years	28(13.2)	0(0)	nd		5(17.9)	.5(.18-1.6)	
<b>Residence</b>							
Urban	130(61.3)	2(1.5)	1*	0.176	20(15.4)	4.8(1.4-6.7)	0.007
Rural	82(38.7)	4(4.9)	3.3(.6-8.3)		3(3.7)	1*	
<b>Marital status</b>							
Single	22(10.4)	1(4.5)	1*	0.078	6(27.3)	1*	0.007
Married	188(88.7)	5(2.7)	.57(.06-5.1)		17(9)	nd	
Separated	2(0.9)	0(0)	nd		0(0)	nd	
<b>Educational status</b>							
Illiterate	72(34)	4(5.6)	5.1(.55-6.4)	0.174	5(6.9)	.65(.21-2)	0.911
Read and write	53(25)	1(1.5)	1.6(.1-2.7)		9(17)	1.78(.65-4.8)	
High school and above	87(41)	1(2.5)	1*		9(10.3)	1*	
<b>Ethnicity</b>							
Amhara	203(95.8)	5(2.5)	1*	0.153	21(10.3)	1*	0.487
Tigre	4(1.9)	0(0)	.1(.01-1.07)		1(25)	2.9(.3-29)	
Others	5(2.3)	1(20)	nd		1(20)	2.2(.23-10.3)	
<b>Religion</b>							
Orthodox	178(84)	5(2.8)	1*	0.315	16(9)	1*	0.243
Muslim	21(9.9)	1(4.8)	1.7(.2-5.6)		6(28.6)	1.2(.14-9.7)	
Others	13(6.1)	0(0)	nd		1(7.7)	4.8(.5-5.5)	
<b>Occupation</b>							
Govt. employeed	42(19.8)	0(0)	1*	0.624	6(14.3)	1*	0.089
Private	21(9.9)	1(4.8)	nd		4(19)	1.4(.35-5.67)	
Daily labors	24(11.3)	1(7)	nd		4(16.7)	1.2(.3-4.76)	
House wife	96(45.3)	3(3.1)	nd		7(7.3)	.47(.15-1.5)	
Others	29(13.7)	1(3.4)	nd		2(3.1)	.44(.08-2.4)	
<b>Income/month</b>							
<500 birr/month	68(32.1)	3(4.4)	3.7(.38-4.6)	0.152	7(10.3)	.73(.27-2)	0.125
500-1000birr/month	63(29.7)	2(3.2)	2.6(.23-3.0)		5(7.9)	.55(.18-1.67)	
>1000birr/month	81(38.2)	1(1.2)	1*		11(13.6)	1*	
Total	212	6(2.83)			23(10.85)		

Note: nd = not determine

## PREVALENCE OF MALARIA AND HIV

From a total of 212 pregnant women screened for malaria only 6 (2.83%) individuals were found to be *Plasmodium* positive, of which *P. falciparum* accounted for 100%. Most of malaria positive patients (4.9%) were in the age range <20 years, 2.8% and 0% in the age range 20-30 years and 30-40 years, respectively. From 212 HIV screened pregnant mothers 23(10.85%) were positive for HIV. A higher number (17.9%) of HIV sero-positive clients were in the age range between 30-40 years followed by 20-30 years (10.5%) and <20 years (7.3%) respectively. From study subjects only 2 (0.94%) were co infected with malaria and HIV. The prevalence of HIV/AIDS in malaria infected patients was high (33.3%) but the prevalence of malaria in HIV positive clients were relatively low (8.7%) (Table 2).

## RISK FACTORS AND MALARIA AND HIV INFECTION

Malaria HIV co-infection was high during second trimester (1.3%) compared to other gestational periods and in Primigravida (1.6%) compared to other parity status. The prevalence of malaria-HIV co-infection was higher among severely malnourished compared with moderately malnourished and well nourished pregnant women. The prevalence of HIV in gestational period and parity status showed almost similar prevalence (Table 3).

The major clinical findings of the ANC attendees on presentation were head ache (72%), fever (67%), and nausea (60%), Vomiting (50%) and chills/shivering (33%). Mean haemoglobin concentration of the study participants was, 12.5 g/dl (ranging from 8.2 g/dl

to 19.4 g/dl) with standard deviation of 2.3. Majority (83.3%) of malaria positive pregnant mothers had fever and vomiting during clinical examination. Also more than half of (66.7%) malaria patients had nausea. Unlike Malaria cases, headache is the most common (73.9%) symptom among HIV positive pregnant mothers and all malaria HIV co infected individuals had Chills/ shivering, fever, nausea, vomiting and headache (Table 3).

## DISCUSSION

The impact of HIV-malaria co-infection among pregnant women is gone be the major problem across the world. The problem of co-infection during pregnancy is worsened by decreased immunity, poverty and drought in Sub-Saharan Africa where the two diseases are highly dominant. Co-infection with HIV and malaria presents specific complications for pregnant women and fetal development. HIV lessens pregnancy-specific malaria immunity normally acquired during the first and second pregnancies (Elizabeth *et al.*, 2002).

The results of this study revealed that malaria parasite prevalence was 2.83% of which *P. falciparum* accounted for 100% and HIV prevalence was 10.85%. The result also showed that malaria HIV coinfection was low (0.94%), only 2 pregnant women were co infected with malaria and HIV. The prevalence of HIV/AIDS in malaria infected patients was high (33.3%) but the prevalence of malaria in HIV positive clients were relatively low (8.7%). Most of malaria positive patients (4.9%) were in the age range <20 years, 2.8% and 0% in the age range 20-30 years and 30-40 years, respectively. A higher number (17.9%) of HIV sero-positive clients were in the age range between 30-40 years followed by 20-

**Table 3: Selected Risk Factors and Prevalence of Malaria and HIV among Pregnant Women at Felege Hiwot Referral Hospital and Addis Zemen Health Center, North Ethiopia, 2012**

Variables	Total sample tested No (%)	Malaria infection No (%)	COR/95% CI	P-value	HIV infection No (%)	COR/95% CI	P- value
<b>Gestational period</b>							
1 <sup>st</sup> trimester			1*		11(12.4)	1*	0.347
2 <sup>nd</sup> trimester	89(42)	3(3.3)	0.78(.126,4.76)	0.62	7(9.2)	1.8(.39,3.6)	
3 <sup>rd</sup> trimester	76(35.8)	2(2.6)	0.62(.06,6.16)		5(10.6)	.85(.25,2.8)	
	47(22.2)	1(2.1)					
<b>Parity status</b>							
Primigravidae	64(33.3)	2(3.1)	1*		9(14.1)	1*	0.682
Gravidae two	90(42.7)	2(2.1)	0.7(.09,5.14)	0.16	9(10)	.68(.25,1.82)	
Multi Gravidae	57(27)	2(3.5)	1.1(.15,8.12)		5(8.8)	.6(0.18 -1.87)	
<b>Nutritional status</b>							
Well nourished	135(64)	5(3.7)	1*		10(7.4)	1*	0.155
Moderately malnourished	62(29.4)	1(1.6)	0.1(0.008,1.17)	0.438	8(12.9)	0.267(0.07-1)	
Severely malnourished	14(6.6)	0(0)	0.136(.02,.9)		5(35.7)	0.144(0.041-.51)	
<b>Use of ITN</b>							
Yes	56(26.1)	1(1.8)	1*	0.21	10(17.85)	1*	0.21
No	156(73.9)	5(3.2)	1.82(0.21,15.9)		13(8.3)	0.42(0.17-1.02)	
<b>Chills/shivering</b>							
Yes	72 (33)	3(4.2)	1(0.39,10.1)	0.408	13(18.%)	1.86(.817, 1.72)	0.319
No	140(67)	3(2.2)	1			10(7.2%)	1
<b>Fever</b>							
Yes	104(49.1)	5(4.8)	1.03(.65,1.67)	0.197	11(10.5)	0.974(0.642,1.475)	0.901
no	108(50.9)	1(0.9)	1		12(11.1)	1	
<b>Nausea</b>							
Yes	127(59.9)	4(3.1)	1.24(.395, 3.88)	0.999	11(8.7)	.74(.4,1.24)	0.215
No	85(40.1)	2(2.3)	1		12(14.1)	1	
<b>Vomiting</b>							
Yes	107(50.5)	5(4.6)	4.6(1.7, 10.75)	0.14	10(9.3)	0.86(0.585,1.27)	0.479
No	105(49.5)	1(0.95)	1		13(12.4)	1	
<b>Headache</b>							
Yes	155(73.1)	4(2.6)	2.5(0.78, 6.67)	0.709	17(10.9)	1.04(0.503,2.251)	0.916
No	57(26.9)	2(3.5)	1		6(10.5)	1	

30 years (10.5%) and <20 years (7.3%), respectively.

The prevalence of malaria, in our study were extremely low (2.83%) compared with a study



from Cameroon (86.5%) (Theresa *et al.*, 2011), Nigeria (33.3%) (Onyenekwea *et al.*, 2007), eastern Sudan (13.7%) (Adam *et al.*, 2005), Uganda (32%) (Brahmbhatt *et al.*, 2008) from the same study subjects. These huge differences could be due to increased awareness and access to malaria prevention and control measures with priority for pregnant women in Ethiopia, study season and endemicity difference of malaria in different area. Prevention and control activities malaria in Ethiopia as guided by the National Strategic Plan which mainly focused on pregnant mothers and under five children. Also current access to antiretroviral and antimalarial drugs is increasing in the sub-region including Ethiopia could be another reason for this huge difference (<http://www.theglobalfund.org/programs/country/countryid=ETH&lang=en>).

As to the risk factors for malaria rural and urban residence did not showed significant association in bi-variate analysis in our study that varies from the Kenyan findings (Van Eijk *et al.*, 2007) which revealed that being a peri-urban residence is a risk factor for malaria infection. This difference in Ethiopia and Kenya may be due to Ethiopia towns are also characterized by poor housing, lack of proper sanitation, poor drainage of surface water, weak health services and wide spread economic disparity, which independently or together facilitate urban malaria transmission (Tilaye and Deressa, 2007).

Age as risk factor in our study has no association for malaria infection which contradicts with the Kenyan study (Van Eijk *et al.*, 2007) that prevails being young age (<21 years) was a risk factor for malaria infection. Likewise parity status was not found to be a risk factor in our study which contradicts with the above the Kenyan findings

that found being a primigravidae is a higher risk for malaria, which may be because of increased awareness of young pregnant women for early marriage and pregnancy in Ethiopia.

On other hand our findings prevailed that primigravidae women and multigravidae women have equal chance of HIV associated malaria infection which contradicts with Kenyan studies that prevails HIV-associated risk of malaria is consistently greater in multigravidae (Erhoeff *et al.*, 1999), this difference may be due to sample size difference in our study and Kenya. The greater HIV-associated risk of malaria in multigravidae may be due to higher risks of malaria in primigravidae and partial immunity of multigravidae for malaria.

The prevalence of HIV/AIDS on our study was high compared with the Cameroon findings (21.1%) [13], similarly, HIV/AIDS-malaria co-infection in our findings was lower than Cameroon finding (17.3%). This difference in prevalence rates could be accounted for by the fact that the majority of participants in the present study (41%) had high school and above educational status and this could have contributed to good knowledge to control and prevent both diseases. It has been previously reported that low level of education influences prevalence rates (Antelman *et al.*, 2000).

HIV infection status (10.8%) in our study showed that a lower prevalence compared with that of Community based Cohort study among pregnant women in rural Uganda (59%) [16]. This difference may be due to increased access for health facilities and expansions of PMTCT service throughout the country, Ethiopia and other factors that contributes HIV prevalence variation in different localities.

Though difficult to directly compare our results with a study done in Hadaya Zone, Southern Ethiopia, the HIV sero-status assessment among malaria suspected ANC clients on our study (10.85%) more than two fold compared with 4.2% HIV prevalence in Hadya Zone, southern Ethiopia [22]. This supports that women have higher risks of HIV infection than the general population in Ethiopia, which may be due to sex tourism and sex commerce. As compared to WHO 2005 report (56.3%) the prevalence of malaria from HIV infected primigravidae from this study was low [23]. This difference might be due to increased access for malaria preventive and control measures in our country in the last five years [24].

On the other hand our result showed a similar HIV prevalence rate (9.2%) to the Ethiopia Ministry of Health Single Point Estimates for 2010, in urban areas, women [25]. As compared to the prevalence of HIV infection among pregnant women in South Africa (29%) our result showed almost a threefold decrease of HIV prevalence [26]. This difference could be due to increased awareness of pregnant women for HIV infection in our country and to some extent decreased access of HIV screening for all women in Ethiopia (for example ,limited mobile voluntary HIV counseling and testing service in the country).

The prevalence of malaria/HIV coinfection in our study (0.94%) lower than a longitudinal study for 18 months conducted in the endemic area of Saki and Ibadan (Adeoti *et al.*, 2012). The results showed that 57.0% mothers and 36.7% babies had microscopically detectable malaria parasites whereas the seroprevalence were 33.0% and 10.7% for mothers and infants respectively. This difference might be due the type of study design

and co endemicity variation these diseases in different geographic areas.

A major limitation of this study was its focus on women attending health facilities. This study did not include women not receiving antenatal care or those delivering in the community. An additional limitation of this study was the cross-sectional design. Cross-sectional studies are prone to variation because they provide only an estimate of the prevalence infections in a short period. However, since in view of the rapidly increasing of malaria and HIV overlapping in the country Ethiopia, this study was the first study in study area and it provides base line information.

## CONCLUSION

The result suggested that prevalence HIV followed *P. falciparum* malaria is considerably high but low prevalence of malaria/ HIV Co-infection among pregnant women in the study area. Therefore, preventive measures of HIV and malaria (chemoprophylaxis and insecticide-treated bednets) may be beneficial in this area for all women irrespective of their age, gestational period or parity. Additionally, Malaria and HIV screening for all pregnant women should be continued to strengthen the diagnosis and treatment at the early stage.

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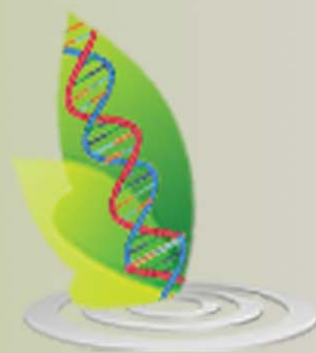
## COMPETING INTERESTS

The authors declare that there is no competing interest.

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