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Effectiveness of Intermittent Preventative Treatment with Sulfadoxine-Pyrimethamine
During Pregnancy on Maternal and Infant Outcomes in Malawi

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Master of Public Health

Global Epidemiology

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By

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B.S. Health: Science, Society and Policy
Brandeis University
2018

Thesis Committee Chair: Kristin M Wall, MS, PhD

An abstract of
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Abstract

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By Amy Zhang

Malaria during pregnancy is associated with low birthweight and other adverse birth
outcomes. Increasing drug resistance may undermine the efficacy of intermittent
preventative treatment (IPTp) with sulfadoxine-pyrimethamine (SP). A cross-sectional study
enrolled HIV-negative women at the time of delivery from July to October 2015 at two
district hospitals in southern Malawi. The exposure of interest was the number of IPTp-SP
doses received during pregnancy, coded as <3 vs ≥3 doses in primary analyses. The primary
outcome was infant birthweight. Secondary outcomes included peripheral and placental
malaria infection and a composite birth outcome indicator comprised of low birthweight,
preterm birth, and small for gestational age. SP resistance markers were assessed by
polymerase chain reaction (PCR). Of 536 women enrolled, 49% received <3 SP doses.
Controlling for gravidity, average infant birthweight among women who received three or
more SP doses was 91.4 g higher than that among women who received less than three
doses of SP (p=0.02). The association between 3+ SP doses (versus <3) and peripheral
infection was marginally protective (adjusted prevalence ratio=0.53, 95% CI: 0.28-1.03,
p = 0.06). Increasing number of SP doses was not statistically significantly associated with
protection against placental malaria infection or the composite birth outcome. A total of 91
(17%) samples were PCR positive; 82 (90.1%) samples were successfully genotoyped. All
harbored the A437G and K540E mutations associated with the quintuple mutant for SP
resistance, which confers high level resistance; only two samples harbored the A581G
mutation associated with the "super resistant" sextuple mutant. None of the samples
harbored mutations at codons 436 or 613. IPTp-SP provides benefit to Malawian pregnant
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Chapter I: Background and Literature Review

Malaria Epidemiology

Malaria is a vector-borne, parasitic disease that contributes significantly to the global burden of disease, putting 3.3 billion people in 97 countries at risk (1). In particular, pregnant women, infants, and children under five are at greatest risk of contracting malaria and developing severe disease. In 2017, the World Health Organization (WHO) estimated there were approximately 219 million cases of malaria worldwide, and 435,000 deaths attributed to malaria (2). Of these deaths, 266,000 (61%) occurred in children under the age of five (2). Although malaria occurs in South America and South Asia as well, the majority of the burden of disease falls on sub-Saharan Africa, which accounts for 85% of malaria cases and 90% of malaria deaths (3).

Five *Plasmodium* species cause all malaria infections in humans: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi*; most cases are caused by the first two species. The parasite infects the liver in order to reproduce asexually and can complete thousands of replication cycles in the human host. The progeny then enters the bloodstream to infect red blood cells. Depending on the host's age and malaria infection history, infection frequently causes disease, marked by fever, which may be accompanied by nausea, chills, malaise, and muscle pain. In severe cases, marked by severe anemia and microvascular obstruction, the disease can be fatal.

The *Plasmodium* parasite is transmitted to humans via the bite of an infected female *Anopheles* mosquito. About half (41 of 70) *Anopheles* species are considered "dominant" malaria vectors, and the *Anopheles gambiae* species is the principle vector in most of Africa (4). The mosquito becomes infected while feeding on an infected human, and the parasites reproduce sexually in the mosquito's gut. The offspring are then injected into the human

host at the mosquito's next blood meal. Because the parasites are reliant upon the mosquito vector, the transmission intensity of malaria depends on where mosquitos live, the density of the mosquito population, and their feeding habits.

The WHO recommends that malaria be diagnosed parasitologically, through microscopy and/or rapid diagnostic tests (RDTs). Microscopy, the gold standard, can be conducted with either a thick or thin blood smear, and requires a trained technician to count the number of parasites found in the smear. The alternative, the RDT, a small disposable device, is used to indicate the presence of *Plasmodium* antigens in the blood, usually obtained from a finger-prick. RDTs perform as well as, and are more readily available than microscopy, which is often difficult to conduct due to the lack of reagents, equipment, and trained staff in low resource settings. If malaria is confirmed, patients are typically prescribed a course of artemisinin-based combination therapy (ACT), the recommended first-line treatment for uncomplicated falciparum malaria in endemic areas. ACTs are also highly effective against other malaria types (3).

There are several established methods for preventing malaria, including insecticide treated nets (ITNs), indoor residual spraying (IRS), and intermittent preventive treatment for pregnant women (IPTp). Following the scale up of ITNs, IRS, and ACTs in Africa, approximately 663 million clinical cases have been prevented with malaria control measures (5). Considerable progress has also been made in vaccine development against malaria, though none have been approved yet for routine use. Following phase III trials of vaccine candidate RTS,S/AS01, vaccine efficacy against clinical malaria after four years among children who received four doses was 36.3% while vaccine efficacy for children who received three doses was 28.3% (6). Vaccine efficacy against clinical malaria in young infants was slightly lower, whereby efficacy for those who received four doses was 25.9% while

efficacy for young infants who received three doses was 18.3% (6). Despite moderate vaccine efficacy, RTS,S/AS01 is the most promising candidate, and has recently moved onto large-scale piloting in Ghana, Kenya, and Malawi.

Malaria in Pregnancy (MiP)

Pregnant women are a particularly vulnerable, high-risk group for malaria. In sub-Saharan Africa, approximately 25 million pregnant women are at risk of being infected with *P. falciparum* every year, with placental infections found in one in four women (7). Among pregnant women, malaria is more common in young mothers, women who are pregnant for the first or second time, and women who are HIV positive (8). Malaria in pregnancy is especially harmful because it can lead to both maternal and infant morbidity and mortality. Malaria in pregnancy increases the risk of maternal anemia, which in severe cases, can lead to congestive heart failure, hemorrhage at delivery, and death (9). In 2009, the WHO estimated that malaria causes over 10,000 maternal deaths every year (9).

The increased susceptibility to malaria in pregnant women can be attributed to the sequestration of infected red blood cells in the placenta and the specific affinity of the parasite to bind to the placenta. The accumulation of infected cells induces an inflammatory response from white blood cells. This can lead to defects in placental circulation and small placenta size, which consequently increases the risk of adverse infant outcomes, including stillbirth, intrauterine growth retardation, preterm delivery, and low birthweight. Growth retardation and preterm birth both contribute to low birthweight of the infant. Low birthweight, defined as less than 2,500 grams at birth, is particularly detrimental because it increases the risk of infant mortality; low birthweight that results from malaria in pregnancy is estimated to cause 100,000 infant deaths in Africa each year (7). *P. falciparum* detected from

a peripheral sample at delivery also increased the odds of stillbirth by 80%; 20% of all still births in sub-Saharan African can be attributed to malaria (10).

In addition to the effects of malaria in pregnancy on maternal health and birth outcomes, infection with malaria also impacts other aspects of infant health. Maternal malaria can negatively affect the transfer of antibodies to the fetus, which inhibits the infant's ability to produce an immune response against common pathogens (8). This leaves infants at increased risk for malaria, pneumonia, and diarrhea. Maternal malaria can also lead to congenital malaria, infant anemia, and other febrile illnesses. In the long term, placental malaria infection has also been found to restrict infant height and weight gain independently from low birthweight (11, 12). Therefore, malaria in pregnancy contributes significantly to both the maternal and infant disease burden and needs to receive continued attention.

Malaria in Malawi

Malaria is a major source of morbidity and mortality in Malawi. Malawi is located in southeastern Africa, and shares borders with Mozambique, Tanzania, and Zambia. Malaria is endemic in 95% of the country, with year-round transmission in most areas that peaks during the rainy season from November to April (13). People who live around Lake Malawi and humid low-lying regions are at higher risk than those who live in the highlands (14). The primary malaria parasite in Malawi is *P. falciparum.*

In 2017, the population of Malawi was approximately 18.6 million, with 5,936,348 reported malaria cases and 3,613 malaria deaths (2). Children bear the greatest burden of malaria in Malawi. In 2013, 22% of child mortality cases were attributed to malaria, the leading cause of death among all children under the age of five (15). According to the 2017 Malaria Indicator Survey, 36% of children 6-59 months old tested positive for malaria by RDTs, and 24.3% of children 6-59 months old tested positive for malaria by microscopy

(16). Although data is scarce regarding the prevalence of malaria in pregnancy, studies from various districts have estimated the prevalence of peripheral or placental infection to range from 5% to 32% (17-19). Overall, trends in malaria morbidity and mortality have been decreasing over the past decade with increasing use of malaria prevention measures.

Policies for MiP Prevention in Malawi

Based on the WHO's recommendations, Malawi uses a three-pronged approach to prevent malaria in pregnancy: the distribution and use of ITNs, prompt diagnosis and treatment of confirmed malaria, and IPTp. The recommended drug for IPTp is sulfadoxine-pyrimethamine (SP), also known as Fansidar. SP is used to clear existing asymptomatic placental malaria infection and to provide post-treatment prophylaxis that lasts around four weeks. IPTp is defined as the administration of at least three doses of SP during antenatal care visits during the second and third trimesters of pregnancy with at least 1 month between doses. Studies since 2004 have shown that IPTp with SP (IPTp-SP) is associated with significant reductions in maternal anemia, low birthweight, and infant mortality (20, 21). Further, the prevalence of adverse birth outcomes decreases with increasing SP doses. Therefore, in 2013, the National Malaria Control Programme of Malawi began to recommend the administration of at least three doses of SP after the first trimester (22).

Challenges to IPTp-SP

Sulfadoxine-pyrimethamine was once a first-line antimalarial drug in sub-Saharan Africa, but *P. falciparum* resistance to SP emerged and forced the switch to ACTs in 2007. While SP is no longer recommended for treatment of clinical malaria, it still confers benefits to pregnant women, so it is used for IPTp. However, the prevalence of SP resistance has been increasing in Eastern and Southern Africa, threatening the efficacy and use of IPTp-SP.

SP resistance is the result of mutations in the parasite's dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes. High-level resistance to SP is caused by a quintuple mutation, a combination of three mutations in the *dhfr* genes (N51I, C59R, S108N) with two mutations in the *dhps* genes (A437G, K540E) (23). This quintuple mutation primarily reduces the duration of the prophylactic effect of the drug, and compromises the ability of SP to clear existing *P. falciparum* infections in asymptomatic pregnant women (21). Despite the presence of the quintuple mutation, SP is still associated with decreases in the prevalence of low birthweight (21). However, the presence of an additional mutation in the *Pfdhps* gene (A581G) creates a sextuple mutation that is even more resistant to SP, and it is associated with increased placental inflammation and parasite growth, and failure of SP to increase birthweight (24).

In 2013, the prevalence of the quintuple mutation exceeded 50% in several East African countries including Mozambique, Tanzania, and Malawi, where the quintuple mutation has reached fixation (19, 25). Although the prevalence of the sextuple mutation in southern Malawi was estimated at 8.4% in 2015, the prevalence of the *Pfdhps* A581G mutation has been increasing in neighboring Tanzania, reaching 55% in some areas in 2007 (24, 25). This is alarming because *Pfdhps* A581G may provide a survival advantage for parasites who already have a quintuple mutation (26). Thus, super resistant parasites with a sextuple mutation could be selected for and become increasingly prevalent over time. Ultimately, widespread highly resistant parasites would impair the use of SP for IPTp and suggests an alternative drug for pregnant women may be necessary soon.

Monitoring of maternal and infant outcomes has revealed challenges to the efficacy of SP in southern Malawi. In 2010, a study in Machinga District found IPTp-SP did not significantly reduce the frequency of placental infection in pregnant women, and even

increased the likelihood of having maternal parasitemia despite having received at least two doses of SP (19). There were also no significant differences in maternal anemia and the prevalence of placental infection between women who had received at least two doses of SP and women who received less than two doses. This suggests that SP is no longer effective at protecting mothers from malaria infections. However, the same study found that SP dosage was still beneficial for infants as it was associated with improved birth outcomes. For mothers who had received at least two doses of SP, there was a significant reduction in low birthweight among multigravidae as well as a significant reduction in small for gestational age among primigravidae (19). The mean birthweight for infants whose mothers received at least two doses of SP also increased by 134 grams (19). Although IPTp-SP has been shown to produce positive outcomes in infants, increasing drug resistance threatens the benefits infants and mothers receive. Therefore, there is a need to continue monitoring IPTp-SP effectiveness in Malawi in order to reduce malaria-related morbidity and mortality among pregnant women and infants.

Chapter II: Manuscript

Abstract

Malaria during pregnancy is associated with low birthweight and other adverse birth outcomes. Increasing drug resistance may undermine the efficacy of intermittent preventative treatment (IPTp) with sulfadoxine-pyrimethamine (SP). A cross-sectional study enrolled HIV-negative women at the time of delivery from July to October 2015 at two district hospitals in southern Malawi. The exposure of interest was the number of IPTp-SP doses received during pregnancy, coded as <3 vs ≥3 doses in primary analyses. The primary outcome was infant birthweight. Secondary outcomes included peripheral and placental malaria infection and a composite birth outcome indicator comprised of low birthweight, preterm birth, and small for gestational age. SP resistance markers were assessed by polymerase chain reaction (PCR). Of 536 women enrolled, 49% received <3 SP doses. Controlling for gravidity, average infant birthweight among women who received three or more SP doses was 91.4 g higher than that among women who received less than three doses of SP (p=0.02). The association between 3+ SP doses (versus <3) and peripheral infection was marginally protective (adjusted prevalence ratio=0.53, 95% CI: 0.28-1.03, p = 0.06). Increasing number of SP doses was not statistically significantly associated with protection against placental malaria infection or the composite birth outcome. A total of 91 (17%) samples were PCR positive; 82 (90.1%) samples were successfully genotoyped. All harbored the A437G and K540E mutations associated with the quintuple mutant for SP resistance, which confers high level resistance; only two samples harbored the A581G mutation associated with the "super resistant" sextuple mutant. None of the samples harbored mutations at codons 436 or 613. IPTp-SP provides benefit to Malawian pregnant

women and their infants, with a higher mean birthweight and less peripheral malaria among women who received three or more doses of SP, even in a setting of high SP drug resistance.

Introduction

In sub-Saharan Africa, approximately 25 million pregnant women are at risk of being infected with *P. falciparum* every year (7). Among pregnant women, malaria is more common in young mothers (<20 years), women who are pregnant for the first or second time, and women who are HIV positive (8). Malaria in pregnancy increases the risk of maternal anemia, which in severe cases can lead to congestive heart failure and maternal death (9).

Malaria in pregnancy not only affects the mother but is also associated with adverse fetal outcomes including low birthweight (<2,500 g at birth), stillbirth, and preterm delivery (<37 weeks' gestation) (7). Parasite sequestration in the placenta can result in growth retardation and preterm birth, both of which contribute to low birthweight of the infant. Low birthweight has significant implications for the infant's future health outcomes, increasing the risk of infant mortality. Malaria associated low birthweight is estimated to cause 100,000 infant deaths in Africa each year (7). Infants born small for gestational age (birthweight below the 10th percentile for infants of the same gestational age) have also been found to be at increased risk for malaria infection after six months of age (27).

In order to reduce the risk of malaria in pregnancy, the World Health Organization recommends the use of intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP), consisting of a treatment dose of SP administered at each antenatal care (ANC) visit from the start of the second trimester, as long as the doses are at least one month apart (28). While SP has not been used for the treatment of malaria in Malawi since 2007 due to widespread resistance to SP (14), it still confers benefits to pregnant women, and remains the only recommended drug for IPTp. However, the

prevalence of highly resistant parasites has been increasing in Eastern and Southern Africa, possibly threatening the efficacy and use of IPTp-SP (24).

High-level resistance to SP is caused by a combination of three mutations in the *dhfr* genes (N51I, C59R, S108N) with two mutations in the *dhps* genes (A437G, K540E), referred to as the quintuple mutation (23). In Malawi, the prevalence of this quintuple mutation increased from 19% in 1997 to 100% in 2005 (29). This quintuple mutation primarily reduces the duration of the prophylactic effect of the drug, and compromises the ability of SP to clear existing *P. falciparum* infections in asymptomatic pregnant women (21). Despite finding nearly 100% prevalence of the quintuple mutation, IPTp-SP is still associated with decreases in the prevalence of low birthweight (21). Of greater concern is an additional sixth mutation in the *Pfdhps* gene (A581G) that creates a "super resistant" genotype. The sextuple mutation is not only associated with increased placental inflammation and parasite growth, but also failure to increase birthweight (24). The prevalence of the sextuple mutation in the Chikwawa and Zomba regions of Malawi between 2010-2012 was 2.7%, while prevalence in Liwonde and Blantyre in 2011 was estimated at 8.4% (24, 30). Until alternatives to IPTp-SP exist, continued monitoring of drug efficacy is vital in the wake of super resistant *P. falciparum*.

In 2011, a cross-sectional survey at Machinga District Hospital among 703 women at delivery found that IPTp-SP did not significantly reduce the prevalence of placental infection, and even increased the likelihood of having maternal parasitemia despite having received at least two doses of SP (19). However, IPTp-SP was associated with improved birth outcomes. Given concern for increasing drug resistance in the region since 2011, it is necessary to reassess the efficacy of IPTp-SP, particularly the effect on birthweight. The prior study also assessed the effects of the previously recommended two-dose regimen,

rather than the updated three-dose regimen. Here, we conducted a secondary analysis of a 2015 cross-sectional study (designed primarily to assess the prevalence of the sextuple mutation and its association with birth outcomes) to evaluate the association between the number of SP doses and infant birthweight in Malawi.

Methods

Study Sites

The study was conducted at two facilities in the Southern Region of Malawi between June and October 2015: Machinga District Hospital and Balaka District Hospital. Balaka District borders Machinga District to the west, across the Shire River. The two district hospitals are 17 kilometers apart. Machinga District Hospital provides care for a catchment area of 369,614 people and has approximately 5,712 deliveries per year. People living in the region are mostly of the Yao ethnic group and make a living from subsistence farming, fishing, and small businesses. Balaka District Hospital provides for a catchment area of 317,324 people and has approximately 3,780 deliveries per year. The districts share similar climate and terrain. Malaria transmission is stable throughout the year, peaking during the rainy season from December to March. The majority of malaria infections are due to *Plasmodium falciparum*. In 2014, the prevalence of malaria in children under the age of 5 in the Southern Region was 37.6% (31). Resistance to SP is high, with nearly 100% prevalence of the quintuple mutation.

Sample Size

This study was designed to investigate if the presence of a sextuple mutation that confers drug resistance in malaria parasites would be associated with decreased infant birthweight and increased parasite densities. Assuming that approximately 10% of women were parasitemic, of which 10% were infected with the sextuple mutant haplotype, a sample

size of 5,000 would have been sufficient to detect a 20% increase in the prevalence of anemia with 80% power. In this secondary analysis of infant birthweight, given that half of women received 3 or more doses of SP and half received 2 or fewer doses, a sample of 500 women was sufficient to detect a 50g difference in mean birthweight with 80% power assuming a standard deviation of 200g in birthweight.

Enrollment and Study Procedures

Women presenting to either facility for delivery were enrolled if they provided informed consent and met all eligibility requirements (singleton pregnancy, IPTp-SP history available, and HIV negative). Upon enrollment, the patient's medical history, insecticide treated bed net (ITN) use, and demographic information were collected on standard forms. Patient ANC cards were examined to record information regarding the total number of ANC visits, the number and timing of IPTp-SP doses, other medications taken such as iron supplementation, HIV status, and syphilis test results.

A maternal finger stick blood sample was obtained prior to delivery and assessed for anemia, defined as hemoglobin levels <11 g/dl, using a HemoCue® Hb 301 machine (HemoCue, Ängelholm, Sweden) (32). A rapid diagnostic test (RDT) was administered to detect malarial infection. Giemsa-stained thick smears were prepared from finger stick blood samples following a standard protocol. A trained technician read the slides to determine species and parasite density. The number of asexual parasites and white blood cells (WBCs) were counted concurrently until 200 WBCs were reached. A smear was determined to be negative if no asexual parasites were identified after 1,000 WBCs were seen. Parasite density was described in terms of the number of asexual parasites per microliter of blood, assuming 8,000 WBC/microliter of blood (33). For quality control of smear readings, slides were read by a second technician. If this second reading was discrepant from the first, the slide was

read by a third technician for a final determination. Placental samples were collected upon delivery from the maternal side of the placenta, and blood from the placenta was prepared for thick smear examination as described above.

Peripheral and placental blood samples were also collected on filter paper for polymerase chain reaction (PCR). Parasite genomic DNAs were extracted from dried blood spots (DBS) using QIAamp DNA Mini kit (Qiagen Inc. Valencia, CA). The parasitic DNAs were genotyped for *Pfdhps* codons 436, 437, 540, 581, 613 using Sanger sequencing. The primers and PCR amplification for *Pfdhfr* gene were performed using previously published methods with modifications (34). The *Pfdhps* gene amplification was performed as previously described (35). Cycle sequencing of purified PCR products was performed using a BigDye® Terminator (v3.1) (Thermo Fisher Scientific, Waltham, MA) cycle sequencing kit on an T100 thermal cycler (Bio-Rad, Hercules, CA). The products from the reaction mixtures was precipitated in 70% ethanol to clean up the dye terminators, rehydrated in 10 µl HiDi formamide, and sequenced using an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Grand Island, NY). Single nucleotide polymorphism (SNP), a single base-pair difference in a DNA sequence, was determined based on the highest peak. In sequences with multiple peaks, mixed genotypes were recorded if peaks height were >30% of the major peak. The single or mixed genotypes were all classified into mutants.

The Ballard examination was used to determine gestational age at delivery (36). Preterm birth was defined as gestational age <37 weeks and 0 days. Small for gestational age (SGA) was defined as <10th percentile of fetal weight for attained gestational age using the intergrowth normogram (37). Infants were weighed within 24 hours of delivery using a digital scale; low birth weight (LBW) was defined as a birth weight <2,500 g. Stillbirths were excluded from analysis.

Exposure of interest

Our exposure of interest was the number of IPTp-SP doses received by participants, which was categorized in two ways. Using the recommended three dose regimen as the cutoff, IPTp-SP was dichotomized as ≥3 vs <3 doses. In order to assess the dose-response effect of SP, dosage was modeled as a continuous variable.

Statistical Analyses

Our primary outcome was infant birthweight, assessed as a continuous variable. Our secondary outcomes included peripheral and placental parasitemia, and adverse birth outcomes including LBW, SGA and preterm delivery (binary variables). A composite birth outcome which included any of SGA, LBW, or preterm delivery was also included in the analysis to increase the number of adverse outcomes measured and thus increase power.

Statistical analysis was done using SAS, version 9.4 (SAS Institute, Cary, NC). We assessed the normality of continuous variables; median and interquartile range (IQR) were reported if they were not normally distributed. Comparisons between exposure groups were made using the χ^2 test or Fisher exact test for categorical variables, and the Kruskal-Wallis test for continuous variables. A two-sided P value of <0.05 was considered statistically significant.

Gravidity was decided *a priori* for inclusion in all models since prior research has indicated that gravidity is associated with both increased SP uptake and birthweight (38, 39). Socioeconomic status tertiles were determined by multiple correspondence analysis of household assets after conducting multiple imputation for missing values. Remaining covariates were assessed for confounding by examining their association with both the exposure and outcome. Covariates that were significantly associated (p<0.05) with both exposure and outcome and which did not show multicollinearity were candidates for

inclusion in the models. Multiple linear regression was used to model the association between IPTp-SP doses and infant birthweight, adjusting for gravidity. Multivariate models included number of IPTp-SP doses (dichotomized as ≥3 vs <3 or continuous) and gravidity (dichotomized as primigravid vs secundigravid/multigravid). Adjusted prevalence ratios (PRs) and 95% CIs measured the association between outcomes of peripheral infection, placental infection, and a composite birth outcome and IPTp-SP doses (≥3 vs <3 or continuous) using Poisson regression with robust standard errors. Parameter estimates were considered statistically significant ($p<0.05$) according to the Wald test.

Ethics

The study was reviewed and approved by the research ethics committee of the University of Malawi College of Medicine. CDC participated under a non-engaged determination from the CDC Human Research Protection Office. Written informed consent was obtained from all participants.

Results

Baseline Characteristics

A total of 536 women were enrolled out of 761 women who were screened. The median patient age was 23 years (IQR: 19-28), with 60% of women under the age of 25 (Table 1). About one-third (36%) of women were primigravid, 17% were secundigravid, and 48% were multigravid. Eighty percent of women owned an insecticide treated net, 65% of whom slept under it the previous night. Women made a median of 3 (IQR: 3-4) ANC visits, and completed a median of 8 (IQR: 5-10) years of school. Fifty-nine (11%) women received less than four years of schooling.

Of 536 women, only 3% received no IPTp-SP, while 11%, 35%, and 51% received 1, 2, and 3 or more doses, respectively. Forty-nine percent of women received <3 doses while

51% received ≥3 doses. When looking at the demographic factors associated with receiving SP, only education was associated with number of SP doses (p=0.01), with women receiving ≥3 doses having attained higher educational levels than women receiving <3 doses. Nearly all IPTp-SP doses were reported to have been delivered at ANC, with only five women reporting receiving SP from a source other than ANC: two obtained ANC from a shop, one from a drug store, one from a hospital, and the last from an unspecified source.

Maternal Outcomes

The prevalence of maternal anemia was not significantly different between those who received ≥3 doses and those who received <3 doses, 27% vs 31% (Table 2). The prevalence of severe maternal anemia was similar regardless of IPTp-SP doses (7%). Maternal hemoglobin levels were also not significantly different between women who received ≥3 doses vs <3 doses, 11.9 g/dL vs 11.7 g/dL (p=0.13).

Overall, 19% of mothers had evidence of malaria: 10.1% by RDT, 7.3% by peripheral smear, 5.3% by placental smear, and 17% by PCR. Malaria was less common among women who received ≥3 vs <3 doses of IPTp-SP: 6.4% vs 14.1% (p=0.003) by RDT; 5.2% vs 9.6% (p=0.05) by maternal peripheral smear; 4.4% vs 6.1% (p=0.37) by placental smear; 16% vs 18% (p=0.42) by PCR; and 18% vs 21% (p=0.26) for any malaria detected by RDT, smear, PCR, or histopathology. The receipt of at least three doses of SP was associated with a decreased prevalence of peripheral malaria infection by smear (adjusted PR (aPR) 0.53; 95% CI: 0.28, 1.03; p=0.06) and a decreased prevalence of placental infection by smear (aPR 0.71; 95% CI: 0.34, 1.50; p=0.38) (Table 4). For every additional dose of SP, the prevalence of peripheral malaria infection decreased by 9% (aPR=0.91; 95% CI: 0.63,

1.33) while the prevalence of placental infection decreased by 12% (aPR=0.88; 95% CI: 0.57, 1.38) (Table 5).

Infant Outcomes

Birthweight was significantly higher among women who had received ≥3 doses of IPTp-SP compared to those who received <3 doses (3,100 g vs 3,000 g, p=0.03) (Table 2). Controlling for gravidity, the average infant birthweight for women who received at least three doses of SP was 91.4 g higher than that of women who received less than three doses of SP (p=0.02) while the average infant birthweight increased by 54.8 g for every additional SP dose (p=0.03) (Table 3). The prevalence of the composite birth outcome was 25% overall, with a lower prevalence among those who received ≥3 doses compared to those who received <3 doses, though this difference was not statistically significant (22% vs 28%, aPR 0.79; 95% CI: 0.56, 1.11; p=0.18) (Table 4). The prevalence of the composite birth outcome decreased by 19% for every additional dose of SP (aPR=0.81, 95% CI: 0.67, 0.99) (Table 5).

Overall, 13% of infants were small for gestational age, 8% of infants were considered low birthweight, and 14% were preterm (Table 2). There was a small but statistically significant difference in mean gestational age at delivery between those who received ≥3 doses vs <3 doses (median 39 weeks vs 38 weeks, p = 0.04). Less than 5% of infants were stillborn, had physical abnormalities, or experienced delivery complications. The prevalence of adverse infant outcomes was lower among women who had received ≥3 doses compared to <3 doses of SP, though this only approached statistical significance for SGA (p= 0.06) (Table 4). When SP doses were modeled continuously, there was a significant decrease in the prevalence of SGA, preterm delivery, and composite birth outcome as dosage increased (Table 5).

PCR Results

Parasite genomic DNAs was extracted and genotyped from 536 patient blood samples. Out of the 536 samples collected from both sites, 91 (17%) were positive for malaria by PCR; 24 out of 232 (10.3%) samples from Balaka District Hospital and 67 out of 304 (22%) samples from Machinga District Hospital. Eighty-two PCR positive samples were successfully genotyped for mutations at *dhps*-436, 437, 540, 581, and 613. All samples harbored mutations at *dhps* 437 and 540, consistent with fixation of the quintuple mutant. Only two (2.4%) samples harbored the A581G mutation, consistent with the sextuple mutant. None of the samples contained mutations at *dhps* codons 436 or 613.

Discussion

Despite concerns that high level parasite resistance to SP in Malawi may threaten the efficacy of IPTp-SP to increase infant birthweight, IPTp-SP continues to provide benefits to infants; infant birthweight was significantly higher among mothers who received at least three doses of IPTp-SP compared to those who received fewer than three doses. The prevalence of a composite adverse birth outcome, maternal peripheral malaria infection at delivery, and placental infection were also lower among those who received at least three doses.

Analysis of the trend-effect for increasing number of SP doses demonstrated a significant effect of three, but not two, doses on birthweight. These results are consistent with previous studies that infant birthweight improves with increasing IPTp-SP uptake. In a previous study at Machinga District Hospital, birthweight increased on average by 134 g among those who received at least two doses compared to those who received less than two doses (19). In a study from Burkina Faso, Gies et. al. reported that for every additional IPTp-SP dose, mean birthweight increased 220 g among primigravidae and 102 g among

secundigravidae (40). Similarly, Kayentao et. al. performed a meta-analysis and found that birthweight increased by an average of 56 g more for infants who received at least three doses compared to two or fewer doses (41).

A three-dose regimen was also more protective than a two-dose regimen in preventing composite birth outcome, SGA, LBW, and peripheral infection. This supports findings from Mali where the prevalence of LBW was halved when women received three doses rather than two doses (42). The prevalence of SGA was also reduced among Zambian women who received three versus two doses of IPTp-SP (43). Despite the additional dose of SP, the proportion of infants who were considered LBW or preterm was significantly greater in this study compared to 2011 regardless of the number of IPTp-SP doses received (p<0.0001). This may be attributed to differences in sampling or food insecurity in Malawi during the 2013/2014 agricultural season followed by flooding in early 2015 (44).

The adjusted analyses did not reveal a significant impact of increased IPTp-SP doses on placental infection. This result is consistent with findings from Malawi, Zambia, and Tanzania (19, 43, 45). Unlike the previous study in Malawi, we did not observe a dose-response decrease in the prevalence of placental malaria (19). While SP helps clear infections, it may not be as effective at preventing new infections especially when the prophylactic effect wears off between doses. The previous study in Malawi also indicated that maternal parasitemia was more common among women who received at least two doses of SP versus less than two doses, but we did not observe a similar association between women who received at least three versus less than three doses (19). The increase observed previously may have occurred because women were infected after receiving SP, while the additional SP doses in our study may have meant that IPTp-SP was delivered closer to delivery and thus still retained some prophylactic effect.

In 2014, Malawi revised its IPTp policy to adhere to the WHO's updated recommendation that women should receive at least three doses of SP rather than two doses during each pregnancy, starting as early as possible in the second trimester (46, 47). IPTp-SP coverage has improved in the area as the 2015-2016 Demographic and Health Survey (DHS) reported that only 30% of women in Balaka and Machinga districts who had a live birth in the two years before the survey received at least three doses (48). While this is an improvement from 2010 when the DHS reported that only 18% of women received at least three doses, there is still a large gap between the number of women who should receive at least three doses and those who do, although Malawi outperformed the average 17% uptake across sub-Saharan Africa (2).

In order to facilitate prenatal care and the administration of IPTp-SP, women are also recommended to attend at least four ANC visits, but there were only slight gains in the proportion of women who attained that; in 2010 46% of women made at least four ANC visits, which rose to 51% in 2015 (48). This indicates that almost half of women are missing opportunities to receive SP. Encouraging women to attend ANC visits and ensuring the administration of IPTp-SP at each visit beginning in the second trimester may improve coverage.

While the quintuple mutation was fixed, we found a 6-percentage point decrease in the proportion of A581G sextuple mutant compared to results from a 2011 study (24). While this was not statistically significantly different, it nonetheless suggests that the prevalence of resistance is not increasing. The fact that BW increased despite the presence of the sextuple mutation in this study supports findings from a meta-analysis by Chico et. al. that IPTp-SP is still beneficial when A581G prevalence is below 10.1% (49). Continued

monitoring of the prevalence of the A581G mutation and IPTp-SP efficacy in the region will be important to inform future IPTp policy.

A limitation of this study is that out of hospital births were excluded by the design of the study. Following a cohort of women from the time of initiation of ANC to delivery would provide a better estimate of the overall effects of IPTp-SP. The finding that three or more doses of IPTp-SP had a positive impact on birthweight when compared to fewer doses is unlikely to be substantially affected by the exclusion of stillbirths/miscarriages. Prior studies have demonstrated that SP is not harmful since it has been shown to reduce adverse outcomes, thus we would not expect a higher number of stillbirths in the arm getting more SP doses. Despite our best attempts to assess for confounding there is potential for residual confounding from unmeasured variables such as disease severity or timing of infection (earlier vs later in pregnancy). We were unable to determine whether women were infected prior to or after receiving SP, which may have limited the effect of SP. We had an unequal distribution of IPTp-SP doses, with only 14 women who received zero doses of SP, which limits the ability to analyze the dose-response effect when comparing to the group of women who received zero doses. It was also not possible to determine the quality of SP doses taken by the women, so there is potential that the drugs were not of high quality, though it is unlikely that the quality of SP varied by study site.

IPTp-SP remains associated with improved birthweight and reduced prevalence of adverse outcomes in infants and their mothers in Southern Malawi. Our results support current IPTp-SP policies and provide evidence that pregnant women continue to benefit from at least three doses of SP. Continued monitoring of SP resistance and efficacy, as well as further exploration of alternative drugs will be necessary though in order to ensure that mothers and infants benefit from IPTp.

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Tables

Table 1. Baseline Characteristics of Enrolled Women and Infants, by Sulfadoxine-Pyrimethamine (SP) Dosage

Variable	No. of SP Doses			P
	Any (n=536)	< 3 (n=262)	≥3 (n=274)	
IPTp doses, n, median (IQR)	3.0 (1.0)	2.0 (1.0)	3.0 (0.0)	<0.0001
Maternal age, y, median (IQR)	23.0 (9.0)	23.0 (10.0)	23.0 (9.0)	0.87
Maternal age, n (%)				
<25	319 (59.6)	155 (48.6)	164 (51.4)	0.91
≥25	216 (40.4)	106 (49.1)	110 (50.9)	
Gravidity, n (%)				
Primi	191 (35.7)	92 (35.2)	99 (36.1)	0.89
Secundi	90 (16.8)	46 (17.6)	44 (16.1)	
Multi	254 (47.5)	123 (47.1)	131 (47.8)	
ANC visits a, median (IQR)	3.0 (1.0)	3.0 (2.0)	4.0 (1.0)	<0.0001
LLIN use last night, n (%)	280 (52.2)	131 (50.0)	149 (54.4)	0.31
Years of schooling, median (IQR)	8.0 (5.0)	7.0 (4.0)	8.0 (5.0)	0.01
Highest School Level, n (%)				
Less than 4 years	59 (11.0)	35 (13.4)	24 (8.8)	0.01
Some primary	220 (41.1)	120 (46.0)	100 (36.5)	
Primary	151 (28.2)	67 (25.7)	84 (30.7)	
Some secondary or higher	105 (19.6)	39 (14.9)	66 (24.1)	
Wealth Status b, n (%)				
Below average	160 (29.9)	82 (31.4)	78 (28.5)	0.73
Average	161 (30.1)	78 (29.9)	83 (30.3)	
Above average	214 (40.0)	101 (38.7)	113 (41.2)	
Floor material, earth/sand c, n (%)	426 (79.6)	208 (79.7)	218 (79.6)	0.97
Roof material, thatch/grass d, n (%)	333 (62.5)	162 (62.5)	171 (62.4)	0.97

Data are number and percent of women or median and IQR. Due to missing values, observations do not add up to total.

IQR, interquartile range; IPTp, intermittent preventive treatment during pregnancy; ANC, antenatal care; LLIN, long lasting insecticide-treated net; SP, sulfadoxine-pyrimethamine.

P-values calculated by the χ2 test (or Fisher exact test) for normal, categorical variables and the Kruskal-Wallis test for non-normal continuous variables.

aANC visits were recorded from patient's antenatal care card.

bWealth status based on asset index; Multiple imputation used to infer missing data values for 22 observations; 1 observation was deleted due to large number of missing values

cEarth/sand considered no flooring in the house compared to wood, ceramic tiles, or cement flooring.

dThatch/grass roofing material for house was compared to plastic or iron sheets.

Table 2. Maternal and Infant Outcomes, by Number of Sulfadoxine-Pyrimethamine (SP) Doses

Outcome	No. of SP Doses						P
	Any (n=536)		< 3 (n=262)		≥3 (n=274)		
	n	%	n	%	n	%	
Birth weight, g, median, IQR	3100	600	3000	500	3100	500	0.03
Secondary infant outcomes							
Composite	133	25.3	73	28.3	60	22.4	0.14
SGA	68	12.8	41	15.7	27	9.9	0.05
LBW	44	8.3	25	9.6	19	7.0	0.28
Preterm delivery	77	14.4	40	15.3	37	13.5	0.56
Gestational age, weeks, median, IQR	38.0	2.0	38.0	2.0	39	2.0	0.04
Stillbirth	4	0.8	1	0.4	3	1.1	0.62
Delivery complications	21	3.9	8	3.1	13	4.8	0.32
Physical abnormality	14	2.7	7	2.7	7	2.6	0.90
Smear positivity, by blood source							
Peripheral infection	39	7.3	25	9.6	14	5.1	0.05
Placental Infection	28	5.3	16	6.1	12	4.4	0.37
Maternal Hb level, g/dL, median, IQR	11.8	2.0	11.7	2.0	11.9	1.9	0.10
Maternal anemia	155	28.9	81	30.9	74	27.0	0.32
Severe maternal anemia	36	6.7	18	6.9	18	6.6	0.89

Data are number and percent of women. Due to missing values, observations do not add up to total.

P-values calculated by the χ2 test (or Fisher exact test) for normal, categorical variables and the Kruskal-Wallis test for non-normal continuous variables.

IQR, interquartile range; SGA, small for gestational age (<10th percentile birthweight for gestational age); LBW, low birthweight (<2,500 g); Preterm delivery (<37 weeks' gestation)

Table 3. Linear models of Sulfadoxine-Pyrimethamine (SP) Dosage Predicting Infant Birthweight

Model 1

Parameters	Estimate (SE)	P
Intercept	3022.1 (28.5)	<0.0001
SP doses (≥3 vs <3)	87.8 (39.9)	0.03

Model 2

Parameters	Estimate (SE)	P
Intercept	2789.1 (73.9)	<0.0001
SP doses (≥3 vs <3)	91.4 (39.6)	0.02
Gravidity (Primigravid vs multigravid)	140.4 (41.3)	0.0007

Model 3

Parameters	Estimate (SE)	P
Intercept	2927.8 (67.8)	<0.0001
SP doses (continuous)	52.4 (24.4)	0.02

Model 4

Parameters	Estimate (SE)	P
Intercept	2693.9 (95.8)	<0.0001
SP doses (continuous)	54.8 (24.4)	0.03
Gravidity (Primigravid vs multigravid)	140.4 (41.3)	0.0009

Table 4. Effect of Sulfadoxine-Pyrimethamine (SP) Dosage on the Prevalence of Adverse Birth Outcomes and Malaria Infection

Outcome	Prevalence, %	Crude Prevalence Ratio (95% CI)	P	Adjusted Prevalence Ratio (95% CI)*	P
Composite birth outcome a					
SP doses, no.					
<3	28.3	Reference		Reference	
≥3	22.4	0.79 (0.56, 1.11)	0.18	0.79 (0.56, 1.11)	0.17
SGA					
SP doses, no.					
<3	15.7	Reference		Reference	
≥3	9.9	0.63 (0.39, 1.03)	0.06	0.63 (0.38, 1.01)	0.06
LBW					
SP doses, no.					
<3	9.6	Reference		Reference	
≥3	7.0	0.73 (0.40, 1.32)	0.30	0.72 (0.40, 1.31)	0.28
Preterm delivery					
SP doses, no.					
<3	15.3	Reference		Reference	
≥3	13.5	0.88 (0.57, 1.38)	0.59	0.88 (0.56, 1.37)	0.57
Peripheral infection					
SP doses, no.					
<3	9.6	Reference		Reference	
≥3	5.2	0.54 (0.28, 1.03)	0.06	0.53 (0.28, 1.03)	0.06
Placental infection					
SP doses, no.					
<3	6.1	Reference		Reference	
≥3	4.4	0.72 (0.34, 1.52)	0.39	0.71 (0.34, 1.50)	0.38

*All models were adjusted for gravidity (primigravid vs multigravid)

a Includes low birthweight, preterm birth, and small for gestational age

Table 5. Effect of Increasing Number of Sulfadoxine-Pyrimethamine (SP) Doses on Adverse Birth Outcomes and Malaria Infection

Outcome	Crude Prevalence Ratio (95% CI)	P	Adjusted Prevalence Ratio (95% CI)*	P
Composite birth outcome	0.82 (0.67,1.00)	0.05	0.81 (0.67, 0.99)	0.04
SGA	0.74 (0.56, 0.97)	0.03	0.74 (0.56, 0.97)	0.03
LBW	0.82 (0.58, 1.17)	0.28	0.82 (0.58, 1.16)	0.26
Preterm delivery	0.77 (0.60, 1.00)	0.05	0.76 (0.59, 0.99)	0.04
Peripheral infection	0.91 (0.63, 1.33)	0.64	0.91 (0.63, 1.33)	0.64
Placental infection	0.89 (0.57, 1.38)	0.61	0.88 (0.57, 1.38)	0.60

*Models were adjusted for gravidity (primigravid vs multigravid)