

Risk factors for placental malaria and associated adverse pregnancy outcomes in Rufiji, Tanzania: a hospital based cross sectional study.

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Abstract

Background: Prevention and treatment of malaria during pregnancy is crucial for reduction of malaria in pregnancy and its adverse outcomes. The spread of parasite resistance to Sulphadoxine-Pyrimethamine (SP) used for Intermittent Preventive Treatment for malaria in pregnancy (IPTp), particularly in East Africa has raised concerns about the usefulness and the reliability of the IPTp regimen. We aimed to assess the effectiveness of two doses of SP in treating and preventing occurrence of adverse pregnancy outcomes.

Methodology: The study was an analytical cross sectional study which enrolled 350 pregnant women from Kibiti Health Centre, South Eastern Tanzania. Structured questionnaires were used to obtain previous obstetrics and medical history of participants and verified by reviewing antenatal clinic cards. Maternal placental blood samples for microscopic examination of malaria parasites were collected after delivery. Data was analyzed for associations between SP dosage, risk for PM and pregnancy outcome. Sample size was estimated based on precision

Results: Prevalence of placental maternal (PM) was 8% among pregnant women (95%CI, 4.4-13.1%). Factors associated with increased risk of PM were primigravidity ($P < 0.001$) and history of fever during pregnancy ($P = 0.02$). Use of at least 2 doses of SP for IPTp during pregnancy was insignificantly associated with reducing the risk PM ($P = 0.08$), low birth weight ($P = 0.73$) and maternal anemia ($P = 0.71$) but associated significantly with reducing the risk of preterm birth ($P < 0.001$).

Conclusion: Two doses of SP for IPTp regime are ineffective in preventing and treating PM and adverse pregnancy outcome. Hence a review to the current IPTp regimen should be considered with possibility of integrating it with other malaria control strategies.

Keywords: Placental malaria, intermittent preventive treatment, Sulphadoxine-Pyrimethamine, Malaria in pregnancy

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Introduction

Despite integrated efforts to control malaria in endemic areas, PM remains a significant cause of maternal and infant mortality and morbidity. Malaria in pregnancy remains a major public health problem especially in sub-Saharan African countries where approximately 25 million pregnant women are at risk of *P. falciparum* infection every year⁷. About 25% of these pregnant

women have evidence of placental parasitaemia during delivery and have increased risk of maternal and perinatal mortality⁸. In Tanzania, the prevalence of PM is 8% which is lower compared to the overall estimated prevalence for Africa¹⁹.

Available literature ascertains that, the parity pattern of malaria susceptibility in highly endemic areas show that primigravidae and secundigravidae (to a lesser extent) are more affected than multigravida²². *P.falciparum* parasites are known to invade the placenta in semi-immune women to cause PM, a condition associated with still-birth, spontaneous abortion, fetal pre-maturity, and maternal anemia¹⁵, low birth weight (LBW). Most of these pregnancy outcomes are important risk factors for neonatal mortality and retarded growth regardless of the factors that affect malaria epidemiology in a given location^{5,15,22,23,29}. Many studies suggest that, multigravida

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women have less risk of PM than primigravida because they have developed immunity to sequestered parasites^{10,11}. In sub-Saharan Africa, 20% of LBW has been attributed to malaria in pregnancy²⁰.

Prevention and treatment of malaria during pregnancy is crucial for reduction of parasitaemia and improving birth outcomes. World Health Organization (WHO 2004) and international Roll Back Malaria (RBM) initiative recommended three cost effective protective measures for all pregnant women, which are the use of IPTp, insecticide-treated mosquito nets and effective case management of malarial illnesses⁴⁰. In Tanzania, these recommendations are implemented through the National Malaria Control Program (NMCP) and up to 2010, 57% of all pregnant women in Tanzania slept under an Insecticide Treated bed-Net (ITN) while 60% of them used at least one dose of IPTp. Sulphadoxine-Pyrimethamine (SP) has been widely used for intermittent preventive treatment during pregnancy (IPTp) as a means to avoid poor outcomes that are associated with PM⁴.

IPTp using SP is recommended in areas of high malaria transmission with low resistance to SP (World Health Organization 2003). In Tanzania, the policy is to offer SP to all pregnant women attending antenatal clinics³⁸ at between 20 and 24 weeks gestation for the first dose and between 28 and 32 weeks for the second dose². The newly proposed regimen that requires administration of monthly IPTp dose from second trimester until delivery has not yet been effectively implemented in the country. It has been documented that, the proportion of pregnant women receiving single dose of SP in Zambia is about twice (60%) as much as those receiving two doses (27%), where single dose proved to be inferior to higher doses^{14,16}.

The changing patterns in malaria epidemiology and pathogenesis may imply major shifts in traditional paradigms of associations between placental malaria and its risk factors on one hand, and its fetal-maternal outcomes on the other hand. We aimed at assessing the prevalence of placental malaria in women living in a high transmission setting, and to determine the risk factors associated with it, including Bednet use and SP prophylaxis as IPTp during pregnancy, and the effects on pregnancy outcomes.

Methods

Study design and area

This study was a hospital based descriptive cross sec-

tional study involving pregnant women, conducted between September and December 2012 in Rufiji district, South-Eastern Tanzania. This is an area of high malaria transmission with prevalence of 20.8% (NBS, 2008). It lies 500 meters below sea level and receives an annual rainfall of 800-1000mm. According to data on malaria incidence from health care facilities, malaria is the most important public health problem for both adult and children in Rufiji District (Goodman et al. 2004). The most common occupation in the study area is subsistence farming, with rice, maize and cassava being the predominant food crops. Residents of the study area are also involved in subsistence animal husbandry, fishing and small-scale trading. Rufiji has very limited tarmac roads, a situation that at times of long rains isolates it from major highways and market centers^{27,28}. Malaria transmission in this region, measured by the mean number of infected bites per person per year (entomological inoculation rate) has been estimated at between 79 and 1,209 infective bites per person per year, and malaria is the leading cause of outpatient diagnoses, and a high proportion of reported inpatient deaths. Pregnant women who attended Kibiti Health Centre (KHC) for delivery were recruited for the study.

Sample size

A minimum sample size of 136 women was estimated to provide a power of 80% with 5% precision. A total of 350 pregnant women with study's inclusion criteria were consecutively recruited from KHC which increased both the power and precision.

Data collection

Data was collected using a structured questionnaire by interviewing participating mothers in the labor ward. This involved mothers who were waiting to enter labor and the ones who are in latent phase of labor. The study's inclusion criteria were women who delivered in the study facility, availability of a placenta for pathological examination and mothers who agreed to participate in the study by providing written consent. Women with serious delivery complications including severe post-partum bleeding, eclampsia and other condition that lead to case referral to a tertiary hospital were excluded. Information regarding biographical, obstetrical and medical history of the participant during pregnancy was recorded. Information on the new born was also recorded including infant birth weight. Gestational length was estimated based on the date of recorded last normal menstrual period (LNMP) or fundal height examination, when LNMP was unknown. Newborns were assessed at delivery. All information of the partici-

pant from antenatal clinic card and medical registry was carefully reviewed by the principal investigator. This information included history of SP use, birth weights of infants and gravidity as well as the use of medications other than SP. Filling of all questions in the questionnaire was completed on the day of interview. Questionnaires completed by study assistants were reviewed in an evening roundup meeting to verify completeness and reliability of filled information. To determine placental malaria (parasitemia), placental blood samples were collected from the maternal side of the placenta by making an incision about 1.5 cm deep on the central maternal side by the pool-biopsy methods³⁴ and hence, about 1 cc of blood sample was collected from a small pool created in this incision after squeezing. Collected blood samples were used to prepare thick and thin blood smears on microscopic glass slides. Prepared smears were then stained with 5% Giemsa's stain for 30 minutes, fixed in absolute methanol for 5 seconds, dried for >45 minutes and examined by two independent microscopists at 100X magnification guided by standard operating procedures (SOPs). Up to 100 High power fields (HPFs) in cases of very scanty malaria on a slide may be read and number of parasites counted against the white cells counted per HPF is recorded. A slide negative after 100 HPFs was read by two microscopists and declared negative. Discrepancy findings were reviewed by a third independent microscopist and results obtained were regarded as decisive. Hemoglobin was measured by using Abbott Cell-Dyn 3700 Hematology Analyzer in which a hemoglobin reading below 10g/dL was considered anemia. In addition a packed cell volume measured by spinning heparinized portion of the placental blood sample (PCV) of 30% was interpreted as anemia.

Primary end points

The primary endpoint of the study was placenta malaria. Secondary outcomes that were assessed under the study include preterm birth, low birth weight and maternal anaemia. Preterm birth was defined as birth before 37 weeks of gestational age. Low birth weight was defined as a birth weight below 2500 g, and maternal anaemia was defined as haemoglobin level below 11 g/dl at the time of labor.

Statistical analysis

Data was analyzed using statistical package for social sciences (SPSS) version 18. Numerical variables were summarized into percentage. Categorical variables were summarized using cross-tabulation to estimate different proportion. The primary outcome was the proportion of women with placenta malaria. The bi-variate models include IPTp, maternal age, gravidity, history of fever in pregnancy, HIV status and ITNs use as explanatory variables. Multivariate analyses were performed using multiple logistic regressions to assess the effects of multiple factors on study's outcome and for controlling of confounders. Differences were considered to be significant at a cutoff point of $p \leq 0.05$.

Ethics

Prior to enrollment, participants were informed on the objectives, procedures and benefits of the study. They were assured on anonymity and confidentiality throughout the study. Written consent was obtained before the participants were enrolled into the study. Approval for this study was obtained from Kilimanjaro Christian Medical University College Ethical Committee (CRERC) on behalf of the National Ethical Review Board of Tanzania and permission to conduct the study authorized by District Medical Officer (DMO) of Rufiji district.

Results

Table 1 summarizes demographic data of study participants. Most (58.9%) of participants were young women aged between 20 and 35 years while those below 20 and above 35 years were 29.1% and 12% respectively. About two thirds (66.3%) were married or cohabiting while 33.7% were single (unmarried, divorced or widowed). One third (33.1%) of the participating mothers were pregnant for the first time (primigravidae) while 15.4%, 13.1% and 12% were pregnant for the 2, 3 and 4 times respectively. Those with 5th pregnancy and above were 26.4%, with 2.3% of these being pregnant for the 10th time. Most of the mothers (63.4%) had primary education, whereas only 7.4% had acquired secondary education and about one third (29.2%) without any formal education. A large proportion of the mothers (97.7%) used ITNs regularly and 52.5% of them took 2 doses of SP for IPTp while the rest either took one dose (40.6%) or did not take SP at all (6.9%).

Table 1 - Socio-demographic and obstetric characteristics (N=350)

Characteristic	Group	Response	% response
Socio-demographic characteristics			
Maternal age	<20 yrs	102	29.1
	20 – 35 yrs	206	58.9
	> 35 yrs	42	12
Marital status	Single	118	33.7
	Married/cohabited	232	66.3
Level of education	No formal education	102	29.2
	Primary level	222	63.4
	Secondary level	26	7.4
Obstetrics characteristics			
Gravidity	Primigravida	116	33.1
	Multigravida	234	66.9
IPTp use	No SP dose	24	6.9
	Single dose	142	40.6
	Double dose	184	52.5
ITN's use	Yes	342	97.7
	No	8	2.3

Abbreviation: IPTp = Intermittent Preventive Treatment for malaria in pregnancy;
ITN = Insecticide Treated bed-Net

Factors associated with PM and prevalence of pregnancy outcomes

Among the 350 pregnant women studied, 28 (8%; 95% CI: 5.5-11.5%) were found to have been positive for PM, while those who gave birth to newborns with low birth weight and those with maternal anemia and preterm birth were 22 (6.3%; 95% CI: 4.1-9.5%), 160 (59.3%; 95% CI: 53.1-65.2%) and 26 (7.4%; 95% CI: 5-10.8%) respectively. By using logistic regression, gravidity and history of fever during pregnancy were closely associ-

ated with PM whereas maternal age, HIV status and ITN use were not. In terms of gravidity, multigravida were 70% less likely to have PM compared to primigravida (OR=0.3; CI 0.1 – 0.7). Pregnant women who had fever during their pregnancy were three times more likely to have PM compared to those without history of fever (OR=3.2; CI 1.3 – 8.1). The study also showed that, maternal age (OR=1.0; CI 0.4 -1.9) and HIV status (OR=1.8; CI 0.3 – 12.2) were neither associated with, nor were they risk factors for PM (Table 2).

Table 2- Factors associated with placenta malaria

Characteristics	Placenta malaria		Crude OR (95%CI)	p-value	Adjusted OR (95%CI)	P-value
	No	Yes				
Maternal age						
< 25 years	172	16				
≥ 25 years	150	12	0.9 (0.6 – 1.9)	0.705	1.0 (0.4 – 1.9)	0.762
Gravidity						
Primigravida	100	16				
Multigravida	222	12	0.3 (0.2 – 0.7)	0.007	0.3 (0.1 – 0.7)	0.005
History of fever						
No	272	18				
Yes	50	10	3.0 (1.3 – 6.9)	0.009	3.2 (1.3 – 8.1)	0.013
HIV status						
Sero-negative	318	26				
Sero-positive	4	2	6.1 (1.1 – 34.9)	0.042	1.8 (0.3 – 12.2)	0.564
ITN use						
No	8	0				
Yes	314	28	-	-	-	-

Abbreviation: P Malaria = Placenta malaria; OR = Odd ratio; ITN = Insecticide Treated bed-Net

The study found that PM did not significantly predispose pregnant women to deliver babies with low birth weight (OR=2.4; CI 0.7 – 8.6). The study also revealed that, pregnant women with PM were three times more likely to develop maternal anemia (OR=3.4; CI 1.3 – 10.2) and four times more likely to have preterm birth (OR=3.8; CI 1.3 – 11.4) compared to the ones without PM (Table 3).

TABLE 3 - Pregnancy outcomes based on status of placenta malaria

Birth outcome	Placenta malaria		Crude OR (95%CI)	p-value	Adjusted OR (95%CI)	P-value
	Yes	No				
Birth weight						
≥ 2.5 kg	24	304				
< 2.5kg	4	18	2.8 (0.9 – 8.9)	0.08	2.4 (0.7-8.6)	0.16
Maternal Hb-level*						
≥ 11g/dl	6	104				
< 11g/dl	20	140	2.5 (0.9– 6.4)	0.061	3.4 (1.3-10.2)	0.01
GA at birth						
≥ 37 weeks	22	302				
<37 weeks	6	20	4.1 (1.5 – 11.3)	0.006	3.8 (1.3-11.4)	0.02

Abbreviations: GA = Gestational age; OR= odds ratio; CI = confidence interval; Hb = Haemoglobin level

*There were 80 participants with no Hb results

Number of IPTp-SP doses as a risk factor for PM
 The study showed that, use of 2 doses of SP during pregnancy did not significantly reduce the risk of developing PM (OR= 0.5; CI 0.2 – 1.1), low birth weight (OR=0.9; 0.3 – 2.1) and maternal anaemia (OR=1.1; CI 0.7 – 1.8). However, 2 doses of SP was significantly associated with reduced risk of preterm birth to as much as ten times when compared to the ones who took single dose or none (OR=0.1; CI 0.05 – 0.4) (Table 4)

TABLE 4 - Effectiveness of IPTp-SP against placental malaria and adverse pregnancy outcome

Birth outcome	IPTp-SP use		Crude OR (95%CI)	p-value	Adjusted OR (95%CI)	P-value
	< 2 doses	2 doses				
Placental malaria						
Negative	148	174				
Positive	18	10	0.5 (0.2 – 1.1)	0.067	0.5(0.2-1.1)	0.08
Birth weight						
2.5kg and above	154	174				
< 2.5kg	12	10	0.7 (0.3 – 1.8)	0.491	0.9 (0.3-2.1)	0.73
Maternal Hb-level						
≥ 11g/dl	60	50				
< 11g/dl	82	78	1.1 (0.7 – 1.9)	0.594	1.1 (0.7-1.8)	0.71
GA age at birth						
≥ 37 weeks	144	180				
<37 weeks	22	4	0.1 (0.05 – 0.4)	0.001	0.1 (0.05-0.4)	< 0.001

Abbreviations: GA = Gestational age; OR= odds ratio; CI = confidence interval; Hb = Haemoglobin level

*There were 80 participants with no Hb results

Discussion

Malaria in pregnancy is known to be a major public health problem that affects millions of women in tropical and subtropical regions. In many parts of Africa, the prevalence of PM is substantially high to about 25%^{9,21}. Our data shows the prevalence of PM to be 28 (8%) in the study site along the coast of Tanzania. The findings are similar to two previous studies that were conducted almost 7 years ago in two districts in Morogoro, Central Tanzania, areas within share similar malaria transmission intensity with Rufiji^{19,25}. This may be an important concern of having a constant prevalence of PM for a period of time despite on-going malaria control interventions in the country.

We show in our study that primigravida were at higher risk of PM compared to multigravida. It is understood from previous studies that primigravida are at a higher risk of PM because of the absence of previous exposure to *P.falciparum* and therefore they have not yet developed protective antibodies that block the adhesion of parasitized erythrocytes to chondroitin sulfate A (CSA) in the placental intervillous space to promote clearance of parasites^{6,11}. Protective immunity to *P. falciparum* malaria is therefore acquired following prolonged exposure in areas of endemic parasite transmission. Primi-

gravidity as a risk factor for PM is a consensus among many studies that usually find it as an important risk factor for PM^{1,12,31}. It is therefore important to prioritize malaria control measures among primigravida. History of fever was found to be a significant predictor of PM in this study. It is most likely in our case that, fever cases that were observed could have been secondary to previous malaria illness and/or PM. However, our study had limitations to differentiate between non malarial fever and confirmed malarial illness during pregnancy period. Fever during pregnancy has also been reported as an important predictor of PM in areas with stable malaria transmission³⁵.

We found in this study that the odds for women with PM to develop maternal anemia and to have preterm births were 3 and 4, respectively. Despite the absence of statistical significance for PM to predispose pregnant women to deliver babies with low birth weights, a weak predisposition of 2.4 times risk was seen. It has been explained in literatures that, parasitized erythrocytes in placenta induce local immunologic responses and complement activation that cause dysregulation of angiogenesis that leads to placental function insufficiency, fetal growth restriction and ultimately low birth weight^{6,36}. PM in this study was found to predispose significantly

pregnant women to maternal anemia ($P=0.01$) and preterm birth ($P=0.02$) but not low birth weight ($P=0.16$). There is a great extent of disparity between findings from different geographical regions. A study done in high malaria transmission areas of eastern Sudan which showed no significant association between PM and low birth weight (Adam et al. 2010). Study done in Accra Ghana by Ofori showed that, PM was significantly associated with low birth weight and maternal anemia³⁰. In another study done by Bako and colleagues in North-Eastern Nigeria where malaria transmission is high, PM was significantly associated with low birth weight and cord parasitaemia³. Study done in Senegal by N'Dao and others showed PM was significantly associated with low birth weight, preterm birth and perinatal mortality²⁶.

In malaria endemic countries like Tanzania, proportions of parasitized erythrocytes in pregnant women are usually higher in the placenta than in peripheral blood^{18,24,32} and with peripheral blood slides examination as the standard diagnostics, the real burden of malaria in pregnancy is almost always underestimated in these areas. Tanzanian National Malaria Control Program implements administration of at least two doses of SP in the second and third trimester as a standard regimen for controlling malaria in pregnancy. We show in our study however that, only half (52.6%) of pregnant women took the recommended two doses of SP. A lesser proportion of 40.5% took a single dose whereas 6.9% never took any SP dose during pregnancy. According to reports from the National Bureau of Standards (NBS) and Tanzania Demographic and Health Survey (TDHS) in 2010, about one third of pregnant mothers (32.3%) took at least two SP doses as recommended by the national policy. Our data show a notable increase of SP use for IPTp from 32.3% in 2010 to 52.6% in 2012 of pregnant women. All these findings possess a major challenge to the newly recommended IPTp regimen that requires more than 3 doses of IPTp to be administered in pregnancy.

In order to determine the effectiveness of SP for IPTp, PM was considered the primary indicator since is determined solely by the presence of parasites in a non multi-factorial manner. However, secondarily, the effectiveness of SP for IPTp was also determined based on occurrence of adverse pregnancy outcome; low birth weight, maternal anemia and preterm birth. We show in

this study that, pregnant women who took two doses of SP were not significantly protected against PM, LBW, MA. Preliminary data from previous studies have suggested a continued trend in reduction of effectiveness of SP for IPTp in many African countries^{13,37,39}.

Our data supports the growing concern over the decreasing effectiveness of the two-dose regimen of SP for IPTp in high malaria transmission regions of East Africa^{17,33}. With a great overlap between malaria and HIV incidences, the usefulness of SP for IPTp is not only currently questionable due to its widespread and increasing resistance, but also its interaction with HIV infections during pregnancy. The continued effectiveness of SP in such areas is therefore challenged by two parameters that must be addressed together if IPTp programs have to be useful.

With growing resistance of the parasite to SP, it is important that the regimen for SP use for IPTp is reviewed. Simply increasing the dose to 3 doses and above may require careful evaluation especially because SP is currently resisted by parasite strains isolated almost all over Tanzania. SP has been a relatively safe drug for use for IPTp, with the advantage of being taken once at a time thus maximizing compliance. The use of most other anti-malarial drugs for IPTp, e.g. ACTs, is challenged by their potential safety concern to fetus. At a point when the use of SP for IPTp is thus challenged, an integrated program that involves the use of alternative drugs, ITNs, IRS, and other strategies could be the most viable approach to controlling malaria during pregnancy.

Conclusion

The main result in our study was that, the use of two doses of SP, among pregnant women in Rufiji in Tanzania could not reduce the risk prevalence of PM as assessed by presence of parasitemia. Furthermore, we observed that SP use for IPTp did not have significantly direct benefit against PM, LBW, MA, although it was protective against pre term birth. Hence special consideration needs to be made when planning for malaria protective measures in pregnancy. The current SP regimen should be reviewed to accommodate the effect of increased resistance by parasites by either increasing the number of doses or devising and integrated interventional program that will include other effective malaria control approaches such as IRS and use of ITNs. Having a small sample size and limited to one study

district are important study limitations of which the finding cannot easily be generalized in the Tanzanian population.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RN and DM conceived the study and carried out data collection and analyses; AJ and JC contributed to the study design and data analysis; RN wrote the first draft; and JC and DM revised and developed the final submitted manuscript version. All authors contributed to the manuscript, read and approved the final submitted version.

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