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A Randomized, Controlled Clinical Trial of Chloroquine as Chemoprophylaxis or Intermittent Preventive Therapy to Prevent Malaria in Pregnancy in Malawi

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Research in context panel

Evidence before this study

Intermittent treatment of malaria during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is an essential component of malaria control in sub-Saharan Africa. With the spread of SP-resistant malaria, there is an urgent need to identify an alternative to SP for the prevention of malaria in this vulnerable population. On 18 Feb 2018, we searched MEDLINE, Embase and the Cochrane Central Register of Controlled Trials for clinical trials investigating malaria preventive treatment during pregnancy. We used the search strategy: (Malaria AND pregnan* AND intermittent) AND (prevent* OR prophyla* OR presumpt* OR chemoprevent* OR chemoprophyla* OR IPT*) AND (placental OR histo* OR Pathology OR malaria OR birth weight OR LBW OR preterm or premature) AND (randomised or clinical or controlled or trial) AND (sulfadoxine and pyrimethamine) without restricting publication language or date.

The search strategy yielded 189 articles. Six studies assessed chloroquine or its combinations but none demonstrated evidence of superiority against SP-IPTp. Five chloroquine studies were conducted in the context of widespread chloroquine resistance but the absence of SP resistance. This is the opposite pattern of what is currently observed in Malawi and the surrounding region. A multicentre trial that assessed IPTp with a fixed-dose combination of chloroquine and azithromycin compared to IPTp with SP showed equivalence between the two interventions but many of the study sites still had high rates of chloroquine-resistant malaria.

Apart from mefloquine, which was poorly tolerated, the only other candidates with demonstrated efficacy superiority over SP-ITPp are artemisinin-based combination therapies (ACTs). However, these drugs may not be suitable replacements for SP. ACTs are the first line treatment for malaria so the very widespread use of these drugs for prevention may lead to the emergence and spread of resistance, thus threatening malaria-endemic countries' ability to treat

those suffering from malaria disease. In addition, the safety of artemisinins in the first trimester of pregnancy has not been definitively established, ACTs may not be the ideal replacement for SP.

Therefore, an efficacious, safe, and well-tolerated alternative to SP-IPTp has not been definitively identified. With new drug regimens being studied, we might also consider whether continuous prophylaxis is preferable to intermittent curative dosing for the prevention of malaria and its adverse outcomes during pregnancy.

Added value of this study

With the return of chloroquine susceptible malaria in Malawi and throughout the region, we had a unique opportunity to evaluate chloroquine, administered either as IPTp or weekly prophylaxis compared to IPTp with SP. In our study, chloroquine given as IPTp (two treatments of chloroquine 600mg on day one, 600mg on day two, and 300mg on day 3 at least four weeks apart), or given as prophylaxis (chloroquine 600mg at enrolment followed by 300mg once every week up to delivery), did not demonstrate superior protection from placental malaria, clinical malaria, low birth weight, or maternal anaemia than SP-IPTp (two doses of 1500 mg SP given at least 4 weeks apart). However, due to an observed background incidence of 15.4% rather than the expected 40%, statistical power was lower than we had anticipated. Our protocol-specified adjusted analyses found that chloroquine as weekly prophylaxis provided modest protection against malaria during pregnancy and was more effective than SP-IPTp in preventing placental malaria infection among women who did not have malaria infection at enrolment, but do not show evidence that chloroquine as IPTp was superior to SP-IPTp. Our study reaffirmed the safety and tolerability profile of chloroquine. Although chloroquine arms had more adverse events than SP-IPTp overall, it did not increase the risk of severe and life-threatening events, and treatment discontinuation was rare.

Implications of all the available evidence

The role of chloroquine as a potential replacement for SP for the prevention of malaria during pregnancy remains to be fully established. However, as the search for alternative regimens continues, chloroquine prophylaxis needs to remain an important consideration for future trials perhaps in higher transmission settings. The unique characteristics making chloroquine a highly desirable option include the increasing radius of chloroquine-susceptible malaria in Africa, the long half-life and the safety and tolerability. Chloroquine also has advantage over artemisinin combination therapies and SP because it can be used in the first trimester, and there is increasing evidence that protecting pregnant women as early as possible in pregnancy, when many of the placental infections are being established, is likely to be beneficial.

Abstract

Background

Sulfadoxine-pyrimethamine (SP) resistance threatens efficacy of intermittent preventive treatment during pregnancy (IPTp) and there is an urgent need to identify alternative regimens. With the return of chloroquine efficacy in Southern Africa, we hypothesized that chloroquine either as IPTp or as chemoprophylaxis would be more efficacious than SP-IPTp for prevention of pregnancy malaria and associated maternal and newborn adverse outcomes.

Methods

We conducted an open label, single-centre randomized clinical trial of chloroquine prophylaxis (600mg day-1, 300mg weekly), chloroquine-IPTp (2 doses of 600mg day-1, 600mg day-2, 300mg day-3) or SP-IPTp (2 doses), to HIV negative first and second gravid women at 20 to 28 weeks gestation, in Malawi. Participants were randomly assigned to receive SP-IPTp, chloroquine-IPTp, and chloroquine-prophylaxis in a 1:1:1 ratio. We used a computer-generated randomization list. Our primary endpoint was placental malaria in participants in the modified intent-to-treat population, comprised of those who were randomized and contributed histopathology data at birth. ClinicalTrials.gov registration: NCT01443130.

Findings

From 2012-2014 we randomized 900 women, 765(85%) contributed histopathology data and were included in the analysis. 14% (108/765) experienced placental malaria, which was lower than the anticipated rate of placental malaria infection. Our primary analysis did not find improved protection from placental malaria by chloroquine as prophylaxis (relative risk (RR)

0.75 [95% confidence interval (CI): 0.48, 1.17]), or as IPTp (RR 1.00 [95% CI: 0.67, 1.50]) compared to SP-IPTp. However, our protocol specified adjusted analysis of the primary outcome found that, women taking chloroquine-prophylaxis experienced 36% lower placental infections than those in SP-IPTp arm (RR 0.64 [95% CI: 0.44, 0.93]). Incidence of clinical malaria, maternal anaemia and low birth weight was not improved by the interventions. There were nine cases of clinical malaria in the SP-IPTp group, four in the chloroquine IPTp group (p = 0.26) and two in the weekly chloroquine group (p = 0.06). There were five cases of maternal anaemia in the SP-IPTp group, fifteen in the chloroquine IPTp group (p = 0.04) and six in the weekly chloroquine group (p = 1.00). There were 31 cases of low birth weight in the SP-IPTp group, 29 in the chloroquine IPTp group (p = 0.78) and 41 in the weekly chloroquine group (p = 0.28). Compared to SP-IPTp both chloroquine arms had more related adverse events overall, but there was no difference in severe or life-threatening events. Adverse events possibly related to study product were experienced by four maternal subjects in the SP-IPTp group, 94 in the chloroquine IPTp group (p < 0.001) and 26 in the weekly chloroquine group (p < 0.001). There were three maternal subjects who experienced severe or life threatening adverse events related to study product. These were all in the chloroquine IPTp group (p = 0.25).

Interpretation

Chloroquine administered as IPTp did not provide better protection from malaria and related adverse effects than SP-IPTp in this setting of high SP-resistance. Protocol-specified adjusted analyses suggest that chloroquine chemoprophylaxis may provide benefit in protecting against malaria during pregnancy.

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INTRODUCTION

Malaria remains one of the most common infectious diseases in sub-Saharan Africa. Pregnant women are uniquely susceptible to malaria because infected erythrocytes sequester in the placenta. Placental malaria infection is associated with maternal anaemia and infant low birth weight.[1] In sub-Saharan Africa, previous studies demonstrated that up to 40% of pregnant women develop placental malaria.[1]

Although malaria during pregnancy can have significantly impact the health of pregnant women and their newborns, it is often asymptomatic and difficult to detect by point-of-care diagnostic methods.[2] Most malaria-endemic countries recommend intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) during the second and third trimesters. SP-IPTp has been shown to diminish the risk of malaria-associated poor pregnancy outcomes but the emergence of SP-resistant malaria is threatening its efficacy hence the urgent need to find replacement.[3]

In determining an alternative to SP-IPTp, a new drug regimen may provide intermittent treatment, as SP did, or provide chemo-prophylaxis to continuously suppress blood stage infection. Chemoprophylaxis is usually administered at lower doses than curative regimens and may permit the persistence or recurrence of low-level parasitaemia that, in pregnant women, could allow for placental sequestration.[4] In contrast, IPTp with a full curative dose of an efficacious drug, may clear infections in all compartments, but would allow recurrent peripheral and placental infection during subsequent infections, especially where transmission rates are high. If infection followed by reliable clearance reverses any adverse effect of placental malaria, then effective IPTp may be as beneficial as preventing all infections. However, given rapid foetal development, even a limited period of acute inflammation and placental dysfunction may adversely impact growth. The maternal and foetal impact of IPTp and chemoprophylaxis have

not been compared because most clinical trials employ different drugs and differences in outcomes are often attributed to variability in the efficacy of the drugs. We had the unique opportunity to conduct a study to elucidate the impact of IPTp vs. chemo-prophylaxis in Malawi.

Chloroquine has well-established dosing regimens for both prophylaxis and treatment,[5] making it one of the few drugs capable of comparing the difference between these two approaches. Chloroquine, owing to extensive use in connective tissue disorders and for malaria prevention, has a well-documented safety and tolerability profile in pregnancy.[6] However, its use for both prevention and treatment was suspended due to widespread resistance in the 1980s and 1990s.[7] The return of chloroquine-susceptible malaria in Malawi[8] allows us to evaluate the potential role of chloroquine in preventing malaria during pregnancy and associated adverse outcomes. The results will ultimately impact malaria policy throughout Africa as the return of chloroquine-susceptible malaria is being reported in Zambia, Tanzania and Senegal. [9-11]

We hypothesized that chloroquine-IPTp or chloroquine chemoprophylaxis would be superior to SP-IPTp in preventing placental malaria infection and the maternal and newborn adverse effects of pregnancy-associated malaria. We therefore performed a randomized controlled clinical trial of chloroquine as IPTp or chloroquine as weekly prophylaxis independently compared to SP-IPTp. Our primary outcome was incidence of placental malaria and our secondary aims included incidence of peripheral malaria infection, clinical malaria illness and maternal and infant adverse outcomes.

METHODS

Study design

Our study was a three-arm, phase 3, open label, randomized controlled clinical trial comparing a weekly prophylactic dose of chloroquine (chloroquine-prophylaxis) and two curative doses of chloroquine (chloroquine-IPTp) to SP-IPTp at Ndirande Health Center, Blantyre, southern Malawi.[12] Malaria infection prevalence in Blantyre at the time of the study was 9% in the peak season[13] and SP resistance was widespread.[14]

The University of Maryland, Baltimore Institutional Review Board, University of Malawi, College of Medicine Research and Ethics Committee, and the Malawi Pharmacy, Medicines and Poisons Board reviewed and approved the study protocol and informed consent forms. We obtained written informed consent from each participant prior to conducting any study procedures.

Study participants

Pregnant women were eligible if they were in their first or second pregnancy, before the 27th week of gestation, attending their first antenatal clinic visit, not yet taken routine SP IPTp, willing to deliver at either the Ndirande Health Centre or Queen Elizabeth Central Hospital in Blantyre, and hoped to remain in the area until 14 weeks after delivery. We excluded women who were HIV infected, receiving any chronic therapy with antimalarial or antifolate drugs, had high risk pregnancies requiring regular supervision of an obstetrician or with known allergies to study drugs. During screening, we evaluated symptomatic women and deferred enrolment if they had clinical malaria illness until after treatment and resolution. We used ultrasound to estimate gestational age at enrolment.

Randomization and masking

Participants were randomly assigned to receive SP-IPTp, chloroquine-IPTp, and chloroquine-prophylaxis in a 1:1:1 ratio. We used a computer-generated block randomization list (blocks of

sizes three and six) prepared by a statistician based at the Statistical and Data Coordinating Center (SDCC) who had no contact with either the participants or study staff caring for the participants. The treatment assignments were concealed using scratch cards adjacent to sequentially listed participant identification numbers.

Those assessing the histopathology and molecular results were blinded to treatment assignments but study participants, clinical staff and the SDCC team were not.

Procedures

Participants in the SP-IPTp group received 1,500 mg sulfadoxine and 75 mg pyrimethamine (Fansidar, Micro Labs,India) twice at least four weeks apart during pregnancy. In the chloroquine-IPTp group, participants received two treatments of chloroquine (ARALEN®, West-Ward Pharmaceutical Corporation, United States) given as 600mg on day one, 600mg on day two, and 300mg on day 3 at least four weeks apart during pregnancy. Participants in the chloroquine-prophylaxis group received 600mg of chloroquine at enrolment followed by 300mg once every week up to delivery.

We administered all study treatments under directly observed therapy and from 20 and 28 weeks gestation based on ultrasound scan. For each foetus, the trained ultrasonographer averaged two sets of biometric measurements to estimate the gestational age. A perinatologist provided training and routine quality control.

We saw participants at least once every four weeks until delivery, then at 2, 6, 10 and 14 weeks postdelivery. We encouraged participants to attend the study clinic when they had health complaints. We attempted to trace participants who failed to attend appointments. At each routine or sick visit, we obtained a comprehensive history including malaria symptoms and

routine obstetric complaints, followed by clinical examination, and management of any ailments. At all routine visits, we collected a dried blood spot on filter paper and measured haemoglobin concentration (HemoCue®). At any visit during which participant had symptoms suggestive of malaria, we prepared and read thin and thick blood smears to facilitate clinical management. We obtained placental biopsies, placental impression smears, and cord blood from deliveries that occurred at the Ndirande Maternity Ward and Queen Elizabeth Central Hospital.

For microscopic detection of malaria infection from the thin and thick blood smears, we stained them with Giemsa stain and two microscopists(MK, RM and others) read them independently to detect the presence of *Plasmodium* parasites. If their readings were discordant, the slide was read by a third expert reader. For molecular detection of malaria infection, we extracted DNA from the filter paper dried blood spots and performed quantitative real-time PCR evaluation for *Plasmodium falciparum* 18S rRNA using University of Maryland malaria research program standard procedures.[15] We performed pyrosequencing on cases of malaria infection detected at enrolment to determine the *P. falciparum* chloroquine resistance transporter genotype at positive 76, the molecular marker for chloroquine-susceptibility.[15] We did not analyse resistance to SP because it is ubiquitous in Malawi and surrounding countries.[14]

For histopathological analysis, we cut the paraffin wax-preserved placental biopsies into four micron thick sections, applied them to glass slides, and then stained with hematoxylin and eosin. The histopathologist based at the University of Malawi, College of Medicine evaluated placental slides for malaria pigment (hemozoin) and malaria parasite to determine acute, chronic or past infection defined as visible parasites but little or no definitive pigment, presence of parasites and pigment, and presence of pigment in the absence of parasites respectively (see examples in Appendix). Ten percent of all the placental slides underwent quality control by a histopathologist based at the Centers for Disease Control and Prevention.

Outcomes

Our primary endpoint was histologically confirmed placental malaria defined as the presence of hemozoin or parasites on a placental biopsy specimen. We also used the outcome of placental malaria measured by either histological or molecular evidence of infection as a secondary definition of placental malaria. Our secondary outcomes were peripheral malaria infection, clinical malaria illness, and adverse outcomes associated with malaria in pregnancy. We recorded episodes of peripheral malaria infection and clinical malaria illness between enrolment and delivery dates.

We defined clinical malaria illness as malaria infection on blood smear in a participant with any one of the following; fever (axillary temperature ≥37·5^{O}C) measured at the clinic, history of fever in the past 48 hours or other symptoms (headache, myalgia, vomiting or weakness) in the last 48 hours. We defined a new case as a clinical malaria episode occurring at least 14 days after the last treatment day of the previous episode. When a maternal clinical malaria illness was diagnosed, we treated the participant according to Malawi national guidelines: they received quinine if in the first trimester and artemether-lumefantrine if in the second or third trimesters. We defined malaria infection as detection of parasites by microscopy or PCR regardless of symptoms. We defined baseline malaria infection as malaria infection at the enrolment visit.

The malaria adverse effects we evaluated included incidence of maternal anaemia (haemoglobin concentration < 10 g/dL), maternal severe anaemia (haemoglobin concentration < 7g/dL), stillbirth and miscarriage (pregnancy loss before and after 28 weeks gestation, respectively), preterm delivery (delivery before 37 weeks gestation), low birth weight (birth weight less than 2500g), intrauterine growth restriction (IUGR, weight <10th percentile

gestational age based on the WHO foetal growth curve), and early infant mortality (death before 14 weeks of age).

Statistical analysis

We calculated the sample size to provide 80% power assuming a 40% incidence of histology-determined placental malaria for women receiving SP-IPTp and a reduction of 33% in either chloroquine treatment arm. Since two separate hypothesis tests were required (i.e., comparing SP-IPTp to each treatment arm), We based the sample size calculation on a two-sided test for difference in proportions with a normal approximation, which led to an estimate of 237 participants per arm, accounting for the Bonferroni correction so each p-value was compared to an alpha of 0·025. Assuming that 20% of enrolled mothers would not contribute a primary outcome, we reached a target sample size of 300 per arm, or 900 in total.

We performed the primary analysis using a modified intention-to-treat (mITT) approach that included all randomized women who contributed placental histology results in the groups to which they were randomized, regardless of the amount of treatment received. We also performed a per-protocol (PP) analysis, excluding any participants who received non-study SP, who were lost to follow-up before delivery, who did not contribute histology results, or who were non-compliant in terms of their allocated treatment arm (missing any dose for chloroquine-IPTp and SP-IPTp arms, or missing 4 or more doses for chloroquine-prophylaxis arm).

For the a priori primary analysis, relative risks of placental malaria in each treatment arm were calculated relative to the SP-IPTp group, and p-values were calculated using Fisher's exact test. Our secondary outcome, placental malaria detected by histopathology or by qPCR, was analysed in the same way.

Our protocol also specified an adjusted analysis of the primary outcome including any of the following potential confounding variables, which are known predictors of placental malaria:[16-19] maternal age, gravidity status, gestational age at enrolment, low body mass index (BMI) (< 18·5 kg/m^2), enrolment during malaria transmission season, use of an insecticide-treated bed net the night before enrolment, baseline anaemia (haemoglobin < 10 g/dL at enrolment), and baseline malaria infection status. Covariates whose relative risks with the outcome had p-values < 0·10 were included in a multivariable model. As an exploratory analysis, we expanded the model to examine interactions between each covariate and treatment assignment. In the expanded model, baseline malaria was the sole covariate whose interaction with at least one treatment variable was statistically significant at the 0·10 level (p = 0.046 in the mITT population). For this reason, we proceeded to fit a model stratified on baseline malaria status. This model is a post-hoc analysis as we had not anticipated any covariate-treatment interactions. We estimated coefficients for the adjusted and stratified models using modified Poisson regression.[20]

For our secondary outcomes, we compared relative risks of peripheral malaria and clinical malaria infection between treatment arms in the intention-to-treat population (including all mothers who were randomized). We also fit Kaplan Meier curves and performed log rank tests comparing time to malaria infection and time to clinical malaria between treatment arms.

We assessed safety and tolerability by comparing unsolicited adverse events between arms from the date the first study product was administered through the final study visit. We graded adverse events depending on severity as defined in the Division of Microbiology and Infectious Diseases (DMID) adult toxicity table of November 2007. Adverse events were listed as mild (grade 1) if they required minimal or no treatment and did not interfere with the patient's daily activities; moderate (grade 2) if they resulted in a low level of inconvenience often interfering

with functioning and requiring minor intervention; Severe (grade 3) if they interrupted usual daily activity and often requiring systemic drug therapy or other treatment; or Life-Threatening (grade 4) if they placed the participant at immediate risk of death.

When reporting each adverse event, we determined based on known properties of the study products, whether the event was causally related to any of the trial treatments. All participants who received treatment and contributed safety data were included in these analyses. We performed analyses using SAS version 9.4 (SAS Institute, USA) and R version 3.4.0.

A Data and Safety Monitoring Board (DSMB) with expertise in clinical trials, malaria in Africa, and biostatistics provided safety oversight. The DSMB reviewed safety data twice during data collection and this included assessment against pre-specified halting criteria. We registered the protocol with ClinicalTrials.gov (NCT01443130).

Role of the funding source

The funder of the study contributed towards the study design, but had no role in data collection, data analysis, data interpretation, or writing of this manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

From February 2012 to May 2014, we screened 1,209 women and randomized 900 to receiving chloroquine-prophylaxis (n=300), chloroquine-IPTp (n=300), or SP-IPTp (n=300) (Figure 1). Eighty-five percent (766/900) of the randomized women provided pregnancy outcome data (Figure 1). Of these, 765 contributed histopathology results, and were included in the mITT analysis for the primary outcome. One participant missing histopathology results did contribute

qPCR results, so was also included in the mITT analysis of the secondary outcome. The PP population comprised of 80% (719/900) of the randomized women. Inclusion in the mITT or PP populations did not differ significantly between treatment arms. However, women who did not contribute a histopathology result tended to be younger at enrolment (19·92 vs. 20·61 years, p = 0·027), have lower gestational age at enrolment (21·90 vs. 22·30 weeks, p = 0·05), and be more likely to have baseline malaria infection (24.44% vs. 18.17%, p=0.087) when compared to those who were successfully followed to delivery and included in primary outcome analysis. Baseline characteristics of participants are summarized in Table 1, and those for participants in the mITT population are in the Appendix, Table A1. All malaria infections that occurred at enrolment were chloroquine-susceptible based on carrying the susceptible form the of the *pfcrt* gene.

Table 2 shows results for our primary and secondary outcomes. The rate of placental malaria in the SP-IPTp arm (15.4%) was much lower than the anticipated rate of 40% that we had used for our sample size calculation. In our primary analysis, the risk of placental malaria detected by histopathology among mothers receiving chloroquine-prophylaxis (30/259 [11.6%]) was lower than those receiving SP-IPTp (15.4%). The relative risk is 0.75 (95% C.I. [0.48, 1.17]) and is not statistically significant (p = 0.24). The relative risk from the model adjusting for potential confounding variables is 0.66 (95% C.I. [0.46, 0.95]) and approaches statistical significance (p = 0.03, compared to a Bonferroni-adjusted significance level of 0.025). The adjusted RR for the secondary outcome, placental malaria detected by positive histopathology or positive qPCR, was 0.64 (95% C.I. [0.46, 0.90]) and was statistically significant (p = 0.01). We did not find evidence that the chloroquine IPTp treatment differed from SP-IPTp in preventing placental malaria (Table 2). Results for PP analysis are similar. (Appendix Table A2). Appendix Tables A3 and A4 include full results from the adjusted models (including covariate effects).

Table 3 shows that in a stratified model on baseline malaria status, chloroquine-prophylaxis has a stronger protective effect in the subgroup of participants without baseline malaria (RR 0.44; 95% CI: 0.23, 0.85; p =0.01) compared to the subgroup of those with baseline malaria (RR 0.94 (95% CI: 0.70, 1.27 p =0.69) (Table 4). Chloroquine-IPTp treatment did not significantly impact either subgroup. In this model, the p-value for the interaction between chloroquine-prophylaxis and baseline malaria is 0.04; it is 0.93 for the interaction between chloroquine-IPTp and baseline malaria. Results for the PP analysis are similar (Appendix Table A5).

We recorded 86 malaria infections during follow up, 15 of which were clinical malaria illness. There was no difference in the risk of malaria infection or clinical malaria illness by treatment arm (Appendix Table A6). Chloroquine-prophylaxis was associated with a longer time to clinical malaria illness compared to SP-IPTp (log rank p=0.03). The time to clinical malaria was similar for chloroquine-IPTp and SP-IPTp (log rank p=0.17). There was no difference in time to malaria infection between the groups.

Malaria-related prenatal and perinatal outcomes included 168 cases (19%) of anaemia during the prenatal period (including 2 severe malaria cases), 26 cases (3%) of anaemia at delivery (none was severe), and one maternal death (Table 4). There were more cases of maternal anaemia at delivery in the chloroquine-IPTp group (15 cases) compared to the SP-IPTp group (5 cases, p=0·04), although the difference is not statistically significant when applying Bonferroni-adjusted significance level of 0.025. There were no significant differences between arms for prenatal anaemia, severe anaemia, or maternal death.

The overall mean birth weight was 2.89 kg (SD = 0.42 kg) and average gestational age at delivery was 38.5 weeks (SD = 2.4 weeks) were similar between treatment arms. The

interventions were not associated with the risk of low birth weight, preterm delivery or smallness for gestational age (Table 7).

Both chloroquine treatment regimens were associated with higher rates of treatment-related adverse events than the SP-IPTp regimen (Table 5). More women in the chloroquine-IPTp (94 women, $p < 0.001$) and chloroquine-prophylaxis (26 women, $p<0.001$) groups experienced at least one treatment-related adverse event than those randomized to SP-IPTp arm (4 women). The most recorded treatment-related adverse events among women were dizziness, vomiting, nausea, palpitations, headache, and abdominal pain. In the SP-IPTp group, 1 woman experienced a Grade 2 or higher treatment-related adverse event, compared to 14 women .3% vs. 5%, $p=0.001$) in the chloroquine-prophylaxis group and 57 women (19%, $p<0.001$) in the chloroquine-IPTp group. Five treatment-related adverse events (4 in chloroquine-IPT and 1 in chloroquine-prophylaxis arms) led to study treatment termination. No significant differences in rates of serious adverse events, between treatment arms were observed.

DISCUSSION

Our open-label randomized controlled clinical trial provides evidence that chloroquine-prophylaxis may be more effective than SP-IPTp in reducing placental malaria in healthy pregnant women in Malawi. In our primary analysis, we did not find evidence of superior protection from histopathology determined placental malaria using either chloroquine-prophylaxis or chloroquine-IPTp versus the current standard of care, SP-IPTp. Our adjusted model found the reduction in placental malaria due to chloroquine-prophylaxis was approached significance for the primary measure of placental malaria by histopathology and was statistically significant when including placental specimens with molecular evidence of infection. Our post-hoc stratified model indicated that the effect was strongest among participants without baseline malaria. Furthermore, compared with SP-IPTp, chloroquine-prophylaxis offered some protection

against clinical malaria illness during pregnancy. Chloroquine-IPTp, however, did not provide any benefit compared to SP-IPTp.

Our stratified analysis suggests that chloroquine-prophylaxis may be more effective than SP-IPTp in preventing new placental malaria infections in pregnant women. However, in the case of existing sequestration of parasites in the placenta, as was the case with women who were infected with malaria when they first presented to antenatal care, the prophylactic doses of chloroquine did not effectively clear the placental infection. As the findings from our stratified model were unanticipated and based on an exploratory analysis, we recommend their validation through confirmatory studies which explicitly investigate the effectiveness of a chloroquine prophylaxis regimen and whether this effect is modified by baseline malaria infection.

One drawback about the use of chloroquine is the higher rate of drug-related adverse reactions compared to SP-IPTp. Most events were mild and dose dependent. The specific complaints and also the higher rate in IPTp compared to prophylaxis were similar to previously described reactions.[21] These complaints rarely resulted in discontinuation of the study treatment. In our study adverse events in the chloroquine arms were mostly mild or moderate (grade 1 or 2) and rarely led to treatment discontinuation. This experience is different from that evaluation of a fixed-dose combination of chloroquine and azithromycin and registered more treatment discontinuations in the intervention arm compared to SP-IPTp arm.[22] It is possible that the intolerance was largely driven by the azithromycin or differences in reporting of safety outcomes. This highlights the need for standardized methods for assessing and reporting safety data from drug trials In addition, because our study was open-label, participant perception of health outcomes may have been influenced by their knowledge of their treatment assignment, and bias may have been introduced in clinical care and adverse event reporting. However, our

primary endpoint of placental malaria, especially given the direct observation of study drugs, is unlikely to have been impacted by this.

Among the maternal and newborn outcomes of malaria during pregnancy that are measured, birth weight is typically considered to be of greatest public health importance. Although we found some evidence that chloroquine prophylaxis may be more effective in preventing placental malaria than SP-IPTp, there was no impact on infant birthweight. The lack of impact of more effective antimalarial drugs on birthweight when compared to SP-IPTp is consistent with many recent findings in which dihydroartemisinin-piperaquine and mefloquine IPTp regimens were more effective in preventing malaria but their use as IPT was not associated with improved birthweight. This suggests that malaria in this setting is not the key driving factor contributing to low birthweight or that SP has an impact on birthweight, perhaps through its broad antimicrobial activity, that is not limited to malaria.[23]

Despite high rates of SP resistant malaria in Malawi,[14] replacing SP-IPTp with chloroquine-IPTp did not provide significantly increased protection against placental malaria, peripheral malaria infection or clinical malaria disease. Larger studies in higher transmission settings have demonstrated that monthly dosing of drugs that are more effective than SP do lead to lower rates of malaria infection and disease.[24] Recent studies employing monthly dihyrdroartemisinin-piperaquine demonstrated better protection from malaria during pregnancy for women receiving the intervention than those receiving monthly SP-IPTp.[25,26] This suggests that increasing the frequency of curative doses from twice during pregnancy to monthly with an effective antimalarial may be an even more optimal approach. This study was conducted before Malawi adopted the World Health Organization recommendation of giving SP IPTp doses at every scheduled antenatal visit in the second and thirrd trimesters, at least one month apart. It is

possible that increasing from two to more doses may lead to improved protection against malaria infection and disease in the chloroquine-IPT group.

Our study had lower power than planned due to a lower rate of placental malaria in the base population (15.4% in SP-IPTp arm) than what we expected during study planning (≥40%). This effectively decreased our power to detect the planned difference (≥33%) by 65% (from 80% down to 28%). Malaria prevalence among pregnant women at our study site declined over the course of the study in association with increased distribution of insecticide-treated bed nets.[12]

As a drug used for frequent IPTp administration, chloroquine has several advantages over dihydroartemisinin-piperaquine. The safety of chloroquine in pregnancy has been well-established due to its widespread use in auto-immune diseases. Exposure to artemisinin compounds during the first trimester is currently not recommended in the World Health Organization treatment guidelines due to early embryonic toxicity identified in some animal studies.[27]

Another key advantage of using chloroquine is avoidance of drug pressure that may promote the development of resistance to either the artemisinin compound or its partner drug when artemisinin-based combination therapy is used for both IPTp and also for treatment of malaria disease in the general population. We have recently demonstrated that SP-resistant malaria in Malawi has persisted and even increased in prevalence with the continued use of SP for IPTp even after its use for the treatment of malaria was discontinued.[14] Although artemisinin resistance has not appeared to emerge in Africa,[28] its arrival is inevitable. It is unclear if using the same or similar drugs for treatment and for IPTp will hasten the emergence or spread of resistance. If a drug or drug combination used in IPTp is not simultaneously used for clinical

malaria case management, then IPTp may actually lower the selection pressure on the first-line drug by decreasing symptomatic cases that require treatment.

In summary, our study did not have enough superiority evidence of chloroquine either as IPTp or as chemoprophylaxis versus SP-IPTp for prevention of malaria during pregnancy and associated maternal and infant adverse outcomes. This was likely secondary to low event rate which reduced our discriminatory power. However, the results of our adjusted analysis and ITT safety analyses suggest that chloroquine prophylaxis remains a valuable alternative to SP worth re-examining in future trials. Our findings are likely generalizable to much of eastern Africa and parts of western Africa where chloroquine-susceptible malaria now predominates. If suppressive levels can be maintainedthrough weekly dosing, chloroquine may provide better protection against clinical malaria disease in pregnant women and parasite sequestration in the placenta than the current standard of care. Most recent studies, with one notable exception, have not found that improved malaria prevention was associated with an increase in birthweight.[24] While birthweight is one of the key public health outcomes to evaluate when identifying a new strategy to prevent malaria during pregnancy, additional outcomes for studies should be considered in evaluating the need to switch from SP-IPTp to a more effective drug.

CONTRIBUTORS

All authors contributed towards study design, study implementation, protocol amendments, and manuscript development. MKL conceived the study, wrote the protocol, acquired funding, and was the principal investigator. THD was the lead investigator at the research site and supervised study implementation with MKL. THD, RGM, JG, PMM, OMN, MK, MN, LT, RM, and FM prepared and supervised all standard operating procedures, and led data collection. PM and OMN oversaw the clinical team and established and maintained the regulatory file and communications. BJW provided foetal biometry training and supervision. LN, PM, and YM

provided specialist obstetrics and gynaecology evaluation of study participants for determination of inclusion criteria and key outcomes. TT performed histopathology examinations. AM provided histopathology training and quality assurance. JK and the Emmes Corporation performed database design and management. THD, MKL, JK and the Emmes Corporation co-wrote the statistical analysis plan. GP, JK, and the Emmes Corporation analysed the data, which was interpreted jointly by THD, MKL, GP, JK, and the Emmes Corporation. THD and MKL drafted the manuscript, and all authors reviewed, revised, approved, and take responsibility for this final version.

DECLARATION OF INTERESTS

We declare that we have no competing interests.

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TABLES AND FIGURES

FIGURE 1: Study Consort

Divala TH, Mungwira RG, Mawindo PM, Nyirenda OM, Kanjala M, Ndaferankhande M, Tsirizani LE, Masonga R, Muwalo F, Boudová S, Potter GE. Chloroquine as weekly chemoprophylaxis or intermittent treatment to prevent malaria in pregnancy in Malawi: a randomised controlled trial. The Lancet Infectious Diseases. 2018 Oct 1;18(10):1097-107. doi:10.1016/S1473-3099(18)30415-8

Table 1: Summary of baseline characteristics for all 900 randomized participants

Covariate	All	Chloroquine prophylaxis	Chloroquine-IPTp	SP-IPTp
Continuous Covariates	**Mean (SD)**			
Maternal Age at Enrolment/Randomization (years)	20.5 (3.3)	20.4 (3.6)	20.7 (3.2)	20.4 (3.1)
Gestational Age at Enrolment by Ultrasound (weeks)	22.2 (2.2)	22.5 (2.2)	22.2 (2.2)	22.0 (2.1)
Categorical Covariates	**n (%)**			
Young Maternal Age at Enrolment/ Randomization (< 20 Years)	382 (42)	141 (47)	111 (37)	130 (43)
Gravidity Status at Enrolment/Randomization	518 (58)	177 (59)	162 (54)	179 (60)
BMI less than 18.5 kg/m^2	7 (1)	1 (<1)	2 (1)	4 (1)
Malaria Transmission Season at Enrolment/Randomization	309 (34)	102 (34)	102 (34)	105 (35)
Used a bed net the night before enrolment	678 (75)	228 (76)	221 (74)	229 (76)
Anaemia (Haemoglobin < 10 g/dL) at Enrolment/Randomization	80 (9)	26 (9)	31 (10)	23 (8)
Malaria Infection at Enrolment/ Randomization	80 (9)	28 (9)	23 (8)	29 (10)

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Table 2: Efficacy of interventions on the incidence of placental malaria, modified intent-to-treat population

	n/N (%)			Relative Risk [95% CI]		P-value	
Type of Infection	**Chloroquine prophylaxis**	**Chloroquine-IPTp**	**SP-IPTp**	**Chloroquine prophylaxis vs. SP-IPTp**	**Chloroquine-IPTp vs. SP-IPTp**	**Chloroquine prophylaxis vs. SP-IPTp**	**Chloroquine-IPTp vs. SP-IPTp**
Positive histopathology (primary outcome)	30/259 (11.6)	39/253 (15.4)	39/253 (15.4)	0.75 [0.48, 1.17]	1.00 [0.67, 1.50]	0.24	1.00
Positive histopathology, Adjusted	-	-	-	0.66 [0.46, 0.95]	0.99 [0.69, 1.41]	0.03	0.94
Positive histopathology or positive qPCR	34/259 (13.1)	45/254 (17.7)	47/253 (18.6)	0.71 [0.47, 1.06]	0.95 [0.66, 1.38]	0.12	0.82
Positive histopathology or positive qPCR, Adjusted	-	-	-	0.64 [0.46, 0.90]	0.94 [0.68, 1.32]	0.01	0.74

NOTE: The adjusted model includes maternal age, gestational age, and indicators for bednet use the night before enrolment, anaemia at enrolment, and malaria infection at enrolment.

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Table 3. Placental malaria (by histology) by treatment arm stratified by baseline malaria, mITT population (adjusted for maternal age, gestational age at enrolment, bed net use, anaemia at enrolment)

	Baseline Malaria (n=65)			**No Baseline Malaria (n=700)**		
Variable	**Estimate**	**95% Confidence Interval**	**P-value**	**Estimate**	**95% Confidence Interval**	**P-value**
Chloroquine prophylaxis	0.942	(0.700, 1.268)	0.693	0.442	(0.230, 0.848)	0.014
Chloroquine-IPTp	0.967	(0.692, 1.352)	0.844	0.995	(0.593, 1.672)	0.986
Maternal Age	0.871	(0.822, 0.923)	<0.001	0.902	(0.841, 0.968)	0.004
Gestational Age (weeks)	0.961	(0.899, 1.026)	0.234	1.103	(0.994, 1.225)	0.065
Bednet Use, Night before Enrollment	1.050	(0.799, 1.380)	0.725	1.267	(0.774, 2.076)	0.347
Anaemia at Enrollment (Hemoglobin < 10 mg)	1.368	(1.070, 1.749)	0.013	2.803	(1.638, 4.796)	<0.001

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Table 4: Number and proportion of subjects experiencing malaria-related prenatal and perinatal outcomes by treatment group in the intention-to-treat population

	ALL		SP-IPTp		Chloroquine-IPTp		Chloroquine prophylaxis		Chloroquine-IPTp vs. SP-IPTp			Chloroquine prophylaxis vs. SP-IPTp		
	N	%	N	%	N	%	N	%	RR	95% CI	P-Value	RR	95% CI	P-Value
Maternal Outcomes														
Prenatal Maternal Anaemia (haemoglobin < 10 g/dL)	168	19	60	20	61	20.3	47	15.7	1.02	(0.74, 1.40)	1	0.783	(0.55, 1.11)	0.200
Prenatal Severe Maternal Anaemia (haemoglobin < 7 g/dL)	2	0	1	0.3	1	0.3	0	0	1	(0.11, 9.55)	1	0	(0, 3.83)	1
Delivery Maternal Anaemia (haemoglobin < 10 g/dL)	26	3	5	1.7	15	5	6	2	3	(1.15, 7.87)	0.038	1.2	(0.39, 3.67)	1
Delivery Severe Maternal Anaemia (haemoglobin < 7 g/dL)	0	0	0	0	0	0	0	0	NA	NA	1	NA	NA	1
Maternal death	1	0	0	0	1	0.3	0	0	NA	NA	1	NA	NA	1

Infant Outcomes														
Low birth weight (<2.5kg)	101	13	31	12	29	11	41	16	0.91	(0.57, 1.45)	0.784	1.29	(0.84, 1.98)	0.257
Preterm Delivery (estimated gestational age < 37 weeks)	69	8	18	7	28	10	23	8	1.50	(0.86, 2.63)	0.168	1.25	(0.69, 2.24)	0.518
IUGR (weight < 10th percentile gestational age based on WHO foetal growth curve)	142	18	52	21	47	18	43	17	0.87	(0.61, 1.23)	0.435	0.80	(0.55, 1.14)	0.255
Stillbirth (estimated gestational age ≥ 28 weeks)	9	1	5	2	1	0	3	1	0.19	(0.03, 1.23)	0.117	0.58	(0.16, 2.19)	0.499
Miscarriage (estimated gestational age < 28 weeks)	6	1	3	1	2	1	1	0	0.64	(0.13, 3.19)	0.681	0.32	(0.05, 2.25)	0.367
Infant Death (< 14 weeks old)	24	3	8	3	10	4	6	2	1.20	(0.50, 2.92)	0.812	0.73	(0.27, 1.99)	0.598

NOTE: RR = relative risk

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Table 5: Number and proportion of maternal subjects experiencing adverse events possibly related to study treatment by treatment group and MedDRA Preferred Term

	SP-IPTp (N= 300)		**Chloroquine-IPTp |(N= 300)**		**Chloroquine prophylaxis (N= 300)**		**Chloroquine-IPTp vs. SP-IPTp**			**Chloroquine prophylaxis vs. SP-IPTp**		
	n	**%**	**n**	**%**	**n**	**%**	**RR**	**95% CI**	**P-value**	**RR**	**95% CI**	**P-value**
Any adverse event	4	1%	94	31%	26	9%	23.5	(9.2, 61.1)	<0.001	6.5	(2.4, 17.7)	<0.001
At least one Grade 3 or higher adverse event	0	0%	3	1%	0	0%	NA	NA	0.249	NA	NA	1
At least two adverse events	1	0%	64	21%	10	3%	64.0	(11.3, 365.5)	<0.001	10.0	(1.7, 60.4)	0.011
Termination of study drug due to treatment-related AE	0	0%	4	1%	1	0%	NA	NA	0.124	NA	NA	1

Dizziness[1]	1	0%	57	19%	7	2%	57.0	(10.1, 326)	<0.001	7.0	(1.1, 43.5)	0.068
Vomiting	0	0%	50	17%	11	4%	NA	NA	<0.001	NA	NA	0.001
Palpitations	1	0%	14	5%	7	2%	14.0	(2.4, 83)	0.001	7.0	(1.1, 43.5)	0.068
Headache	1	0%	17	6%	2	1%	17.0	(2.9, 100)	<0.001	2.0	(0.3, 15.2)	1
Nausea	2	1%	15	5%	3	1%	7.5	(1.9, 29.3)	0.002	1.5	(0.3, 7.5)	1
Abdominal pain	0	0%	7	2%	2	1%	NA	NA	0.015	NA	NA	0.499

NOTE: RR = relative risk

[1] The six most frequent related events are displayed.

Figure 1 Consort Diagram

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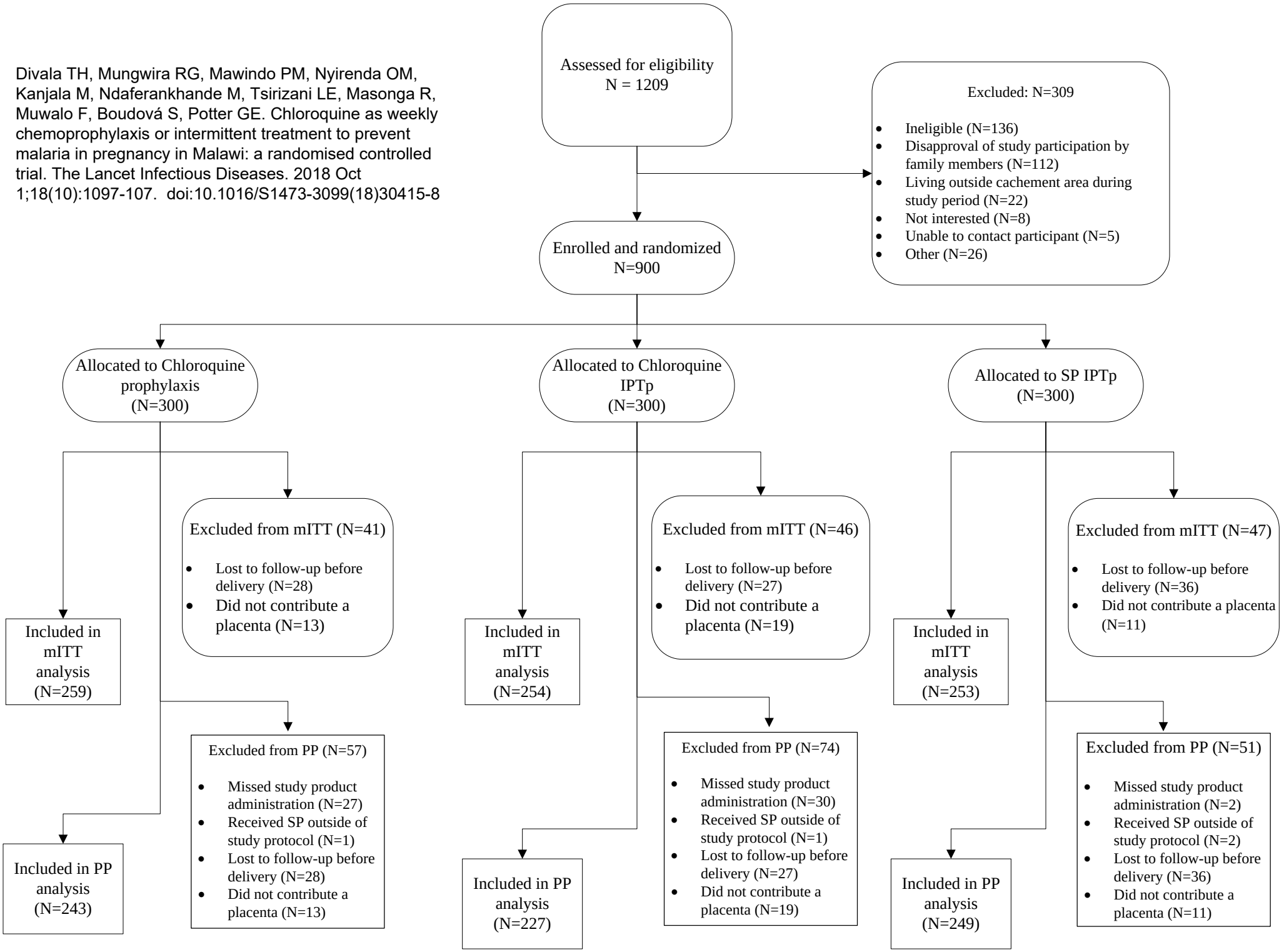

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Appendix

Table A1. Summary of Baseline Characteristics for Participants in the Modified Intent-to-Treat Population

Covariate	All Treatment Groups	Chloroquine Weekly	Chloroquine IPTp	SP IPTp
Continuous Covariates	**Mean (SD)**			
Maternal Age at Enrollment/Randomization	20.6 (3.4)	20.4 (3.7)	20.9 (3.3)	20.5 (3.2)
Gestational age by ultrasound (weeks)	22.3 (2.2)	22.5 (2.2)	22.3 (2.2)	22.1 (2.1)
Categorical Covariates	**n (%)**			
Young Maternal Age at Enrollment/Randomization (< 20 Years)	316 (41)	123 (47)	86 (34)	107 (42)
Gravidity Status at Enrollment/Randomization	438 (57)	154 (59)	135 (53)	149 (59)
BMI (weight/height2) less than 18.50	5 (1)	1 (<1)	2 (1)	2 (1)
Malaria Transmission Season at Enrollment/Randomization	265 (35)	87 (34)	89 (35)	89 (35)
Used a bednet last night	582 (76)	196 (76)	193 (76)	193 (76)
Anemia (Hemoglobin < 10 mg) at Enrollment/Randomization	70 (9)	25 (10)	26 (10)	19 (8)
Malaria Infection at Enrollment/Randomization	65 (8)	24 (9)	20 (8)	21 (8)

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Table A2: Efficacy of interventions on the incidence of placental malaria, per-protocol population

	n/N (%)			Relative Risk [95% CI]		P-value[1]	
Type of Infection	**Chloroquine prophylaxis**	**Chloroquine-IPTp**	**SP-IPTp**	**Chloroquine prophylaxis vs. SP-IPTp**	**Chloroquine-IPTp vs. SP-IPTp**	**Chloroquine prophylaxis vs. SP-IPTp**	**Chloroquine-IPTp vs. SP-IPTp**
Positive histopathology	27/243 (11.1)	35/226 (15.5)	39/249 (15.7)	0.71 [0.45, 1.12]	0.99 [0.65, 1.50]	0.15	1.00
Positive histopathology, Adjusted	-	-	-	0.64 [0.44, 0.93]	0.98 [0.68, 1.42]	0.02	0.92
Positive histopathology or positive qPCR	31/243 (12.8)	40/227 (17.6)	46/249 (18.5)	0.69 [0.45, 1.05]	0.95 [0.65, 1.40]	0.08	0.91
Positive histopathology or positive qPCR Adjusted	-	-	-	0.64 [0.44, 0.91]	0.95 [0.67, 1.34]	0.01	0.76

Divala TH, Mungwira RG, Mawindo PM, Nyirenda OM, Kanjala M, Ndaferankhande M, Tsirizani LE, Masonga R, Muwalo F, Boudová S, Potter GE. Chloroquine as weekly chemoprophylaxis or intermittent treatment to prevent malaria in pregnancy in Malawi: a randomised controlled trial. The Lancet Infectious Diseases. 2018 Oct 1;18(10):1097-107. doi:10.1016/S1473-3099(18)30415-8

Table A3. Efficacy of interventions on the incidence of placental malaria (by histopathology) adjusting for baseline characteristics, mITT population

	Univariate analysis, N=765		Multivariable analysis, N=765	
Variable	**Unadjusted RR (95% Confidence Interval)**	**P-value**	**Adjusted RR (95% Confidence Interval)**	**P-value**
Chloroquine Prophylaxis	0.75 [0.48, 1.17]	0.244	0.66 [0.46, 0.95]	0.027
Chloroquine-IPTp	1.00 [0.66, 1.50]	1.000	0.99 [0.69, 1.41]	0.936
Maternal Age (years)	0.88 [0.83, 0.93]	<.001	0.89 [0.84, 0.93]	<.001
Gravidity Status (Primagravid)	1.13 [0.79, 1.61]	0.531		.
Gestational Age (weeks)	1.07 [0.99, 1.16]	0.080	1.05 [0.98, 1.13]	0.140
BMI less than 18.50[†]	0 [0, 3.10]	1.000		.
Malaria Transmission Season	0.98 [0.68, 1.42]	1.000		.
Bednet Use, Night before Enrollment	0.69 [0.48, 1.00]	0.068	1.17 [0.85, 1.61]	0.332
Anemia at Enrollment (Hemoglobin < 10 mg)	3.82 [2.71, 5.38]	<.001	2.02 [1.45, 2.80]	<.001
Baseline Malaria	8.30 [6.25, 11.0]	<.001	6.43 [4.74, 8.73]	<.001

[†]Score interval was used due to small cell counts.

Divala TH, Mungwira RG, Mawindo PM, Nyirenda OM, Kanjala M, Ndaferankhande M, Tsirizani LE, Masonga R, Muwalo F, Boudová S, Potter GE. Chloroquine as weekly chemoprophylaxis or intermittent treatment to prevent malaria in pregnancy in Malawi: a randomised controlled trial. The Lancet Infectious Diseases. 2018 Oct 1;18(10):1097-107. doi:10.1016/S1473-3099(18)30415-8

Table A4. Efficacy of interventions on the incidence of placental malaria (by histopathology) adjusting for baseline characteristics, PP population

	Univariate analysis, N=718		Multivariable analysis, N=718	
Variable	**Unadjusted RR (95% Confidence Interval)**	**P-value**	**Adjusted RR (95% Confidence Interval)**	**P-value**
Chloroquine Prophylaxis	0.71 [0.45, 1.12]	0.147	0.64 [0.44, 0.93]	0.021
Chloroquine-IPTp	0.99 [0.65, 1.50]	1.000	0.98 [0.68, 1.42]	0.924
Maternal Age (years)	0.88 [0.83, 0.93]	<.001	0.88 [0.84, 0.93]	<.001
Gravidity Status (Primagravid)	1.15 [0.80, 1.67]	0.516		.
Gestational Age (weeks)	1.07 [0.98, 1.16]	0.121	1.05 [0.97, 1.12]	0.215
BMI less than 18.50[†]	0 [0, 3.51]	1.000		.
Malaria Transmission Season	0.99 [0.67, 1.44]	1.000		.
Bednet Use, Night before Enrollment	0.67 [0.46, 0.99]	0.047	1.17 [0.84, 1.63]	0.342
Anemia at Enrollment (Hemoglobin < 10 mg)	3.73 [2.61, 5.32]	<.001	1.95 [1.39, 2.72]	<.001
Baseline Malaria	7.99 [5.95, 10.7]	<.001	6.21 [4.52, 8.53]	<.001

[†]Score interval was used due to small cell counts.

Divala TH, Mungwira RG, Mawindo PM, Nyirenda OM, Kanjala M, Ndaferankhande M, Tsirizani LE, Masonga R, Muwalo F, Boudová S, Potter GE. Chloroquine as weekly chemoprophylaxis or intermittent treatment to prevent malaria in pregnancy in Malawi: a randomised controlled trial. The Lancet Infectious Diseases. 2018 Oct 1;18(10):1097-107. doi:10.1016/S1473-3099(18)30415-8

Table A5. Placental Malaria (by histology) by treatment arm, stratified by baseline malaria, PP Population

	Baseline Malaria (n=61)			No Baseline Malaria (n=657)		
Variable	**Estimate**	**95% Confidence Interval**	**P-value**	**Estimate**	**95% Confidence Interval**	**P-value**
CQ Weekly	0.918	(0.675, 1.248)	0.586	0.431	(0.219, 0.848)	0.015
CQ IPTp	0.924	(0.645, 1.325)	0.668	1.021	(0.603, 1.728)	0.938
Maternal Age	0.863	(0.809, 0.920)	<0.001	0.899	(0.836, 0.966)	0.004
Gestational Age (weeks)	0.956	(0.891, 1.027)	0.218	1.095	(0.984, 1.220)	0.097
Bednet Use, Night before Enrollment	1.081	(0.811, 1.442)	0.594	1.233	(0.744, 2.046)	0.416
Anemia at Enrollment (Hemoglobin < 10 mg)	1.365	(1.045, 1.783)	0.022	2.613	(1.504, 4.540)	<0.001

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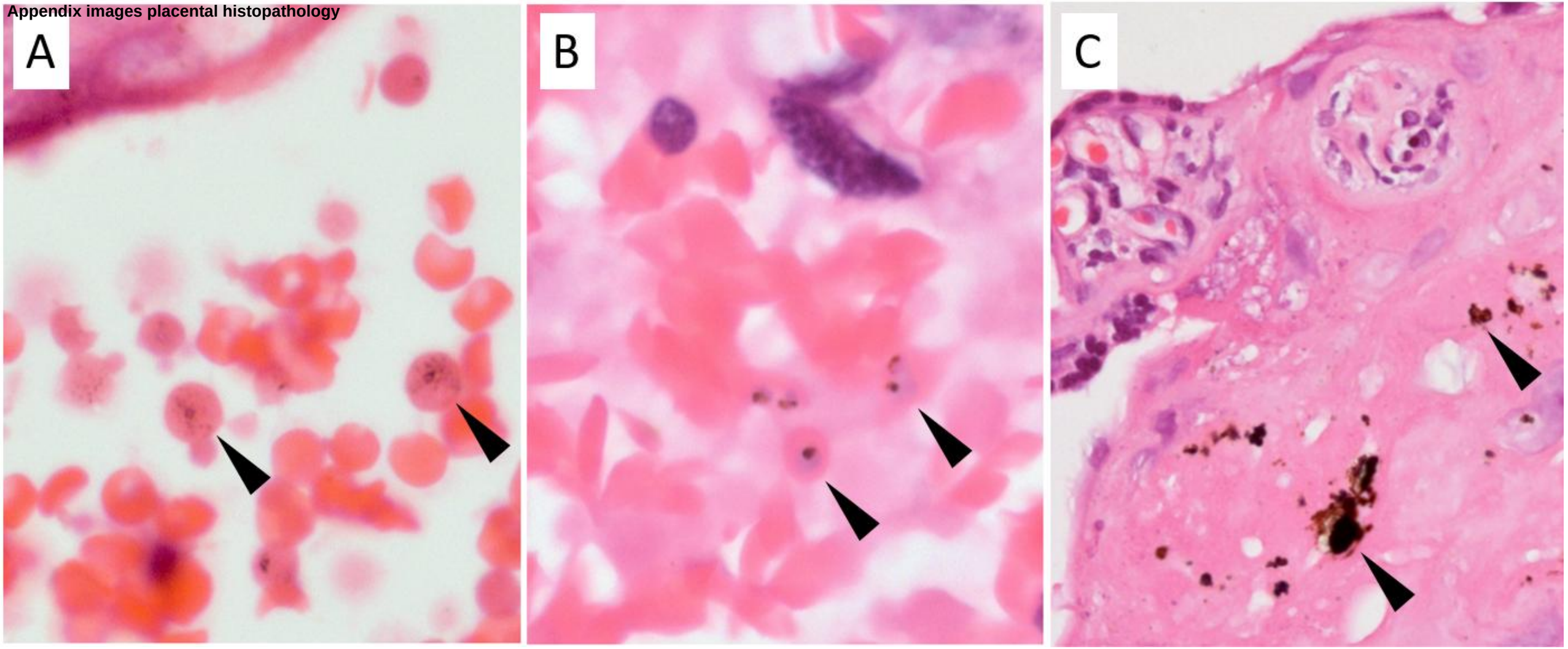
Table A6: Efficacy of interventions on the incidence of malaria infection and clinical malaria

	Variable	Arm	N	Events	Incidence	RR (95% CI)	P-value
Intention to treat	Malaria infection during pregnancy	SP-IPTp	300	33	33/300 (11.0%)	NA	NA
		Chloroquine-IPTp	300	27	27/300 (9.0%)	0.82 (0.51, 1.32)	0.45
		Chloroquine prophylaxis	300	26	26/300 (8.7%)	0.79 (0.49, 1.28)	0.41
	Clinical malaria during pregnancy	SP-IPTp	300	9	9/300 (3.0%)	NA	NA
		Chloroquine-IPTp	300	4	4/300 (1.3%)	0.44 (0.15, 1.34)	0.26
		Chloroquine prophylaxis	300	2	2/300 (0.7%)	0.22 (0.05, 0.90)	0.06
Per protocol	Malaria infection during pregnancy	SP-IPTp	260	27	27/260 (10.4%)	NA	NA
		Chloroquine-IPTp	243	19	19/243 (7.8%)	0.75 (0.43, 1.31)	0.36
		Chloroquine prophylaxis	254	21	21/254 (8.3%)	0.80 (0.47, 1.37)	0.45
	Clinical malaria during pregnancy	SP-IPTp	260	5	5/260 (1.9%)	NA	NA
		Chloroquine-IPTp	243	1	1/243 (0.4%)	0.21 (0.03, 1.37)	0.22
		Chloroquine prophylaxis	254	2	2/254 (0.8%)	0.41 (0.09, 1.81)	0.45

NOTE: Malaria infection includes those with positive parasitaemia by blood smear and/or parasitaemia by PCR and/or clinical malaria from enrolment day to delivery day. Confidence intervals are score intervals, and p-values were calculated with Fisher's Exact Test. One subject was excluded from the analysis of clinical malaria due to missing smear result.

Appendix images placental histopathology



Hematoxylin and eosin stained sections of formalin-fixed paraffin-embedded placental tissue. A) Uninfected red blood cells (RBC) from an uninfected placenta. Fine pigment precipitate can be seen (arrowheads) which is a processing artifact that can be mistaken for parasitized RBC. B) Parasitized RBC (arrowheads) in a case of active placental malaria. C) Malaria pigment (hemozoin) (arrowheads) persisting in fibrin in a case of past placental malaria.

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