

Prevalence of asymptomatic *Plasmodium falciparum* infection, anaemia and use of ITNs among pregnant women yet to receive IPTp in parts of southern Ghana

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Abstract

Background: Asymptomatic malaria in pregnancy evades most fever-based surveillance systems yet causes significant morbidities such as anaemia in the pregnant woman and low birth weight in the neonate.

Objective: This study determined the prevalence of asymptomatic malaria and its association with anaemia among pregnant women who are yet to receive their first dose of Sulphadoxine-Pyrimethamine (SP) as intermittent preventive treatment of malaria in pregnancy (IPTp). Information on the use of insecticide treated nets (ITNs) as vector control by the pregnant women was also sought.

Methods: This is a cross-sectional hospital-based study conducted in the Western Region of Ghana. Pregnant women at gestational ages, 16-26 wk were included. A structured questionnaire was used to collect vital information from the participants. *Plasmodium* parasitaemia was determined by rapid diagnostic test (MRDT), microscopy and species-specific nested polymerase chain reaction (PCR). Anaemia was classified using the level of haemoglobin.

Results: A total of 413 antenatal clinic attendants were recruited. Prevalence of asymptomatic *Plasmodium falciparum* infection was 13.1% by MRDT, 10.1% by microscopy and 13.8% by PCR. The mean haemoglobin was 10.73 g/dL. Prevalence of anaemia was 40.49% and the mean parasite density was 149.6 parasite/μL. Pregnant women with asymptomatic malaria were 4 times more at risk of being anaemic (adjusted odds ratio with 95% confidence interval: 4.42, 1.82 -10.70) than those who did not have malaria. There was statistically significant negative correlation between parasite density and anaemia ($r = 0.0028$, $p = 0.02$).

Conclusion: Asymptomatic *P. falciparum* infection was found among some of the pregnant women and the presence of the parasites make them 4 times at increased risk of developing anaemia. Anaemia when occurring amongst such women was significantly worsened by increasing parasitaemia.

Keywords: Anaemia, *Plasmodium falciparum*, pregnancy, prevalence

INTRODUCTION

Relative to non-pregnant women, pregnant women have an increased risk of malaria infection, independent of previously acquired immunity [1]. A pregnant woman attracts twice the number of *Anopheles gambiae* complex, the predominant African malaria parasite-carrying mosquito compared to her non-pregnant counterparts [2,3]. In most of these women, the infection with *Plasmodium falciparum*, the most lethal of all the *Plasmodium* parasites, remain

asymptomatic especially in areas of high *P. falciparum* transmission. Thus, pregnancy tends to increase the risk of asymptomatic malaria especially in regions with high transmission intensity [4]. The prevalence of asymptomatic malaria is therefore higher in pregnant women (especially in primi and secundi-gravidae women) compared to non-pregnant women and men. Malaria infection in pregnancy is a major public health problem, especially in sub-Saharan Africa. The condition poses significant risks for the mother, the developing fetus and subsequently, the offspring. Malaria in pregnancy increases the risk of low birth weight [5,6] which is the single most important risk factor for perinatal, neonatal, and infant death [7-9].

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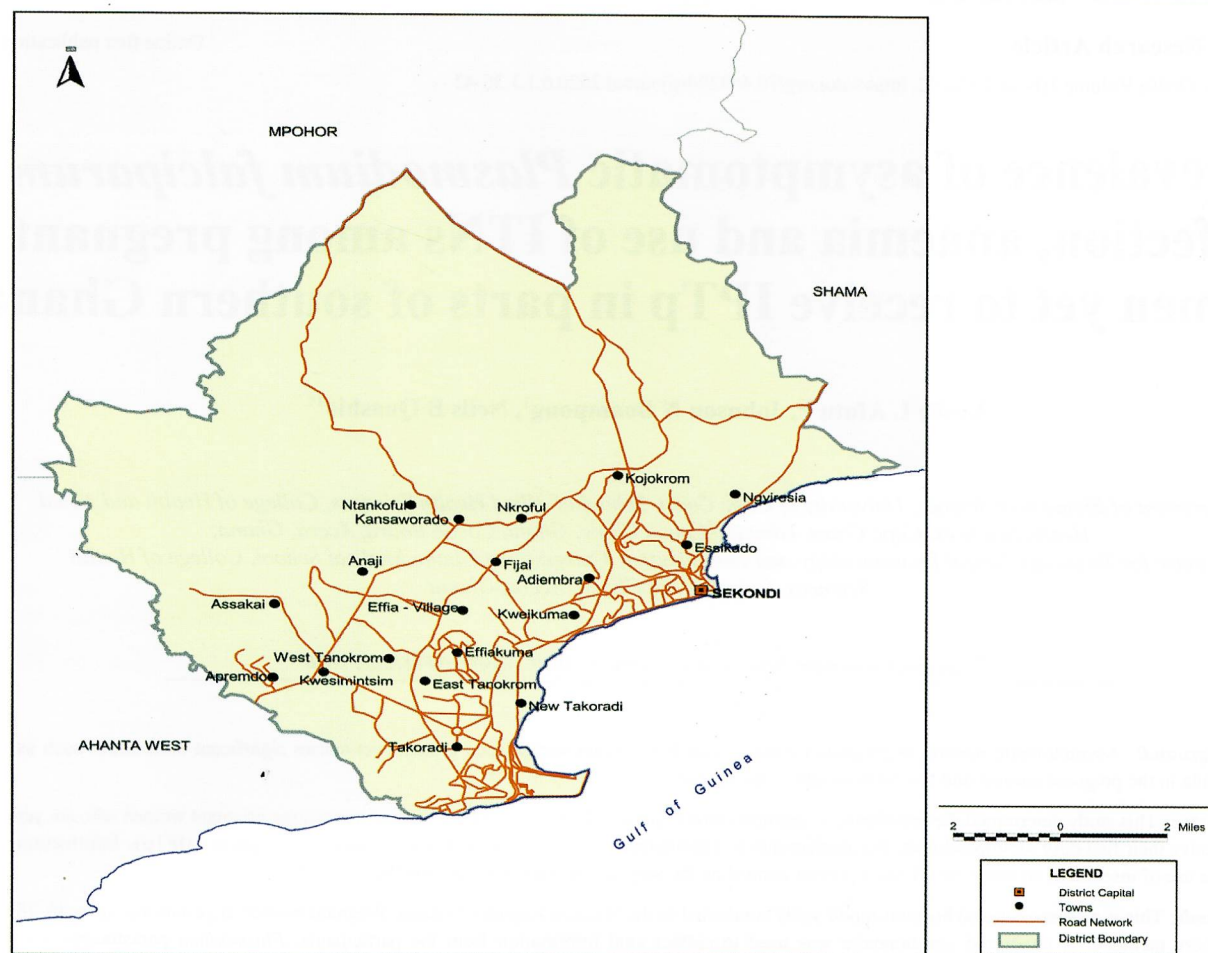


Figure 1: Map of Sekondi-Takoradi metropolis. (Source: Ghana Statistical Service report, 2014).

In areas of unstable malaria transmission, malaria in pregnancy has been associated with preterm deliveries, still births, neonatal deaths as well as miscarriages [10]. Apart from the afore-mentioned, malaria in pregnancy is also a known cause of maternal anaemia which is said to contribute significantly, directly or indirectly, to maternal morbidity and mortality. This anaemia typically occurs over a background of physiological anaemia of pregnancy due to increased blood volume and poses a significant risk of severe anaemia in pregnancy. In poor socio-economic regions of the world, maternal anaemia may further be worsened by micronutrients (such as iron, folate and vitamins) deficiency as well as infection with geo-helminths. Both symptomatic and asymptomatic malaria infections can cause anaemia. While anaemia from acute symptomatic *falciparum* infection is due to an increased removal from the circulation of parasitized and to a greater extent, non-parasitized erythrocytes [11], anaemia from chronic asymptomatic malaria infection may be compounded by decreased bone marrow production of functional red blood cells due to the direct inhibitory effects of parasites [12] and cytokines [13,14] along with dysregulation of erythropoietin and iron metabolism. Several studies have investigated the prevalence and consequences (in this case, in causing maternal anaemia) of acute

symptomatic *P. falciparum* infections in pregnant women in Ghana and South Sahara Africa for that matter. Data on the prevalence of and consequences of asymptomatic *falciparum* malaria (which is undetected by fever based surveillance systems) however remain scanty. The objective of this study was to investigate the prevalence of asymptomatic *Plasmodium* parasitaemia and the factors affecting it in antenatal attendants who were yet to receive their first dose of Sulphadoxine-Pyrimethamine (SP) as intermittent preventive treatment of malaria in pregnancy (IPTp) in hospitals located in the Western Region of Ghana. The study also ascertained how asymptomatic parasitaemia correlated with maternal anaemia in order to emphasize the need and importance of malaria control in pregnancy. The use of insecticide treated nets (ITNs) as vector control measure by the pregnant women was also determined.

MATERIALS AND METHODS

Study area

The study was carried out in Sekondi-Takoradi, the administrative capital of the Western Region of Ghana (Figure 1). It is located in the South-Eastern part of the Western Region and lies on latitude 4.91°N and longitude 1.77°W. The climate of

the Metropolis is equatorial, with an average annual temperature of about 22°C. Rainfall is bimodal, with the major season occurring between March and July and the minor season occurring between August and November. The mean annual rainfall is about 1,380 mm [15]. Malaria transmission rate in this area is nearly stable all year round with rates increasing in the rainy seasons, mainly in the months of May, June and July. The incidence of malaria in the general population is 28.7% whilst that in pregnant women is 1.84% [16].

Study facilities

Two (2) health facilities were chosen for the study. These were the Takoradi Hospital and the Essikado Hospital. Takoradi Hospital is the metropolitan hospital with an obstetric bed capacity of 18. The average monthly antenatal care attendance is about 1200 and average monthly new antenatal attendees is 120. The Essikado hospital is a well-attended sub-district hospital. It serves mostly women in the relatively lower socio-economic suburbs of the metropolis. The average monthly antenatal attendance is about 1050, with 100 of them being new attendees.

Study protocol

The study was a hospital-based cross-sectional study. It was conducted during the rainy season, April-June. Those included in the study were pregnant women of any age and parity who were at 16-26 wk of gestation, yet to receive their first SP dose and had axillary temperatures less than 37.5°C. Those with a history of receipt of antimalarial in the past month and those known to have HIV infection were excluded from the study. Socio-demographic and obstetric characteristics of study participants were determined by a structured questionnaire. Blood samples were taken from study participants for malaria rapid diagnostic tests (MRDT), microscopic detection of the presence of parasites and species-specific PCR. Haemoglobin (Hb) levels of participants were also determined.

Sample collection

Procedures for obtaining the samples were adequately explained to participants. After an aseptic preparation, about 3 mL of venous blood was obtained from each participant using a sterile disposable hypodermal syringe fitted with a 23-gauge needle and dispensed from the syringe barrel into sterile ethylenediaminetetraacetic acid tubes for further processing. These samples obtained were used for MRDT, microscopy after staining with Giemsa and determination of Hb concentrations. While dispensing the blood from the syringe barrel, about 10 µL of the blood was used to make circular blood blots, usually about 2 cm, on filter papers. These blood blotted filter papers were allowed to air dry, put into individual plastic envelopes, appropriately labeled and stored at 27°C for DNA extraction and amplification.

Laboratory analysis

Detection of *Plasmodium falciparum* infection was by microscopy, rapid diagnostic tests (RDTs) and polymerase chain reaction (PCR).

Microscopic determination of parasitaemia. Parasite detection by microscopy was done after staining prepared thick and thin blood smears with Giemsa stain. Blood slides were

examined using light microscopy at 1,000 x magnification. One hundred microscopic fields were examined in the thick smear before concluding that a blood slide was negative. All slides were read independently by two experienced microscopists. If there occurred any discrepancy in the reading, a third and a more experienced microscopist confirmed diagnosis. Parasite density per microlitre (P/µL) of blood was computed.

Detection of parasite by RDT. MRDTs were also performed to detect the presence of the parasite. The SD Bioline Malaria Ag Pf® detecting Histidine Rich Protein-2 (HRP-2) was used.

Molecular detection of Parasite. Detection of malaria infection and identification of *Plasmodium* species were done using species-specific primer nested PCR amplification of the genomic DNA. This additional method of determining the presence of malaria was necessary since microscopy in pregnancy is less sensitive because of high possibility of placental sequestration by the parasites and the internal validity problems associated with the use of MRDT. DNA was extracted by the classic Chelex method. Extracted DNA was stored at -32°C until used. Species-specific PCR was conducted on all samples. It involved the use of both genus and species-specific primers in a 25 µL reaction mixture in all cases. This consisted of 12.5 µL of the DreamTaq green® premix; 0.3 µL each of the forward and reverse primers; 10.2 µL nuclease free water and 2 µL of extracted DNA. The nested 1 PCR involved the use of the genus-specific primers; rPLU1: 5'TCA AAG ATT AAG CCA TGC AAG TGA3' and rPLU5: 5' CCT GTT GTT GCC TTA AAC TCC3' under the following reaction conditions: Initial denaturation at 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 1 min and extension at 72°C for 1 min. Final elongation was at 72°C for 4 min. The nested 2 amplifications involved the use of *P. falciparum* specie-specific primers: rFAL1: 5' TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT3' and rFAL2: 5' ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC3'. Reaction conditions for nested 2 were identical to that of nested 1 except that the annealing temperature in step 3 was 56°C. PCR mixture without a DNA template was used as negative control and PCR-confirmed *P. falciparum* 3D7 laboratory clones donated by Professor Neils Quashie of the University of Ghana was used as reference. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide and the DNA fragments detected using UV light.

Haemoglobin concentration. The Hb concentrations for participating women were estimated using a Mindray® automated haematology analyzer. Anaemia was characterized using Hb concentrations as follows: mild, Hb 9.0-10.9 g/dL; moderate, Hb 7.0-8.9 g/dL; and severe, Hb < 7.0 g/dL [17].

Statistical analysis

A minimum sample size of 384 was calculated with a desired accuracy set at 5% and a confidence interval of 95%. Four hundred and thirteen (413) participants were however recruited into the study. Data from laboratory analysis, as well as those from socio-economic parameters were entered, stored and analyzed using SPSS version 21 and Stata/MP version 13. Data analyses were by both descriptive and inferential statistical methods. A multivariate regression analysis was used to measure the risk posed by ITNs usage, maternal age, gravidity, marital

status and level of education to the prevalence of asymptomatic malaria infection: It was also used to measure the risk posed by asymptomatic malaria to the prevalence of maternal anaemia. Results were expressed in odds or adjusted odds ratio (OR or aOR respectively), with 95% confidence interval (CI). All $p < 0.05$ were considered significant.

RESULTS

Socio-demographic characteristics of participants

In all, 413 pregnant women, aged 18 to 39 yr. (mean = 28.02 yr.) participated in the study. Their mean gravidity was 2.0. One hundred and eight (108) of the participants representing 26.2% were primigravidae. The mean gestational age was 19.7 wk.

Malaria vector control measures among participants

It was observed that 64.8% (160) of the pregnant women owned and used ITNs as their mode of malaria vector control (Figure 2). Of the 35.2% (146) who were not using the ITNs, 13.3% actually owned the nets. Altogether, the total ITN coverage in terms of ownership was 78.1% (323). Twenty-eight percent (28.3%, 117) sprayed their rooms with mosquito insecticide sprays as their measure of mosquito control. Four (0.9%) of the participants were not using any form of vector control measure and about 26 (6.12%) used other forms of mosquito control such as mosquito repellents, mosquito coils, and wearing of protective clothing. The reasons for not using the ITNs included rooms being too hot; not being appreciative of its use as a method to control malaria; the perception that there are no mosquitoes in the area of residence; and inability to mount the nets.

Prevalence of asymptomatic malaria infection

Prevalence of asymptomatic *P. falciparum* infection amongst the pregnant women studied was 13.1% by MRDT, 10.1% by microscopy and 13.8% by PCR. The mean parasite density was 149.6 parasite/ μ L. Five (1.2%) of the samples showed presence of gametocytes. All the parasites detected were *P. falciparum*.

Factors Influencing the Prevalence of asymptomatic malaria infection

Pregnant women who did not use ITNs were twice likely to have asymptomatic malaria compared to those who did (OR, 2.41; 95% CI, 1.14-5.02). Other factors that predicted positively for asymptomatic *P. falciparum* infection in the pregnant women included age below 20 yr. (OR, 4.4; 95% CI, 21-89) and primigravidae (OR, 2.8; 95% CI, 1.5-5.5). Marital status and level of education however did not influence the risk of asymptomatic malaria infection.

Prevalence of anaemia

Mean Hb of participating women was 10.73 g/dL. The total prevalence of anaemia among participants was 40.47%: 24.47% being mild; 14.82% being moderate and 1.20% being severe (Figure 3). Pregnant women who had asymptomatic *falciparum* infection were 4 times at risk of being anaemic (aOR, 4.42; 95% CI, 1.82-10.70) compared to those who did not have the infection. There was a statistically significant negative correlation between parasite density and Hb level ($r = -0.0028$, $p = 0.02$) amongst the pregnant women with asymptomatic malaria (Figure 4).

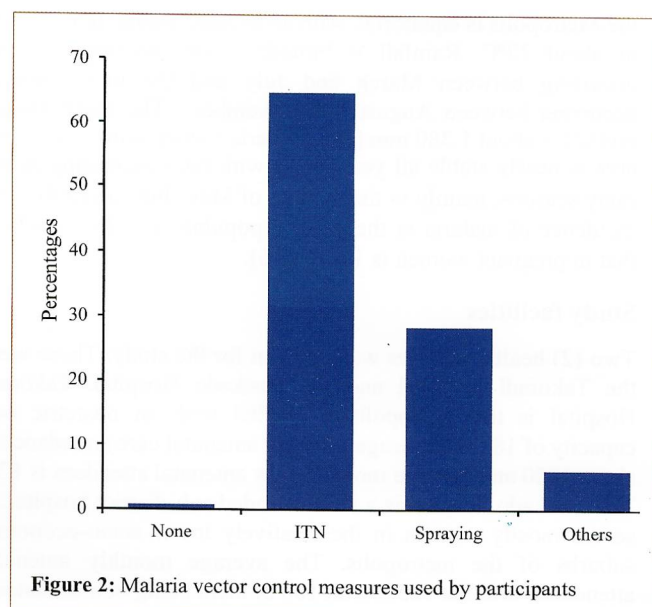


Figure 2: Malaria vector control measures used by participants

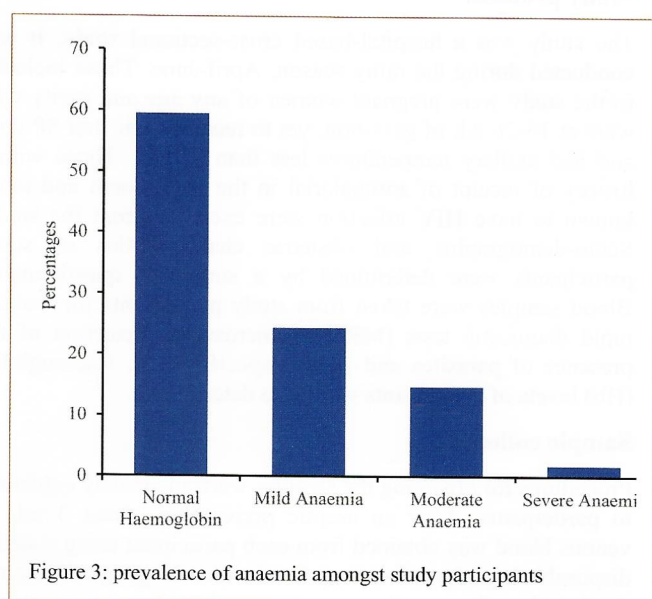


Figure 3: prevalence of anaemia amongst study participants

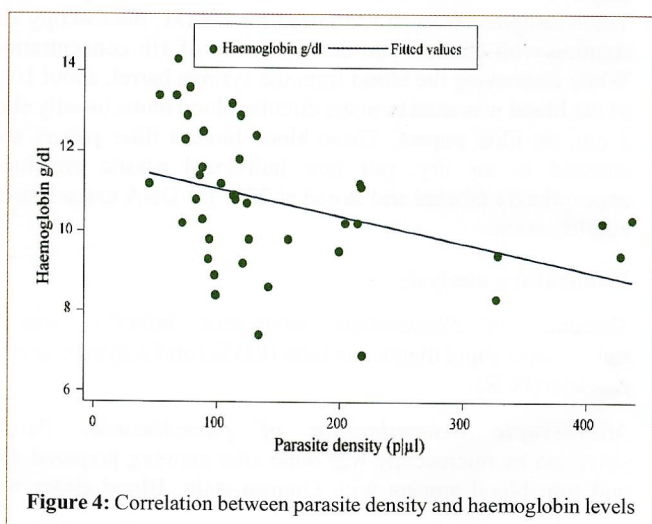


Figure 4: Correlation between parasite density and haemoglobin levels

Relationship between Parity and Parasite Density

A significant negative correlation was observed between participants' parity and parasite density ($r = -0.0053$; $p < 0.001$) (Figure 5).

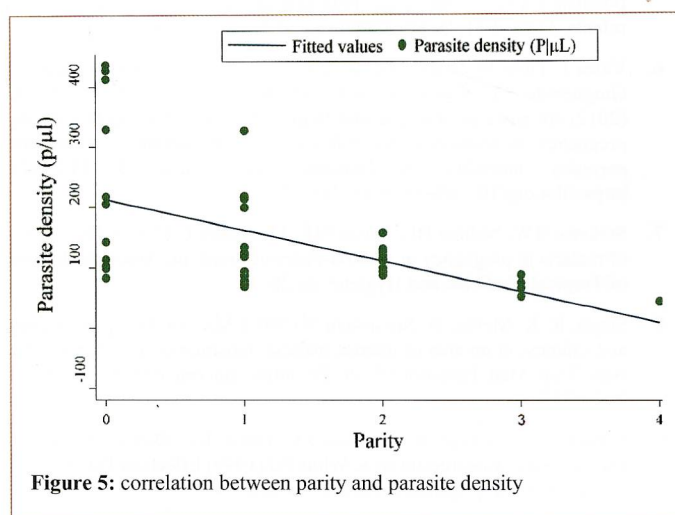


Figure 5: correlation between parity and parasite density

DISCUSSION

The study determined the prevalence of asymptomatic malaria and its association with anaemia among pregnant women who were yet to receive their first dose of SP as IPTp. The higher prevalence recorded using MRDT compared to microscopy, could be due to the persistence of the Pf-HRP-2 even after parasite clearance [18-20]. Meanwhile, the higher prevalence recorded using PCR was expected since PCR is generally more sensitive than both microscopy and MRDT in detecting *Plasmodium* parasitaemia [21]. PCR therefore remains the most effective method of diagnosis of malaria parasitaemia. However due to operational constraints and cost, it could only be used for research purposes. The asymptomatic malaria prevalence by microscopy and MRDT were lower than the 24% and 30% recorded respectively in a similar study in neighboring Burkina Faso [22]. The Burkina Faso study, unlike the current, included all pregnant women who had received any number of doses of SP as IPTp. The difference is therefore unexpected, since the prevalence of malaria in pregnancy, whether symptomatic or asymptomatic should be higher in those who were yet to receive any dose of SP. This odd observation could probably be due to the differences in the extent of applications of other malaria control measures, mainly the usage of ITNs and indoor residual spraying in the study areas being compared [23].

The prevalence of 10.1% by microscopy was however twice as higher as the 5% recorded in a similar study in Madina, in the Ga East Municipality, Ghana [24]. The difference could be due to the fact that whilst samples for the current study were collected from April to July (peak of the rainy season, higher malaria transmission) that of the Madina study were collected from July to August when the frequency and intensity of rains might be reducing hence decrease in rate of transmission. Parasite densities were low, averaging 149.6 parasite/μL in all infected pregnant women. This is because *P. falciparum*-infected asymptomatic individuals generally tend to have low

submicroscopic parasite densities due to pre-immunity especially in malaria endemic regions [25,26]. Placental sequestration of parasitized red blood cells during pregnancy also leads to an absence of, or low grade of peripheral parasitaemia. Though 78.1% of the participants owned ITNs, only 64.8% were using them. This coverage is lower than the projected 80% for endemic regions fixed in 2010 [27] and currently pegged at 100% in the same region [28]. It is also lower than the current national ITN coverage of 96% in Ghana [28]. There is very strong evidence for the efficiency of ITNs in preventing malaria infection and its consequences on pregnancy, as reported by Gamble et al. in a Cochrane review in 2009 [29] and recently by Eisele et al. (2012) [30].

The WHO recognizes ITNs as one of the most cost-effective ways of controlling malaria in vulnerable groups. In this study, pregnant women who did not use ITNs were twice as likely to have asymptomatic malaria compared to those who used it (OR, 2.41; 95% CI, 1.14-5.02). There is the need therefore, to strengthen efforts to scale up ITN coverage amongst pregnant women in this part of the country since they together with children < 5 yr. are at the most risk to malaria infection. Some reasons why most of these women who had the nets but were not using them included, lack of appreciation of its importance, feeling warm when sleeping in it, not knowing how to mount it and the perception that there are no mosquitoes in their neighborhood. All these barriers can be surmounted simply by intensified user education. Other factors that predicated positively for *P. falciparum* parasitaemia are maternal age below 20 yr. and primigravidity. At a younger age, these women are still in the process of acquiring natural immunity to pregnancy related malaria [31], making them to have a higher risk than their older counterparts. Again, with successive pregnancies, women in malaria endemic regions tend to develop immunity against pregnancy related malaria. This explains why primigravidae who are yet to develop this immunity were relatively susceptible to malaria infection. Though being married and having a higher level of education is expected to give impetus to maternal health promotion and therefore prevention of malaria infection, no significant association was found between marital status and level of education on one side and malaria infection in pregnancy on the other.

Overall, the study observed a high rate of anaemia of 42.3%. This correlated strongly with the presence of asymptomatic *P. falciparum* infection in these pregnant women. This phenomenon has also been observed in similar studies in Ikot Ekpene in Nigeria [32], Ouagadougou in Burkina Faso [23] and Kinshasa in the Democratic Republic of Congo [33]. Chronic asymptomatic malaria infection causes anaemia by decreasing bone marrow production of functional red blood cells as a result of the direct inhibitory effects of parasites [12] and cytokines [13,14] as well as dysregulation of erythropoietin and iron metabolism [13,14]. An interesting observation made in this study is the worsening of anaemia associated with increasing parasite density. This observation was in contrast with findings made in Tanzania by Marchant et al. (2002) [34] and in the Democratic Republic of Congo by Matangila et al. (2014) [33]. Our inability to ascertain the contributions of factors such as infection with helminthes and micronutrients (such as iron, folate and vitamins) to anaemia was a limitation to this study.

Conclusion

This study firmly confirms a high level of anaemia among all the antenatal attendees who were yet to receive their first dose of SP as IPTp in part of the Western Region of Ghana. The anaemia was strongly associated with asymptomatic *P. falciparum* infection. Though using ITNs was seen to protect against the risk of asymptomatic *P. falciparum* infection, its coverage and usage in the study area was low. There is therefore the need to intensify education on the use of this control method as well as find innovative ways to minimize infection with *P. falciparum* during pregnancy.

DECLARATIONS

Ethical considerations

The study was conducted according to the Helsinki Declaration on Research regarding human subjects. The protocol for the study was reviewed and approved by the Ghana Health Service's Ethical Review Committee (GHS-ERC). Approval was also sought and given by respective Medical Superintendents of the health facilities used in the study. Participants' consent was also sought before administering questionnaires and taking blood samples.

Funding

None

Competing interests

No potential conflict of interest was reported by the authors

Author contributions

LLA, JNB and NBQ designed the study. LLA carried out the research work. He also analyzed and interpreted all data and prepared the manuscript. JNB and NBQ supervised the research and participated in drafting and editing of the manuscript. All authors read and approved the final draft of the manuscript.

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Availability of data

Data is available upon request to corresponding author

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