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Prenatal malaria exposure and the risk of adverse birth outcomes : a cohort of pregnant women in from Northern Region of Ghana.

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Abstract

Objective Malaria remains endemic in most of Sub-Saharan Africa, wreaking havoc on pregnant women and resulting in morbidity and poor birth outcomes. The purpose of this study was to assess the relationships between malaria infection and adverse birth outcomes in prenatal women in the Northern Region of Ghana.

Design

A prospective cohort study of women with singleton pregnancies at 28 weeks gestational age and above were recruited between July 2018 and May 2019.

Setting Four hospitals in Northern region of Ghana

Primary outcome measures were low birth weight (LBW), preterm birth, and perinatal deaths

Results

A total of 1323 pregnant women completed the study out of 1626 recruited. Their average age was 27.3 ± 5.2 years. The incidence of malaria in this population was 9.5% (95% confidence interval [CI]: 7.9-11.1). After adjusting for newborn admissions to the neonatal Intensive care Unit (NICU), parity, maternal age, and Glucose-6-phosphate dehydrogenase (G6PD); women who were exposed to malaria during the third trimester of pregnancy had 1.45 times (95%CI 1.20 - 1.78) higher risk of premature delivery. They further had 1.93 times [95% CI 1.11 - 63.41] higher chance of giving birth to infants with low birth weight (LBW), regardless of their socioeconomic status.

Conclusion
This study confirms a high frequency of prenatal malaria which critically increases risk of both preterm and LBW delivery. A decisive policy by the Ministry of Health (MoH) to eradicate or minimize perinatal malaria is needed to contribute to the prevention of LBW and adverse pregnancy outcomes.

Keywords: Malaria, RDT, prenatal, pregnancy, preterm, LBW, northern Region, Ghana.

Strengths and limitations of this study

This study until now is first prospective cohort study with relatively a large sample size to examine the link between maternal malaria at third trimester and birth outcomes in Tamale., Ghana.

It gives a detailed information on malaria and adverse birth outcomes which is not readily available due challenges of under reporting, analysis and application of surveillance data as result of poor record linkage.

This study was designed as an independent appraisal of birth outcomes among people with prenatal malaria in light of these difficulties

Rapid Diagnostic Test (RDT) was used to diagnose malaria in pregnancy as routine procedure in state-licensed laboratory practitioners which may be a limitation

We were also unable to account for the effects of malaria at early stages of pregnancy on birth outcomes

Introduction

Of the US$ 2.7 billion invested in malaria control and elimination efforts, only 30% was invested by malaria-endemic countries which were used for malaria control activities and patient care.(1) These global efforts and investments to curb malaria seem to have worked for China, El Salvador, Iran, Malaysia, and Timor-Leste with each reporting zero indigenous cases in 2018.(1) However, the narrative in Africa remains considerably different as malaria continues to threaten many populations in the sub-region, especially among pregnant women.

In malaria-endemic areas in Sub-Saharan Africa, women face significant risks throughout their pregnancy. Examples of these risks pregnant women are exposed to include low birth weight (LBW), premature birth, and spontaneous abortions.(2) Prenatal malaria infection is responsible for 5-12% of LBW and accounted for between 75,000 to 200,000 infant deaths each year (3). In sub-Saharan Africa, 11 million women were infected with malaria in 2018 resulting in about 872,000 neonates being born with LBW.(1) In 2018, the Central and Western Africa sub-regions reported the highest prevalence of malaria in pregnant women each with 35% prevalence. In addition, West Africa had the highest frequency of LBW due to malaria.(1)

Twelve years after adopting the WHO policy recommendation on malaria by some countries (4), the WHO continues to prioritize vector control options [(Indoor Residual Spray (IRS) and Insecticide Treated Net (ITN)] for mosquito bite prevention and sulfadoxine-pyrimethamine (SP) for malaria infections suppression. As a result, for pregnant women in endemic nations, Intermittent Preventive Treatment (IPT) with SP became a preventive and control method to the national malaria eradiction strategy. In practice, and regardless of her malarial diagnosis, every pregnant woman is presumptively administered SP as early as the second trimester. While avaialble data suggests that in 2018, around 60% and 31% of women got their dosages during the first and third trimesters, respectively,(1) SP continues to be considered helpful in reducing the burden of malaria in pregnancy. Especially since most developing countries rely on RDTs to diagnose malaria, which have limitations such as deletions in the (pfhrp2/3) genes of clinical isolates, which make these parasites invisible to RDTs but only identifiable under a microscope.(1) Hence, malaria parasites can escape detection in some pregnant women.

Malaria cases increased by half a million in Ghana in 2018 compared to the year before.(1) Regarding treatment, a research conducted in a war hospital in the Upper East Region found that children born to mothers on artemether–lumefantrine (ISTp-AL) had a lower risk of malaria than those delivered to mothers on sulfadoxine/pyrimethamine (IPTp-SP).(5) Yet, malaria prevalence and poor birth outcomes were 9.0 percent and 22.2 percent, respectively, in Kumasi.(6) In Navrongo, IPT3 uptake was 76 percent, while IPT5 uptake was 16 percent, with women who received at least three doses having better health outcomes.(6) Given that Ghana is in an endemic malaria zone, these studies highlight implementation gaps as well as provide information that are useful to improve our malaria prevention policies and programs. Unfortunately, there is a dearth in the Ghanaian literature relating to the role of malaria infection in poor birth outcomes in pregnant women in urban settlements in Northern Ghana.

Furthermore, due to insufficient linkages between malaria control and prenatal care data collection, progress in attaining malaria control among pregnant women has been slow.(7) In addition, inconsistencies in data management practices were discovered during a data quality evaluation in several health institutions, posing problems in data reporting, analysis, and application.(8) Therefore, the accuracy of aggregate data collected from these facilities through surveillance is compromised by these discrepancies. We designed this prospective cohort research as an independent appraisal of birth outcomes among people with prenatal malaria in light of these difficulties. This study sought to provide considerably more detailed information on the links between prenatal malaria infection and poor birth outcomes in pregnant women in northern Ghana.

Methods and Analysis

Setting

The data for this sub-study was drawn from a prospective cohort study that took place in four hospitals in Ghana's Northern region to assess how different fuel types affected pregnancy outcomes and baby respiratory problems. This study's techniques and follow up process are already published.(9) Briefly, for this present study, we included third-trimester singleton pregnant women at all four hospitals recruited between July 2018 and May 2019. Once they were recruited, we followed them up until delivery to collect the birth outcome data.

Study Procedure

Baseline data was collected at recruitment using a structured questionnaire at the antenatal care (ANC) centers of each hospital. Pregnancy outcome data were collected at the labour wards of all the hospitalsby trained research assistants using a predesigned questionnaire. Except for those with a Glucose-6-phosphate Dehydrogenase (G6PD) deficiency, all pregnant women in the study were given sulfadoxine/pyrimethamine (IPTp-SP).

Laboratory Procedure

Malaria Parasite

The SD BIOLINE Malaria Ag P.f Rapid Diagnostic Tests Kits (RDTs) for malaria were used in all hospitals.(10) with specificity and sensitivity of 99.5% and 99.7% respectively. The principal investigator (PI) monitored and observed laboratory practices in each health facility to ensure they conformed to both the manufacturers guide and the fundamental laboratory principles for the test. The RDT test kits were used to identify malaria presence in peripheral blood according to the manufacturer's guide. A test is positive if the test and control bands were both positive (show pink lines) and negative when only the control band is positive (show pink line).(10)

Haemoglobin estimation – All hospitals used a blood analyzer to estimate full blood count (FBC) andHemoglobin (Hb) was extracted from the FBC of pregnant women. Blood sample (5 milliliters) for Hb estimation was collected into an ethylenediamine tetraacetic acid (EDTA) tube, and was mixed with an EDTA anticoagulant. In this study, anaemia was defined as Hb < 9.0 g/dL.

Glucose-6-phosphate Dehydrogenase (G6PD) Test - The methaemoglobin reduction test was used in all hospitals for the screening of pregnant women for G6PD. The test outcome was reported as No defect/normal, Partial defect or Full defect.

Data Collection

Computer-assisted personal interviewing (CAPI) was used to gather all of the data, which was done using the kobo collect android app. The data collecting procedure is described in details elsewhere.(9)

Outcome Variable

The major outcomes of this study were low birth weight (LBW), preterm birth, and perinatal death. These were all gathered during delivery in the various hospitals' labour wards. On the seventh day, women were contacted by mobile phone to inquire about the baby's well-being in order to ensure that the infant was still alive. This was done to prevent neonatal mortality following discharge from the hospital. Preterm birth was defined as <37 weeks gestational age, while LBW was defined as <2.5 kilograms as previously published. (9)

Objective

To appraise the association of birth outcomes among people with prenatal malaria. The exposure variable was a positive RDT test verifying that a pregnant woman had malaria during her third trimester or just before birth.

Statistical Analysis

Data was exported from the kobo collect application database into an MS Excel sheet, cleaned and transferred into STATA 13 for analysis. Individual covariates were added into the simple log bionmial regression model, and a significant variable of > 0.05 or near significant were retained in the multiple log binomial regressions to evaluate the potential associations between adverse birth outcomes and malaria infection. Some adjusted confounders included in the adjusted models included G6PD, parity, maternal age and Socio Economic Status (SES), assets from 2014 Ghana Demographic Health Survey were used for calculation of total assets,dividing the total into quantiles, we considered less the 50 quantile as poor, between 50 and 75 as moderately rich and 75 and beyond as rich.(9) Missing data of more than 10% from any

observation were dropped in order not to open the study to bias, single manual imputation was used to address some missing data based on previous patterns of questions.(11) Sensitivity analysis was assesd in both univariate and multiple logistic regression model in comparison with log bionomial regression model.

Patient and Public Involvement

Patients were not involved in the design of this study.

Results

Figure 1 shows an elaborate details of plan for follow up during this study which was published in Hussein et al., 2020, at baseline 1626 third trimester preganant women were recuited with 1323 women completed the study.The age of pregnant women ranged between 15-48 years, 59.1% were between 25-34 years. Only about 1.0% of them were unmarried, nearly 7.0% had at least diploma education with 75.4% having primary or no education. Also, 6.6% were formally employed, with 63.1% being traders. About 31.1% were rich, with 42.4% living in rural settings while 3.3% lived in semi-detached houses (Table 1).

On medical history, 14.8% had four or more children. The incidence of malaria in this cohort was 9.5% (95% confidence interval [CI]: 7.9-11.1). About 6.4% tested positive for sickle cell and, out of these, 50.0% of them checked for their genotypes were SS. About 9.1% of the women were severely anaemic with haemoglobin levels of less than 9 g/dl within their third trimester of pregnancy while 4.7% had G6PD full defect (Table 1).

The incidence of preterm birth among women with malaria was 52.0%. Also, the prevalence of LBW was 10.4% among women with malaria and 5.1%, in women without malaria. In both mothers with and without malaria, newborn death and live birth were equally 1.6 percent. (Table 2).

The risk of preterm birth among women with malaria was 1.45 times (CI: 95% (1.20-1.78) compared to those without malaria after adjusting for parity, maternal age, G6PD deficiency and neonatal admissions (Table 3). The risk of LBW was 1.93 times [CI 95% (1.11-63.41)] in women with malaria compared to those without malaria after adjusting for parity, maternal age, and socio-economic status (Table 4). The risk of having perinatal mortality was 1.02 times [CI: 95% (0.26 – 4.01) in women with malaria compared to women without malaria after adjusting for the caesarian section (Table 5).

Sensitivity analysis in both univariate and multiple logistic regression model did not change the directions or strengths of the estimates compared with log binomial regression model for LBW, even though odds ratio marginally exaggerated the relative risk to some magnitude (Table 6)

Discussion

In this study, 59.1% of the women were between the ages of 25 and 34, suggesting a youthful distribution. Our population's illiteracy rate was high, at around 75.4 percent, which may explain why 63.1 percent were traders since trading in Ghana is traditionally associated with women with low educational status.(12,13) The proportion of malaria in pregnant women was nearly 10% in this study. The study also investigates the associations between RDT-detected malaria and preterm delivery, LBW, and perinatal death.

Even though our study differed from other studies in design, relating to the biological specimens examined, mode of diagnosis, period of malaria infestation during pregnancy and different confounding factors, it also found similar results with some previous research.(14–16) This study found perinatal malaria to be significantly related with preterm birth, LBW and perinatal mortality after adjusting for parity, maternal age, G6PD, SES, neonatal admissions at birth and type of housing. Vogel and colleagues reported similar findings where exposure to malaria increased odds of spontaneous preterm term birth by 1.67 times based on their secondary analysis of data from 22 low-and middle-income countries.(14) Also, Van den Broek and colleagues reported similar findings as maternal malaria significantly increased the risk of preterm birth by 1.99 times.(17) In Tanzania, similar studies show that maternal malaria parasites in red blood cells were linked to a 3.2 higher risk of premature delivery (15). In comparison to individuals who did not have placental site malaria, the chances of preterm delivery rose by between 4.7 and 5.6 percent. (18,19). Outside Africa, a research in Brazil found that *Plasmodium falciparum* (*P. falciparum*) species were substantially linked with preterm delivery during pregnancy, despite the fact that they accounted for less than 40% of the total.(16) Some research, however, found non-significant correlations that differed from this one. For example, a recently published comprehensive study of malaria at delivery in Uganda used three different methods of detecting parasites, including peripheral and placental blood microscopy, placental blood loop mediated isothermal amplification (LAMP), and placental histopathology, and found no statistically significant link between malaria and preterm birth for any of the methods.(20)

While we did not distinguish between malaria species in this study, prior research has indicated that malaria, regardless of the malaria species, might induce poor birth outcomes. In Malawi, placental blood *P. falciparum* infection raised the risk of LBW by approximately 1.7 times.(21)

Also Nyirjesy et al. discovered a 3.7-fold higher incidence of LBW in pregnant women who had malaria in 1993.(22) A *P. vivax* during pregnancy was reported to enhance the risks of LBW by 1.4 times in a Brazilian research, where Plasmodium (P.) vivax constituted around 64% of malaria species.(16) In a Brazilian study, where *Plasmodium (P.) vivax* represented about 64% of malaria species, *P. vivax* during pregnancy was found to increase the odds of LBW by 1.4 times.(16) Additionally, about 33% and 19% of women with placental malaria delivered LBW babies compared with those without placental malaria in Nigeria and Uganda respectively. (19,23)

However, we observed no significant increased odds for perinatal mortality in both adjusted and unadjusted models, despite the fact that women who delivered through caserian sections (CS) were nearly 5 times more likely to suffer perinatal mortality. In contrast, other studies, such as one conducted in Zaire in 1993, reported that maternal malaria with chloroquine prophylaxis increased the risk of perinatal death by 12 times after adjusting for parity and prenatal clinic visits.(22) Also, *P falciparum* and *P. vivax* malaria during pregnancy increased the hazard of neonatal mortality by 2.6 times and 1.9 times respectively.(24) The odds of mortality significantly increased by 5 times among infants born to mothers with acute placental infections.(25)

Multiple variables, such as parity, mother age, SES, and maternal BMI, can lead to negative birth outcomes. Therefore in this study, we were able to adjust for those significant confounders in the multiple log binomial regression and keep those that were significant. Women who had several pregnancies were protected against preterm delivery. This is apparently due to the extra protection provided by antibodies in subsequent pregnancies against parasites variant surface antigen VAR2CSA. (26,27)

The pathway that connects the mother to the child during pregnancy may influence the survival of the feotus at birth or even beyond since the placenta supplies nutrients to the baby through the umbilical cord. Thus, Ouédraogo and colleagues found a significant association between umbilical cord parasiteamia level and maternal peripheral blood parasiteamia.(28) Although we used RDT with peripheral/capillary blood, our findings were consistent with the majority of studies that used placental site malaria.(26,27) This might be because peripheral blood infections could promote parasite sequestration at the placenta and activate antibody-antigen immune

responses, which can cause complications during delivery.(26,27) Furthermore, Kapisi et al. (2017) found that women who had a high burden of malaria, defined as two or more episodes of symptomatic malaria, had a 14-fold increased risk of placental malaria by blood microscopy and a four-fold increased risk of Loop-mediated isothermal amplification (LAMP). This might indicate that our group had a greater malaria burden, correlating with previous research using a different technique of diagnosis.(18)

This study benefitted from a large sample size. Our search until date, showed no study examining the link between maternal malaria at delivery and birth outcomes in Tamale and its surroundings in Ghana's Northern Region. In this cohort, we utilized RDTs to diagnose malaria, although its sensitivity was 19% lower than microscopic examination of peripheral blood and placental blood for malaria; nevertheless, RDTs outperformed microscopy in identifying malaria in other pregnant settings.(29,30) Moreover, RDT is useful in settings like ours because it produces results quickly, requires less training and equipment, and experiences little to no power interruptions. As a consequence, it is essential in detecting malaria, particularly in health institutions with limited human resources and equipment. Nonetheless, one possible limitation of our study was that we depended on the routine laboratories in each hospital to gather data on malaria. While we made efforts to observe and monitor adherence to standard testing protocols, we are unable to control for possible measurement bias among the laboratory personnel for the exposure variable (malaria). However, it is worth mentioning that using the RDT method to diagnose malaria in pregnancy is routine procedure in the context and performed by state-licensed laboratory practitioners. In addition, hospital laboratories follow the Ministry of Health/Ghana Health Services standard operating standards for diagnosis. We were also unable to account for the effects of malaria at early stages of pregnancy on birth outcomes. Despite this, our study is comparable with similar studies in this subject matter.

In conclusion, maternal malaria within third trimester of pregnancy may be a determinant of LBW and preterm birth. This indicates that malaria is still a major contributor to some adverse birth outcomes in the northern region of Ghana.

Data availability statement

Data are available upon reasonable request.

Ethical Approval

Approval was given by the ethical review committees of the Ghana Health Services and the Tehran University of Medical Sciences. Ethical Numbers IR.TUMS.SPH.REC.1396.4066 and GHS-ERC respectively, 010/12/17. Information sheets were provided to participants with concerns of risk adequately discussed, signed or thumb printed informed consent was obtained from each particpnat before recruitment.

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sectors.

Contributors HH: Investigation, writing draft, data analysis. MS:Writing review & editing. MY: Conceptualization, Validation. M.S.H: Visualization, Resources. MAS, Project administration. PDA, Data curation AF: Supervision, Funding acquisition. All authors reviewed and approved the final manuscript:

Competing interests None Declared

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Table 1: Baseline characteristicas of pregnant women

Variables	Delivery period
	Frequency (%) n= 1323
Maternal age (mean ±SD)	27.3±5.2
15-24	394 (29.8)
25-34	782 (59.1)

35-48	147 (11.1)
Marital status	
Married	1310 (99.0)
Unmarried	13 (1.0)
Ethnicity	
Mole Dagbani	1274 (96.3)
Others	49 (3.7)
Maternal education	
Primary/no education	997 (75.4)
JHS/Middle school	147 (11.1)
SHS/Technical/Vocational	89 (6.7)
At least Diploma	89 (6.8)
Maternal Occupation	
No employment	302 (22.9)
Trader	834 (63.1)
Laborer	74 (5.6)
Factory/Industry	24 (1.8)
Formal employment	87 (6.6)
Socio economic status	
Poor	473 (35.8)
Moderately rich	439 (33.2)
Rich	411 (31.1)
Residence	
Urban	762 (57.6)
Rural	561 (42.4)
Housing	
Separate house	68 (5.14)
Semi-detached	43 (3.25)
Compound house (sandcrete)	850 (64.25)
Compound house (mud)	362 (27.36)
Parity	
First pregnancy	633 (47.9)
2-3 pregnancies	494 (37.3)
4 or more pregnancies	196 (14.8)
Medical History	
Malaria	
Positive	125 (9.5)
Negative	1198 (90.5)
Sickle cell	
Positive	77 (6.4)
Negative	1120 (93.6)
Genotype	
AS	24 (36.4)
SC	9 (13.6)
SS	33 (50.0)
Anaemia	

<9 g/dl	120 (9.1)
≥9 g/dl	1203 (90.9)
Glucose-6-phosphate Dehydrogenase (G6PD)	
No defect	1029 (86.9)
Partial defect	98 (8.3)
Full defect	56 (4.7)
Malaria	
Yes	125 (9.5)
No	1198 (90.5)

Table 2: Incidence of pregnancy outcome and malaria

Pregnancy Outcomes n=1323	**Negative**	**Positive**	**Total**
	Freq (%)	Freq (%)	
Preterm birth			
Term	780 (65.1)	60 (48.0)	840
Preterm	418 (34.9)	65 (52)	483
Birth weight			
Normal birth weight	1137 (94.9)	112 (89.6)	1249
Low birth weight	61 (5.1)	13 (10.4)	74
Type of delivery			
Live birth	1176 (98.4)	123 (98.4)	1299
Neonatal mortality	19 (1.6)	2 (1.6)	21

Table 3: Log bionomial regression of preterm and malaria

Preterm	**Crude RR (CI)**	**Pvalue**	**Adjusted (CI) RR"**"**	**Pvalue**
Malaria				
No	1		1	
Yes	1.49 (1.24-1.23)	<0.001	1.45 (1.20- 1.78)	<0.001

Parity				
First pregnancy	1		1	
2-3 pregnancy	0.89 (0.78-1.04)	0.150	0.87 (0.74-1.02)	0.087
4 or more	0.72 (0.57-0.91)	0.006	0.74 (0.57-0.96)	0.025
Maternal Age				
15-24 years	1			
25-34 years	1.14 (0.98-1.35)	0.106	1.19 (1.01-1.42)	0.039
35-48 years	0.81 (0.61-1.09)	0.181	0.93 (0.67-1.29)	0.670
G6PD				
Normal	1		1	
Partial defect	1.29 (1.03-1.60)	0.025	1.27 (1.02- 1.59)	0.036
Full defect	1.04 (0.74-1.48)	0.803	0.98 (0.67-1.44)	0.935
Neonatal admission at birth				
No	1		1	
Yes	1.47 (1.13-1.91)	0.004	1.39 (1.07. 1.83)	0.015

** significant confounders adjusted in the multiple log binomial model

**parity, maternal age, G6PD, Neonatal admissions at birth

Table 4: Log bionomial of LBW and malaria

LBW	Crude RR (CI)	Pvalue	Adjusted RR(CI)"**"	Pvalue
Malaria				
No	1		1	

Yes	2.04 (1.16-3.61)	0.014	1.93 (1.11-3.41)	0.020
Parity				
First pregnancy	1		1	
2-3 pregnancy	1.19 (0.72-1.97)	0.482	1.29 (0.78-2.16)	0.317
4 or more	1.66 (0.94- 293)	0.084	2.01 (1.05-3.88)	0.037
Maternal Age				
15-24 years	1			
25-34 years	0.80(0.49-1.31)	0.378	0.72 (0.43-1.20)	0.207
35-48 years	0.75 (0.33-1.66)	0.459	0.48 (0.19-1.20)	0.117
Socio-economic status				
Poor	1		1	
Moderately rich	0.59 (0.35-1.00)	0.051	0.63 (0.37-1.08)	0.093
Rich	0.54 (0.31-0.94)	0.029	0.57(0.32-0.99)	0.046

** significant confounders adjusted in the multiple log binomial model
**parity, Socio-economic status

Table 5: Log bionomial of perinatal mortality and malaria

Perinatal mortality	Crude RR (CI)	P value	Adjusted RR(CI)"**"	Adjusted Models P value
Malaria				
No	1		1	
Yes	1.01 (0.24-4.27)	0.993	1.02 (0.26-4.01)	0.983
Mode of delivery				
Normal	1		1	
Ceasarian section	4.99 (1.88-13.27)	0.001	4.98(1.95-14.36)	0.001

**significant confounders adjusted in the multiple log binomial model
**Mode of delivery

Table 6: Sensitivity analysis using logistic regression for LBW and malaria

LBW	Crude OR (CI)	Pvalue	Adjusted OR(CI)"**"	Pvalue

Malaria				
No	1		1	
Yes	2.16 (1.15-4.06)	0.016	2.06 (1.08-3.93)	0.027
Parity				
First pregnancy	1		1	
2-3 pregnancy	1.21 (0.71-2.05)	0.480	1.32 (0.76-2.29)	0.322
4 or more	1.71 (0.91- 3.23)	0.093	2.13 (1.03-4.39)	0.041
Maternal Age				
15-24 years	1			
25-34 years	0.79(0.47-1.32)	0.373	0.70 (0.41-1.22)	0.210
35-48 years	0.72 (0.30-1.71)	0.457	0.45 (0.17-1.19)	0.109
Socio-economic status				
Poor	1		1	
Moderately rich	0.57 (0.33-0.99)	0.050	0.60 (0.34-1.07)	0.082
Rich	0.51 (0.29-0.93)	0.028	0.54(0.29-0.98)	0.044

** significant confounders adjusted in the multiple log binomial model

**parity, Socio-economic status

Figure 1 : Follow-up plan

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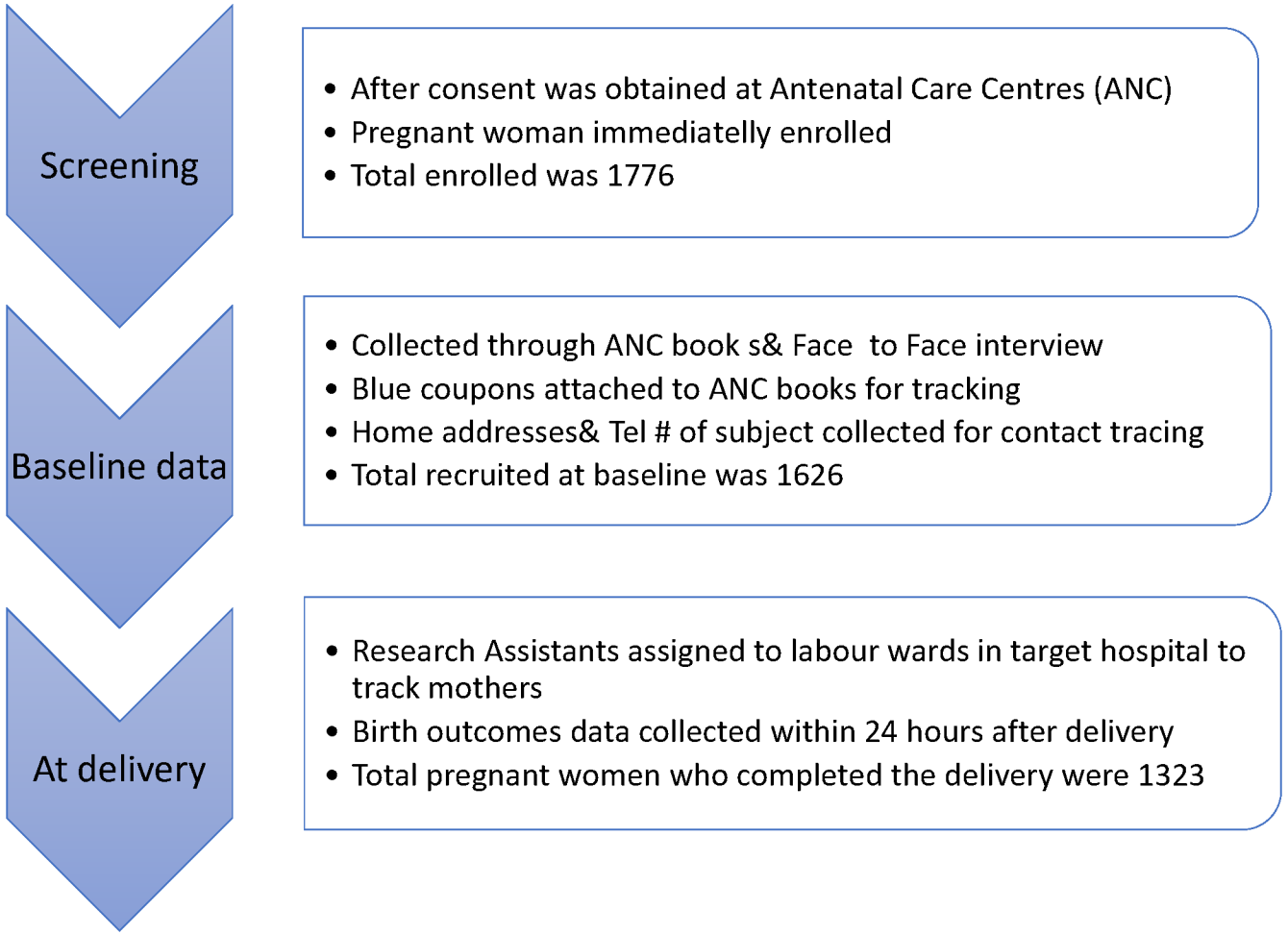

Figure 1 : Plan for follow up

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8 9 8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	9

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Prenatal malaria exposure and risk of adverse birth outcomes: a prospective cohort study of pregnant women in the Northern Region of Ghana.

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Abstract

Objective Malaria remains endemic in most of Sub-Saharan Africa, wreaking havoc on pregnant women and resulting in morbidity and poor birth outcomes. The purpose of this study was to assess the relationship between malaria and adverse birth outcomes in prenatal women in the Northern Region of Ghana.

Design

A prospective cohort study with of singleton pregnancies at 28 weeks of gestational age and above were recruited between July 2018 and May 2019.

Setting Four hospitals in the northern region of Ghana

Primary outcome measures were low birth weight (LBW), preterm birth, and perinatal deaths.

Results

A total of 1323 pregnant women completed the study out of 1626 recruited. Their average age was 27.3 ± 5.2 years. The incidence of malaria in this population was 9.5% (95% confidence interval [CI]: 7.9-11.1). After adjusting for newborn admissions to the neonatal intensive care Unit (NICU), parity, maternal age, and glucose-6-phosphate dehydrogenase (G6PD); women who were exposed to malaria during the third trimester of pregnancy had 2.02 times (95%CI 1.36 – 2.99) higher odds of premature delivery. Furthermore, they had 2.06 times [95% CI 1.09 - 3.93] higher chance of giving birth to babies with low birth weight (LBW), regardless of their socioeconomic status.

Conclusion
This study confirms a prenatal malaria which critically increases odd of both preterm and LBW delivery. A decisive policy by the Ministry of Health (MoH) to eradicate or minimize perinatal malaria is needed to contribute to the prevention of LBW and adverse pregnancy outcomes.

Keywords: Malaria, RDT, prenatal, pregnancy, preterm, LBW, northern Region, Ghana.

Strengths and limitations of this study

Until now, this study is first prospective cohort study with a relatively large sample size to examine the link between maternal malaria in the third trimester and birth outcomes in Tamale, Ghana.

It provides detailed information on malaria and adverse birth outcomes which is not readily available due to the challenges of under reporting, analysis, and application of surveillance data as a result of poor record linkage.

This study was designed as an independent evaluation of birth outcomes among people with prenatal malaria in light of these difficulties

Rapid Diagnostic Test (RDT) was used to diagnose malaria in pregnancy as a routine procedure in state-licensed laboratory practitioners, which may be a limitation.

We were unable to account for the effects of malaria at in the early stages of pregnancy on birth outcomes.

Introduction

In 2020, Over 600 000 people died of malaria, about 95% from sub-Saharan Africa.(1) Malaria exposure can be life threathening to people with low immunity, especially pregnant women and young children. It also presents an economic cost to family and government with a direct cost of about $ 12 billion per year.(2)

In malaria-endemic areas in sub-Saharan Africa, women face significant risks throughout their pregnancy. Examples of these risks pregnant women are exposed to include low birth weight (LBW), premature birth, and spontaneous abortions.(3) Prenatal malaria is responsible for 5-12% of LBW and accounts for between 75,000 to 200,000 infant deaths each year (4). In sub-Saharan Africa, 11 million women were infected with malaria in 2018, resulting in approximately 872,000 newborns born with LBW.(5) In 2018, the Central and Western Africa subregions reported the highest prevalence of malaria in pregnant women, each with 35% prevalence. Furthermore, West Africa had the highest frequency of LBW due to malaria.(5) In particular, the effect of malaria exposure on fetal growth was observed during third trimester of pregnancy regardless of period the exposure.(6)

Malaria cases increased by half a million in Ghana in 2018 compared to the year before.(5) Regarding treatment, , a research conducted in a War Memorial Hospital in the Upper East Region found that children born to mothers on artemether–lumefantrine (ISTp-AL) had a lower risk of malaria than those delivered to mothers on sulfadoxine/pyrimethamine (IPTp-SP).(7) Yet, malaria prevalence and poor birth outcomes were 9.0 percent and 22.2 percent, respectively, in Kumasi.(8) In Navrongo, uptake of Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPT3) i.e uptake of three doses was 76 percent, while uptake of 5 doses (IPT5) was 16 percent, with women who received at least three doses having better health outcomes.(8) Given that Ghana is in an endemic malaria zone, these studies highlight implementation gaps as well as provide information that are useful to improve our malaria prevention policies and programs. Unfortunately, there is a dearth in the Ghanaian literature

relating to the role of malaria in poor birth outcomes in pregnant women in urban settlements in Northern, Ghana.

Furthermore, due to insufficient linkages between malaria control and prenatal care data, progress in attaining malaria control among pregnant women has been slow.(9) In addition, inconsistencies in data management practices were discovered during a data quality evaluation in several health institutions, posing problems in data reporting, analysis, and application.(10) Therefore, the precision of aggregate data collected from these facilities through surveillance is compromised by these discrepancies. We designed this prospective cohort research as an independent evaluation of birth outcomes among people with prenatal malaria in light of these difficulties. This study sought to provide considerably more detailed information on the links between prenatal malaria and poor birth outcomes in pregnant women in northern Ghana.

Methods and Analysis

Setting

Data for this substudy was drawn from a prospective cohort study that took place in four hospitals in Ghana's Northern region. Three of the hospitals are located in Tamale, the Northern Regional Capital, the fourth largest city in Ghana, while the fourth hospital is located in the Savelgu Municipality bordered by Tamale to the West: These areas are located within the Guinea Savannah belt. (11) The P. falciparum peripheral parasitemia prevalence in pregnant women in Northern Savanna Zone ranged between 26% and 13.4% from 2013 to 2019 respectively.(12)

Design

The study was designed from a parent cohort study that sought to assess how different cooking fuel types influenced pregnancy outcomes and infant respiratory problems, this was the primary research question, (13,14) the present study answers a secondary research question about prenatal malaria exposure and the risk of adverse birth outcomes. Therefore sample size calculation was based on proportion of developing the outcome (respiratory symptoms) with cooking fuel type as an exposure. However, the present study design was added to leverage on the advantages of the large sample size. As our main results were statistically significant, we assumed that the sample size for this study was reasonable.

The study recruited third trimester pregnant women, who were primary cooks, non-smokers and carried singleton pregnancies. The process began in July 2018 and ended by May 2019. The main study was planned for three phases of data collection. At the beginning of the study, women were screened and recruited. In phase one, during the third trimester, we collected demographic, medical history, exposure data for the primary objective (fuel type) and exposure for secondary objective (malaria). The endpoint for this study was birth outcome; and this data was collected in the labor wards of various hospitals during phase two. The final part was the collection of baby data in phase three followed up.(13)

The original study encountered a methodological shortcoming during its implementation, as we initially assumed recruited pregnant women will return to the hospitals they attended ANC (i.e recruitment center) to deliver. However, few months into the study, we observed most of them did not return to deliver and given that we were not financially capable to follow them up, we replaced them with women who strictly agreed to return to the recruitment center to give birth. This increased our initial sample size from 1472 published in (15) to 1776, more details can be found in. (13)

Study Procedure

Baseline data was collected at recruitment using a structured questionnaire at the antenatal care (ANC) centers of each hospital. Gestational age was ascertained through an ultrasound, therefore our study relied on midwife validated gestational age. Pregnancy outcome data was collected at the labour wards of all hospitals by trained research assistants using a predesigned questionnaire. Except for those with a glucose-6-phosphate dehydrogenase (G6PD) deficiency, all pregnant women in the study received at least one sulfadoxine / pyrimethamine (IPTp-SP).

Laboratory Procedure

RDT malaria diagnosis

The SD BIOLINE Malaria Ag P.f Rapid Diagnostic Test Kits (RDTs) for malaria were used in all hospitals.(16) with specificity and sensitivity of 99.5% and 99.7%, respectively. The principal investigator (PI) and research assistants (RA) made efforts to observe and monitor adherence to standard testing protocols in each hospital to ensure that they complied with both the manufacturer's guide and the fundamental laboratory principles for the test. RDT was used to determine whether participants had parasitemia in peripheral blood. Still, we were unable to control for possible measurement bias among laboratory personnel for malaria.

Haemoglobin estimation – All hospitals used a blood analyzer to estimate the full blood count (FBC) and hemoglobin (Hb) was extracted from the FBC of pregnant women. Blood sample (5 milliliters) for Hb estimation was collected into an ethylenediamine tetraacetic acid (EDTA) tube and was mixed with an EDTA anticoagulant. In this study, anemia was defined as Hb < 11.0 g/dL.

Glucose-6-phosphate dehydrogenase (G6PD) Test - The methaemoglobin reduction test was used in all hospitals for the screening of pregnant women for G6PD. The test result was reported as No defect/normal, Partial defect or Full defect.

Data collection

Computer-assisted personal interviewing (CAPI) was used to gather all data, which was done using the Kobo Collect Android app. The data collection procedure is described in detail elsewhere.(13)

Outcome variable

The main outcomes of this study were low birth weight (LBW), preterm birth, and perinatal death. These were all gathered during delivery in the various hospitals' labour wards. On the seventh day, women were contacted by mobile phone to inquire about the baby's well-being to ensure that the infant was still alive, and to capture neonatal mortality after discharge from the hospital. Preterm birth was defined as <37 weeks gestational age, while LBW was defined as <2.5 kilograms as previously published. (13)

Objective

To appraise the association of birth outcomes among people with prenatal malaria. The exposure variable was a positive RDT test verifying that a pregnant woman had malaria during her third trimester or just before birth.

Statistical analysis

Data was exported from the kobo collect application database into an MS Excel sheet, cleaned and transferred into STATA 13 for analysis. Individual confounders were added into the simple logistic regression model, and a significant variable of > 0.05 or near significant were retained in the multiple logistic regressions to evaluate the potential associations between adverse birth outcomes and malaria . For each model, we set out adjust potential confounders such maternal age at birth, Neonatal admissions at birth, mode of delivery, marital status, parity, G6PD,

genotype, anemia, Socio Economic Status (SES) , drinking of alcohol, and maternal respiratory condition, and initially added to logistic regression with significance set 0.05%. Those with significance were retained in the multiple logistic regression model, and non-significant ones were dropped. Therefore genotype, anemia, respiratory condition and drinking of alcohol were all dropped during the initial univariate analysis, and thats why some were found in the final models while others were not. Maternal age was however non-significant, but was retained in the models, given its relevance as a confounder and its association with the adverse birth outcomes (17,18) . For the SES, assets from the 2014 Ghana Demographic Health Survey were used for the calculation of total assets,dividing the total into quantiles, we considered less than 50 quantiles poor, from 50 to 75 as moderately rich and at least 75 as rich.(13) Missing data of more than 10% from any observation were dropped in order not to open the study to bias, a single manual imputation was used to address some missing data based on previous patterns of questions.(19) Sensitivity analysis was assessed in both univariate and multiple log binonmial regression model compared to logistic regression model used in the main analysis.

Patient and Public Involvement

Patients were not involved in the design of this study.

Results

Figure 1 shows an elaborate detail of the plan for follow up during this study which was published in Hussein et al., 2020, at baseline 1626 third trimester pregnant women were recruited with 1323 women completing the study.The age of pregnant women ranged between 15-48 years, 59.1% were between 25-34 years. (Table 1).

For medical history, 14.8% had four or more children. The incidence of malaria in this cohort was 9.5% (95% confidence interval [CI]: 7.9-11.1). About 6.4% tested positive for sickle cell and, out of these, 50.0% of them who checked for their genotypes were SS. About 47.9% of the women were severely anaemic with haemoglobin levels of less than 11 g/dl within their third trimester of pregnancy, while 4.7% had G6PD full defect (Table 1).

The incidence of preterm birth among women with malaria was 52.0%. Moreover the prevalence of LBW was 10.4% among women with malaria and 5.1% among women without malaria. In both mothers with and without malaria, newborn death and live births were equally 1.6 percent. (Table 2).

Pregnant women with malaria had 2.02 times (CI: 95% (1.39-2.93) incresead odds of preterm birth compared to those without malaria after adjusting for parity, maternal age, G6PD deficiency and neonatal admission (Table 3). Furthermore, pregnant women with malaria had 2.06 times increased odds [CI 95% (1.09-3.93)] of LBW compared to those without malaria after adjusting for parity, maternal age, and socio-economic status (Table 4). Lastly, pregnant women with malaria had 1.02 times non significant odds [CI: 95% (0.26 – 4.01) of perinatal mortality compared to women without malaria after adjusting for cesarean section. Moreso, women who delivered through cesarean section had 5 times higher odds of perinatal mortality in comparison to those without cesarean section (Table 5).

Sensitivity analysis in both univariate and multiple log binomial regression model for model did not change the direction or strength of the estimates compared with the logistic regression model for preterm birth, even though odds ratio marginally exaggerated the relative risk to some magnitude. (Table 6)

Discussion

In this study, 59.1% of the women were between the ages of 25 and 34, suggesting a youthful distribution. The illiteracy rate was high, at around 75.4%, which may explain why 63.1 percent were traders, since trading in Ghana is traditionally associated with women with low educational status.(20,21) The proportion of malaria in pregnant women was nearly 10% in this study. The study also investigated the associations between malaria and preterm delivery, LBW, and perinatal death.

Although our study differed from other studies in design, biological samples examined, mode of diagnosis, period of malaria infestation during pregnancy, and different confounding factors, it still found results similar to some previous research.(22–24) This study found that prenatal malaria was significantly related with preterm birth and LBW after adjusting for parity, maternal age, G6PD, Socio Economic Sstatus, and neonatal admissions at birth; and non-significantly associated with perinatal mortality after adjusting for caesarian section. Indeed, Nkwabong et al, found that third trimester malaria had an increased risk of preterm birth by five times and LBW by 2.8 times which resonated with our study.(25) Vogel and colleagues reported similar findings where exposure to malaria increased odds of spontaneous preterm term birth by 1.67 times based on their secondary analysis of data from 22 low-and middle-income countries.(22) Also, Van den Broek and colleagues reported similar findings as maternal malaria significantly increased the risk of preterm birth by 1.99 times.(26) In Tanzania, similar studies showed that maternal malaria parasites in red blood cells were linked to a 3.2 higher risk of premature delivery.(23) In comparison to individuals who did not have placental site malaria, the chances of preterm delivery rose by between 4.7 and 5.6 percent. (27,28). Outside of Africa, a research in Brazil found that *Plasmodium falciparum species* (*P. falciparum)* were substantially linked with preterm delivery during pregnancy, despite the fact that they accounted for less than 40% of the

total.(24) Some research, however, found nonsignificant correlations that differed from this one. For example, a recently published comprehensive study of malaria at delivery in Uganda used three different methods of detecting parasites, including peripheral and placental blood microscopy, placental blood loop-mediated isothermal amplification (LAMP), and placental histopathology, and found no statistically significant link between malaria and preterm birth for any of the methods.(29)

Although we did not distinguish between malaria species in this study, previous research has indicated that malaria, regardless of the malaria species, might induce poor birth outcomes. In Malawi, placental blood *P. falciparum* raised the risk of LBW by approximately 1.7 times.(30) Nyirjesy et al. discovered a 3.7-fold higher incidence of LBW in pregnant women who had malaria in 1993.(31) Additionally, about 33% and 19% of women with placental malaria delivered LBW babies compared to those without placental site malaria in Nigeria and Uganda respectively. (28,32)

However, we did not observe significant increases in the odds of perinatal mortality in both adjusted and unadjusted models, despite the fact that women who delivered by caserean sections (CS) were nearly five times more likely to suffer perinatal mortality. In contrast, other studies, such as the one conducted in Zaire in 1993, reported that maternal malaria with chloroquine prophylaxis increased the risk of perinatal death by 12 times after adjusting for parity and prenatal clinic visits.(31) Also, *P falciparum* malaria during pregnancy increased the hazard of neonatal mortality by 2.6 times.(33) The odds of mortality significantly increased by 5 times among infants born to mothers with acute placental infections.(34)

Multiple variables, such as parity, mother age and SES can lead to negative birth outcomes. Therefore, in this study, we were able to adjust for those significant confounders in the multiple logistic regression and keep those that were significant. Women who had several pregnancies were protected against preterm delivery. This is apparently due to the extra protection provided by antibodies in subsequent pregnancies against the parasite variant surface antigen VAR2CSA. (35,36)

Moreover, the effect of malaria exposure on fetal growth was observed during third trimester of pregnancy regardless of the period of exposure and has been blamed for poor birth

outcomes.(6,25,37) this may be because the pathway that connects mother to the child during pregnancy may influence the survival of the fetus at birth or even beyond, since the placenta supplies nutrients to the baby through the umbilical cord. Thus, Ouédraogo and colleagues found a significant association between umbilical cord parasitemia level and maternal peripheral blood parasitemia.(38) Also, malaria in pregnancy may have induced excessive stimulation and dysregulated hemoglobin-scavenging system; and bioavailabliblity of nitric oxide and L-arginine which may be associated poor vascular development and adverse birth outcomes.(39) Although we used RDT with peripheral blood, our findings were consistent with the majority of studies using placental site malaria.(35,36) This could be because peripheral blood infections could promote parasite sequestration in the placenta and activate antibody-antigen immune responses, which can cause complications during delivery.(35,36) Furthermore, Kapisi et al. (2017) found that women who had a high burden of malaria had a 14-fold increased risk of placental malaria by blood microscopy and a four-fold increased risk of loop-mediated isothermal amplification (LAMP). This could indicate that our group had a higher malaria burden, correlating with previous research using a different diagnosis technique of diagnosis.(27)

This study benefitted from a large sample size. Our search to date did not show any study examining the link between maternal malaria at delivery and birth outcomes in Tamale and its surroundings in the Northern Region of Ghana. In this cohort, we used RDT to diagnose malaria, although its sensitivity was 19% lower than microscopic examination of peripheral blood and placental blood for malaria and as such a limitation; however, RDT outperformed microscopy in identifying malaria in other pregnant settings.(40,41) Moreover, RDT is useful in settings like ours because it produces results quickly, requires less training and equipment, and experiences little to no power interruptions. As a consequence, it is essential to detect malaria, particularly in health institutions with limited human resources and equipment. Nonetheless, one possible limitation of our study was that we depended on the routine laboratories in each hospital to gather data on malaria. While we made efforts to observe and monitor adherence to standard testing protocols, we are unable to control for possible measurement bias among laboratory personnel for the exposure variable (malaria). However, it is worth mentioning that using the RDT method to diagnose malaria in pregnancy is a routine procedure in our context and performed by licensed laboratory practitioners, who also follow the standard operating procedure for diagnosis. We were also unable to account for the effects of malaria at the early stages of

pregnancy on birth outcomes, since we conveniently included women in their third trimester who had done at least one RDT. Moreover, our inability to account for number of doses of SP uptake by pregnant women denied us the opportunity to measure its confounding effect between malaria and birth outcome. Despite this, our study is comparable with similar studies on this subject matter.

In conclusion, maternal malaria within the third trimester of pregnancy may be a major contributor to LBW and preterm birth in the northern region of Ghana.

Data availability statement

Data are available upon reasonable request.

Ethical Approval

The approval for this study was given by the ethical review committees of Ghana Health Services, and the Tehran University of Medical Sciences. Ethical Numbers IR.TUMS.SPH.REC.1396.4066 and GHS-ERC, respectively, 010/12/17. Information sheets were provided to participants with concerns of risk, adequately discussed, and signed or thumb printed informed consent was obtained from each participant prior to recruitment.

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Contributors HH: Investigation, writing the draft and data analysis. MS: Writing review & editing. MY: Conceptualization, validation. M.S.H: Visualization, Resources. MAS, Project administration. PDA:data curation, AF: Supervision, Acquisition of funds. All authors reviewed and approved the final manuscript:

Competing interests None Declared

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Table 1: Baseline characteristics of pregnant women

Variables	Delivery period
	Frequency (%) n= 1323
Maternal age (mean ±SD)	27.3±5.2
15-24	394 (29.8)
25-34	782 (59.1)
35-48	147 (11.1)
Marital status	
Married	1310 (99.0)
Unmarried	13 (1.0)
Ethnicity	
Mole Dagbani	1274 (96.3)
Others	49 (3.7)
Maternal education	
Primary/no education	997 (75.4)
JHS/Middle school	147 (11.1)
SHS/Technical/Vocational	89 (6.7)
At least Diploma	89 (6.8)
Maternal Occupation	
No employment	302 (22.9)
Trader	834 (63.1)
Laborer	74 (5.6)
Factory/Industry	24 (1.8)
Formal employment	87 (6.6)
Socio economic status	
Poor	473 (35.8)
Moderately rich	439 (33.2)
Rich	411 (31.1)
Residence	
Urban	762 (57.6)
Rural	561 (42.4)
Housing	
Separate house	68 (5.14)
Semi-detached	43 (3.25)
Compound house (sandcrete)	850 (64.25)
Compound house (mud)	362 (27.36)
Alcohol	

Yes	4 (0.3)
No	1319 (99.7)
Medical History	
Parity	
First pregnancy	633 (47.9)
2-3 pregnancies	494 (37.3)
4 or more pregnancies	196 (14.8)
Malaria	
Positive	125 (9.5)
Negative	1198 (90.5)
Sickle cell	
Positive	77 (6.4)
Negative	1120 (93.6)
Genotype	
Hb AS	24 (36.4)
Hb SC	9 (13.6)
Hb SS	33 (50.0)
Anaemia	
<11 g/dl	582 (47.9)
≥11 g/dl	633 (52.1)
Sulfadoxine pyrimethamine (SP) Usage	
Yes	1137 (88.9)
No	154 (11.9)
Glucose-6-phosphate Dehydrogenase (G6PD)	
No defect	1029 (86.9)
Partial defect	98 (8.3)
Full defect	56 (4.7)
Respiratory Condition	
Yes	10 (0.8)
No	1,313 (99.2)

**** Hb = Hemoglobin**

Table 2: Incidence of pregnancy outcome and malaria

Pregnancy Outcomes n=1323	**Negative**	**Positive**	**Total**
	Freq (%)	Freq (%)	
Preterm birth			
Term	780 (65.1)	60 (48.0)	840
Preterm	418 (34.9)	65 (52)	483
Birth weight			
Normal birth weight	1137 (94.9)	112 (89.6)	1249
Low birth weight	61 (5.1)	13 (10.4)	74

Type of delivery			
Live birth	1176 (98.4)	123 (98.4)	1299
Neonatal mortality	19 (1.6)	2 (1.6)	21

Table 3: Log bionomial regression of preterm and malaria

Preterm	Crude OR (CI)	Pvalue	Adjusted (CI) OR"**"	Pvalue
Malaria				
No	1		1	
Yes	2.02 (1.39-2.93)	<0.001	2.02 (1.36- 2.99)	<0.001
Parity				
First pregnancy	1		1	
2-3 pregnancy	0.83 (0.65-1.07)	0.147	0.79 (0.61-1.03)	0.087
4 or more	0.59 (0.42-0.85)	0.004	0.62 (0.42-0.93)	0.021
Maternal Age				
15-24 years	1			
25-34 years	1.23 (0.96-2.85)	0.102	1.35 (1.02-1.77)	0.034
35-48 years	0.75 (0.49-1.13)	0.170	0.92 (0.57-1.48)	0.720
G6PD				
Normal	1		1	
Partial defect	1.55 (1.02-2.35)	0.039	1.54 (1.01- 2.37)	0.047
Full defect	1.07 (0.61-1.87)	0.803	0.98 (0.54-1.76)	0.934
Neonatal admission at birth				
No	1		1	
Yes	2.10 (1.17-3.79)	0.004	1.98(1.09. -3.60)	0.025

** Significant confounders adjusted in the multiple log binomial model

**parity, maternal age, G6PD, neonatal admissions at birth

Table 4: Logistic regression of LBW and malaria

LBW	Crude OR (CI)	Pvalue	Adjusted OR(CI)"**"	Pvalue
Malaria				
No	1		1	
Yes	2.04 (1.16-3.61)	0.014	2.06 (1.09-3.93)	0.027
Parity				
First pregnancy	1		1	
2-3 pregnancy	1.21 (0.71-2.06)	0.480	1.32 (0.76-2.28)	0.322
4 or more	1.72 (0.91- 3.23)	0.093	2.13 (1.03-4.39)	0.041
Maternal Age				
15-24 years	1			
25-34 years	0.79(0.47-1.32)	0.373	0.70 (0.41-1.22)	0.210
35-48 years	0.72 (0.30-1.71)	0.457	0.45 (0.17-1.19)	0.109
Socio-economic status				
Poor	1		1	
Moderately rich	0.57 (0.33-0.99)	0.050	0.60 (0.34-1.07)	0.082
Rich	0.52 (0.28-0.93)	0.028	0.54(0.29-0.98)	0.044

** Significant confounders adjusted in the multiple log binomial model

**parity, socioeconomic status

Table 5: Logistic regression of perinatal mortality and malaria

Perinatal mortality	Crude OR (CI)	P value	Adjusted OR(CI)"**"	Adjusted Models P value
Malaria				

No	1		1	
Yes	1.01 (0.23-4.37)	0.993	1.02 (0.26-4.01)	0.983
Mode of delivery				
Normal	1		1	
Ceasarian section	5.18 (1.88-14.26)	0.001	5.17(1.87-14.36)	<0.001

**Significant confounders adjusted in the multiple log binomial model

**Mode of delivery

Table 6: Sensitivity analysis using log bionomial regression for preterm and malaria

Preterm	Crude RR (CI)	Pvalue	Adjusted (CI) RR"**"	Pvalue
Malaria				
No	1		1	
Yes	1.49 (1.24-1.23)	<0.001	1.45 (1.20- 1.78)	<0.001
Parity				
First pregnancy	1		1	
2-3 pregnancy	0.89 (0.78-1.04)	0.150	0.87 (0.74-1.02)	0.087
4 or more	0.72 (0.57-0.91)	0.006	0.74 (0.57-0.96)	0.025
Maternal Age				
15-24 years	1			
25-34 years	1.14 (0.98-1.35)	0.106	1.19 (1.01-1.42)	0.039
35-48 years	0.81 (0.61-1.09)	0.181	0.93 (0.67-1.29)	0.670
G6PD				
Normal	1		1	
Partial defect	1.29 (1.03-1.60)	0.025	1.27 (1.02- 1.59)	0.036
Full defect	1.04 (0.74-1.48)	0.803	0.98 (0.67-1.44)	0.935
Neonatal admission at birth				
No	1		1	
Yes	1.47 (1.13-1.91)	0.004	1.39 (1.07. 1.83)	0.015

** Significant confounders adjusted in the multiple log binomial model

**parity, maternal age, Glucose-6-phosphate Dehydrogenase (G6PD), Neonatal admissions at birth

Figure 1 : Follow-up Plan

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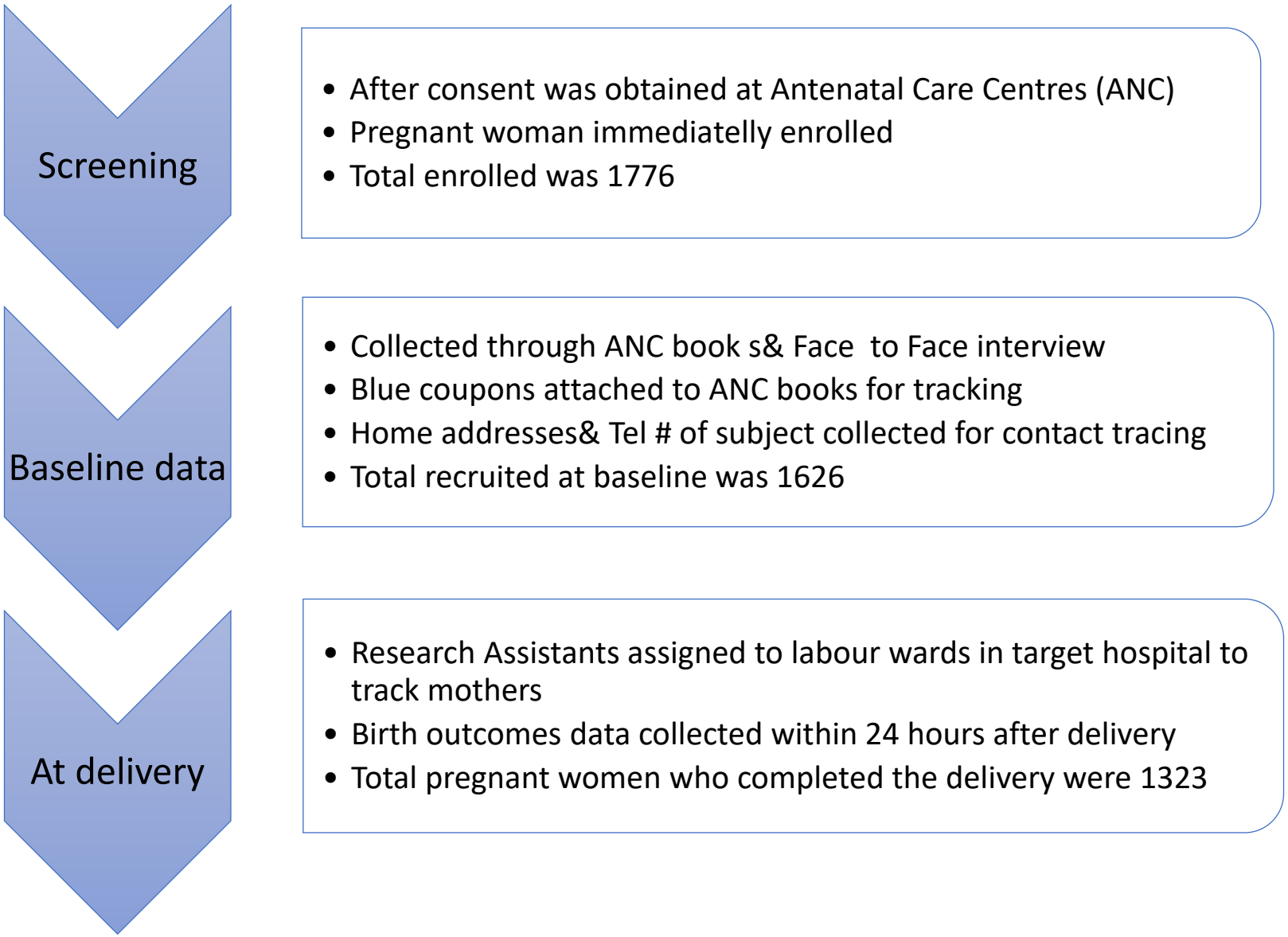

Figure 1 : Plan for follow up

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10 10 10
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Prenatal malaria exposure and risk of adverse birth outcomes: a prospective cohort study of pregnant women in the Northern Region of Ghana.

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Abstract

Objective Malaria remains endemic in most of Sub-Saharan Africa, wreaking havoc on pregnant women and resulting in morbidity and poor birth outcomes. The purpose of this study was to assess the relationship between malaria and adverse birth outcomes in prenatal women in the Northern Region of Ghana.

Design

A prospective cohort study of singleton pregnancies at 28 weeks of gestational age and above were recruited between July 2018 and May 2019 from four public hospitals in the northern region of Ghana.

Primary outcome measures: low birth weight (LBW), preterm birth, and perinatal death.

Results

A total of 1323 pregnant women completed the study out of 1626 recruited. Their average age was 27.3 ± 5.2 years. The incidence of malaria in this population was 9.5% (95% confidence interval [CI]: 7.9-11.1). After adjusting for newborn admissions to the neonatal intensive care Unit (NICU), parity, maternal age, and glucose-6-phosphate dehydrogenase (G6PD); women who were exposed to malaria during the third trimester of pregnancy had 2.02 times (95%CI 1.36 – 2.99) higher odds of premature delivery. Furthermore, they had 2.06 times [95% CI 1.09 - 3.93] higher chance of giving birth to babies with low birth weight (LBW), regardless of their socioeconomic status.

Conclusion
This study confirms a prenatal malaria which critically increases odds of both preterm and LBW delivery. A decisive policy by the Ministry of Health (MoH) to eradicate or minimize perinatal malaria is needed to contribute to the prevention of LBW and adverse pregnancy outcomes.

Keywords: Malaria, RDT, prenatal, pregnancy, preterm, LBW, northern Region, Ghana.

Strengths and limitations of this study

Until now, this study is the first prospective cohort study with a relatively large sample size to examine the link between maternal malaria in the third trimester and birth outcomes in Tamale, Ghana.

It provides detailed information on malaria and adverse birth outcomes which is not readily available due to the challenges of under reporting, analysis, and application of surveillance data as a result of poor record linkage.

This study was designed as an independent evaluation of birth outcomes among people with prenatal malaria in light of these difficulties

Rapid Diagnostic Test (RDT) was used to diagnose malaria in pregnancy as a routine procedure in state-licensed laboratory practitioners, which may be a limitation.

We were unable to account for the effects of malaria at in the early stages of pregnancy on birth outcomes.

Introduction

Malaria claimed over 600,000 lives in 2020, with Sub-Saharan Africa accounting for 95% of deaths.(1) Malaria exposure can be fatal to persons with inadequate immunity, particularly pregnant women and small children. It also has an economic cost to the family and the government, with a direct cost of around $ 12 billion every year.(2)

In malaria-endemic areas in sub-Saharan Africa, women face significant risks throughout their pregnancy. Examples of these risks pregnant women are exposed to include low birth weight (LBW), premature birth, and spontaneous abortions.(3) Prenatal malaria is responsible for 5-12% of LBW and accounts for between 75,000 to 200,000 infant deaths each year (4). In sub-Saharan Africa, 11 million women were infected with malaria in 2018, resulting in approximately 872,000 newborns born with LBW.(5) In 2018, the Central and Western Africa subregions reported the highest prevalence of malaria in pregnant women, each with 35% prevalence. Furthermore, West Africa had the highest frequency of LBW due to malaria.(5) In particular, the effect of malaria exposure on fetal growth was observed during the third trimester of pregnancy regardless of period the exposure.(6)

Malaria cases increased by half a million in Ghana in 2018 compared to the year before.(5) Regarding treatment, a research conducted in a War Memorial Hospital in the Upper East Region found that children born to mothers on artemether–lumefantrine, intermittent screening and treatment of malaria in pregnancy (ISTp-AL) had a lower risk of malaria than those delivered to mothers on sulfadoxine/pyrimethamine, intermittent preventive treatment of malaria in pregnancy (IPTp-SP).(7) Yet, malaria prevalence and poor birth outcomes were 9.0 percent and 22.2 percent, respectively, in Kumasi.(8) In Navrongo, uptake of Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPT3) i.e uptake of three doses was 76 percent, while uptake of 5 doses (IPT5) was 16 percent, with women who received at least three doses having better health outcomes.(8) Given that Ghana is in an endemic malaria zone, these studies highlight implementation gaps as well as provide information that are useful to improve our malaria prevention policies and programs. Unfortunately, there is a dearth in the Ghanaian literature relating to the role of malaria in poor birth outcomes in pregnant women in urban settlements in Northern Ghana.

Furthermore, due to insufficient linkages between malaria control and prenatal care data, progress in attaining malaria control among pregnant women has been slow.(9) In addition, inconsistencies in data management practices were discovered during a data quality evaluation in several health institutions, posing problems in data reporting, analysis, and application.(10) Therefore, the precision of aggregate data collected from these facilities through surveillance is compromised by these discrepancies. We designed this prospective cohort research as an independent evaluation of birth outcomes among people with prenatal malaria in light of these difficulties. This study sought to provide considerably more detailed information on the links between prenatal malaria and poor birth outcomes in pregnant women in northern Ghana.

Methods and Analysis

Setting

Data for this substudy was drawn from a prospective cohort study that took place in four hospitals in Ghana's Northern region. Three of the hospitals are located in Tamale, the Northern Regional Capital, the fourth largest city in Ghana. The fourth hospital is located in the Savelegu Municipality bordered by Tamale to the west. These areas are located within the Guinea Savannah belt,(11) with little seasonal variations in prevalence as such, Oheneba-Dornyo and collegues found the prevalence of malaria to be positively correlated with rainfall with nearly a borderline significance. (12) The P. falciparum peripheral parasitemia prevalence in pregnant women in Northern Savanna Zone ranged between 26% and 13.4% from 2013 to 2019, respectively.(13)

Design

The study was designed from a parent cohort study that sought to assess how different cooking fuel types influenced pregnancy outcomes and infant respiratory problems, these were the primary research questions,(14,15) , the present study answers a secondary research question about prenatal malaria exposure and the risk of adverse birth outcomes. Therefore the sample size calculation was based on proportion of developing the outcome (respiratory symptoms) with cooking fuel type as an exposure. However, the present study design was added to leverage on the advantages of the large sample size. As our main results were statistically significant, we assumed that the sample size for this study was reasonable.

The study recruited third trimester pregnant women, who were primarily cooks, non-smokers and carried singleton pregnancies. The process began in July 2018 and ended in May 2019. The main study was planned for three phases of data collection. At the beginning of the study, women were screened and recruited. In phase one, during the third trimester, we collected demographics, medical history, exposure data for the primary objective (fuel type), and exposure for secondary objective (malaria). The endpoint for this study was birth outcome; and this data was collected in the labor wards of various hospitals during phase two. The final part was the collection of baby data in phase three followed up.(15)

The original study encountered a methodological shortcoming during its implementation, as we initially assumed recruited pregnant women will return to the hospitals they attended ANC (i.e a recruitment center) to deliver. However, few months into the study, we observed most of them did not return to deliver and given that we were not financially capable to follow them up, we replaced them with women who strictly agreed to return to the recruitment center to give birth. This increased our initial sample size from 1472 to 1776 as published in, (16) consequently, we followed up 1323 pregnant women in this study, more details can be found in. (15)

Study Procedure

Baseline data was collected at recruitment using a structured questionnaire at the antenatal care (ANC) centers of each hospital. Gestational age was ascertained through an ultrasound, therefore our study relied on a midwife validated gestational age. Pregnancy outcome data was collected at the labour wards of all hospitals by trained research assistants using a predesigned questionnaire. Only 88% received at least one sulfadoxine / pyrimethamine (IPTp-SP).

Laboratory Procedure

RDT malaria diagnosis

The SD BIOLINE Malaria Ag P.f Rapid Diagnostic Test Kits (RDTs) for malaria were used in all hospitals.(17) with specificity and sensitivity of 99.5% and 99.7%, respectively. The principal investigator (PI) and research assistants (RA) made efforts to observe and monitor adherence to standard testing protocols in each hospital to ensure that they complied with both the manufacturer's guide and the fundamental laboratory principles for the test. RDT was used to determine whether participants had parasitemia in peripheral blood. Still, we were unable to control for possible measurement bias among laboratory personnel for malaria.

Haemoglobin estimation – All hospitals used a blood analyzer to estimate the full blood count (FBC) and hemoglobin (Hb) was extracted from the FBC of pregnant women. Blood sample (5 milliliters) for Hb estimation was collected into an ethylenediamine tetraacetic acid (EDTA) tube and was mixed with an EDTA anticoagulant. In this study, anemia was defined as Hb < 11.0 g/dL.

Glucose-6-phosphate dehydrogenase (G6PD) Test - The methaemoglobin reduction test was used in all hospitals for the screening of pregnant women for G6PD. The test result was reported as No defect/normal, partial defect or Full defect.

Data collection

Computer-assisted personal interviewing (CAPI) was used to gather all data, which was done using the Kobo Collect Android app. The data collection procedure is described in detail elsewhere.(15)

Outcome variable

The main outcomes of this study were low birth weight (LBW), preterm birth, and perinatal death. These were all gathered during delivery in the various hospitals' labour wards. On the seventh day, women were contacted by mobile phone to inquire about the baby's well-being, to ensure that the infant was still alive, and to capture neonatal mortality after discharge from the hospital. Preterm birth was defined as <37 weeks gestational age, while LBW was defined as <2.5 kilograms as previously published. (15)

Objective

To appraise the association of birth outcomes among people with prenatal malaria. The exposure variable was a positive RDT test verifying that a pregnant woman had malaria during her third trimester or just before birth.

Statistical analysis

Data was exported from the kobo collect application database into an MS Excel sheet, cleaned and transferred into STATA 13 for analysis. Individual confounders were added into the simple logistic regression model, and a significant variable of > 0.05 or near significant were retained in the multiple logistic regressions to evaluate the potential associations between adverse birth outcomes and malaria . For each model, we set out to adjust potential confounders such maternal age at birth, Neonatal admissions at birth, mode of delivery, marital status, parity, G6PD, genotype, anemia, Socio Economic Status (SES) , drinking of alcohol, and maternal respiratory condition, and initially added to logistic regression with significance set 0.05%. Those with significance were retained in the multiple logistic regression model, and nonsignificant ones were dropped. Therefore, genotype, anemia, respiratory condition, and drinking of alcohol were

all dropped during the initial univariate analysis, and thats why some were found in the final models while others were not. Maternal age was however non-significant, but was retained in the models, given its relevance as a confounder and its association with the adverse birth outcomes.(18,19) For the SES, assets from the 2014 Ghana Demographic Health Survey were used for the calculation of total assets,dividing the total into quantiles, we considered less than 50 quantiles poor, from 50 to 75 as moderately rich and at least 75 as rich.(15) Missing data of more than 10% from any observation were dropped in order not to open the study to bias, a single manual imputation was used to address some missing data based on previous patterns of questions.(20) Sensitivity analysis was assessed in both univariate and multiple log binonmial regression model compared to logistic regression model used in the main analysis.

Patient and Public Involvement

Patients were not involved in the design of this study.

Results

Figure 1 shows an elaborate detail of the plan for follow up during this study which was published in Hussein et al., 2020, at baseline, 1626 third trimester pregnant women were recruited with 1323 women completing the study.The age of pregnant women ranged between 15-48 years, 59.1% were between 25-34 years. (Table 1).

For medical history, 14.8% had four or more children. The incidence of malaria in this cohort was 9.5% (95% confidence interval [CI]: 7.9-11.1). About 6.4% tested positive for sickle cells and, out of these, 50.0% of them who checked for their genotypes were SS. About 47.9% of the women were anaemic with haemoglobin levels of less than 11 g/dl within their third trimester of pregnancy, while 4.7% had G6PD full defect (Table 1).

The incidence of preterm birth among women with malaria was 52.0%. Moreover, the prevalence of LBW was 10.4% among women with malaria and 5.1% among women without malaria. In both mothers with and without malaria, newborn death and live births were equally 1.6 percent. (Table 2).

Pregnant women with malaria had 2.02 times (CI: 95% (1.39-2.93) incresead odds of preterm birth compared to those without malaria after adjusting for parity, maternal age, G6PD deficiency and neonatal admission (Table 3). Furthermore, pregnant women with malaria had 2.06 times increased odds [CI 95% (1.09-3.93)] of LBW compared to those without malaria after adjusting for parity, maternal age, and socio-economic status (Table 4). Lastly, with the odds of 1.02 [CI: 95% (0.26 – 4.01), there was no difference between pregnant women with malaria and those without malaria for perinatal mortality after adjusting for cesarean section. Moreso, women who underwent cesarean section had a 5 times greater risk of perinatal death than those who did not have cesarean section (Table 5).

Sensitivity analysis in both the univariate and multiple log binomial regression model for model did not change the direction or strength of the estimates compared with the logistic regression

model for preterm birth, even though odds ratios marginally exaggerated the relative risk to some magnitude. (Table 6)

Discussion

In this study, malaria was found in nearly 10% of pregnant women. The study also investigated the associations between malaria and preterm birth, low birth weight, and perinatal mortality.

Prenatal malaria was found to be substantially linked with preterm birth and LBW after correcting for parity, mother age, G6PD, socioeconomic status, and neonatal hospitalization at birth, but not with perinatal death after adjusting for caesarian section. Indeed, Nkwabong et al. previously reported that third trimester malaria increased the chance of preterm delivery by five times and LBW by 2.8 times, which was consistent with our findings.(21) Similarly, Vogel and colleagues found that exposure to malaria increased the risk of spontaneous preterm term birth by 1.67 times with a secondary analysis of data from 22 low- and middle-income countries.(22) Van den Broek and colleagues similarly conclude from their data that, maternal malaria significantly increased the risk of preterm birth by 1.99 times.(23) In Tanzania, similar studies have shown that malaria parasites in the mothers's red blood cells are associated with 3.2 times the risk of premature birth.(24) Compared to people without placental malaria, pre-term birth rates increased by 4.7% to 5.6%, among pregnant women with placental malaria. (25,26)

In Brazil, researchers reported that *Plasmodium falciparum species* (*P. falciparum*) were significantly associated with preterm births, albeit accounting for less than 40% of the total.(27) In contrast, some authors suggest that such correlations zre nonsignificant. For example, a recent comprehensive study of malaria at birth in Uganda that studied three different parasite detection methods, including peripheral and placental blood microscopy, placental blood loop-mediated isothermal amplification (LAMP), and placental histopathology, and found no statistically significant link between malaria and preterm birth for any of the methods. (28) In this study, it was not possible to distinguish between the various species of malaria. However, previous studies have shown that regardless of the species, malaria can lead to poor birth outcomes. For example, *P. falciparum* in placental blood increased the risk of LBW by around 1.7 times in Malawi (29) and about 3.7-fold increase in Zaire. (30) Furthermore, in Nigeria and

Uganda, approximately 33% and 19% of mothers with placental malaria, respectively, delivered LBW babies compared to those without placental malaria. (26,31)

One interesting aspect that emerged from the analysis is the finding that women who delivered via caserean section (CS) were about five times more likely to suffer perinatal mortality. Notwithstanding, we found that there was no significant increase in the odds of pregnancy mortality in both adjusted and unadjusted models. In contrast, other studies, such as the one conducted in Zaire in 1993, reported that maternal malaria with chloroquine prophylaxis increased the risk of perinatal death by 12 times after adjusting for parity and prenatal clinic visits.(30) In addition, *P falciparum* malaria during pregnancy increased the risk of neonatal deaths by 2.6 times.(32) Infants delivered to moms with acute placental infections had a fivefold risk of death. (33)

Multiple variables such as gender, mother's age, and SES can result in adverse birth outcomes. Therefore, in this study, we adjusted for these significant confounders in the multiple logistic regressions and maintain the significant confounders. Women with multiple pregnancies are protected from premature birth. This is apparently due to the extra protection provided by antibodies in subsequent pregnancies against the parasite variant surface antigen VAR2CSA. (34,35)

Furthermore, regardless of the period of exposure, the effect of malaria exposure on fetal development was detected during the third trimester of pregnancy and has been blamed for poor birth outcomes.(6,25,36) This might be because the pathway that connects the mother to the kid throughout pregnancy may impact the fetus's survival at delivery or even beyond, since the placenta delivers nutrition to the newborn via the umbilical cord. For example, Ouédraogo and his colleagues found a connection between umbilical cord parasitemia and maternal peripheral blood parasitemia.(37) Also, malaria in pregnancy may have induced excessive stimulation and dysregulated hemoglobin-scavenging system; and bioavailabliblity of nitric oxide and L-arginine which may be associated poor vascular development and adverse birth outcomes.(38) Although we used RDT with peripheral blood, our findings were consistent with the majority of studies on placental malaria.(34,35) This is because peripheral blood infections may promote the sequestration of parasites in the placenta and activate immune reactions of antibodies and antigens that may cause complications during delivery..(34,35) Furthermore, Kapisi et al. (2017)

found that women who had a high burden of malaria had a 14-fold increased risk of placental malaria by blood microscopy and a four-fold increased risk of loop-mediated isothermal amplification (LAMP). This could indicate that our group had a higher malaria burden, correlating with previous research using a different diagnosis technique of diagnosis.(25)

This study benefited from the large size of the sample.. To date, we have not found any studies that examined the relationship between maternal malaria during delivery and the results of birth in Tamale in the northern region of Ghana.. In this cohort, RDT was used to diagnose malaria, although the sensitivity to malaria was 19% lower than a microscopic examination of peripheral and placenta blood. However, RDT has been reported to have outperformed microscopy in identifying malaria in other settings.(36,39) Furthermore, RDT is useful in environments like ours, because it produces quick results, requires fewer training and equipment, and has virtually no power interruption.. Consequently, especially in hospitals with limited human resources and equipment, rapid malaria detection techniques are imperative for initiating care.

A potential drawback of our study was that we relied on standard laboratories in each hospital to collect malaria data. While we took every attempt to observe and monitor compliance with established testing techniques, we were unable to account for any measurement bias among laboratory staff for the exposure variable (malaria). However, it should be noted that the RDT method for malaria diagnosis during pregnancy is used routinely in our context and is performed by state-licensed laboratory professionals for diagnosis ng malaria in our popualtion. We cannot also explain the impact of malaria during the early stages of pregnancy on birth outcomes, since we only included pregnant women their third trimester as long as they had done at least one RDT prior to recruitment. This study also failed to account for the number of doses of SP uptake by pregnant women .This denied us the opportunity to measure its confounding effect on malaria and birth outcomes. Nevertheless, our research is comparable to similar studies on the subject matter.

Taken together, these findings indicate that maternal malaria within the third trimester of pregnancy may be a major contributor to LBW and preterm birth in the northern region of Ghana.

Data availability statement

Data are available upon reasonable request.

Ethical Approval

The approval for this study was given by the ethical review committees of Ghana Health Serviceand the Tehran University of Medical Sciences. Ethical Numbers IR.TUMS.SPH.REC.1396.4066 and GHS-ERC, respectively, 010/12/17. Information sheets were provided to participants with concerns of risk, adequately discussed, and signed or thumb printed informed consent was obtained from each participant prior to recruitment.

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Contributors HH: Investigation, writing the draft and data analysis. MS: Writing review & editing. MY: Conceptualization, validation. M.S.H: Visualization, Resources. MAS, Project administration. PDA:data curation, AF: Supervision, Acquisition of funds. All authors reviewed and approved the final manuscript:

Competing interests None Declared

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Table 1: Baseline characteristics of pregnant women

Variables	Delivery period
	Frequency (%) n= 1323
Maternal age (mean ±SD)	27.3±5.2
15-24	394 (29.8)
25-34	782 (59.1)
35-48	147 (11.1)
Marital status	
Married	1310 (99.0)
Unmarried	13 (1.0)
Ethnicity	
Mole Dagbani	1274 (96.3)
Others	49 (3.7)
Maternal education	
Primary/no education	997 (75.4)
JHS/Middle school	147 (11.1)
SHS/Technical/Vocational	89 (6.7)
At least Diploma	89 (6.8)
Maternal Occupation	
No employment	302 (22.9)
Trader	834 (63.1)
Laborer	74 (5.6)
Factory/Industry	24 (1.8)
Formal employment	87 (6.6)
Socio economic status	
Poor	473 (35.8)
Moderately rich	439 (33.2)
Rich	411 (31.1)
Residence	
Urban	762 (57.6)
Rural	561 (42.4)
Housing	
Separate house	68 (5.14)
Semi-detached	43 (3.25)
Compound house (sandcrete)	850 (64.25)
Compound house (mud)	362 (27.36)
Alcohol	
Yes	4 (0.3)
No	1319 (99.7)

Medical History	
Parity	
First pregnancy	633 (47.9)
2-3 pregnancies	494 (37.3)
4 or more pregnancies	196 (14.8)
Malaria	
Positive	125 (9.5)
Negative	1198 (90.5)
Sickle cell	
Positive	77 (6.4)
Negative	1120 (93.6)
Genotype	
Hb AS	24 (36.4)
Hb SC	9 (13.6)
Hb SS	33 (50.0)
Anaemia	
<11 g/dl	582 (47.9)
≥11 g/dl	633 (52.1)
Sulfadoxine pyrimethamine (SP) Usage	
Yes	1137 (88.9)
No	154 (11.9)
Glucose-6-phosphate Dehydrogenase (G6PD)	
No defect	1029 (86.9)
Partial defect	98 (8.3)
Full defect	56 (4.7)
Respiratory Condition	
Yes	10 (0.8)
No	1,313 (99.2)

**** Hb = Hemoglobin**

Table 2: Incidence of pregnancy outcome and malaria

Pregnancy Outcomes n=1323	**Negative**	**Positive**	**Total**
	Freq (%)	Freq (%)	
Preterm birth			
Term	780 (65.1)	60 (48.0)	840
Preterm	418 (34.9)	65 (52)	483
Birth weight			
Normal birth weight	1137 (94.9)	112 (89.6)	1249
Low birth weight	61 (5.1)	13 (10.4)	74
Type of delivery			
Live birth	1176 (98.4)	123 (98.4)	1299

Neonatal mortality	19 (1.6)	2 (1.6)	21

Table 3: Logistic regression of preterm and malaria

Preterm	Crude OR (CI)	Pvalue	Adjusted (CI) OR"**"	Pvalue
Malaria				
No	1		1	
Yes	2.02 (1.39-2.93)	<0.001	2.02 (1.36- 2.99)	<0.001
Parity				
First pregnancy	1		1	
2-3 pregnancy	0.83 (0.65-1.07)	0.147	0.79 (0.61-1.03)	0.087
4 or more	0.59 (0.42-0.85)	0.004	0.62 (0.42-0.93)	0.021
Maternal Age				
15-24 years	1			
25-34 years	1.23 (0.96-2.85)	0.102	1.35 (1.02-1.77)	0.034
35-48 years	0.75 (0.49-1.13)	0.170	0.92 (0.57-1.48)	0.720
G6PD				
Normal	1		1	
Partial defect	1.55 (1.02-2.35)	0.039	1.54 (1.01- 2.37)	0.047
Full defect	1.07 (0.61-1.87)	0.803	0.98 (0.54-1.76)	0.934
Neonatal admission at birth				
No	1		1	
Yes	2.10 (1.17-3.79)	0.004	1.98(1.09. -3.60)	0.025

** Significant confounders adjusted in the multiple log binomial model

**parity, maternal age, G6PD, neonatal admissions at birth

Table 4: Logistic regression of LBW and malaria

LBW	Crude OR (CI)	Pvalue	Adjusted OR(CI)"**"	Pvalue
Malaria				
No	1		1	
Yes	2.04 (1.16-3.61)	0.014	2.06 (1.09-3.93)	0.027
Parity				
First pregnancy	1		1	
2-3 pregnancy	1.21 (0.71-2.06)	0.480	1.32 (0.76-2.28)	0.322
4 or more	1.72 (0.91- 3.23)	0.093	2.13 (1.03-4.39)	0.041
Maternal Age				
15-24 years	1			
25-34 years	0.79(0.47-1.32)	0.373	0.70 (0.41-1.22)	0.210
35-48 years	0.72 (0.30-1.71)	0.457	0.45 (0.17-1.19)	0.109
Socio-economic status				
Poor	1		1	
Moderately rich	0.57 (0.33-0.99)	0.050	0.60 (0.34-1.07)	0.082
Rich	0.52 (0.28-0.93)	0.028	0.54(0.29-0.98)	0.044

** Significant confounders adjusted in the multiple log binomial model
**parity, socioeconomic status

Table 5: Logistic regression of perinatal mortality and malaria

Perinatal mortality	Crude OR (CI)	P value	Adjusted OR(CI)"**"	Adjusted Models P value
Malaria				
No	1		1	

Yes	1.01 (0.23-4.37)	0.993	1.02 (0.26-4.01)	0.983
Mode of delivery				
Normal	1		1	
Ceasarian section	5.18 (1.88-14.26)	0.001	5.17(1.87-14.36)	<0.001

**Significant confounders adjusted in the multiple log binomial model

**Mode of delivery

Table 6: Sensitivity analysis using log bionomial regression for preterm and malaria

Preterm	Crude RR (CI)	Pvalue	Adjusted (CI) RR"**"	Pvalue
Malaria				
No	1		1	
Yes	1.49 (1.24-1.23)	<0.001	1.45 (1.20- 1.78)	<0.001
Parity				
First pregnancy	1		1	
2-3 pregnancy	0.89 (0.78-1.04)	0.150	0.87 (0.74-1.02)	0.087
4 or more	0.72 (0.57-0.91)	0.006	0.74 (0.57-0.96)	0.025
Maternal Age				
15-24 years	1			
25-34 years	1.14 (0.98-1.35)	0.106	1.19 (1.01-1.42)	0.039
35-48 years	0.81 (0.61-1.09)	0.181	0.93 (0.67-1.29)	0.670
G6PD				
Normal	1		1	
Partial defect	1.29 (1.03-1.60)	0.025	1.27 (1.02- 1.59)	0.036
Full defect	1.04 (0.74-1.48)	0.803	0.98 (0.67-1.44)	0.935
Neonatal admission at birth				
No	1		1	
Yes	1.47 (1.13-1.91)	0.004	1.39 (1.07. 1.83)	0.015

** Significant confounders adjusted in the multiple log binomial model

**parity, maternal age, Glucose-6-phosphate Dehydrogenase (G6PD), Neonatal admissions at birth

Figure 1 : Follow-up Plan

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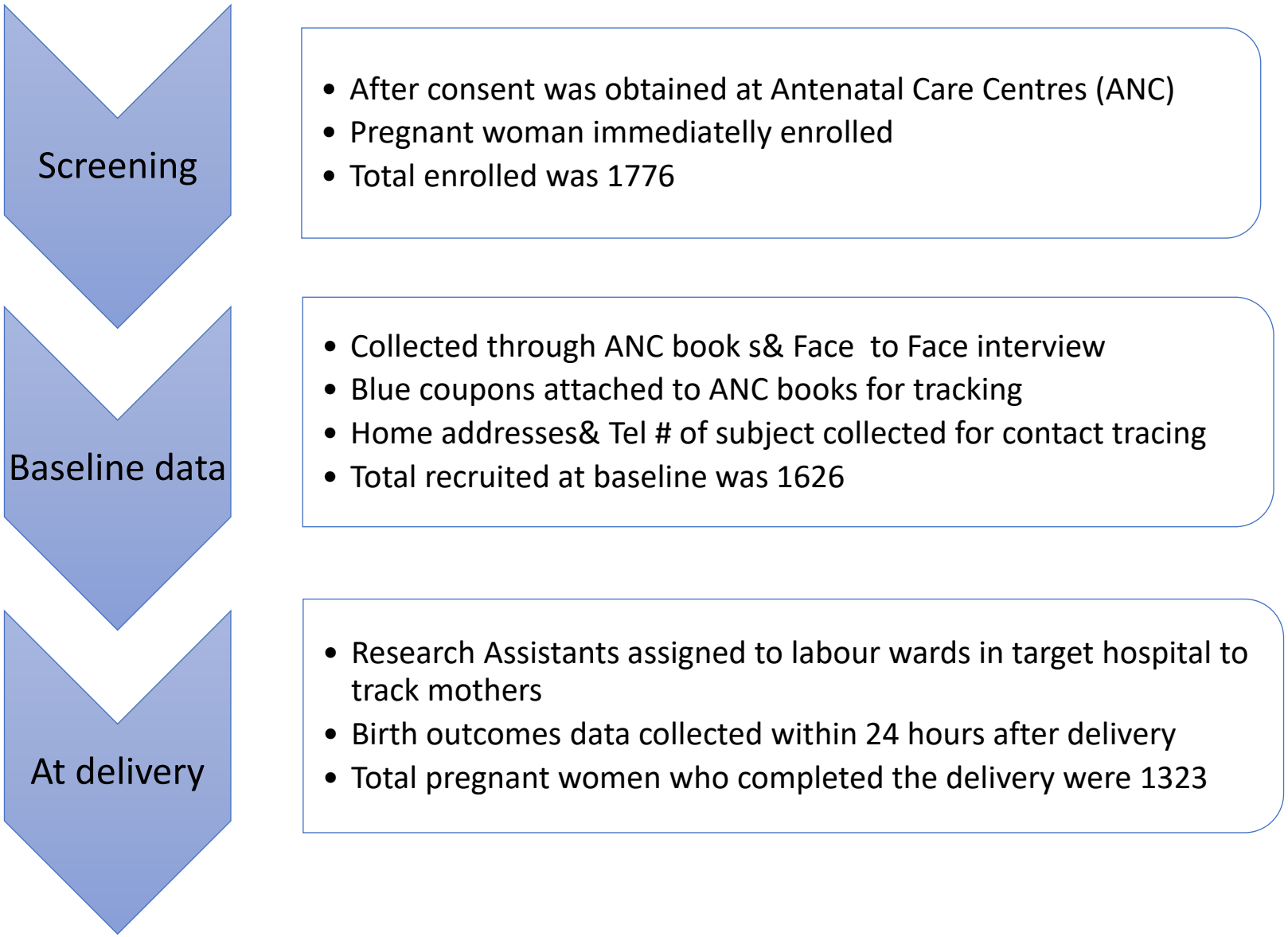

Figure 1 : Plan for follow up

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10 10 10
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Prenatal malaria exposure and risk of adverse birth outcomes: a prospective cohort study of pregnant women in the Northern Region of Ghana

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Abstract

Objective: Malaria remains endemic in most of sub-Saharan Africa and has negative impacts on pregnant women, resulting in morbidity and poor birth outcomes. The purpose of this study was to assess the relationship between malaria and adverse birth outcomes in prenatal women in the Northern Region of Ghana.

Design: A prospective cohort study of singleton pregnancies at 28 weeks of gestational age and above were recruited between July 2018 and May 2019 from four public hospitals in the Northern Region of Ghana.

Outcome measures: Low birth weight (LBW), preterm birth and perinatal death.

Results: A total of 1323 pregnant women completed the study out of the 1626 recruited. Their average age was 27.3 ± 5.2 years. The incidence of malaria in this population was 9.5% (95% confidence interval [CI]: 7.9–11.1). After adjusting for newborn admissions to the Neonatal Intensive Care Unit (NICU), parity, maternal age, and glucose-6-phosphate dehydrogenase (G6PD), women who were exposed to malaria during the third trimester of pregnancy had 2.02 times (95% CI 1.36–2.99) higher odds of premature delivery. Furthermore, they had 2.06 times [95% CI 1.09–3.93] higher chance of giving birth to babies with LBW, irrespective of their socioeconomic status. With an odds ratio of 1.02 (95% CI 0.26 – 4.01), there was no difference between pregnant women with malaria and those without malaria for perinatal mortality after adjusting for cesarean section.

Conclusion: This study confirms that prenatal malaria increases the odds of both preterm and LBW deliveries. A decisive policy to eradicate or minimize perinatal malaria is needed to contribute to the prevention of LBW and adverse pregnancy outcomes.

Keywords: Malaria, RDT, prenatal, pregnancy, preterm, LBW, Northern Region, Ghana

Strengths and limitations of this study

- This was a prospective cohort study done to investigate the relationship between maternal malaria in the third trimester and birth outcomes in Tamale, Ghana.
- The study provides detailed information on malaria and adverse birth outcomes that is not otherwise readily available due to the challenges of underreporting and poor record linkage for the use surveillance data.
- Rapid Diagnostic Tests was used to diagnose malaria in pregnancy as a routine procedure in state-licensed laboratory practitioners, which may be a limitation.
- We were unable to account for the effects of malaria at in the early stages of pregnancy on birth outcomes.

Introduction

Malaria claimed over 600,000 lives in 2020, with sub-Saharan Africa accounting for 95% of deaths.(1) Malaria exposure can be fatal to persons with inadequate immunity, particularly pregnant women and small children. It also has an economic cost to the family and the government, with a direct cost of around $ 12 billion every year.(2)

In malaria-endemic areas in sub-Saharan Africa, women face significant risks throughout their pregnancy. Examples of these risks pregnant women are exposed to include low birth weight (LBW), premature birth, and spontaneous abortions.(3) Prenatal malaria is responsible for 5-12% of LBW and accounts for between 75,000 to 200,000 infant deaths each year. (4) In sub-Saharan Africa, 11 million women were infected with malaria in 2018, resulting in approximately 872,000 newborns born with LBW.(5) In 2018, the Central and Western Africa subregions reported the highest prevalence of malaria in pregnant women, each with 35% prevalence. Furthermore, West Africa had the highest frequency of LBW due to malaria.(5) In particular, the effect of malaria exposure on fetal growth was observed during the third trimester of pregnancy regardless of period the exposure.(6)

Malaria cases increased by half a million in Ghana in 2018 compared to the year before.(5) Regarding treatment, a research conducted in a War Memorial Hospital in the Upper East Region found that children born to mothers on artemether–lumefantrine, intermittent screening and treatment of malaria in pregnancy (ISTp-AL) had a lower risk of malaria than those delivered to mothers on sulfadoxine/pyrimethamine, intermittent preventive treatment of malaria in pregnancy (IPTp-SP).(7) Yet, malaria prevalence and poor birth outcomes were 9.0 percent and 22.2 percent, respectively, in Kumasi.(8) In Navrongo, uptake of Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPT3) i.e uptake of three doses was 76 percent, while uptake of 5 doses (IPT5) was 16 percent, with women who received at least three doses having better health outcomes.(8) Given that Ghana is in an endemic malaria zone, these studies highlight implementation gaps as well as provide information that are useful to improve our malaria prevention policies and programs. Unfortunately, there is a dearth in the

Ghanaian literature relating to the role of malaria in poor birth outcomes in pregnant women in urban settlements in northern Ghana.

Furthermore, due to insufficient linkages between malaria control and prenatal care data, progress in attaining malaria control among pregnant women has been slow.(9) In addition, inconsistencies in data management practices were discovered during a data quality evaluation in several health institutions, posing problems in data reporting, analysis, and application.(10) Therefore, the precision of aggregate data collected from these facilities through surveillance is compromised by these discrepancies. We designed this prospective cohort research as an independent evaluation of birth outcomes among people with prenatal malaria in light of these difficulties. This study sought to provide considerably more detailed information on the links between prenatal malaria and poor birth outcomes in pregnant women in northern Ghana.

Methods

Setting

Data for this substudy was drawn from a prospective cohort study that took place in four hospitals in Ghana's Northern Region. Three of the hospitals are located in Tamale, the Northern Regional Capital, the fourth largest city in Ghana. The fourth hospital is located in the Savelegu Municipality bordered by Tamale to the west. These areas are located within the Guinea Savannah belt,(11) with little seasonal variations in prevalence as such, Oheneba-Dornyo and collegues found the prevalence of malaria to be positively correlated with rainfall with nearly a borderline significance. (12) The *Plasmodium falciparum* peripheral parasitemia prevalence in pregnant women in the Northern Savanna Zone ranged between 26% and 13.4% from 2013 to 2019, respectively.(13)

Design

The study was designed from a parent cohort study that sought to answer the primary research questions of whether or not different cooking fuel types influenced pregnancy outcomes and infant respiratory problems. (14,15) Therefore the original sample size calculation was based on the proportion of pregnant women developing the outcome (respiratory symptoms) with cooking fuel type as an exposure. The present study answers a secondary research question about the relationship between prenatal malaria exposure and the risk of adverse birth outcomes. Thus this study design leverages on the advantages of the large sample size of the original prospective cohort study. As our main results were statistically significant, we assumed that the sample size for this study was reasonable.

The study recruited pregnant women in their third trimester, who primarily cooked their family meals, were non-smokers and were confirmed to carry singleton pregnancies. The process began in July 2018 and ended in May 2019. The main study was planned for three phases of data collection. At the beginning of the study, women were screened and recruited. In phase one, during the third trimester, we collected demographics, medical history, exposure data for the

primary objective (fuel type), and exposure for secondary objective (malaria). The endpoint for this study was birth outcome; and this data was collected in the labor wards of the various hospitals during phase two. The final part was the collection of baby data in phase three followed up.(15)

The original study encountered a methodological shortcoming during its implementation, as we initially assumed recruited pregnant women will return to the hospitals they attended ANC (i.e a recruitment center) to deliver. However a few months into the study, we observed that most of them did not return to deliver and given the project's limited funding we were unable to follow them up. Therefore we replaced them with women who strictly agreed to return to the recruitment center to give birth. This increased our initial sample size from 1472 to 1776 as published in. (16) Cconsequently, we followed up 1323 pregnant women in this study, more details can be found in (15).

Procedures

Baseline data was collected at recruitment using a structured questionnaire at the antenatal care (ANC) centers of each hospital. Gestational age was routinely ascertained through an ultrasound during ANC, therefore our study relied on a midwife validated gestational age. Pregnancy outcome data was collected at the labour wards of all hospitals by trained research assistants using a predesigned questionnaire. Only 88% of our final sample received at least one sulfadoxine / pyrimethamine (IPTp-SP).

Laboratory procedure

RDT malaria diagnosis

The SD BIOLINE Malaria Ag P.f/Pan Rapid Diagnostic Test Kits (RDTs) for malaria were used in all hospitals (17) with specificity and sensitivity of 99.5% and 99.7%, respectively. The principal investigator (PI) and research assistants (RA) made efforts to observe and monitor adherence to standard testing protocols in each hospital to ensure that they complied with both the manufacturer's guide and the fundamental laboratory principles for the test. RDT test was performed during the third trimester whenever possible to determine whether or not our study

participants had parasitemia in peripheral blood. Still, we were unable to control for possible measurement bias among laboratory personnel for malaria.

Haemoglobin estimation – All hospitals used a blood analyzer to estimate the full blood count (FBC) and hemoglobin (Hb) was extracted from the FBC results of all participating pregnant women. Blood sample (5 milliliters) for Hb estimation was collected into an ethylenediamine tetraacetic acid (EDTA) tube and was mixed with an EDTA anticoagulant. In this study, anemia was defined as Hb < 11.0 g/dL.

Glucose-6-phosphate dehydrogenase (G6PD) test - The methaemoglobin reduction test was used in all hospitals for the screening of pregnant women for G6PD. The test result was reported as No defect/normal, partial defect or Full defect.

Data collection

Computer-assisted personal interviewing (CAPI) was used to gather all data, which was done using the Kobo Collect Android app. The data collection procedure is described in detail elsewhere.(15)

Outcomes

The main outcomes of this study were low birth weight (LBW), preterm birth, and perinatal death. These were all gathered during delivery in the various hospitals' labour wards. On the seventh day, women were contacted by mobile phone to inquire about the baby's well-being, to ensure that the infant was still alive, and to capture neonatal mortality after discharge from the hospital. Preterm birth was defined as <37 weeks gestational age, while LBW was defined as <2.5 kilograms as previously published. (15)

Objective

To appraise the association of birth outcomes among people with prenatal malaria. The exposure variable was a positive RDT test verifying that a pregnant woman had malaria during her third trimester or just before birth.

Statistical analysis

Data was exported from the kobo collect application database into an MS Excel sheet, cleaned and transferred into STATA 13 for analysis. Individual confounders were added into the simple logistic regression model, and a significant variable of > 0.05 or near significant were retained in the multiple logistic regressions to evaluate the potential associations between adverse birth outcomes and malaria. For each model, we set out to adjust potential confounders such as maternal age at birth, neonatal admissions at birth, mode of delivery, marital status, parity, G6PD status, genotype, anemia, socioeconomic status (SES), drinking of alcohol, and maternal respiratory condition, and initially added to logistic regression with significance set at $p \leq 0.05$. Those with significance were retained in the multiple logistic regression model, and nonsignificant ones were dropped. Consequently, genotype, anemia, respiratory condition, and drinking of alcohol were all dropped during the initial univariate analysis, and that is why some were found in the final models while others were not. Maternal age was however non-significant, but was retained in the models, given its relevance as a confounder and its previously established association with adverse birth outcomes. (18,19) For the SES, assets from the 2014 Ghana Demographic Health Survey were used for the calculation of total assets. We divided the total SES scores into quantiles andconsidered all scores less than 50 quantiles as poor, from 50 to 75 as moderately rich and at least 75 or more as rich.(15) Missing data of more than 10% from any observation were dropped in order not to open the study to bias, a single manual imputation was used to address some missing data based on previous patterns of questions.(20) Sensitivity analysis was assessed in both univariate and multiple log binonmial regression model compared to logistic regression model used in the main analysis.

Patient and public involvement

None.

Results

Figure 1 shows an elaborate detail of the plan for follow up during this study which was published in Hussein et al., 2020. At baseline, 1626 third trimester pregnant women were recruited with 1323 women completing the study.The age of pregnant women ranged between 15-48 years, 59.1% were between 25-34 years.

Table 1: Baseline characteristics of pregnant women

Variables	Delivery period
	Frequency (%) n= 1323
Maternal age (mean ±SD)	27.3±5.2
15-24	394 (29.8)
25-34	782 (59.1)
35-48	147 (11.1)
Marital status	
Married	1310 (99.0)
Unmarried	13 (1.0)
Ethnicity	
Mole Dagbani	1274 (96.3)
Others	49 (3.7)
Maternal education	
Primary/no education	997 (75.4)
JHS/Middle school	147 (11.1)
SHS/Technical/Vocational	89 (6.7)
At least diploma	89 (6.8)
Maternal occupation	
No employment	302 (22.9)
Trader	834 (63.1)
Laborer	74 (5.6)
Factory/industry	24 (1.8)
Formal employment	87 (6.6)
Socioeconomic status	
Poor	473 (35.8)
Moderately rich	439 (33.2)
Rich	411 (31.1)
Residence	
Urban	762 (57.6)
Rural	561 (42.4)
Housing	
Separate house	68 (5.14)
Semi-detached	43 (3.25)
Compound house (sandcrete)	850 (64.25)
Compound house (mud)	362 (27.36)
Alcohol	
Yes	4 (0.3)
No	1319 (99.7)
Medical history	
Parity	
First pregnancy	633 (47.9)
2-3 pregnancies	494 (37.3)
4 or more pregnancies	196 (14.8)
Malaria	

Positive	125 (9.5)
Negative	1198 (90.5)
Sickle cell	
Positive	77 (6.4)
Negative	1120 (93.6)
Genotype	
Hb AS	24 (36.4)
Hb SC	9 (13.6)
Hb SS	33 (50.0)
Anaemia	
<11 g/dl	582 (47.9)
≥11 g/dl	633 (52.1)
Sulfadoxine pyrimethamine (SP) usage	
Yes	1137 (88.9)
No	154 (11.9)
Glucose-6-phosphate dehydrogenase (G6PD)	
No defect	1029 (86.9)
Partial defect	98 (8.3)
Full defect	56 (4.7)
Respiratory Condition	
Yes	10 (0.8)
No	1,313 (99.2)

Hb = Hemoglobin

For medical history, 14.8% had a parity of four or more children. The incidence of malaria in this cohort was 9.5% (95% confidence interval [CI]: 7.9-11.1). About 6.4% tested positive for sickle cells and, out of these, 50.0% of them who checked for their genotypes were sickled (SS). About 47.9% of the women were anaemic with haemoglobin levels of less than 11 g/dl within their third trimester of pregnancy, while 4.7% had G6PD full defect (Table 1).

Table 2: Incidence of pregnancy outcome and malaria

Pregnancy outcomes n=1323	**Negative**	**Positive**	**Total**
	Freq (%)	Freq (%)	
Preterm birth			
Term	780 (65.1)	60 (48.0)	840
Preterm	418 (34.9)	65 (52)	483

Birth weight			
Normal birth weight	1137 (94.9)	112 (89.6)	1249
Low birth weight	61 (5.1)	13 (10.4)	74
Type of delivery			
Live birth	1176 (98.4)	123 (98.4)	1299
Neonatal mortality	19 (1.6)	2 (1.6)	21

The incidence of preterm birth among women with malaria was 52.0%. Moreover, the prevalence of LBW was 10.4% among women with malaria and 5.1% among women without malaria. In both mothers with and without malaria, newborn death and live births were equally 1.6 percent (Table 2).

Table 3: Logistic regression of preterm and malaria

Preterm	Crude OR (CI)	P value	Adjusted (CI) OR*	P value
Malaria				
No	1		1	
Yes	2.02 (1.39-2.93)	<0.001	2.02 (1.36- 2.99)	<0.001
Parity				
First pregnancy	1		1	
2-3 pregnancy	0.83 (0.65-1.07)	0.147	0.79 (0.61-1.03)	0.087
4 or more	0.59 (0.42-0.85)	0.004	0.62 (0.42-0.93)	0.021
Maternal age				
15-24 years	1			
25-34 years	1.23 (0.96-2.85)	0.102	1.35 (1.02-1.77)	0.034
35-48 years	0.75 (0.49-1.13)	0.170	0.92 (0.57-1.48)	0.720
G6PD				
Normal	1		1	
Partial defect	1.55 (1.02-2.35)	0.039	1.54 (1.01- 2.37)	0.047
Full defect	1.07 (0.61-1.87)	0.803	0.98 (0.54-1.76)	0.934
Neonatal admission at birth				
No	1		1	
Yes	2.10 (1.17-3.79)	0.004	1.98(1.09. -3.60)	0.025

*Significant confounders adjusted in the multiple log binomial model
*Parity, maternal age, G6PD, neonatal admissions at birth

Pregnant women with malaria had 2.02 times (CI: 95% (1.39-2.93) incresead odds of preterm birth compared to those without malaria after adjusting for parity, maternal age, G6PD deficiency and neonatal admission (Table 3).

Table 4: Logistic regression of LBW and malaria

LBW	Crude OR (CI)	P value	Adjusted OR (CI)*	P value
Malaria				
No	1		1	
Yes	2.04 (1.16-3.61)	0.014	2.06 (1.09-3.93)	0.027
Parity				
First pregnancy	1		1	
2-3 pregnancy	1.21 (0.71-2.06)	0.480	1.32 (0.76-2.28)	0.322
4 or more	1.72 (0.91- 3.23)	0.093	2.13 (1.03-4.39)	0.041
Maternal age				
15-24 years	1			
25-34 years	0.79(0.47-1.32)	0.373	0.70 (0.41-1.22)	0.210
35-48 years	0.72 (0.30-1.71)	0.457	0.45 (0.17-1.19)	0.109
Socioeconomic status				
Poor	1		1	
Moderately rich	0.57 (0.33-0.99)	0.050	0.60 (0.34-1.07)	0.082
Rich	0.52 (0.28-0.93)	0.028	0.54(0.29-0.98)	0.044

*Significant confounders adjusted in the multiple log binomial model
*Parity, socioeconomic status

Furthermore, pregnant women with malaria had 2.06 times increased odds [CI 95% (1.09-3.93)] of LBW compared to those without malaria after adjusting for parity, maternal age, and socioeconomic status (Table 4).

Table 5: Logistic regression of perinatal mortality and malaria

Perinatal mortality	Crude OR (CI)	P value	Adjusted OR (CI)*	Adjusted models P value
Malaria				
No	1		1	
Yes	1.01 (0.23-4.37)	0.993	1.02 (0.26-4.01)	0.983
Mode of delivery				
Normal	1		1	
Ceasarian section	5.18 (1.88-14.26)	0.001	5.17(1.87-14.36)	<0.001

*Significant confounders adjusted in the multiple log binomial model
*Mode of delivery

Lastly, with an OR of 1.02 (95% CI 0.26 – 4.01), there was no difference between pregnant women with malaria and those without malaria for perinatal mortality after adjusting for

cesarean section. Women who underwent cesarean section had a 5 times greater risk of perinatal death than those who did not have cesarean section (Table 5).

Table 6: Sensitivity analysis using log bionomial regression for preterm and malaria

Preterm	Crude RR (CI)	P value	Adjusted (CI) RR*	P value
Malaria				
No	1		1	
Yes	1.49 (1.24-1.23)	<0.001	1.45 (1.20- 1.78)	<0.001
Parity				
First pregnancy	1		1	
2-3 pregnancy	0.89 (0.78-1.04)	0.150	0.87 (0.74-1.02)	0.087
4 or more	0.72 (0.57-0.91)	0.006	0.74 (0.57-0.96)	0.025
Maternal age				
15-24 years	1			
25-34 years	1.14 (0.98-1.35)	0.106	1.19 (1.01-1.42)	0.039
35-48 years	0.81 (0.61-1.09)	0.181	0.93 (0.67-1.29)	0.670
G6PD				
Normal	1		1	
Partial defect	1.29 (1.03-1.60)	0.025	1.27 (1.02- 1.59)	0.036
Full defect	1.04 (0.74-1.48)	0.803	0.98 (0.67-1.44)	0.935
Neonatal admission at birth				
No	1		1	
Yes	1.47 (1.13-1.91)	0.004	1.39 (1.07. 1.83)	0.015

*Significant confounders adjusted in the multiple log binomial model
*Parity, maternal age, Glucose-6-phosphate dehydrogenase (G6PD), neonatal admissions at birth

Sensitivity analysis in both the univariate and multiple log binomial regression model for model did not change the direction or strength of the estimates compared with the logistic regression

model for preterm birth, even though odds ratios marginally exaggerated the relative risk to some magnitude (Table 6).

Discussion

In this study, malaria was found in nearly 10% of pregnant women. The study also investigated the associations between malaria and preterm birth, low birth weight, and perinatal mortality.

Prenatal malaria was found to be substantially linked with preterm birth and LBW after correcting for parity, mother age, G6PD, socioeconomic status, and neonatal hospitalization at birth, but not with perinatal death after adjusting for caesarian section. Indeed, Nkwabong et al. previously reported that third trimester malaria increased the chance of preterm delivery by five times and LBW by 2.8 times, which was consistent with our findings.(21) Similarly, Vogel and colleagues found that exposure to malaria increased the risk of spontaneous preterm term birth by 1.67 times with a secondary analysis of data from 22 low- and middle-income countries.(22) Van den Broek and colleagues similarly conclude from their data that, maternal malaria significantly increased the risk of preterm birth by 1.99 times.(23) In Tanzania, similar studies have shown that malaria parasites in the mothers's red blood cells are associated with 3.2 times the risk of premature birth.(24) Compared to people without placental malaria, pre-term birth rates increased by 4.7% to 5.6%, among pregnant women with placental malaria. (25,26)

In Brazil, researchers reported that *Plasmodium falciparum species* (*P. falciparum*) were significantly associated with preterm births, albeit accounting for less than 40% of the total.(27) In contrast, some authors suggest that such correlations zre nonsignificant. For example, a recent comprehensive study of malaria at birth in Uganda that studied three different parasite detection methods, including peripheral and placental blood microscopy, placental blood loop-mediated isothermal amplification (LAMP), and placental histopathology, and found no statistically significant link between malaria and preterm birth for any of the methods. (28) In this study, it was not possible to distinguish between the various species of malaria. However, previous

studies have shown that regardless of the species, malaria can lead to poor birth outcomes. For example, *P. falciparum* in placental blood increased the risk of LBW by around 1.7 times in Malawi (29) and about 3.7-fold increase in Zaire. (30) Furthermore, in Nigeria and Uganda, approximately 33% and 19% of mothers with placental malaria, respectively, delivered LBW babies compared to those without placental malaria. (26,31)

One interesting aspect that emerged from the analysis is the finding that women who delivered via caserean section (CS) were about five times more likely to suffer perinatal mortality. Notwithstanding, we found that there was no significant increase in the odds of pregnancy mortality in both adjusted and unadjusted models. In contrast, other studies, such as the one conducted in Zaire in 1993, reported that maternal malaria with chloroquine prophylaxis increased the risk of perinatal death by 12 times after adjusting for parity and prenatal clinic visits.(30) In addition, *P falciparum* malaria during pregnancy increased the risk of neonatal deaths by 2.6 times.(32) Infants delivered to mothers with acute placental infections had a fivefold risk of death. (33)

Multiple variables such as gender, mother's age, and SES can result in adverse birth outcomes. Therefore, in this study, we adjusted for these significant confounders in the multiple logistic regressions and maintain the significant confounders. Women with multiple pregnancies are protected from premature birth. This is apparently due to the extra protection provided by antibodies in subsequent pregnancies against the parasite variant surface antigen VAR2CSA. (34,35)

Furthermore, regardless of the period of exposure, the effect of malaria exposure on fetal development was detected during the third trimester of pregnancy and has been blamed for poor birth outcomes.(6,25,36) This might be because the pathway that connects the mother to the kid throughout pregnancy may impact the fetus's survival at delivery or even beyond, since the placenta delivers nutrition to the newborn via the umbilical cord. For example, Ouédraogo and his colleagues found a connection between umbilical cord parasitemia and maternal peripheral blood parasitemia.(37) Also, malaria in pregnancy may have induced excessive stimulation and dysregulated hemoglobin-scavenging system; and bioavailabliblity of nitric oxide and L-arginine which may be associated poor vascular development and adverse birth outcomes.(38) Although we used RDT with peripheral blood, our findings were consistent with the majority of studies on

placental malaria.(34,35) This is because peripheral blood infections may promote the sequestration of parasites in the placenta and activate immune reactions of antibodies and antigens that may cause complications during delivery.(34,35) Furthermore, Kapisi et al. (2017) found that women who had a high burden of malaria had a 14-fold increased risk of placental malaria by blood microscopy and a four-fold increased risk of loop-mediated isothermal amplification (LAMP). This could indicate that our group had a higher malaria burden, correlating with previous research using a different diagnosis technique of diagnosis.(25)

This study benefited from the large size of the sample.. To date, we have not found any studies that examined the relationship between maternal malaria during delivery and the results of birth in Tamale in the Northern Region of Ghana.. In this cohort, RDT was used to diagnose malaria, although the sensitivity to malaria was 19% lower than a microscopic examination of peripheral and placenta blood. However, RDT has been reported to have outperformed microscopy in identifying malaria in other settings.(36,39) Furthermore, RDT is useful in environments like ours, because it produces quick results, requires fewer training and equipment, and has virtually no power interruption. Consequently, especially in hospitals with limited human resources and equipment, rapid malaria detection techniques are imperative for initiating care.

A potential drawback of our study was that we relied on standard laboratories in each hospital to collect malaria data. While we took every attempt to observe and monitor compliance with established testing techniques, we were unable to account for any measurement bias among laboratory staff for the exposure variable (malaria). However, it should be noted that the RDT method for malaria diagnosis during pregnancy is used routinely in our context and is performed by state-licensed laboratory professionals for diagnosing malaria in our popualtion. We cannot also explain the impact of malaria during the early stages of pregnancy on birth outcomes, since we only included pregnant women in their third trimester as long as they had done at least one RDT prior to recruitment. This study also failed to account for the number of doses of SP uptake by pregnant women. This denied us the opportunity to measure its confounding effect on malaria and birth outcomes. Nevertheless, our research is comparable to similar studies on the subject matter.

Taken together, these findings indicate that maternal malaria within the third trimester of pregnancy may be a major contributor to LBW and preterm birth in the Northern Region of Ghana.

Data availability statement

Data are available upon reasonable request.

Ethical approval

The approval for this study was given by the ethical review committees of the Ghana Health Service and the Tehran University of Medical Sciences. The ethical approval numbers are GHS-ERC010/12/17 and IR.TUMS.SPH.REC.1396.4066, respectively. Information sheets with accompanying verbal clarifications were provided to adequately explain all concerns of risk to the study participants. A signed or thumb printed informed consent was obtained from each participant prior to recruitment.

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Contributors

HH: Investigation, writing the draft and data analysis. MS: Writing review & editing. MY: Conceptualization, validation. M.S.H: Visualization, Resources. MAS, Project administration. PDA: data curation, AF: supervision and acquisition of funds. All authors reviewed and approved the final manuscript:

Competing interests

None declared.

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Figure 1 : Follow-up plan

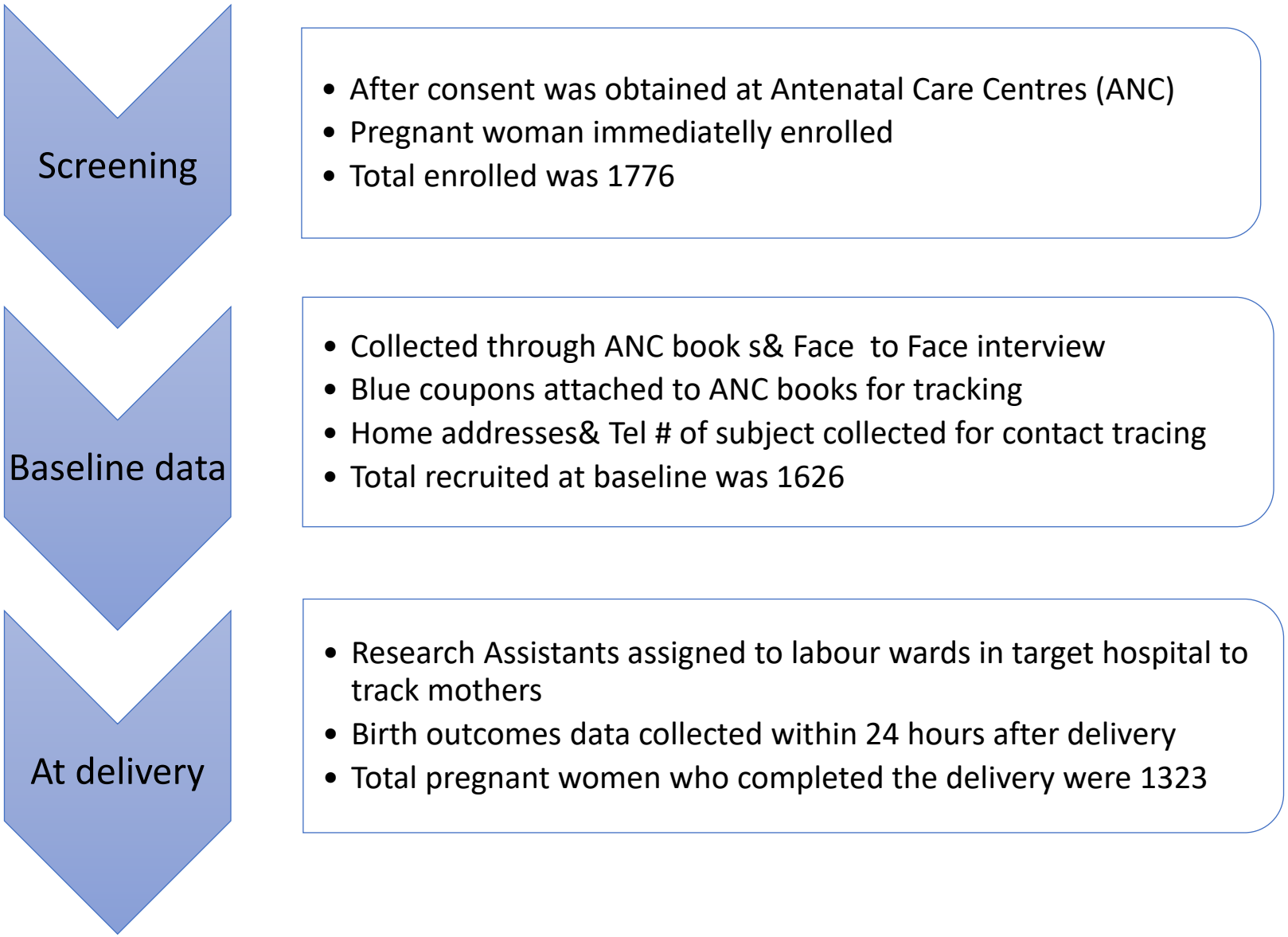

Figure 1 : Plan for follow up

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10 10 10
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.