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Association between the presence of DHPS and DHFR resistant markers and the level of parasitaemia, preterm birth and low birth weight among pregnant women in Ogun state, Nigeria

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Abstract
Objective: There have been reports of resistance to Intermittent Preventive Therapy with Sulphadoxine-Pyrimethamine, probably due to the accumulation of mutations at the dihydrofolate reductase and dihydropteroate synthase genes of the malaria parasite. This study aimed to determine the association between the presence of dihydrofolate reductase markers and dihydropteroate synthase markers and birth outcomes (parasitaemia, low birth weight and preterm delivery) among pregnant women in Ogun State, Nigeria.
Methodology: This was a cross-sectional study of pregnant women, 28- and 40-week gestational age with malaria parasitaemia, who were screened for malaria parasitaemia with rapid diagnostic test kits, thick and thin blood films for microscopy, and DNA analysis using PCR during the antenatal clinic. Gestational age at delivery and birth weight were obtained from the delivery records. Data were entered into SPSS version 22.0. Bivariate analysis of categorical variables was done using the Chi-square or Fisher's exact test as appropriate. The significance level was set at $p<0.05$.
Result: Of the 270 women, only 30 (11.1%) of the participants had parasitaemia on microscopy. The mean parasite density was 5,540 microliter/ml ±1,090.66. The mean gestational age at delivery was 39 weeks 3 days ±1.67, while the mean birthweight was 3.1kg ±0.46. Bivariate analysis showed statistically significant associations between the presence of resistance markers to sulphadoxine-pyrimethamine and the occurrence of microscopic parasitaemia and adverse birth outcomes (preterm delivery and low birth weight).
Conclusion: The presence of Triple, quadruple and quintuple resistant markers may be associated with severe parasitaemia (> 5000/mcl), preterm delivery, and low birthweight.

Keywords: Malaria, Low birth weight, Preterm delivery, Parasitaemia

Plain English summary

Malaria can cause problems in pregnancy, such as small babies, going into labour before 9 completed months, a shortage of blood and making women very unwell in pregnancy. To tackle this problem, the World Health Organisation has recommended that pregnant women use sulphadoxine-pyrimethamine

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from the early second trimester and can be used till delivery at least 4 weeks apart. However, it has been noticed that the malaria parasite has developed some resistance to this medication. The resistance is caused by genetic changes in the genetic component of the malaria parasite, which affects the way the drug is processed by the parasite. The study discovered that there is an association between malaria parasite resistance to sulphadoxine-pyrimethamine and delivering small babies, early delivery before due date and high levels of parasite in blood. Despite this, the World Health Organisation has still recommended the use of the medication because it still offers some protection in pregnancy.

Introduction

Malaria is a global health challenge. Two hundred and forty-seven million malaria cases were recorded in 2021, which shows an increase compared to the previous year, where 245 million cases were recorded (1). Most of this increase was from countries in the WHO African region. Nigeria accounted for 27% of the cases recorded globally, and when combined with the Democratic Republic of Congo, Uganda, and Mozambique, the four countries accounted for almost 50% of the cases globally (1).

Pregnant women are susceptible to malaria during pregnancy because pregnancy causes a temporary immunosuppression (2). In addition, Plasmodium falciparum binds to the placental surface through a unique adhesion factor, var2csa, that binds to the chondroitin sulphate of the placenta (3). This leads to increased inflammation and reduced blood flow to the placenta, which in turn increases foetal and maternal morbidity (3). In the World Malaria Report for the year 2022, it was estimated that there were 40 million pregnancies in 38 countries with high and moderate transmission in the WHO African region. Thirty-two per cent of these pregnancies were exposed to malaria, and West Africa had the highest exposure to malaria during pregnancy (1). Complications from malaria in pregnancy (MiP) include anaemia, low birthweight, preterm delivery, pulmonary oedema, intrauterine death, foetal growth restriction (4).

Strategies to prevent and reduce the complications of malaria in pregnancy advocated by WHO include the use of insecticide-treated nets, intermittent preventive therapy with sulphadoxine pyrimethamine and effective case management of Malaria in pregnancy. Malaria in pregnancy would have resulted in 96100 children with low birthweight in the WHO African subregion with moderate to high transmission if there were no pregnancy-specific intervention to prevent malaria in pregnancy (1). WHO has recommended that IPTp with SP should be administered at each scheduled antenatal visit using directly observed therapy (DOT). The first dose should be administered as early as possible during the second trimester, with subsequent doses administered at least one month apart. It can be administered up to the time of delivery (5).

In countries where IPTp is implemented, it is estimated to have prevented 457,000 low birth weight (1). This effect of sulphadoxine-pyrimethamine is being threatened by resistance to the medication by the malaria parasite. Resistance primarily arises due to the accumulation of single-nucleotide polymorphisms (SNPs) within the dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) genes (6). These SNPs lead to amino acid substitutions, rendering the parasite less susceptible to certain antimalarial medications. Key mutations at the *Plasmodium falciparum* dhfr gene, namely N51I1, C59R, and S108N, are commonly referred to as the triple mutation, while mutations in the *Plasmodium falciparum* dhps gene at codons A437G and G540E are known as the double mutation. The significance of these mutations lies in the fact that various combinations of these mutant alleles can occur, ranging from double mutations to septuple mutations, each associated with varying degrees of resistance to antimalarial drugs. The higher the number of mutant alleles present, the stronger the resistance observed. This creates a substantial challenge in the efforts to reduce the effect of malaria in pregnancy, as the effectiveness of commonly used antimalarial drugs for IPTp is compromised due to the widespread presence of these mutations. Therefore, the study aims to describe the association between the presence of mutant parasites and parasitaemia, low birthweight and preterm delivery.

Methodology

Study design and setting

This was a cross-sectional study. The study was carried out in a rural setting. It was carried out in Ikenne Local Government and Remo North Local Government, Ogun State. Ikenne Local Government is composed of five towns, which are Ikenne, Ilishan, Iperu, Ogere and Irolu. It has an area of 144km^2 with a population of 118,735 at the 2006 census. It has 10 public primary health centres, 3 public secondary health centres, and one private tertiary hospital. Remo North Local Government is composed of six towns, which are Isara, Ode-Remo, Ipara, Akaka, Ilara and Orile-Oko. It has an area of 199km^2 with a population of 59,911. It has 12 public primary health centres and one secondary health centre.

One facility was selected from each local government for the study, i.e., Babcock University Teaching Hospital in Ikenne local government and Isara State Hospital in Remo North local government. These hospitals were selected by convenience sampling because they have the largest pool of pregnant women in the selected local governments. In Babcock University Teaching Hospital, the antenatal clinic attendance in the 12 months preceding this study was 2,348, while that of Isara State Hospital was 1,486, with new antenatal bookings of 313 and 247, respectively.

Participants

Antenatal records of women attending a clinic at the study sites were used to identify pregnant women between 28 and 40 weeks of gestation. Pregnant women who were less than 28 weeks, women who did not give their consent, and those who were HIV positive or had hypersensitivity to SP were excluded. Inclusion criteria were Pregnant women between 28 weeks gestation and 40 weeks gestation, pregnant women who test positive to either rapid diagnostic test for malaria parasite, thick and thin blood film, or malaria parasite genotype using PCR, must be attending antenatal care in the study site, must have consented to the study, must be willing to deliver at study sites and pregnant women that have no contraindication to sulphadoxine-pyrimethamine use.

Sample size

Sample size calculation: The sample size was calculated using the Leslie Kish formula.

$n = Z^2p\ (1-p)/d2$

Where N is the sample size, Z is the standard normal deviation corresponding to a 95% confidence level, which is 1.96. P is the maternal prevalence of the *pfdhfr* mutation at codon 108 among pregnant women in Lagos State, Nigeria, and the value is 80% (0.8) (8). d is the degree of accuracy, which is set at 0.05, i.e., 95%

$N = 1.96^2 \times 0.8\ (1-0.8)/0.05^2$

N = 245.7i.e.,246

An additional 10% to account for likely loss to follow up = 24.6. Therefore, the required sample is approximately 270.6, i.e. 271.

Hypotheses

Null hypothesis

There is no association between plasmodium falciparum resistance to sulphadoxine-pyrimethamine and the occurrence of asymptomatic malaria in pregnancy, low birth weight, or preterm delivery.

Alternate hypothesis: There is an association between plasmodium resistance to sulphadoxine-pyrimethamine and the occurrence of asymptomatic malaria in pregnancy, low birth weight, or preterm delivery.

Collection procedure

A venipuncture was done on recruited participants, and about 4 ml of blood was obtained for a rapid diagnostic test for malaria parasite, thick and thin blood film, and molecular studies to detect malaria parasite. If any one of the tests was positive for malaria, the participant was enrolled in the study. For participants enrolled, a structured questionnaire was used to obtain information about the participant's sociodemographic status, her obstetric history, use of sulphadoxine-pyrimethamine for IPTp in index pregnancy, history of malaria symptoms in index pregnancy, and treatments received. Gestational age was confirmed by an early ultrasound scan from the antenatal records.

The SD BIOLINE Malaria antigen *Plasmodium falciparum* test was used for the rapid diagnostic test for P. falciparum malaria parasite. The kit is an in vitro immunochromatographic, rapid assay designed for the qualitative detection of histidine-rich protein II (HRP-II) antigen of malaria plasmodium falciparum in human whole blood. The procedure was carried out and the result interpreted according to the manufacturer's instructions.

Thick and thin blood film for malaria microscopy was made on clean slides following the World Health Organisation standard operating procedure for Giemsa staining of malaria blood films (7). Reporting of the film was done by 2 experienced microscopists, and a third reviewer was invited in case of discordance to ensure quality control.

Genomic studies were carried out in the Centre for Advanced Molecular Research and Biotechnology (CAMRAB) laboratory, Babcock University Teaching Hospital. The laboratory's standard operating procedure for DNA extraction was strictly followed. Blood samples of patients with positive malaria parasites were subjected to DNA analysis. DNA extraction was done using the Quick DNA^{TM} miniprep plus kit according to the manufacturer's instructions.

The DNA extracted was then amplified using a Polymerase chain reaction (PCR). The reaction was carried out in a final volume of 20 microliters. Added to the extracted DNA was 0.02 U/microliter of Taq polymerase (QIAGEN, Hilden, Germany), 250 nM primers, 125uM deoxynucleoside triphosphate, 1.5 mm MgCl, and buffer solution to create a suitable environment for the Taq polymerase. The final solution went through three different cycles in the PCR machine, and the PCR thermal profile was set at 95^0C for 1 minute in the first cycle, 60^0C for 2 minutes in the second cycle, and 72^0C for 2

minutes in the third cycle, which was repeated 45 times. During the first cycle, the DNA was denatured, breaking down the hydrogen bonds and converting the double-stranded DNA to a single-stranded DNA. Two genus-specific primers which are (-rPLU5-, (CCTGTTGTTGCCTTAAACTTC) and –rPLU6-, (TTAAAATTGTTGCAGTTAAAACG) and species-specific primers which are (-rFAL1-, (TTAAACTGGTTTGGGAAAACCAAATATATT) and –rFAL-, (ACACAATGAACTCAATCATGACTACCCGTC) would bind to the target DNA if present in the second cycle. In the third cycle, Taq polymerase synthesised a new DNA strand complementary to the DNA template using the deoxynucleoside triphosphate as building blocks, thus amplifying the target DNA. The final PCR product was visualised using gel electrophoresis, stained with ethidium bromide. Positive and negative controls were included for each PCR reaction and gel electrophoresis reading to ensure quality control. The same process was used to identify mutations in the P. falciparum dihydrofolate reductase (*pfdhfr*) and P. falciparum dihydropteroate synthase (*pfdhps*) using M1 (5'TTTATGATGGAACAAGTCTGC3') and M5 (5'AGTATATACATCGCTAACAGA3') as primers for the *pfdhfr* gene with the reaction condition set at 94^0C for 2 minutes, 94^0C for 1 minute, 45^0C for 1 minute and then 72^0C for 2 minutes repeated 35 times.

Birth outcomes

The study subjects continued their routine antenatal clinic visits and use of SP following the WHO guideline. A white sticker with an identification number specific to the subject was used to tag the folders of enrolled patients. This was used to trace and identify enrolled patients at delivery. Birth outcomes such as low birth weight and gestational age at delivery were recorded at delivery.

Statistical Methods.

Data was entered into SPSS version 22.0 (California, USA). Frequency was calculated using simple mathematical methods. Missing data were not included when calculating the frequency. The total number analysed was indicated for each of the variables. Bivariate analysis of categorical variables was done using the chi-square or Fisher's exact test as appropriate. The significance level was set at $p < 0.05$

Result

During the period of study, 571 pregnant women were recruited out of whom 270 women with malaria parasitaemia were enrolled for the study. Two hundred and sixty-two (97%) women were positive by PCR, 114 (42.2%) by RDT, while 30 (11.1%) had positive microscopy. The mean age of participants was 29.49±5.51 years, while the mean gestational age at recruitment was 33.62±3.66 weeks. The modal gravidity was primigravidae (94, 35.9%), and the majority had tertiary education (144, 53.7%). Most of the respondents (180, 66.7%) had used at least one dose of sulphadoxine pyrimethamine (SP) for IPT at the time of enrolment, and 30.4% reported sleeping under insecticide-treated nets the night before. One hundred and thirty-six women (50.4%) had been treated for malaria at least once in their index pregnancy before enrolment, while 44 (16.4%) reported symptoms as ongoing at the time of recruitment. Other general characteristics of the participants are represented in Table 1.

Table 1: General characteristics of participants enrolled in the study

Parameters	Frequency	Percentages
Level of Education (n-268)		
Tertiary	144	53.7
Secondary	88	32.8
Primary	32	11.9
No formal education	4	1.5
Marital status (n-270)		
Married	264	97.8
Single	6	2.2
Parity (n-262)		
P_0	94	35.9
P_1	88	33.6
P_2	46	17.6
P_3	22	8.4
$\geq P_4$	12	4.6
Doses of SP used for IPT (n-270)		
0	90	33.3
1	66	24.4
2	42	17.0

3	46	15.6
4	20	7.4
5	6	2.2
Use of insecticide in the last one month (n-266)		
Yes	144	54.1
No	122	45.9
Use of residual spray in the last one month (n-268)		
Yes	266	99.3
No	2	0.7

Association between parasite-resistant markers and parasitaemia.

Only 30 (11.1%) of the participants had parasitaemia on microscopy. The mean parasite density was 5,540 microliter/ml ±1,090.66. A Pearson Chi-Square test showed that there was a significant association between the presence of P. falciparum resistance markers and the presence of parasitaemia. Triple CIRNL+SGKAA and quadruple CIRNL+SGKAA combination alleles showed more association with parasitaemia> 5,000 microliter/ml.

Table 2: Association between resistant markers and parasitaemia.

Resistant markers	No parasitaemia	< 500 parasite/mcl	500-5000 parasites/mcl	>5000 parasites/mcl	Total	P Value
CIRNL+SGKAA						
Positive	118 (85.5%)	4 (2.9%)	12 (8.7%)	4 (2.9%)	138 (100%)	. 247
Negative	108 (88.5%)	0 (0.0%)	12 (9.8%)	2 (1.6%)	122 (100%)	
CIRNL+SGKAA						
Positive	50 (67.6%)	2 (2.7%)	16 (21.6%)	6 (8.1%)	74 (100%)	<.001
Negative	176 (94.6)	2 (2.1%)	8 (4.3%)	0 (0.0%)	186 (100%)	
CIRNL+SGKAA						
Positive	12 (60.0%)	0 (0.0%)	6 (30%)	4 (30%)	20 (100%)	.001
Negative	204 (88.7%)	4 (1.7%)	18 (7.8%)	2 (1.7%)	230 (100%)	
CIRNL+SGKAA						
Positive	46 (69.7%)	2 (30%)	12 (18.2%)	6 (9.1%)	66 (100%)	<.001
Negative	180 (92.8%)	2 (1.0%)	12 (6.2%)	0 (0.0%)	194 (100%)	
CIRNL+SGKAA						
Positive	10 (62.5%)	0 (0.0%)	4 (25.0%)	2 (12.5%)	16 (100%)	.005
Negative	206 (88.0%)	4 (1.7%)	20 (8.5%)	4 (1.7%)	234 (100%)	
CIRNL+SGKAA						
Positive	32 (66.7%)	2 (4.2%)	10 (20.8%)	4 (8.3%)	48 (100%)	<.001
Negative	194 (91.5%)	2 (0.9%)	14 (6.6%)	2 (0.9%)	212 (100%)	
CIRNL+SGKAA						
Positive	4 (40.0%)	0 (0.0%)	4 (40.4%)	2 (20.0%)	10 (100%)	<.001
Negative	212 (87.6%)	4 (1.7%)	20 (8.3%)	4 (1.7%)	240 (100%)	
CIRNL+SGKAA						
Positive	4 (50.0%)	0 (0.0%)	4 (50.0%)	0 (0.0%)	10 (100%)	<.001
Negative	212 (87.6%)	4 (1.7%)	20 (8.3%)	6 (2.5%)	240 (100%)	

Association between parasite-resistant markers, low birth weight and preterm delivery.

The mean gestational age at delivery was 39 weeks 3 days ±1.67, while the mean birthweight was 3.1kg ±0.46. Quadruple haplotype CIRNL+SGKAA and quintuple haplotype CIRNL+SGKAA showed association with preterm delivery (P <.001 and .006, respectively, Fisher's Exact test). On the other hand, was presence of triple CIRNL+SGKAA, quadruple CIRNL+SGKAA, and quintuple CIRNL+SGKAA combination alleles showed association with low birthweight using Fisher's Exact test. Tables 3 and 4 show the association between resistant markers and birth outcomes

Table 3: Association between parasite-resistant markers and preterm delivery

Resistant markers	Gestational age at delivery ≥ 37 weeks	Gestational age at delivery between 28-36 weeks+6 days	Total	P Value (Fisher's Exact test)

CIRNL+SGKAA				
Positive	62 (83.8%)	12 (16.2%)	74 (100%)	016
Negative	174 (93.5%)	12 (6.5%)	186 (100%)	
CIRNL+SGKAA				
Positive	11 (61.1%)	7 (38.9%)	18 (100%)	<.001
Negative	206 (92.8%)	16 (7.2%)	222 (100%)	
CIRNL+SGKAA				
Positive	56 (84.8%)	10 (15.2%)	66 (100%)	.051
Negative	180 (92.8%)	14 (7.2%)	194 (100%)	
CIRNL+SGKAA				
Positive	9 (64.3%)	5 (35.7%)	14 (100%)	.006
Negative	208 (92.0%)	18 (8.0%)	226 (100%)	
CIRNL+SGKAA				
Positive	43 (89.6%)	5 (10.4%)	48 (100%)	.465
Negative	193 (91.0%)	19 (9.0%)	212 (100%)	
CIRNL+SGKAA				
Positive	8 (80.8%)	2 (20.0%)	10 (100%)	.247
Negative	218 (90.8%)	22 (9.2%)	240 (100%)	
CIRNL+SGKAA				
Positive	6 (75.0%)	2 (25.0%)	8 (100%)	.173
Negative	220 (90.9%)	22 (9.1%)	242 (100%)	

Table 4: Association between parasite-resistant markers and low birth weight.

Resistant markers	Birth weight >2.5kg	Birth weight <2.5kg	Total	P value (Fisher's exact test)
CIRNL+SGKAA				
Positive	13 (9.4%)	125 (90.6%)	138 (100%)	1.000
Negative	11 (9.0%)	111 (91.0%)	122 (100%)	
CIRNL+SGKAA				
Positive	14 (18.9%)	60 (81.1%)	74 (100%)	.001
Negative	10 (5.4%)	176 (94.6%)	186 (100%)	
CIRNL+SGKAA				
Positive	2 (11.1%)	18 (88.9%)	20 (100%)	.661
Negative	19 (8.5%)	205 (91.5%)	224 (100%)	
CIRNL+SGKAA				
Positive	14 (21.2%)	52 (78.8%)	66 (100%)	<.001
Negative	10 (5.2%)	184 (94.8%)	236 (90.8%)	
CIRNL+SGKAA				
Positive	2 (14.3%)	12 (85.7%)	14 (100%)	.348
Negative	19 (8.3%)	209 (91.7%)	228 (100%)	
CIRNL+SGKAA				
Positive	9 (18.8%)	39 (81.3%)	48 (100%)	0.23
Negative	15 (7.1%)	197 (92.9%)	212 (100%)	
CIRNL+SGKAA				
Positive	0 (0.0%)	10 (100%)	10 (100%)	.606
Negative	23 (9.6%)	217 (90.4%)	240 (100%)	
CIRNL+SGKAA				
Positive	0 (0.0%)	23 (9.5%)	8 (100%)	1.000
Negative	8 (100%)	219 (90.5%)	242 (100%)	

Discussion

The study set out to determine if there are associations between the presence of dihydrofolate reductase and dihydropteroate synthase resistant markers and parasitaemia, low birth weight and preterm delivery. The prevalence of microscopic parasitaemia in this study was low at 11% but still higher compared to a prevalence of 7% reported in Lagos (9). Also, the mean parasite density of 5,540 parasites/mcl is higher compared to 733 parasites/mcl reported in Ghana (10). This may be so because there is an association between the presence of parasite-resistant markers and parasitaemia. All combinations of mutant haplotypes had an association with parasitaemia except for the triple *pfdhps* mutation. This correlates with a study reported in Ghana in which a mutation at *pfdhps* was not associated with treatment failure in children.

The effect of the presence of resistant markers to SP on low birthweight has been controversial. There was an association between the presence of triple haplotype CIRNL+SGKAA, quadruple haplotype CIRNL+SGKAA, and quintuple haplotype CIRNL+SGKAA combination allele and low birth weight in this study. Similar findings have been reported in a meta-analysis, where the increase in the prevalence of *pfdhps* 540 and 581 was associated with reduced effectiveness of the drug in preventing low birth weight and clearing parasitaemia (11). However, the drug seems to be significantly protective against low birthweight in areas where the *pfdhps*540 mutation is 90% or higher and *pfdhps581* is less than 10% (11). Additionally, IPTp-SP still reduces low birth weight by 7- 10% in areas where the resistant quintuple-mutant is fixed (11).

The fact that IPTp-SP is still having some effectiveness in preventing low birth weight in areas where there is reduced clearance of parasitaemia may suggest that suppression rather than radical clearance of P falciparum malaria parasite is needed to prevent the complications of malaria on placental growth and function. It has also been suggested that there may be alternate pathways in which the drug acts to prevent low birthweight aside from its antimalarial activities, and this is not affected by parasite resistance (11). No association was observed between the sextuple and septuple combination allele and low birthweight. This is surprising as the presence of high parasite resistance has been associated with low birth weight in other studies (12). This may be due to the low prevalence of mutations at *pfdhfr* observed in this study. In areas where the sextuple mutation is associated with low birth weight, the prevalence of *pfdhfr* mutations is > 90% and is almost reaching fixation in some instances (12). Also, 46% of pregnant women have had 3 doses of IPTp-SP at the time of recruitment into the study, and IPTp-SP has been shown to still be moderately effective in preventing low birth weight in areas of high resistance.

The connection between mutations in the pfdhfr and pfdhps genes and the effectiveness of IPTp-SP is not well understood (13). Most research on the efficacy of IPTp-SP has been conducted in regions with either very low or very high prevalence of the quintuple mutant, which is a form of the malaria parasite resistant to SP. For example, in Ghana, where triple mutations are present but not the quintuple mutations, an in vivo study confirmed that IPTp-SP is effective (10). Conversely, in the Benin Republic, where the quintuple mutation's prevalence was initially less than 10% before IPTp-SP treatment, one study found that 11% of patients experienced parasitological failure after one month of follow-up (14). Unfortunately, the small sample size of this Benin study made it impossible to determine the significance of the relationship between the mutated haplotypes and outcomes like low birth weight and maternal anaemia (13).

On the opposite end of the spectrum is Malawi, where 100% of parasites from pregnant women exhibited the quintuple haplotype. In Malawi, a study found that despite the complete penetration of the haplotype, IPTp-SP still demonstrated a protective effect dependent on the dose among first-time pregnant women. This effect was associated with a positive impact on a composite of outcomes, including small for gestational age babies, preterm delivery, and low birth weight, despite the high prevalence of the quintuple mutant (15).

In summary, the connection between the prevalence of these mutations and the effectiveness of IPTp-SP in preventing malaria during pregnancy is intricate and can differ depending on the specific region and mutation prevalence. Further research is necessary to gain a comprehensive understanding of how IPTp-SP functions in diverse contexts.

Although there were associations between resistant parasite markers and birth outcomes, confounders such as pre-eclampsia, previous preterm delivery, and iatrogenic preterm delivery that may also cause preterm delivery and low birth weight were not controlled for in this study. Also, the significance of the inference above is limited by the cross-sectional nature of the study.

Conclusion

The presence of Triple, quadruple and quintuple resistant markers had a significant association with severe parasitaemia (> 5000/mcl), preterm delivery, and low birthweight. Therefore, there is an urgent need to find an effective alternative to SP, as there is evidence of growing resistance to this medication. While that is being done, IPTp-SP should still be used in preventing adverse pregnancy outcomes due to malaria in malaria-endemic regions.

List of Abbreviations

Dhfr: Dihydrofolate reductase
Dhps: Dihydropteroate synthase
IPT: Intermittent preventive therapy
IPTp-SP: Intermittent preventive therapy with sulphadoxine-pyrimethamine
LBW: Low birth weight.
MiP: Malaria in pregnancy
Pf: Plasmodium falciparum
WHO: World Health Organisation
SP: Sulphadoxine-pyrimethamine

Declarations

Ethical approval

Approval for the study was obtained from the Babcock University Health Research Ethics Committee (BUHREC 494/19) before the commencement of the study. The Declaration of Helsinki ethical principles, developed by the World Medical Association, were also followed during the conduct of this study.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

The raw data sets used and analysed during the current study are available from the corresponding author on request.

Competing interests.

The authors declare that they have no competing interests.

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Authors' contributions

AO contributed to conception and design, collecting, analysis and interpretation of data, draft writing and revising and gave approval for the final version of the article. OA contributed to the conception and design of the study, analysis and interpretation of data, draft writing and revising it critically for intellectual content. IJ contributed to the collection of data, data analysis and interpretation, revising the draft of the article and making a major intellectual contribution. Also gave approval for the final version of the article. AA Contributed to data analysis and interpretation, revising the draft of the article, and making major intellectual contributions. PT revised the draft of the article and made intellectual contributions. AT contributed to revising the article and made intellectual contributions.

References.

1. World Health Organization. Stronger collaboration for an equitable and resilient recovery towards the health-related Sustainable Development Goals, incentivizing collaboration: 2022 progress report on the Global Action Plan for Healthy Lives and Well-being for All. World Health Organization; 2022 May 18.
2. Schantz-Dunn J, Nour NM. Malaria and pregnancy: a global health perspective. Reviews in obstetrics and gynecology. 2009;2(3):186.
3. Sundararaman SA, Odom John AR. Prevention of malaria in pregnancy: the threat of sulfadoxine-pyrimethamine resistance. Frontiers in Pediatrics. 2022 Aug 18;10:966402. https://doi.org/10.3389/fped.2022.966402
4. Marchesini P, Crawley J. Reducing the burden of malaria in pregnancy. MERA IV, Supporting Agency–Roll Back Malaria, WHO. 2004 Jan.
5. WHO. Intermittent preventive treatment in pregnancy (IPTp). Geneva: World Health Organization; 2018. www.who.int/malaria/adate assessed: 28/11/2018
6. Gutman J, Mwandama D, Wiegand RE, Abdallah J, Iriemenam NC, Shi YP, Mathanga DP, Skarbinski J. In vivo efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitaemia in pregnant women in Machinga District, Malawi. Malaria Journal. 2015 Dec;14:1-9. https://doi.org/10.1186/s12936-015-0710-7
7. WHO. Giemsa staining of malaria blood film. MM-SOP-07A. 2016
8. Iwalokun BA, Iwalokun SO, Adebodun V, Balogun M. Carriage of mutant dihydrofolate reductase and dihydropteroate synthase genes among Plasmodium falciparum isolates recovered from pregnant women with asymptomatic infection in Lagos, Nigeria. Medical Principles and Practice. 2015 Jun 2;24(5):436-43. https://doi.org/10.1159/000430987
9. Agomo CO, Oyibo WA, Sutherland C, Hallet R, Oguike M. Assessment of markers of antimalarial drug resistance in Plasmodium falciparum isolates from pregnant women in Lagos, Nigeria. PloS one. 2016 Jan 25;11(1):e0146908. https://doi.org/10.1371/journal.pone.0146908
10. Mockenhaupt FP, Bedu-Addo G, Eggelte TA, Hommerich L, Holmberg V, von Oertzen C, Bienzle U. Rapid increase in the prevalence of sulfadoxine-pyrimethamine resistance among Plasmodium falciparum isolated from pregnant women in Ghana. The Journal of infectious diseases. 2008 Nov 15;198(10):1545-9. https://doi.org/10.1086/592455
11. van Eijk AM, Larsen DA, Kayentao K, Koshy G, Slaughter DE, Roper C, Okell LC, Desai M, Gutman J, Khairallah C, Rogerson SJ. Effect of Plasmodium falciparum sulfadoxine-pyrimethamine resistance on the effectiveness of intermittent preventive

therapy for malaria in pregnancy in Africa: a systematic review and meta-analysis. The Lancet Infectious Diseases. 2019 May 1;19(5):546-56. https://doi.org/10.1016/S1473-3099(18)30732-1

12. Mbonye AK, Birungi J, Yanow SK, Shokoples S, Malamba S, Alifrangis M, Magnussen P. Prevalence of Plasmodium falciparum resistance markers to sulfadoxine-pyrimethamine among pregnant women receiving intermittent preventive treatment for malaria in Uganda. Antimicrobial Agents and Chemotherapy. 2015 Sep;59(9):5475-82. https://doi.org/10.1128/AAC.00507-15
13. Tan KR, Katalenich BL, Mace KE, Nambozi M, Taylor SM, Meshnick SR, Wiegand RE, Chalwe V, Filler SJ, Kamuliwo M, Craig AS. Efficacy of sulphadoxine-pyrimethamine for intermittent preventive treatment of malaria in pregnancy, Mansa, Zambia. Malaria journal. 2014 Dec;13:1-8. https://doi.org/10.1186/1475-2875-13-227
14. Moussiliou A, De Tove YS, Doritchamou J, Luty AJ, Massougbodji A, Alifrangis M, Deloron P, Ndam NT. High rates of parasite recrudescence following intermittent preventive treatment with sulphadoxine-pyrimethamine during pregnancy in Benin. Malaria journal. 2013 Dec;12:1-9. https://doi.org/10.1186/1475-2875-12-195
15. Rogerson SJ, Chaluluka E, Kanjala M, Mkundika P, Mhango C, Molyneux ME. Intermittent sulfadoxine-pyrimethamine in pregnancy: effectiveness against malaria morbidity in Blantyre, Malawi, in 1997–1999. Transactions of the Royal society of Tropical Medicine and Hygiene. 2000 Sep 1;94(5):549-53. https://doi.org/10.1016/S0035-9203(00)90083-X