

## ORIGINAL ARTICLE

# Malaria and intestinal parasites in pregnant and non-pregnant women: a comparative study at the University Hospital, Kumasi, Ghana

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In sub-Saharan African countries, both malaria and intestinal helminth infections are endemic and co-infection commonly occurs. It is estimated that over a third of the world's population, mainly in the tropics and sub-tropics are infected with parasitic helminths and *Plasmodium* species thus often leading to co-infections. This cross-sectional study was conducted to assess the prevalence of malaria and intestinal parasites in a sample of 760 study participants comprising 380 pregnant women and 380 non-pregnant women attending the University Hospital in Kumasi, Ghana. Blood and stool samples were analyzed for malaria and intestinal parasites using Giemsa staining technique and direct wet mount method respectively. The overall prevalence of malaria infection, intestinal parasite infection, and malaria-intestinal parasite co-infection was 73 (9.6%), 43 (5.6%), and 10 (1.3%) respectively. Malaria infection was higher in pregnant women (12.6%) compared to non-pregnant women (6.6%). Non-pregnant women recorded higher intestinal helminth infection (10%) than pregnant women (1.3%). No case of co-infection was recorded among the pregnant women. The study suggests a higher susceptibility to malaria infection when compared to their non-pregnant counterpart with an association between malaria parasite and intestinal helminths in non-pregnant women.

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## INTRODUCTION

Parasitic diseases caused by protozoa and helminths are major causes of human diseases in most countries. It is estimated that over a third of the world's population, mainly in the tropics and sub-tropics are infected with parasitic helminths and *Plasmodium* species (*P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*) (de Silva *et al.*, 2003) which leads to co-infections (Petney and Andrews, 1998). Infection by intestinal helminth is associated with socio-economic indicators such as poor sanitation and inappropriate disposal of sewage (Yatich *et al.*, 2009). Malaria infection in pregnancy is twice that in non-pregnant women due to physiological changes and suppressed immunity during pregnancy (Lindsay *et al.*, 2000).

In sub-Saharan African countries, both malaria and intestinal helminth infections are endemic and co-infection commonly occurs. In Nigeria, a study examining malaria and helminth co-infection in pregnancy demonstrated that over 45% of *Plasmodium*-infected pregnant women also harbored various intestinal helminths. This co-infection was associated with low haemoglobin level especially among primigravid women (Egwunyenga *et al.*, 2001). Many hypotheses have been presented to explain potential co-infection with malaria and intestinal helminths.

It has been suggested that helminth infection stimulates the Th2 cytokine response which possibly predominates and down-modulates Th1 cytokines in malaria-helminth co-infection (Torre *et al.*, 2002). Th1 response inhibits IFN- $\gamma$  during the blood and liver stages of malaria infection (Torre *et al.*, 2002) which could explain the worsening of

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anemia in malaria-helminth co-infection (Basavaraju and Schant, 2006). Helminth infection thus creates a cytokine milieu favorable for the production of non-cytophilic antibodies, thus making individuals more susceptible to clinical malaria (Mwangi *et al.*, 2006). During helminth infection, T regulatory cells are amplified, and if present in sufficient numbers, could induce a non-specific suppression of immunity (Yazdanbakhsh *et al.*, 2001), making individuals susceptible to infections such as malaria; however, malaria may also exacerbate the consequences of helminth infection (Mwangi *et al.*, 2006).

Information on co-infection of malaria and intestinal helminths in pregnant and non-pregnant women is limited. Therefore it is imperative to investigate malaria and intestinal parasites among the vulnerable pregnant women and their non-pregnant counterparts.

### MATERIALS AND METHODS

#### *Study area and population*

This cross-sectional study was conducted to investigate malaria and intestinal helminth infection in pregnant and non-pregnant women. The study was conducted at the University Hospital, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Kumasi is the second largest city in Ghana, located in the rainforest zone of West Africa with a population of 1.5 million inhabitants of which 51% are women (GSS, 2002). A total of 760 study subjects comprising 380 pregnant women and 380 non-pregnant women were enrolled.

#### *Blood sample collection and examination*

Blood samples were collected by venipuncture by a trained phlebotomist after thorough disinfection of the venipuncture site with 70% ethanol into vacutainer® EDTA (ethyl diamine tetra acetic acid) tubes and this was used to prepare thick and thin films.

#### *Thin film preparation*

Five microlitres (5  $\mu$ L) of blood was placed on the clean glass slide and a clean smooth edged spreader used to prepare the thin film. The spreader was

drawn back to touch the drop of blood and the blood allowed to extend along the edge of the spreader. Holding the spreader at an angle of about 30°, the drop of blood was spread to make a film about 40-50mm in length. After air drying, the slide was fixed with absolute methanol and stained with 10% giemsa for 10 minutes. The thin film was read for speciation of *Plasmodium* parasite.

#### *Thick film preparation*

Thick films were prepared on the other half of the glass slide using 10  $\mu$ L of blood to evenly spread to cover an area of 15 x 15mm of the slide. The smear was stained with 10% giemsa for 10 minutes. The slides were read by a trained microscopist. All the procedures for the preparation of both thick and thin films were as described by Cheesbrough (2005).

#### *Stool sample collection and examination*

Stool samples for the determination of intestinal parasites were collected from pregnant and non-pregnant women in wide-mouthed sterile containers. A smear was prepared from each of the fresh samples by emulsifying about 2mg of the stool sample on a clean glass slide and a drop of lugol's iodine added. The sample was covered with a cover slip and observed using low power (x10) and high power (x40) objectives for identification of protozoan trophozoites and cyst, helminth ova and larvae. The specimen were examined microscopically using the direct wet mount technique as described by Cheesbrough, (2005).

#### *Statistical analyses*

Data were entered into Microsoft Office Excel 2007 and read out into Epi Info version 7 (<http://www.cdc.gov/epiinfo/index.html>). The results were presented as simple frequencies and percentages and odds ratio estimated where appropriate using chi-square. Ethical clearance for the study was obtained (Ref: CHRPE/Student/195/10) from the Ethical Review Committee of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Permission to undertake the study at the University Hospital, KNUST was granted by the hospital manage-

ment and the head of the laboratory with a written and signed consent from each study participant enrolled.

**RESULTS**

A total of 760 women comprising 380 pregnant women and 380 non-pregnant women were recruited into the study. Out of the 380 pregnant women enrolled, 48 (12.6%) were infected with malaria parasites. The *plasmodium* species identified in the blood films of the pregnant women were *plasmodium falciparum* (41/380; 10.8%), *plasmodium ovale* (5/380; 1.3%), and *plasmodium malariae* (2/380; 0.5%). Among the 380 non-pregnant women, 25 (6.6%) had malaria with percentage distributions as follows: *plasmodium falciparum* (19/380; 5.0%), *plasmodium ovale* (4/380; 1.1%) and *plasmodium malariae* (2/380; 0.5%) were examined in the non-pregnant women. The study showed a total malaria infection rate of 9.6% (73/760) with the least prevalence of 6.6% (25/380) being observed in non-pregnant women. Pregnant women were two times more likely to have malaria than non-pregnant women (OR=2.05). There was a significant association between pregnancy and malaria parasitaemia (p = 0.005) (Table 1).

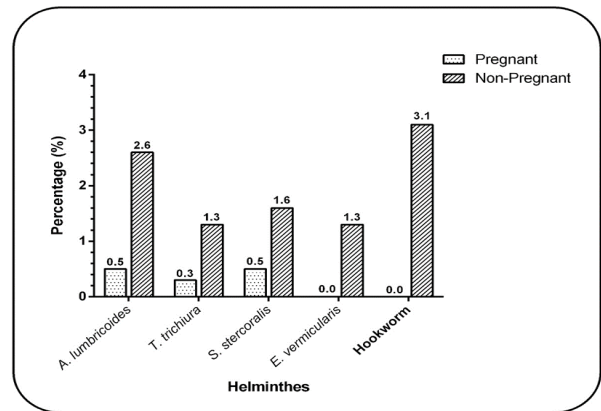
Intestinal helminthes were found in 5 (1.3%) and 38 (10.0%) pregnant women and non-pregnant women

**Table 1: Overall prevalence of malaria in pregnant and non-pregnant women (n=380)**

Parameters	Pregnant	Non-Pregnant	OR (p-value)
<b>With malaria</b>	48(12.6)	25(6.6)	2.1(0.005)
<b>Without malaria</b>	332(87.4)	355(93.4)	

respectively. Among the pregnant women, *Ascaris lumbricoides* (2/380; 0.5%), *Trichiuris trichiura* (1/380; 0.3%), and *Strongyloides stercoralis* (2/380; 0.5%) were identified in the stool samples. However, *A. lumbricoides* (2.6%), *Enterobius vermicularis* (1.3%), *T. trichiura* (1.3%), *S. stercoralis* (1.6%), and Hookworm (3.1%) were also identified in the stool samples from the non-pregnant women (Figure 1). A total of 43 (5.6%) pregnant and non-pregnant women were infected with intestinal helminthes with the highest

infection rate in non-pregnant women. No cases of co-infection were observed in the 380 pregnant women examined. However, 10 (2.6%) non-pregnant women were observed to have helminth co-infection with only *Plasmodium falciparum* as follows: 4 (1.0%) *A. lumbricoides*, 1 (0.3%) *S. stercoralis*, and 5 (1.3%) Hookworms. The least and highest co-infection rates were observed in *S. stercoralis* and Hookworm infection respectively (Table 2).



**Figure 1: Prevalence of intestinal helminthes in pregnant and non-pregnant women**

**Table 3: Co-infection of malaria parasite and intestinal helminthes in pregnant and non-pregnant women**

Parameters	Pregnant	Non-Pregnant
Malaria + <i>A. lumbricoides</i>	0 (0.0)	4 (1.0)
Malaria + <i>S. stercoralis</i>	0 (0.0)	1 (0.3)
Malaria + Hookworms	0 (0.0)	5 (1.3)

**DISCUSSION**

The study demonstrated that malaria parasitaemia is higher in pregnant women than non-pregnant women. Women living in malaria-endemic areas have an increased risk of *P. falciparum* infection during pregnancy (Bouyou-Akotet et al., 2003). Although parasite prevalence and density are higher among pregnant women compared with non-pregnant women, infection with *P. falciparum* is usually asymptomatic (Marchesini and Crawley, 2004). The high prevalence (12.6%) of malaria in pregnant women in this study confirmed another study in Ghana by Mockenhaupt et al., (2000) with a rate of

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63%. Malaria infection in pregnancy is twice that in non-pregnant women due to physiological changes and suppressed immunity during pregnancy (Lindsay *et al.*, 2000). This may suggest the reason for high prevalence of malaria during pregnancy.

An overall prevalence of 5.6% was observed for intestinal parasites infection in pregnant women and non-pregnant women. Hookworm and *A. lumbricoides* were the most common intestinal helminths in the study participants. Another study in Ghana by Yatich *et al.*, (2010) recorded a higher prevalence of 25.7% intestinal parasites in pregnant women compared to the current study where 1.3% pregnant women were infected with intestinal parasites. This result conforms to study by Yatich *et al.*, (2010) where the most common intestinal helminth in pregnant women was *A. lumbricoides*. Its infection rate was higher (12.3%) compared to this study where 1.3% pregnant women were infected. Infection by intestinal helminth may be associated with socio-economic indicators such as poor sanitation and inappropriate disposal of sewage (Yatich *et al.*, 2009).

In pregnant women, co-infection was not recorded in this study. But non-pregnant women showed 10 (2.6%) co-infection of malaria and intestinal parasites. This observation contradicts a study in Nigeria by Ekejiindu *et al.*, (2010) whereby 13 (13%) and 6 (6%) pregnant and non-pregnant women had co-infection respectively. Mwangi and his colleagues suggested that helminth infection creates a cytokine milieu favorable to the production of non-cytophilic antibodies, thus making individuals more susceptible to clinical malaria (Mwangi *et al.*, 2006). Moreover, it is also thought that the presence of T-regulatory cells is amplified during helminth infection, persuading a non-specific suppression and individuals become susceptible to malaria infection (Yazdanbakhsh *et al.*, 2001).

Women in many sub-Saharan African countries consume soil while pregnant. A study in Kenya recorded that 73% of pregnant women ate soil often (Geissler *et al.*, 1999) and this might contribute to intestinal helminth infections (Geissler *et al.*, 1998).

However, this study reported zero prevalence of malaria-helminth co-infection in Ghanaian pregnant women. Improper sewage disposal and poor sanitation could cause high prevalence of intestinal helminth infections. Awareness created by agencies educating pregnant women may cause the low rate of co-infection in this study.

## CONCLUSION

There was an association between intestinal helminths and malaria infection in non-pregnant women. Pregnant women are twice more susceptible to malaria and reported zero co-infection rate in this study. The study also revealed higher prevalence of intestinal helminth infection and co-infection in non-pregnant women.

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## COMPETING INTERESTS

The authors declare that they have no competing interests.

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