

Prevalence and associated factors of *Plasmodium falciparum* and soil transmitted helminth infections among pregnant women in Osun state, Nigeria

Olusola Ojurongbe¹, Patricia Nkem Okorie², Rofiat Labake Opatokun¹, Taiwo Adetola Ojurongbe³, Victor Olatunji Mabayoje¹, Olugbenga Adekunle Olowe¹, Oluwaseyi Adegboyega Adeyeba¹

1. Ladoke Akintola University of Technology, PMB 4400, Osogbo, Nigeria.
2. Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan.
3. Osun State University, Mathematical and Physical Sciences.

Abstract

Background: *Plasmodium falciparum* and soil transmitted helminth (STHs) infection are widespread in sub-Saharan Africa, where co-infection is also common. This study assessed the prevalence of these infections and their risk factors among pregnant women in Osogbo, Nigeria.

Methods: A total of 200 pregnant women attending the antenatal clinic were recruited. *Plasmodium falciparum* was detected using thick and thin film methods, while formol ether concentration method was used for STHs detection. A questionnaire was used to investigate the possible risk factors associated with acquisition of malaria and helminth infections.

Results: The prevalence of *P. falciparum*, STHs and their co-infection was 29.5%, 12% and 5% respectively. *P. falciparum*, STHs and *P. falciparum* + STHs co-infection was significantly higher in primigravidae (52.5% vs 58.3% vs 80%) than in seconggravidae (18.6% vs 25.0% vs 20%) and multigravidae (28.8% vs 16.7% vs 0%) ($p=0.02$). Prevalence associated factors identified for *P. falciparum* was age ($p=0.0001$) while gravidity ($p=0.02$) was identified for *P. falciparum* + STHs co-infection.

Conclusion: High prevalence of *P. falciparum* and helminth infections was observed among the pregnant women with primigravidae being the most susceptible to co-infection. There is an urgent need to implement an effective malaria and STHs preventive method for this high risk population.

Keywords: *P. falciparum*, STHs, Co-infection, pregnant women, Nigeria.

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Introduction

Malaria and soil transmitted helminths (STHs) infections are the most important parasitic infections in sub-Saharan Africa, where a significant proportion of the populations including pregnant women are exposed to these

infections. In Nigeria the coverage of antenatal care is put at 61% and maternal mortality rate is put at over 560/100,000 pregnant women annually¹. Malaria caused by *Plasmodium falciparum* is one of the major problems encountered by these pregnant women living in malaria endemic areas of Nigeria². Pregnant women have a higher density of parasitaemia and have more complications of *P. falciparum* infection than non-pregnant women³. *Plasmodium falciparum* infection contributes significantly to maternal anaemia, low birth weight of infants, intrauterine growth retardation, preterm deliveries and infant mortality in sub-Saharan Africa^{3,4,5}. Similarly, STHs infections are widely distributed in tropical and sub-tropical areas

Corresponding author:

Olusola Ojurongbe,
Ladoke Akintola University of Technology,
PMB 4400, Osogbo, Nigeria.
Email: oojurongbe@lautech.edu.ng

of the developing countries⁶. STHs infections are associated with cognitive impairment and lowered educational achievement, anaemia, stunted growth, physical and mental development, malnutrition and responsible for about one million deaths per year⁷. Co-infection of *P. falciparum* and STHs is common given the spatial coincidence of risk between malaria and helminths infections among individual living in Africa^{8,6}.

A major impact of malaria and STHs infections is anaemia which serves as a public health problem in the tropics⁹. *Plasmodium falciparum* has been shown to contribute significantly to anaemia in both pregnant women and young children using a number of mechanisms that includes haemolysis and phagocytosis^{3,10}. Blood loss resulting from hookworm infection is considered as the main cause of anaemia¹¹. Therefore the combination of *P. falciparum* and STHs (mostly hookworm infection) infection is considered as a strong indicator of moderate to severe anaemia¹². Iron deficiency anaemia due to STHs infection depends on many factors, including the iron status of the individual and the bioavailability of iron in the diet. Most African women live on diets with poor bioavailable iron which results in low iron stores and could predispose helminth positive pregnant women to iron deficiency anaemia¹³. In addition, many other factors like environmental conditions, educational status, adherence to preventive measures could directly affect the prevalence of malaria and helminths in sub-Saharan Africa.

Few studies have reported the occurrence, interaction and risk factors of *P. falciparum* and helminths infection in pregnant women in Nigeria. Epidemiological surveillance and impact of both *P. falciparum* and STHs remain poorly defined among pregnant women in Nigeria. This study was therefore conducted to investigate the prevalence and risk factors of *P. falciparum* malaria and/or helminth co-infection in pregnant women in Osogbo, South Western, Nigeria.

Methodology

Study area

The study was carried out between October 2012 and May 2013 at the State General Hospital, Ikirun and Osogbo, Osun State, Nigeria. These communities are in urban areas in Osun State Nigeria. Osogbo is the state capital with a landed area of about 835 hectare and population projection of over 3 Million people as at 2006 population

census. The climate in Osogbo is tropical with two seasons; October to February (dry season) and March to July rainy season. The average daily temperature is 32°C with a minimum temperature of 19°C and a maximum temperature of 35.9°C¹⁴. Malaria transmission is usually intense in the raining season in these two study areas.

Study population and recruitment

This was a cross-sectional study of pregnant women attending antenatal clinic. Inclusion criteria for participations were: pregnant women attending antenatal clinics, absence of general danger signs (for example vomiting, inability to sit or stand); absence of signs of severe and complicated *falciparum* malaria; absence of recent history of convulsion; absence of hyperpyrexia (i.e. axillary temperature > 39.5°C) and informed consent of the participating women. The women were adequately informed on the benefit and risks of the study and their consent was sought verbally. Only consented women were recruited into the study. Ethical approval was obtained from Ethical committee of the State Hospital at the Hospital Management Board, Osogbo. After verbal consent, questionnaire were administered to collect information on age, parity, gestational age, toilet facilities, malaria prevention methods and history of drugs taken. The number of pregnant women included in this study was determined using the formula.

$$n = \frac{1.96^2 p(1-p)}{d^2}$$

The prevalence of *P. falciparum* was estimated as 35% based on the average value of a wide range of publications in this area. The marginal error was considered as 5% using 95% confidence level (standard value 1.96); the minimum sample size was determined to be approximately 188 and subsequently 200 was collected. This research was reviewed and approved by the Ethical Review Committee of the Osun State Ministry of Health, Osogbo.

Sample collection and analysis

Blood was collected from the thumb with a sterile lancet onto two separate slides after cleaning the thumb with methylated spirit. The slides were used for the examination and estimation of malaria parasite from thick and thin film preparation. Blood was also collected into heparinised capillary tube for PCV estimation. A wide mouth

clean specimen bottle with tight screw cover was individual provided for each participant for collection of stool sample in the morning of the day of their antenatal clinic visit. The sample was collected early in the morning and processed less than 12 hours when it was passed. The stool samples were visualized macroscopically. Direct saline and formol-ether concentration method of stool samples were prepared and microscopically examined using 10× and 40× objectives¹⁵.

Data analysis

Data analysis was performed using SPSS version 16.0. Chi-square tests and descriptive statistics were used to describe the differences in socio-demographic characteristics. P-value ≤ 0.05 was considered significant. Point estimation of prevalence of malaria and STHs infection was calculated based on the blood and stool sample results. The comparison of prevalence of *P. falciparum*, STHs and

P. falciparum + STHs co-infection between malaria and STHs was performed using chi square test. Odds ratios (OR) with a 95% confidence interval were computed to compare the strength of association.

Results

General characteristics of the study population

A total of 200 pregnant women with a mean age of 26.5 years (range:15-40) were enrolled in this study (Table 1). The mean gestational age of the pregnant women at enrolment was 17.4 weeks (range: 8-36 weeks). The mean packed cell volume (PCV) was 35.94±6.50. The overall-prevalence of *P. falciparum* and STHs was 29.5% (59/200) and 12% (24/200) respectively (Table 1). Out of the 59 malaria positive pregnant women, 24.5% (n=49) were positive for *P. falciparum* only, 6.5% (n=13) were positive for STHs only while 5.0% (n=10) were co-infected with *P. falciparum* and STHs. Two (1%) of the STHs infected women had co-infection of hookworm+*A. lumbricoides*

Table 1 Participants characteristics and prevalence of malaria and helminthic infection among pregnant and non- pregnant women

<i>Characteristics</i>	<i>Pregnant (N = 200)</i>
Mean Age (yrs) ±SD	26.5±5.3
Mean Gestational Age (weeks) ±SD	17.4±5.8
Mean packed cell volume ±SD	35.9±6.5
Mean weight ±SD	58.8±8.2
Prevalence of <i>P. falciparum</i>	29.5% (59/200)
Prevalence of Intestinal helminth	12.0% (24/200)
i. <i>Ascaris lumbricoides</i>	10.0% (20/200)
ii. <i>Hookworm</i>	2.0% (4/200)
iii. <i>Strongyloides stecoralis</i>	1.0% (2/200)
Malaria/helminth co-infection	5.0% (10/200)
Hookworm/ <i>Ascaris</i> co-infection	1.0% (2/200)
Primigravida vs Multigravida	78 vs 122

Prevalence of *P. falciparum* and STHs

The prevalence of *P. falciparum*, STHs and *P. falciparum*+STHs co-infection with respect to age, gravidity and gestational age is shown in Table 2. The highest prevalence of *P. falciparum* infection was recorded in the age group >30 years (49.2%) followed by age group 25 – 29 years (30.5%) and the least in age group <20 (5.1%) and the difference was statistically significant ($p=0.001$). Prevalence of *P. falciparum* and STHs infection was high-

er among primigravidae, but the difference was not statistically significant. For *P. falciparum*+STHs co-infection the primigravidae (80%) had the highest prevalence compared to secongravidae (20%) and multigravidae (0%) and the difference was statistically significant ($p=0.02$). Those in the second trimester of pregnancy had the highest prevalence of *falciparum* (57.6%), STHs (70.8) and *P. falciparum*+STHs co-infection (70%) but the difference was not statistically significant.

Table 2: Prevalence of Malaria and intestinal helminth infection among pregnant by age, parity, gestational age and PCV

Characteristics	No. Examined	<i>P. falciparum</i> +ve (%)	p-value	Helminth +ve (%)	p-value	<i>P. falciparum</i> + Helminth (%)	p-value
Age group (yrs)							
<20	12	3 (5.1)		0		0	
20-24	57	9 (15.3)	0.001*	10 (41.7)	0.08	2 (20.0)	0.39
25-29	71	18 (30.5)		11 (45.8)		6 (60.0)	
≥30	60	29 (49.2)		3 (12.5)		2 (20.0)	
Gravidity							
Primigravidae	78	31 (52.5)		14 (58.3)		8 (80.0)	
Secongravidae	60	11 (18.6)	0.22	6 (25.0)	0.09	2 (20.0)	0.02*
Multigravidae	62	17 (28.8)		4 (16.7)		0 (0.0)	
Gestational age							
First trimester	63	20 (33.9)	0.23	4 (16.7)		3 (30.0)	0.71
Second trimester	120	34 (57.6)		17 (70.8)	0.38	7 (70.0)	
Third trimester	17	5 (8.5)		3 (12.5)		0	
PCV							
> 30%	156	49 (83.1)		17 (70.8)		7 (70.0)	
≤ 30%	44	10 (17.0)	0.35	7 (29.2)	0.367	3 (30.0)	0.53

*Values are statistically significant ($p \leq 0.05$)

Association between *P. falciparum* and intestinal helminth

The association between *P. falciparum* and STHs infection among the pregnant women is shown in Table 3. Out of the 59 pregnant women that were positive for malaria, 10 (16.9 %) were co-infected with STHs and the difference was not statistically significant ($p = 0.23$). In the final model, children infected with helminths in this study are almost twice as likely to have *P. falciparum* infection

than those without helminth infection (OR=1.85 (95% confidence interval CI: 0.77-4.45). An increase odds of *P. falciparum* was also observed among children infected with *A. lumbricoides* when compared to those not infected (OR =2.13; CI: 0.83– 5.44). Although a similar observation to those of *A. lumbricoides* was observed for hookworm and *S. stercoralis*, only one observation was recorded in both cases which makes the analysis not extremely reliable (Table 3).

Table 3: Relationship between malaria and intestinal helminth infection among pregnant women

Helminth infection	P. falciparum infection (%)		Odds Ratio (95% CI)	p-value
	Positive n=59 (%)	Negative n=141 (%)		
Any intestinal helminth infection	10 (16.9)	14 (9.9)	1.85 (0.77-4.45)	0.23
<i>Ascaris lumbricoides</i>	9 (15.3)	11(7.8)	2.13 (0.83– 5.44)	0.12
Hookworm	1 (1.7)	3 (2.1)	0.79 (0.08- 7.78)	1.00
<i>Strongyloides</i>	1 (1.7)	1(0.7)	2.41 (0.15– 39.25)	0.50
<i>A. lumbricoides</i> / Hookworm co-infection	1 (1.7)	1 (0.7)	2.41 (0.15-39.25)	0.50

Influence of demographic and attitudinal practice on *P.falciparum* and helminth infection

Table 4 shows the influence of demographic characteristics and attitudinal practice on *P.falciparum* and STHs infection in the study population. Age was significantly associated with *P.falciparum* infection ($P=0.0001$) among pregnant women in the study area. Education, employment status, toilet facility type, gravidity and gestational age were not significantly associated with *P. falciparum* infection. Age, education, employment status, toilet facility type and gestational age were not significantly associated with *P. falciparum* infection. None of the factors was found to be significantly associated with STHs infection. The highest prevalence of STHs was among pregnant

women aged 25-29 years (45.8%; 11/24), unemployed (41.7%; 10/24) and self-employed (41.7%;10/24), primigravidae (58.3%; 14), those who used pit latrines (58.3%; 14/24) and those who were in the second trimester of pregnancy (70.8%; 17/24). Of the 24 pregnant women positive for helminths, 7 (29.2%) were anaemic while 17% (10/59) of the malaria positive pregnant women were also anaemic with PCV $\leq 30\%$ (Table 3). The highest prevalence of malaria was found among women aged >30 yrs (49.2%), unemployed (47.5%), and those that used pit latrines (49.2%). Three of the 10 pregnant women with *P. falciparum* helminth co-infection were anaemic. Amongst the 10 pregnant women co infected with *P. falciparum* and STHs, the highest prevalence was observed

among pregnant women aged 25-29 years (60%), unemployed (50%), those who used pit latrines (50%), those in the second trimester of pregnancy (70%) and those who had not taken any sulfadoxine-pyrimethamine (SP) for malaria prevention (70%) but the differences were not

statistically significant (table 3). Prevalence of *P. falciparum* was also higher among primigravidae (52.5%), women in the second trimester of pregnancy (57.6%) and women who had not received any SP for malaria prevention (55.9%) (Table 4).

Table 4: Risk factors associated with malaria and helminths infection among pregnant women

Characteristics	No. Examined 200	Malaria n=59 (%)	p-value	Helminth n = 24 (%)	p-value	Malaria/ Helminth n=10 (%)	p-value
Education							
None	23	5 (8.5)		2 (8.3)		0	
Primary	37	14 (23.7)		4 (16.7)		3 (30.0)	
Junior Sec	65	14 (23.7)	0.18	7 (29.2)	0.823	2 (20.0)	0.71
≥Senior Sec	75	26 (44.1)		11 (45.8)		5 (50.0)	
Employment Status							
Unemployed	76	28 (47.5)		10 (41.7)		5 (50.0)	
Employed	44	11 (11.6)	0.20	4 (16.7)	0.79	1 (10.0)	0.58
Self-employed	80	20 (33.9)		10 (41.7)		4 (40.0)	
Toilet facilities							
Pit latrine	96	29 (49.2)		14 (58.3)		5 (50.0)	
Water closet	78	23 (39.0)	0.95	10 (41.7)	0.12	5 (50.0)	0.43
None	26	7 (11.9)		0		0	
SP Usage							
No	106	33 (55.9)		16 (66.7)		7 (70.0)	
Yes	94	26 (44.1)	0.64	8 (33.3)	0.15	3 (30.0)	0.267
Use of prevention methods							
No	38	9 (15.3)		4 (16.7)		3 (30.0)	
Yes	162	50 (84.7)	0.43	20 (83.3)	0.76	7 (70.0)	0.36

Discussion

This study presents data on the prevalence of *P. falciparum* and STHs co-infection and some possible associated risk factors in pregnant women in South Western, Nigeria. *P. falciparum* is a major cause of mortality in pregnant women and their foetus and it is endemic in many parts of the world where its co-occurrence with STHs has been reported¹⁶. This study observed that malaria is still highly prevalent among pregnant women in Nigeria. The 29.5% prevalence of *P. falciparum* recorded in this study was similar to the prevalence of 21.9% reported in Ilorin Nigeria, 21.9% in Cameroon¹⁷, and 36.3% in Ghana¹⁸. However the prevalence was lower than the reports from some other parts of Nigeria (Kebbi (41.6%)¹⁹, Ethiopia (11.6%)²⁰ and Libreville (Gabon) (57%)²¹. Also, a study in Ango-

la and Sudan recorded a prevalence of 8.6% and 13.7% respectively among pregnant women^{22,23}. The differences in the prevalence of *P. falciparum* in pregnancy may be attributed to various reasons like sample size, abundance of vector but also primarily linked availability of effective control measures in the endemic areas.

Although WHO recommends two doses of sulfadoxine-pyrimethamine (IPTp-SP) for malaria prophylaxis in pregnancy²⁴, 53% of the women in this study did not received any SP prescription during Ante-Natal Care (ANC) and only 47.0% received one or two doses. Use of IPTp has been shown to decrease *Plasmodium* parasitemia²⁵ but unfortunately the implementation of this recommendation has been sub-optimal in many endemic countries as

observed in our study. Improving the coverage of pregnant women receiving IPTp in malaria endemic region, like in Nigeria is essential in order to reduce the mortality associated with malaria in pregnancy.

An overall prevalence of 12.0% was observed for STHs infections amongst pregnant women in this study. This shows the susceptibility of the pregnancy state to infection which could be due to impaired immunity²⁵. The prevalence observed in our study is lower compared to what was reported in Ghana (25.7%)¹⁸, Ethiopia (41%)²⁰ and Kenya (13.8%)²⁶. The relatively low prevalence of STHs in this study could be an indication of improved sanitation and proper sewage disposal and improved toilet facilities as shown by the fact that 39% of the participant had improved toilet facilities. The prevalence of *A. lumbricoides* (15.3%) was higher than those of other helminths, an observation that has been made in other studies in North Central and South South region of Nigeria (19.1%)²⁷. However, the prevalence of *A. lumbricoides* recorded here was higher than 5.0% recorded in South Eastern Nigeria²⁸ and Kenya²⁶.

Only, 5% of the participants in this study were co-infected with *P. falciparum* and STHs of the women who had malaria while 12% had some type of worm infection. Awareness of the importance of co-infection is increasing and suggestions have been made that helminth infection may influence susceptibility to other infections including malaria (Nacher 2001; Bentwich, 2000). This association may vary by geographical region, the local species and the risk factors for the parasites being studied²⁹. In this study, although no significant association was observed in the interaction between helminth and malaria, the odds revealed that pregnant women infected with helminths, more precisely *A. lumbricoides* were almost two times as likely to have *P. falciparum* infection compared to those without *A. lumbricoides* infection. This is similar to our previous observation in studies conducted among school children in different locations in Nigeria^{6,30}. Also, similar association was previously observed among cohort of pregnant women in Ghana¹⁸. Although the main reason for this observation is yet to be elucidated, the general belief is that helminths drive the type 2 (Th2) immune response which down-regulates the Th1 anti-malarial immune response, resulting in increased risk of malaria infection. This explanation may not hold true in all circumstances as other authors have conflicting reports

on how helminths regulate *P. falciparum* infection. More studies are therefore needed to expound on these conflicting observations.

Although, the prevalence of *P. falciparum*, STHs and co-infection was higher among the primigravidae compared to secondigravida and multigravida, no significant association was observed between primiparity and *P. falciparum* infection. However, primiparity was significantly associated with *P. falciparum* and STHs coinfection. Primiparity has been identified as a risk factor for *P. falciparum* infection during pregnancy^{3,21,25,29}.

Majority of the pregnant women were not anaemic and so had a PCV value of 30% or more. A study in Ethiopia recorded anaemia prevalence of 53.9% among pregnant women³¹. Anaemia (PCV \leq 30%) was not associated with *P. falciparum* infection, STHs infection and *P. falciparum* + STHs co-infection among the pregnant women studied. Lack of association between hookworm and anaemia has been reported in Brazil¹¹. Hookworm may cause anaemia particularly in pregnant women³² and hemoglobin levels have been found to be lowest in pregnant women who had helminth or malaria infections³³.

Age was identified as a risk factor for malaria in pregnancy while gravidity was a risk factor for *P. falciparum* + STHs co-infection. Previous studies have identified the risk factors for malaria in pregnancy as age and primigravidae^{10,21,25}. However, a lack of association between age or parity and malaria has been reported in Sudan²². No risk factor was identified for helminth infection in this study. Any risk factors (if present) in this study might not have been detected in this study given the low number of women who were infected with helminths (especially with hookworm). Again the use of one stool sample from each woman is a limiting factor as the proportion of women with low-intensity infection could have been mis-classified as uninfected since multiple samples are required for accurate detection³⁴. Future study with larger sample size is therefore recommended to help further investigate the impact anemia and other risk factor in pregnant women.

The risk factors for helminth infections depend on the route of transmission and life cycle of the helminth, and are related to the state of hygiene, sanitation and environmental conditions (temperature and humidity)^{16,35}. It is also worth noting that pregnant women in the rural

communities who are the most vulnerable to malaria and helminth infection do not attend antenatal clinics. The implication of this is that higher prevalence of malaria, helminth and malaria helminth coinfection could be recorded in a rural community based study. Also other studies have shown other risk factors associated with STHs infection include the absence of garbage bin in the household and age of the mother at the time of marriage, house floor type or handwash^{33,36,37}. However, data for these factors were not included in the study and may have an effect which was not observed. Poor education and unemployment have also been identified as risk factors for both helminth and *P. falciparum* infections³³.

Conclusion

The results suggest that *P. falciparum* and STHs infection and their co-infection could constitute a serious problem among women attending antenatal clinic (ANC) with primigravida being more parasitized than the multigravida. More effort should be placed on the control and prevention of malaria and intestinal helminths among pregnant women in endemic areas in order to reduce the associated risks and burden.

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Disclosures and ethics

The authors have no competing interests in the manuscript. The authors also confirmed that this article is unique and not under consideration or published in any other journal.

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