Effectiveness of Intermittent Preventive Treatment With Dihydroartemisinin-Piperaqunine Against Malaria in Pregnancy in Tanzania: A Randomized Controlled Trial

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Intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) to prevent malaria and adverse birth outcomes is threatened by *Plasmodium falciparum* resistance to sulfadoxine-pyrimethamine. We investigated the effectiveness of intermittent preventive treatment in pregnancy with monthly dihydroartemisinin-piperaquine (IPTp-DHP) as an alternative option to IPTp-SP. A total of 956 malaria-free (malaria rapid diagnostic test (MRDT) negative) pregnant women from moderate malaria transmission areas in Tanzania were enrolled and randomized to receive monthly IPTp-DHP (n = 478) or IPTp-SP (n = 478) and followed for maternal and birth outcomes. The primary outcome was the prevalence of histopathologically confirmed placental malaria (active or past infection). Secondary outcomes were overall malaria at delivery, symptomatic-malaria, parasitemia during pregnancy, and adverse birth outcomes as a composite of spontaneous-abortion, premature birth, stillbirth, and low birth weight (LBW) fetal anemia. Outcome differences between treatment groups were expressed as the protective efficacy (PE), defined as 1-prevalence ratios or 1-incidence rate ratio. The prevalence of histopathologically confirmed placental malaria was significantly lower in IPTp-DHP (2.5%, 12/478) than IPTp-SP (8.2%, 39/478); PE = 69% (95% confidence interval (CI): 42–84, P < 0.001). The prevalence of maternal malaria at delivery was significantly lower in IPTp-DHP (8.2%) than IPTp-SP (18.2%, P < 0.001). The incidence per person-years at risk for symptomatic-malaria (0.02 vs. 0.12, P = 0.002) and parasitemia during pregnancy (0.28 vs. 0.67, P < 0.001) were significantly lower in the IPTp-DHP group than in the IPTp-SP group. The prevalence of any adverse birth outcomes (composite) was not significantly (P = 0.06) different between IPTp-DHP (17.9%) and IPTp-SP (23.8%). However, the prevalence of LBW (4.6% vs. 9.6%, P = 0.003) was significantly lower in IPTp-DHP compared with IPTp-SP. We report superior protective efficacy of monthly IPTp-DHP against malaria in pregnancy and LBW than IPTp-SP.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Intermittent preventive treatment in pregnancy with monthly dihydroartemisinin–piperaquine (IPTp-DHP) is safe and prevents malaria in pregnancy more than the standard of care intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) in high malaria transmission settings when initiated early in the second trimester (≤ 20 weeks gestational age). But data from a moderate malaria transmission area is lacking.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This randomized clinical trial compared the effectiveness of monthly IPTp-DHP against the standard of care IPTp-SP initiated during the second and third trimester of pregnancy from an area with moderate malaria transmission.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Compared with the standard IPTp-SP, we report significantly higher protective efficacy of IPTp-DHP against malaria during pregnancy, malaria at delivery, and improves infant birth weight in moderate malaria transmission settings.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This study provides an evidence-based recommendation of monthly IPTp-DHP against malaria in pregnancy and low birth weight for policymakers to revise IPTp strategies and guidelines in malaria-endemic areas with high rates of P-*falciparum* resistance to sulfadoxine-pyrimethamine.

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Malaria in pregnancy is a significant public health problem affecting more than 11 million pregnant women in sub-Saharan Africa.[1] *Plasmodium falciparum* infection causes maternal illness, anemia, premature birth, stillbirth, low birthweight (LBW), and associated mortality.[1–3] To prevent malaria during pregnancy, the World Health Organization (WHO) recommends intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) in addition to insecticide-treated nets (ITNs) use and effective case treatment.[4] However, the high prevalence of *P. falciparum* resistance to sulfadoxine-pyrimethamine (SP)[5,6] compromises the effectiveness of IPTp-SP in sub-Saharan Africa.[5,7,8]

The spread of *P. falciparum* resistance to SP has prompted an urgent need for alternative strategies to control malaria in pregnancy. Different studies compared intermittent preventive treatment in pregnancy (IPTp) with amodiaquine, amodiaquine-SP, mefloquine, chloroquine-azithromycin, and azithromycin-SP vs. IPTp-SP.[9–12] However, these alternatives failed to replace IPTp-SP due to poor tolerance. Furthermore, intermittent screening and treatment in pregnancy (ISTp) using artemisinin-based combination therapy (ACTs) was also investigated. ISTp with artemether-lumefantrine in West Africa,[13] and dihydroartemisinin-piperaquine (DHP) in Malawi and Kenya[14,15] were not superior to IPTp-SP. Based on findings from these trials, ISTp was not recommended by the WHO due to the limited sensitivity of malaria rapid diagnostic tests.[16]

Nevertheless, ACTs provide attractive features for IPTp. For instance, a systematic review reported that ACTs had the lowest parasitological failure rates than SP when used for the treatment or chemoprevention of malaria in pregnancy.[17] Besides, previous randomized controlled trials have shown that ACTs were safe and effective for treating malaria during the second and third trimesters.[18,19] However, in sub-Saharan Africa, most malaria infections in pregnancy are asymptomatic. This necessitates the need to expand the role of ACTs to IPTp. Among the ACTs, DHP is the most attractive option for this treatment approach given its safety, efficacy, once-daily dosing, and the longest post-treatment prophylactic effect.[20]

So far, three clinical trials from high malaria transmission intensity areas in East Africa explored the use of DHP for the prevention of malaria in pregnancy. Two previous trials indicated that intermittent preventive treatment in pregnancy with monthly dihydroartemisinin-piperaquine (IPTp-DHP) resulted in a significant reduction of malaria infection during pregnancy compared with IPTp-SP.[15,21] The third study compared monthly IPTp-SP vs. IPTp-DHP initiated at 16 or 20 weeks of gestation reported that monthly IPTp-DHP is safe and result in a better malaria protective efficacy, but no significant improvements in birth outcomes compared to IPTp-SP.[22] However, in many sub-Saharan African countries, including Tanzania, most pregnant women initiate their first antenatal care (ANC) visit after 20 weeks of gestational age.[23] Therefore, it is unclear whether monthly IPTp-DHP commenced during the second and third trimesters would still be effective in preventing malaria during pregnancy. Furthermore, data from the moderate malaria transmission area is currently lacking and is crucial, given the fact that transmission intensity affects the effectiveness of the intervention. Moreover, more data are needed on the impact of IPTp-DHP on improving birth outcomes. Thus, larger sample size trials from different geographical locations with high levels of parasite resistance to SP are needed to provide further evidence-based recommendations for policy change as stressed by the WHO Malaria Policy Advisory Committee.[24]

In a randomized clinical trial, this study compared the effectiveness of monthly DHP against the standard SP for intermittent prevention of malaria in pregnancy and adverse birth outcomes in a moderate malaria transmission setting in southeast Tanzania.

METHODS

Study design and setting

This was an open-label, two-group parallel randomized controlled trial to investigate the protective efficacy of IPTp-DHP vs. IPTp-SP against malaria in pregnancy and adverse birth outcomes. The study was conducted at Kibiti Health Center, Kibiti district, in the Coast region southeast Tanzania, where the prevalence of quintuple and sextuple haplotypes conferring resistance to SP were reported to be 90.2% and 1%, respectively.[6,25]

Study participants

The study targeted pregnant women attending their first ANC at the study site. The inclusion criteria were HIV-uninfected, age 16 years or older, malaria negative (rapid diagnostic test (RDT)), gestational age of ≥ 13 weeks, willing and able to give informed consent. Exclusion criteria were a history of malaria for the past 1 month, clinical malaria, and severe anemia. Women with patent malaria were excluded because they were treated with artemether-lumefantrine, which may compromise the study intervention.

Randomization and blinding

The randomization list was generated by using permuted blocks of different sizes (4 or 8) to ensure balance and unpredictability overall. An independent statistician performed a computer-generated randomization list. A set of sequentially numbered, opaque, sealed, indistinguishable envelopes containing either active intervention or standard were prepared. Enrolled pregnant women were randomly assigned (1:1) by the allocation of the next sequentially numbered envelope. Midwives and clinicians responsible for delivery and collection of birth outcomes, and laboratory scientists responsible for processing and analysis of samples for outcome measures were blinded to treatment group allocation.

Study procedures

Pregnant women in the intervention group received IPTp-DHP (D-ARTEPP; Guilin Pharmaceutical Co. Ltd, China). Participants received 3 tablets, each containing 40 mg of dihydroartemisinin and 320 mg of piperaquine once a day for 3 consecutive days. The first dose was given as directly observed therapy at the ANC. Participants were emphasized to take the remaining second and third doses at home 24th and 48th hours after the first dose, respectively. Self-reported adherence at the day 7 visit since enrollment was used to assess the adherence of drugs administered at home. The control arm received the standard of care consisting of IPTp-SP with a single dose of 3 tablets, each containing 500 mg of sulfadoxine and 25 mg of pyrimethamine (Orodar; Elys Chemical Industries Ltd., Kenya) as directly observed therapy at the ANC. If vomiting occurred within the first 30 minutes, the full dose was repeated. At enrollment, participants received long-lasting ITNs for their beds.

Participants follow-up

Regular study visits were scheduled monthly, where a finger-prick blood sample was collected for detection of malaria parasites (RDT

and polymerase chain reaction (PCR)) and determination of hemoglobin (Hb) level. At each monthly ANC visit, participants in either the IPTp-SP group or IPTp-DHP group received their respective study drugs. During unscheduled visits, all participants who presented with fever (temperature ≥ 37.5°C) or a history of fever in the previous 24 hours were screened for malaria using RDT and microscopy.

At delivery, a standardized assessment form was completed, including newborn sex, congenital anomalies, measurement of birth weight, and any adverse birth outcomes. Maternal venous blood, placental blood, and cord blood were collected in EDTA tubes and screened for malaria (RDT, microscopy, and PCR). Furthermore, two pieces of placental biopsy from the maternal side (2 cm^3) were collected immediately after delivery and fixed with 10% buffered formalin for histopathological malaria detection.

After delivery, participants were followed for 6 weeks, where any adverse events, including congenital anomalies, were assessed. All trial data were double entered into electronic case report forms database Census and Survey Processing System version 7 (CSPro 7; US Census Bureau, Suitland, MD).

Diagnosis of anemia

Hemoglobin concentrations were determined from maternal peripheral finger-pricks blood at enrollment, during each scheduled ANC visit, and from maternal-venous blood at delivery using digital HemoCue Hb 201+ analyzer (HemoCue AB, Angelholm, Sweden). Maternal anemia was confirmed when maternal blood Hb level was < 11 g/dL.[26] Fetal anemia was confirmed when cord blood Hb was < 12.5 g/dL.[27]

Detection of malaria by RDT and microscopy

Malaria *Pf*/PAN (HRP2/pLDH) Ag Combo RDTs (Care start, ACCESS BIO Somerset, NJ) was used for the detection of malaria. RDTs were performed and read according to the manufacturer's instructions. Thick blood smears stained with 2% Giemsa were prepared and read by experienced laboratory technicians. A thick blood smear was considered malaria negative when the examination of 100 high-power fields did not reveal asexual parasites and/or gametocytes.

Detection of malaria by histopathology

Placental tissues were processed and analyzed according to the standard procedures as previously described.[28,29] Histopathological slides were read in duplicate by two independent readers. Discrepant readings were taken to a third reader, and conclusive results were based on two readers. Evidence for positive placental malaria was concluded when malaria parasites indicating active infection and/or malaria pigments indicating past infection were observed.

Detection of malaria by real-time PCR

Three circles of 3 mm in diameter from each dried blood spots were punched for genomic DNA isolation using QIAamp DNA blood micro kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. The 7500 Fast real-time PCR system (Applied Biosystems, Foster City, CA) was used to screen for *Plasmodium* infection (*P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*) targeting the 18S rRNA gene, as described previously.[30] Briefly, the master mix for a single reaction included species-specific probes and forward primers for all four *Plasmodium* species used in combination with a conserved reverse primer. The *P. ovale*-, *P. malariae*-, *P. vivax*-, and *P. falciparum*-probes, each labeled with a distinct fluorophore and Mustang Purple (Applied Biosystems), were used as the reference dye. Each multiplex PCR reaction was performed in duplicate in a final volume of 15 µL per well containing 3 µL DNA, 7.5 µL of TaqMan multiplex master mix (Applied Biosystems), 0.3 µL (10 µmol/L) of species-specific forward primers, 0.75 µL (10 µmol/L) of the reverse primer, 0.15 µL (10 µmol/L) of the species-specific probe, passive reference dye Mustang Purple, and DNA/RNA-free water. The samples were run using 45 cycles of PCR starting with initial denaturation at 95°C for 20 seconds, followed by thermal cycles of 95°C for 3 seconds and 60°C for 20 seconds. Standards, negative, and species-specific positive controls were included on each plate. The assay was optimized to detect all species simultaneously.

Study outcomes

The primary outcome was the prevalence of histopathologically confirmed placental malaria, defined as the presence of any malaria parasites and/or malaria pigment in the placental tissue at delivery. A post hoc secondary analysis for histopathological placental malaria considering active and past infections was done. Secondary outcomes included (i) any malaria at delivery detected by histopathology, RDT, microscopy or PCR from a maternal, cord, or placental blood (ii) prevalence of symptomatic malaria during pregnancy, (iii) prevalence of asymptomatic malaria (parasitemia) during pregnancy, (iv) anemia during pregnancy (Hb level, < 11 g/dL), and (v) any adverse birth outcomes, including spontaneous abortion (< 28 weeks of gestational age), fetal anemia (cord blood Hb < 12.5 g/dL), stillbirth (≥ 28 weeks of gestational age), LBW (birth weight < 2,500 g), preterm delivery (delivery < 37 weeks gestational age), and congenital anomaly.[22] Any adverse birth outcome was defined by a composite of LBW, premature birth, spontaneous abortion, stillbirth, and fetal anemia. Additionally, we did a post hoc analysis on adverse birth outcome by excluding fetal anemia. Assessment of drug safety was done by monitoring the prevalence of vomiting after administration of study drugs and the incidence of adverse events after initiation of drug intake up to 6 weeks post-partum.

Statistical analysis

The sample size was calculated, as described previously.[31] Based on previous data,[32] we assumed a 10% prevalence of histopathological placental malaria in pregnant women who received IPTp-SP. Considering the previous trial[21] that reported 45% protective efficacy of IPTp-DHP in a high malaria transmission area, we anticipated the prevalence of histopathological placental malaria to be 4% in the IPTp-DHP group, which is a 60% reduction in the prevalence of histopathological placental malaria as compared to IPTp-SP. To detect such a difference with 80% statistical power at the 2-sided 5% level of significance and 15% loss to follow up, a total of 956 pregnant women were needed (478 women per each group).

Primary outcome data were analyzed according to intention to treat population in which data from all participants allocated to a treatment group at enrollment were used. A supportive secondary analysis was done using per protocol analysis considering data from participants who completed the study and primary outcome (histopathological malaria) data were collected at delivery (**Figure 1**). In a post hoc analysis, we compared the associations between treatment groups and the efficacy outcomes by gravidity. Depending on suitability, the χ^2 test or Fisher's exact test were used to compare proportions between groups. For continuous variables, an independent *t*-test was used to compare means of normally distributed data, whereas the Mann-Whitney *U* Test was used to compare mean ranks of skewed data between the treatment groups. Rates of malaria detection at each ANC visit over time during pregnancy were compared using the Kaplan–Meier plot and log-rank test. Incidence measures were compared using Poison regression model for count data measured during the follow-up period. Incidence rate ratios (IRR) were defined as the incidence of outcomes in the intervention IPTp-DHP divided by the incidence in the control IPTp-SP group. Prevalence ratios were defined as the measures of outcomes at enrollment or at delivery in the intervention IPTp-DHP group divided by the prevalence in the control IPTp-SP group. Point estimates of differences between the treatment groups were expressed as the protective efficacy (PE), defined as 1-prevalence ratio or 1-IRR. Statistical Package for Social Sciences (SPSS) software (IBM, Armonk, NY) was used for data analysis. The significance level was set at 0.05, and the confidence level at 95%. A *P* value of < 0.05 was considered to indicate statistical significance. In addition, comparisons of study outcomes between the

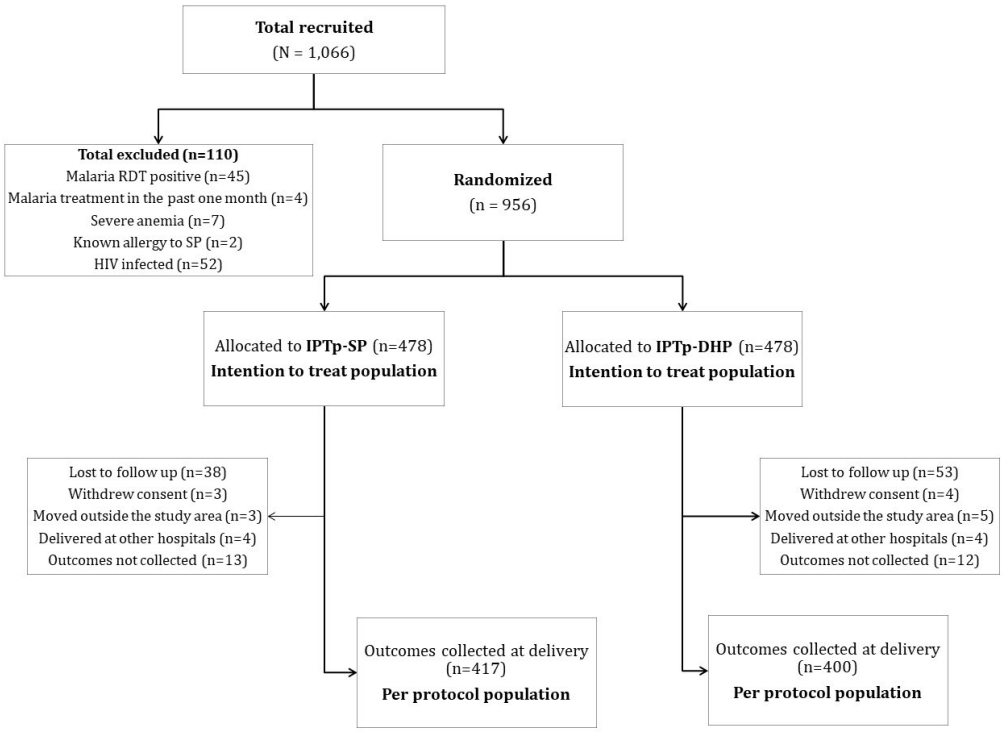

Figure 1 Study flow chart.

two treatment groups were made by including and excluding women who had sub-patent malaria (parasitemia detected by PCR but not recognized by RDT or microscopy) at enrollment.

Ethics approval

Ethical approval was granted by the Institutional Review Boards of the Tanzania National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/2342), and the Muhimbili University of Health and Allied Sciences (MUHAS; 2016-06-07/AEC/Vol.XI/2). Written informed consent was obtained from all study participants before enrollment. This clinical trial was registered at the WHO | Pan African Clinical Trial Registry (PACTR201612001901313) before participants' recruitment.

RESULTS

Between January 2017 and May 2019, 956 pregnant women were enrolled, randomly assigned to 2 treatment groups and followed until delivery (**Figure 1**). Baseline characteristics were similar between the two treatment groups (**Table 1**).

Malaria during pregnancy

The incidence of symptomatic malaria and parasitemia during pregnancy is shown in **Table 2**. During the follow-up period, the incidence of symptomatic malaria was significantly lower in the IPTp-DHP group compared with IPTp-SP group (**Table 2**). Equally, rates of parasitemia at each scheduled ANC by RDT and/or PCR was significantly lower in the IPTp-DHP group compared with the IPTp-SP group (hazard ratio, 0.35, 95% confidence interval (CI): 0.22 to 0.55; see **Table ,2 Figure 2**). There was no significant effect modification by gravidity for both symptomatic malaria and parasitemia at each scheduled ANC visit (**Figure 3**).

Anemia during pregnancy

There was no significant difference in the prevalence of anemia between the IPTp-SP and IPTp-DHP groups at enrollment (**Table 1**), during ANC, and at delivery (**Table 3**). Similarly, 1.5% (7/478) women in the IPTp-DHP group had severe anemia (Hb < 7 g/dL) at delivery compared with 1.1% (5/478) in the IPTp-SP group with no significant difference. Two cases of severe anemia in the IPTp-SP group and one case in the IPTp-DHP group were associated with malaria at delivery. There was no significant effect modification by gravidity for maternal anemia during pregnancy (**Figure 3**).

Malaria at delivery

At delivery, compared to IPTp-SP, IPTp-DHP was significantly associated with a lower prevalence of histopathological placental malaria (active or past infection; **Table 2**). In post hoc secondary analysis, IPTp-DHP was significantly associated with lower prevalence of active histopathological placental malaria compared with IPTp-SP (**Table 2**). However, no significant difference was found for the past histopathological placental malaria (pigment) between the two treatment groups (**Table 2**). The detection of malaria in the placental blood, maternal venous blood, and cord blood using RDT, microscopy, and PCR were significantly lower in the IPTp-DHP group compared with the IPTp-SP group (**Table 2**). Furthermore, the prevalence of any malaria at delivery was significantly lower in the IPTp-DHP group than in the IPTp-SP group (**Table 2**). There was no significant effect modification by gravidity on any malaria during pregnancy and at delivery (**Figure 3**).

In a separate secondary analysis, where data from women with sub-patent malaria at enrollment were excluded, IPTp-DHP was

Table 1 Characteristics of study participants at baseline and during pregnancy

Characteristics		IPTp-SP (*n* = 478)	IPTp-DHP (*n* = 478)
Age category *n* (%)	Adolescent (< 20 years)	95 (19.9)	94 (19.7)
	Young (20–34 years)	294 (61.5)	298 (62.3)
	Adult (> 35 years)	89 (18.6)	86 (18)
Gravidity *n* (%)	Primigravida	128 (26.8)	115 (24.1)
	Secundigravida	105 (22)	108 (22.6)
	Multigravida	245 (51.2)	255 (53.3)
ANC *n* (%)	Early (13–20 weeks)	220 (46)	213 (44.6)
	Late (≥ 21 weeks)	258 (54)	265 (55.4)
Trimesters *n* (%)	Second (13–27 weeks)	435 (91)	433 (90.6)
	Third (≥ 28 weeks)	43 (9)	45 (9.4)
Education level *n* (%)	No formal education	95 (19.9)	91 (19)
	At least primary education	383 (80.1)	387 (81)
Baseline ITNs use *n* (%)	Yes	345 (72.2)	350 (73.2)
	No	133 (27.8)	128 (26.8)
ITNs use during pregnancy *n* (%)	Yes	435 (91)	431 (90.2)
	No	43 (9)	47 (9.8)
Anemia at enrollment, Hb < 11 g/dL *n* (%)		286 (59.8)	289 (60.5)
Mean age in years (SD)		26.6 (7)	26.8 (8)
Mean hemoglobin g/dL (SD)		10.4 (1.4)	10.3 (1.3)
Median parity, number of live births (range)		2 (0–9)	2 (0–9)
Median gestational age in weeks (range)		21 (14–32)	22 (14–32)
Median body temperature in °C (range)		37 (34.3–37)	37 (34–37.2)
Median height in cm (range)		151 (146–164)	151 (145–168)
Median body weight in kg (range)		54 (39–95)	54 (38–95)
Median IPTp doses received (range)		3 (1–5)	3 (1–5)

ANC, antenatal care; IPTp-DHP, intermittent preventive treatment in pregnancy with dihydroartemisinin-piperaquine; IPTp-SP, intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine; ITNs, insecticide-treated bed nets; range, minimum-maximum.

also associated with significant reduction in symptomatic malaria and parasitemia during pregnancy and any malaria at delivery by all methods as compared to IPTp-SP (**Figure 4**).

Adverse birth outcomes

The prevalence of any adverse birth outcomes did not differ significantly between the IPTp-DHP and IPTp-SP groups (**Table 3**). There was no significant effect of modification by gravidity on any adverse birth outcome (**Figure 3**). However, in a post hoc analysis when the composite adverse birth outcome was analyzed by excluding fetal anemia, IPTp-DHP was significantly associated with lower prevalence of any adverse outcomes compared with IPTp-SP (**Table 3**). Similarly, IPTp-DHP was significantly associated with a lower prevalence of LBW compared to IPTp-SP (**Table 3**). The mean difference in birth weight was 55 grams (95% CI: 19–93) being higher in the DHP arm $P = 0.004$. When data from women who had sub-patent malaria at enrollment were excluded in the analysis, no significant difference in any adverse birth outcomes was found between IPTp-DHP and IPTp-SP groups, but the prevalence of LBW became significantly lower in IPTp-DHP group than the IPTp-SP group (**Figure 4**).

Safety and tolerability

There was no significant difference in the prevalence of adverse drug events between the treatment groups. A total of 5.2% of women (25/478) who received IPTp-SP experienced adverse drug events compared with 3.7% (18/478) women who received IPTp-DHP ($P = 0.26$). Nausea was the most common reported adverse drug event with no significant difference between the IPTp-SP group (5.0%, 24/478) and the IPTp-DHP group (3.1%, 15/478), $P = 0.14$. In addition, vomiting occurred in 0.4% of women (2/478) who received IPTp-SP compared with 0.6% of women (3/478) in the IPTp-DHP group with no significant difference; Fischer's exact test $P = 0.66$. One incidence of moderate skin rash was reported in the IPTp-SP group. There were no neonatal congenital anomalies, neonatal, or maternal deaths observed at delivery and at 6 weeks post-delivery in both groups.

DISCUSSION

The present randomized controlled trial evaluated the effectiveness of monthly IPTp-DHP vs. the standard IPTp-SP for the prevention of malaria during pregnancy and adverse birth outcomes. Our main finding shows that, compared with IPTp-SP, monthly IPTp-DHP displays significantly higher protective efficacy against (i) histopathologically confirmed placental malaria and parasitemia at delivery and (ii) symptomatic malaria and parasitemia during pregnancy, (iii) although no significant differences in the prevalence of any adverse birth outcome between the two treatment groups were found, the incidence of LBW was significantly lower in the IPTp-DHP group than in the IPTp-SP group. To our knowledge, this is the first randomized clinical trial to investigate the effectiveness of IPTp-DHP for the prevention of malaria in pregnancy and adverse birth outcome from a setting with moderate malaria transmission intensity and high *P. falciparum* resistance to SP, and also the first to report significantly higher protective efficacy of monthly IPTp-DHP against adverse birth outcomes especially LBW.

Compared with the standard IPTp-SP, our result indicates that monthly IPTp-DHP significantly reduced the risk of malaria during pregnancy. The incidence of asymptomatic malaria (parasitemia) during pregnancy was significantly lower in the IPTp-DHP group, with 59% more protective efficacy as compared to IPTp-SP. Furthermore, IPTp-DHP significantly reduced the risk of symptomatic malaria during pregnancy, with 86% protective efficacy compared with IPTp-SP. The significantly higher protective efficacy of IPTp-DHP against malaria during pregnancy could be explained by the long malaria prophylactic effect associated with long piperaquine elimination half-life.[33] The piperaquine component in DHP has the longest elimination half-life (about 30 days) among the partner drugs in the currently recommended ACTs.[33] The long elimination half-life of piperaquine provides long post-treatment

Table 2 Malaria detection during pregnancy and at delivery between the treatment groups

		Intention to treat analysis						Per protocol analysis					
		IPTp-SP (*n* = 478)		IPTp-DHP (*n* = 478)		Protective efficacy (95% CI)		IPTp-SP (*n* = 417)		IPTp-DHP (*n* = 400)		Protective efficacy (95% CI)	
Samples screened for malaria	Methods	*n*	%	*n*	%		*P* value	*n*	%	*n*	%		*P* value
Placental tissue	Histopathology (active and past infection)	39	8.2	12	2.5	69 (42 to 84)	< 0.001	39	9.4	12	3	68 (40 to 83)	< 0.001
	Histopathology (only active infection)	29	6.1	6	1.3	80 (51 to 92)	< 0.001	29	7	6	1.5	78 (49 to 91)	< 0.001
	Histopathology (only past infection)	10	2.1	6	1.3	40 (−64 to78)	0.31	10	2.4	6	1.5	37 (−71 to 77)	0.34
Placental blood	RDT	11	2.3	2	0.4	82 (18 to 96)	0.03	11	2.6	2	0.5	81 (15 to 96)	0.02
	Microscopy	10	2.1	2	0.4	80 (9 to 96)	0.04	10	2.4	2	0.5	79 (5 to 95)	0.02
	PCR	34	7.1	10	2.1	71 (41 to 85)	< 0.001	34	8.2	10	2.5	69 (39 to 85)	< 0.001
	RDT, microscopy or PCR	38	7.9	12	2.5	68 (40 to 83)	< 0.001	38	9.1	12	3	67 (38 to 83)	< 0.001
Placental tissue and blood	Histopathology, RDT, microscopy, or PCR	64	13.4	21	4.4	67 (47 to 80)	< 0.001	64	15.3	21	5.3	66 (45 to 79)	< 0.001
Maternal blood at delivery	RDT	12	2.5	3	0.6	75 (12 to 93)	0.03	12	2.9	3	0.8	74 (8 to 92)	0.02
	Microscopy	9	1.9	1	0.2	89 (13 to 98)	0.04[a]	9	2.2	1	0.2	85 (10 to 98)	0.02[a]
	PCR	39	8.2	17	3.6	56 (24 to 75)	0.003	39	9.4	17	4.3	55 (21 to 74)	0.005
	RDT, microscopy, or PCR	42	8.8	18	3.8	57 (27 to 75)	0.002	42	10.1	18	4.5	55 (24 to 74)	0.002
Cord blood	RDT	11	2.3	2	0.4	82 (18 to 96)	0.03	11	2.6	2	0.5	81 (15 to 96)	0.005
	Microscopy	10	2.1	2	0.4	80 (9 to 96)	0.04 [a]	10	2.4	2	0.5	79 (5 to 95)	0.02[a]
	PCR	25	5.2	11	2.3	66 (12 to 78)	0.02	25	6	11	2.8	54 (8 to 77)	0.03
	RDT, microscopy, or PCR	25	5.2	11	2.3	66 (12 to 78)	0.02	25	6	11	2.8	54 (8 to 77)	0.03
Any malaria at delivery[b]	Histopathology, RDT, microscopy, or PCR	87	18.2	39	8.2	55 (36 to 71)	< 0.001	87	20.9	39	9.8	52 (32 to 66)	< 0.001
Malaria during pregnancy[c]	Symptomatic malaria	14	(0.12)	2	(0.02)	86 (37 to 97)	0.002	14	(0.13)	2	(0.02)	85 (34 to 97)	0.003
	Parasitemia by RDT	40	(0.34)	12	(0.01)	70 (43 to 84)	< 0.001	40	(0.38)	12	(0.12)	69 (40 to 84)	< 0.001
	Parasitemia by RDT and/or PCR	80	(0.67)	33	(0.28)	59 (38 to 72)	< 0.001	80	(0.77)	33	(0.33)	57 (35 to 71)	< 0.001

Unless otherwise noted, the *P* values were based on χ^2 test.
95% CI, 95% confidence interval; IPTp-DHP, intermittent preventive treatment in pregnancy with dihydroartemisinin-piperaquine; IPTp-SP, intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine; PCR, polymerase chain reaction; RDT, rapid detection test.
[a]Fisher's exact test. [b]Any malaria defined by the detection of malaria parasites and/or pigments in the placental tissue by histology, or parasitemia in the placental blood, maternal blood, and cord blood by RDT, microscopy, or PCR. [c]Number of events (incidence per person-year at risk)

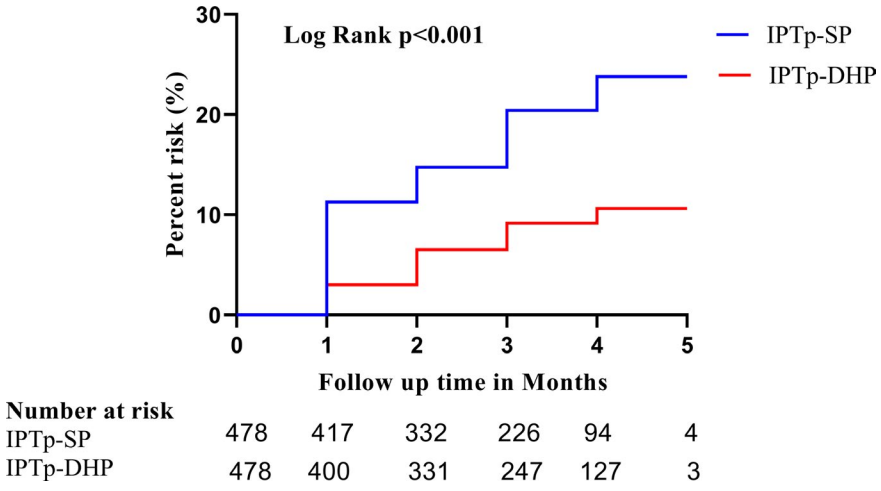

Figure 2 Kaplan–Meier plot with log rank test showing hazard proportions for malaria detection at each antenatal care visit over time during pregnancy stratified by the treatment groups. IPTp-DHP, intermittent preventive treatment in pregnancy with monthly dihydroartemisinin-piperaquine; IPTp-SP, intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine.

prophylaxis, which clears parasite infection and reduces the risk of malaria reinfection.[33] On the other hand, the high prevalence of *P. falciparum* resistance to SP in the study area[6,25] could partly explain the higher risk and burden of malaria in the IPTp-SP group.

At delivery, IPTp-DHP was associated with significantly higher protective efficacy against maternal malaria, compared with IPTp-SP. The prevalence of histopathological placental malaria was significantly lower in the IPTp-DHP group with 69% protective efficacy as compared to the IPTp-SP group. DHP might have cleared malaria parasites and hemozoin pigments in the placenta,[34] thus contributed to significantly lower histopathological placental malaria in the IPTp-DHP group compared with the IPTp-SP group. However, the lack of significant difference for the past histopathological placental malaria between the two treatment groups could reflect old infections that were present before women were randomized. Furthermore, the considerably higher protective effect of malaria infection in the maternal venous blood at delivery, cord blood, and placental blood in the IPTp-DHP group as compared to the IPTp-SP group might be due to reduced risk of malaria infection associated with the long prophylactic effect of piperaquine. In addition, the higher prevalence of quintuple mutations in the area could explain the higher malaria burden in the IPTp-SP group.[6,25]

Our findings indicate that IPTp-DHP was superior to IPTp-SP for the prevention of malaria during pregnancy in an area with moderate malaria transmission intensity and high *P. falciparum* resistance to SP. Our results are similar to previous trials from high malaria transmission areas of Kenya and Uganda that reported higher malaria protective efficacy of IPTp-DHP initiated early in the second trimester against malaria in pregnancy.[15,21,22] Our study adds additional evidence to the literature indicating that monthly IPTp-DHP is superior to IPTp-SP commenced during the early or late second trimester or early third trimester. The significant reduction of malaria infection in pregnant women using monthly IPTp-DHP found in our study is consistent with the findings of the previous trials from Uganda.[21,22] Although caution should be taken when comparing results from different settings, our findings provide further evidence that monthly DHP is a better option to replace SP as IPTp in areas of moderate malaria transmission intensity and high SP parasite resistance.

The observed higher protective efficacy and a significant reduction in risk of malaria infection associated with IPTp-DHP corroborated well with the observed reduced risk for LBW in the same cohort. IPTp-DHP was significantly associated with a lower risk of LBW compared with IPTp-SP. Additionally, in the analysis of adverse birth outcomes that excluded fetal anemia, IPTp-DHP was significantly associated with lower prevalence of adverse birth outcomes as compared with IPTp-SP. This result differs from the previous trials that reported no significant improvement in birth outcomes associated with IPTp-DHP.[15,21,22] Such inconsistent findings could be due to variation in the study design, sample size, and study settings, particularly malaria transmission intensity. We report significantly higher protective efficacy of IPTp-DHP against malaria during pregnancy, malaria at delivery, any adverse birth outcome, and LBW from a moderate malaria transmission area. The mean birth weight was 55 grams (95% CI; 19–93) higher in the DHP arm as compared with the SP arm. The association of malaria in pregnancy and LBW is related to intrauterine growth restriction in several studies.[2] Therefore, the significantly lower risk of LBW in the IPTp-DHP group might be due to the lower risk of malaria infection in pregnant women receiving IPTp-DHP compared with IPTp-SP.

A previous study reported that about 50% of malaria infections at the first ANC visit is sub-patent (not detected by RDT or microscopy).[35] It is therefore expected that IPTp clears such infections in pregnant women. In this study, the primary analysis, which included pregnant women with sub-patent malaria at enrollment, resulted in relatively higher IPTp-DHP protective efficacy against LBW (51%, $P = 0.003$) than when women with sub-patent malaria are excluded (46%, $P = 0.027$) as compared with IPTp-SP. The observed increase in protective efficacy on LBW after including women with sub-patent malaria at enrollment may indicate higher efficiency of DHP to clear sub-patent malaria and its associated

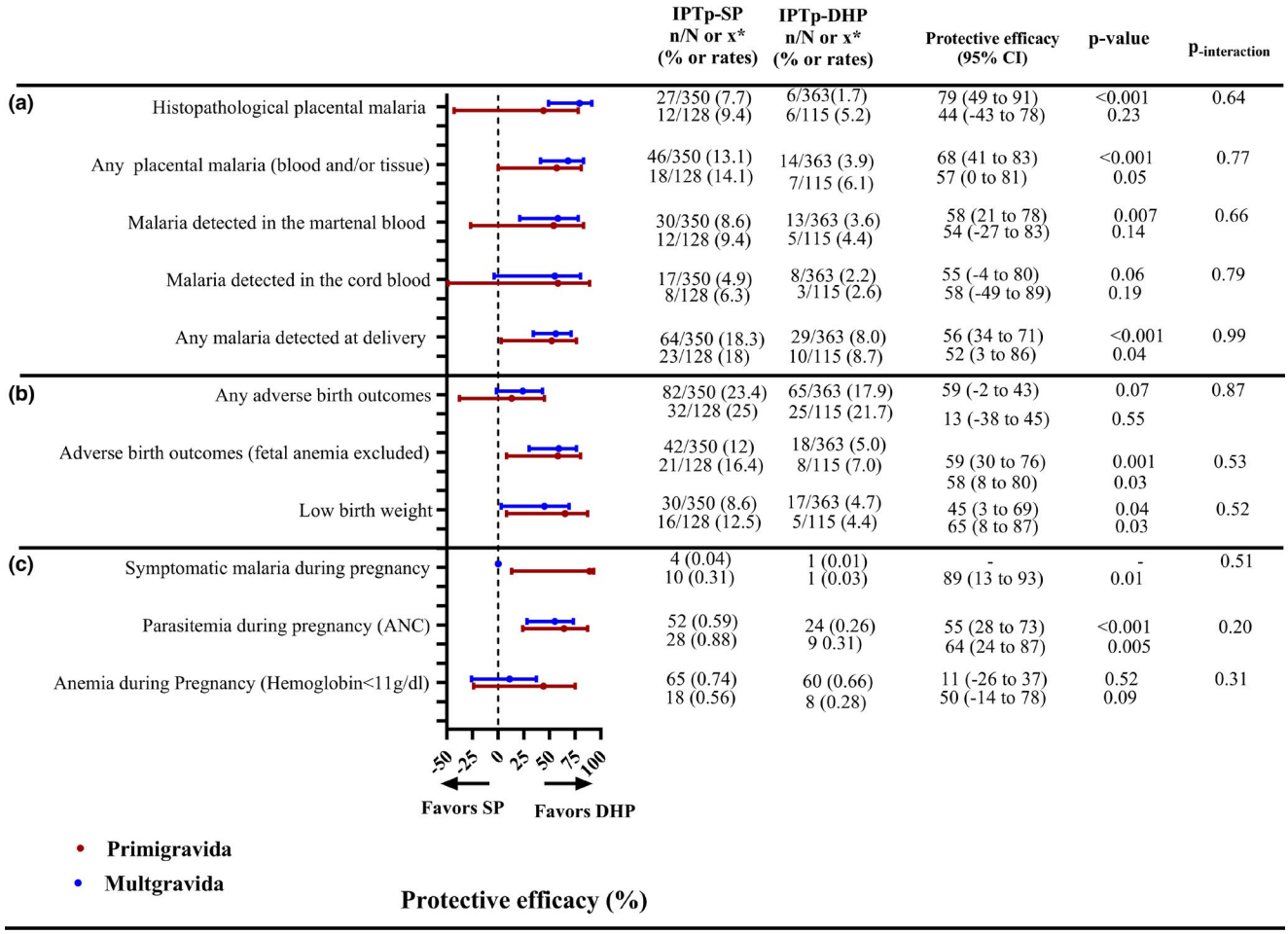

Figure 3 Protective efficacy of the study drugs stratified by gravidity; A: malaria outcomes collected at delivery; B: adverse birth outcomes collected at delivery; C: outcomes collected during pregnancy (incidence measures). ANC, antenatal care; CI, 95% confidence interval; IPTp-DHP, intermittent preventive treatment in pregnancy with dihydroartemisinin-piperaquine; IPTp-SP, intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine; rates, incidence per person-year at risk; x*, the number of women with at least one event.

adverse birth outcomes compared with SP. Measurable difference in adverse birth outcomes, especially LBW, is one of the reasons to influence policy change for IPTp.[36] The results of this trial, therefore, provide insight into the impact of IPTp-DHP in improving LBW from a setting of moderate malaria transmission intensity.

The overall risk of maternal anemia during pregnancy did not differ significantly between the IPTp-DHP and IPTp-SP groups. This finding is contrary to the previous trials from Kenya and Uganda that reported IPTp-DHP significantly reduced the risk of maternal anemia during pregnancy compared with IPTp-SP.[15,21,22] However, malaria is only one of the causes of anemia in sub-Saharan Africa. Therefore, other causes of anemia, such as nutrition, adherence to oral iron, and folic acid supplementation during pregnancy, may differ across geographical settings. In addition, excluding women with patent malaria at enrollment might have contributed to this observation, because malaria is a high-risk factor for anemia. Besides, the impact of IPTp-DHP on anemia in the present study might have been modified by other factors, such as malaria transmission intensity or gravidity.

When evaluating drugs for routine use, especially during pregnancy, safety is one of the important considerations. As reported previously in other trials,[15,21,22] both IPTp-SP and IPTp-DHP were well-tolerated, with no significant differences in this study. Incidences of nausea reported in this study should be interpreted with caution because it is usually common during pregnancy.

Study limitations

As one of the limitations, this study did not assess the effect of repeated DHP doses on corrected QT (QTc) prolongation. A study from Uganda has reported that the QTc prolongation with repeated DHP was not associated with any cardiac adverse events.[22] In addition, the WHO has indicated that DHP has a low risk of cardiotoxicity.[37] Because the trial was done in an area with moderate malaria transmission intensity, results may not be generalized to areas with higher malaria transmission intensities. Furthermore, the study volunteers were screened at enrollment, and those who were malaria positive (by MRDT) were excluded and treated with artemether-lumefantrine following the national malaria treatment guidelines. Hence, our study participants may not be representative of the ANC

Table 3 Adverse birth outcomes between the treatment groups

	Intention to treat analysis						Per protocol analysis					
	IPTp-SP (*n* = 478)		IPTp-DHP (*n* = 478)		Protective efficacy (95% CI)	*P* value	IPTp-SP (*n* = 417)		IPTp-DHP (*n* = 400)		Protective efficacy (95% CI)	*P* value
Outcomes	*n/N*	%	*n/N*	%			*n/N*	%	*n/N*	%		
Composite adverse outcomes	114/478	23.8	90/478	17.9	21 (−1 to 38)	0.06	114/417	27.3	90/400	21.5	18 (−5 to 36)	0.11
Composite adverse outcomes (excluding fetal anemia)	63/478	13.2	26/478	5.4	59 (36 to 73)	< 0.001	63/417	15.1	26/400	6.5	57 (33 to 72)	< 0.001
LBW (birth weight < 2,500 g)	46/478	9.6	22/478	4.6	51 (22 to 73)	0.003	46/410	11.2	22/400	5.5	51 (20 to 70)	0.004
Premature birth (birth < 37 weeks)	10/478	2.1	4/478	0.8	60 (−26 to 87)	0.12	10/417	2.4	4/400	1	58 (−32 to 87)	0.12
Still birth (≥ 28 weeks)	6/478	1.3	0/478	0	—	—	6/417	1.4	0/400	0	—	—
Fetal anemia (cord blood Hb < 12.5 g/dL)	78/478	16.3	69/478	14.6	22 (−19 to 34)	0.42	78/417	18.7	69/400	17.2	8 (−24 to 31)	0.59
Spontaneous abortion (≤ 28 weeks)	1/478	0.2	0/478	0	—	—	1/417	0.2	0/400	0	—	—
Pre-eclampsia	1/478	0.2	0/478	0	—	—	1/417	0.2	0/400	0	—	—
Delivery by cesarean section	8/478	1.7	3/478	0.6[a]	62 (−40 to 90)	0.15	8/417	1.9[a]	3/400	0.8	61 (−46 to 90)	0.16
Anemia during pregnancy (Hb < 11 g/dL)[b]	83	(0.70)	68	(0.57)	18 (−13 to 40)	0.22	83	(0.80)	68	(0.68)	15 (−18 to 38)	0.33
Anemia at delivery (Hb < 11 g/dL)	337/478	70.5	315/478	65.9	7 (−2 to 14)	0.12	337/417	80.8	315/400	78.8	3 (−4 to 10)	0.46

Unless otherwise noted, the *P* values were based on χ^2 test.
95% CI = 95% confidence interval; IPTp-DHP, intermittent preventive treatment in pregnancy with dihydroartemisinin-piperaquine; IPTp-SP, intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine; LBW, low birth weight.
[a]Fisher's exact test [b]Number of events (incidence per person-year at risk); spontaneous abortion (< 28 weeks of gestational age), fetal anemia (cord blood Hb < 12.5 g/dL), stillbirth (≥ 28 weeks of gestational age), LBW (birth weight < 2,500 g), preterm delivery (delivery < 37 weeks gestational age), and maternal anemia (Hb < 11 g/dL), Any adverse birth outcome was defined by a composite of LBW, premature birth, spontaneous abortion, stillbirth, and fetal anemia.

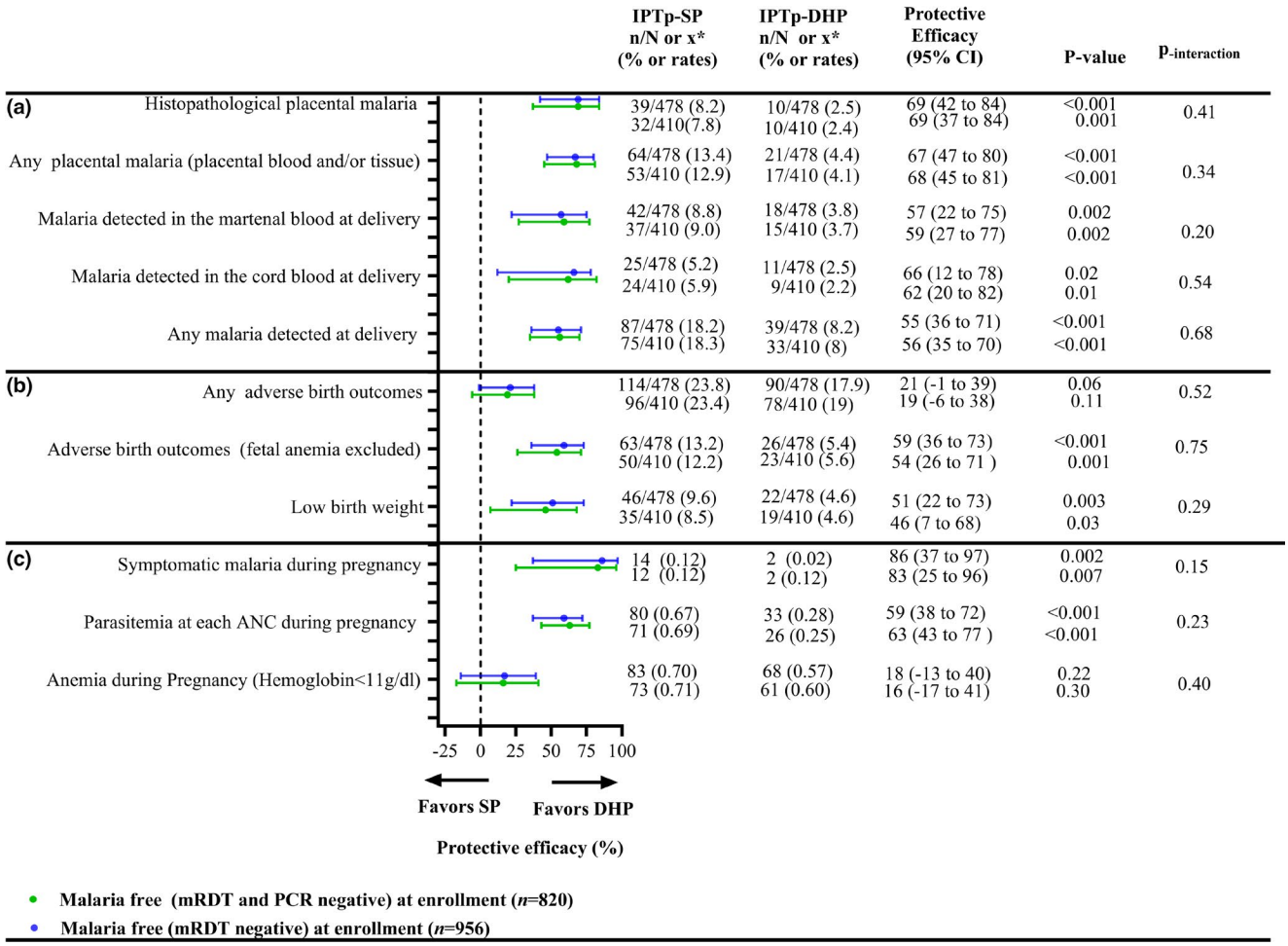

Figure 4 Comparison of overall protective efficacy of the study drugs stratified by study population (with or without sub-patent malaria at enrolment). A: Malaria outcomes collected at delivery; B: adverse birth outcomes collected at delivery; C: outcomes collected during pregnancy (incidence measures). ANC, antenatal care; CI, 95% confidence interval; DHP, dihydroartemisinin–piperaquine; IPTp-DHP, intermittent preventive treatment in pregnancy with dihydroartemisinin-piperaquine; IPTp-SP, intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine; MRDT, malaria rapid detection test; PCR, polymerase chain reaction; rates, incidence per person-year at risk; SP, sulfadoxine–pyrimethamine; x* = the number of women with at least one event.

population because only women who did not have patent malaria were included. Although a similar significantly higher PE of IPTp-DHP than IPTp-SP was found by including or excluding data from women with sub-patent malaria infections at enrollment (**Figure 4**), women with patent malaria might have different characteristics and risk factors for malaria and associated adverse birth outcomes.

CONCLUSIONS

In a setting with moderate malaria transmission intensity, we report significantly higher protective efficacy of monthly IPTp-DHP against malaria during pregnancy and at delivery compared with monthly IPTp-SP. The significant malaria reduction associated with IPTp-DHP corroborates well with the observed significantly reduced risk of LBW compared with IPTp-SP. These results provide further evidence to support the use of IPTp-DHP as an alternative to IPTp-SP, especially in areas with a high level of *P. falciparum* resistance to SP.

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CONFLICTS OF INTEREST

All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

E.M.M. wrote the manuscript. E.M.M., O.M., A.A.K., and E.A. designed the research. E.M.M., O.M., A.A.K., and E.A. performed the research. E.M.M., O.M., A.A.K., and E.A. analyzed the data.

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