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The effect of hemoglobinopathy traits on maternal anemia and the role of placental malaria parasitemia

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Abstract

Background: Prevalent hemoglobinopathy traits, sickle cell trait (SCT) and hemoglobin C trait (HbAC), prevent severe malaria while causing mild structural erythrocyte modifications. However, limited high-quality evidence on carrier traits on pregnancy outcomes has precluded clinical guidance. We aimed to assess the effects of SCT and HbAC on maternal anemia, a risk factor for adverse pregnancy outcomes, and mediation by placental malaria in Benin.

Methods: This longitudinal observational cohort study utilized data from 895 women enrolled in randomized control trial in Benin (MiPPAD). The effects of each carrier trait on maternal anemia at delivery and during pregnancy with mediation by placental parasitemia were estimated in mediation analyses. This secondary data analysis was approved as non-human subjects research based on a completed study registered with ClinicalTrials.gov, NCT0081142, which compared the efficacy of two antimalarials, sulfadoxine-pyrimethamine and mefloquine.

Findings: The risk of maternal anemia at delivery was greater among patients with SCT [relative risk (RR) = 1.19 (95%CI 0.99-1.45)] but was not mediated by placental parasitemia [1.00 (0.99, 1.02)]. The effect of SCT on maternal anemia was not found in early and mid-pregnancy. Maternal anemia at delivery was not associated with HbAC [RR = 0.98 (0.68-1.41)] overall and was not mediated by placental parasitemia [0.96 (0.66,1.38)].

Interpretation: Pregnant participants with SCT, but not HbAC, had a slightly higher risk of maternal anemia at delivery in comparison to pregnant participants without hemoglobinopathies. Placental parasitemia was not strongly associated with either trait and did not mediate associations. Carrier traits may have distinct relationships with hematological parameters during pregnancy and should be further studied.

Key Words: Malaria, Pregnancy, Sickle Cell Trait, Mediation, Anemia, Placental Parasitemia, Hemoglobinopathies

Declaration

Ethics approval and consent to participate

Ethical approval for the parent study (MiPPAD) providing the data for this study was obtained by multiple institutional review boards (IRB). MiPPAD was approved by the IRB of the Beninese Ethical Committee of the Faculté des Sciences de la Santé (FSS), the Committee of Ethical Research of the Applied Biomedical Sciences Institute (CER-ISBA) in Benin, the IRB of New York University (NYU) in the United States (IRB #09-1253), and the Research Institute for Development's (IRD) Consultative Ethics Committee in France. The primary trial was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants in the MiPPAD trial with thumbprints provided by those unable to read or write.

Consent for Publication

Not applicable (no individual-level data).

Competing Interests

Authors have no competing interests as defined by BMC, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Fundings

T32, National Heart, Lung, and Blood Institute (T32HL129982).

Availability of data and materials

Data used to support the findings of this study are available from the MiPPAD trial, but restrictions apply to the availability of these data and are not publicly available.

Introduction

Maternal anemia during pregnancy is a significant global health concern and is associated with an increased risk of maternal, neonatal, and infant morbidities, such as low birthweight, preterm birth, and reduced infant motor function(1-4). The role of genetic blood traits, particularly sickle cell trait (SCT) and hemoglobin C trait (HbAC), in the etiology of anemia remains understudied. SCT and HbAC are hemoglobinopathy carrier traits that affect hemoglobin structure and are among the most common hemoglobin variants, having regional frequencies of up to 40% and 20%, respectively, in sub-Saharan Africa due to their protection against severe malaria(5, 6). SCT and HbAC cells may deform under certain conditions. Malaria is a known cause of hemolytic anemia, but conversely some studies have shown the carrier traits may increase the risk of anemia, a known complication of the homozygous hemoglobinopathies (4, 7-9). While the relationship between SCT and peripheral malaria has been established, the relationship between placental parasitemia and carrier traits remains understudied(4).

Gaining a better understanding of the relevance of carrier traits to maternal health is a significant need(10-12). We conducted a study leveraging a randomized controlled trial of malaria prevention in Benin, with detailed information on malaria episodes, parasitemia during pregnancy, hemoglobin variants, and anemia(13). This population offers a unique opportunity to interrogate the relationship between hemoglobinopathy carrier status, malaria, and maternal anemia, taking advantage of the longitudinal design and repeated measurements of parasitemia and anemia over the course of pregnancy. By applying a mediation model, we distinguish direct effects of hemoglobin variants from those operating through malaria, thereby offering insight into the etiologic mechanisms that link red cell polymorphisms to pregnancy outcomes. This analytic approach enhances the potential generalizability of our findings beyond malaria-endemic regions by clarifying when and under what conditions these associations may manifest. This research may inform antenatal care strategies for pregnant persons in regions of high malaria burden.

Methods

Study Design and Population. The Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) trial was a randomized controlled trial that compared the efficacy of two antimalarials, sulfadoxine-pyrimethamine and mefloquine, for use as intermittent preventative treatments in pregnancy (IPTp) (NCT0081142, Registration Date: 12/18/2008) (14, 15). Between January 2010 and October 2011, the study enrolled pregnant women who were less

than 28 weeks of gestation with a permanent residence in the study area, a negative human immunodeficiency virus (HIV) test at recruitment, no known allergies to sulfa or mefloquine drugs, no treatment for malaria using these drugs within 4 weeks of recruitment, and no history of severe malarial disease. Participants contributed data at two study visits during the prenatal period, when each dose of IPTp was administered. In addition, data on emergency or other antenatal health care visit after enrollment, and at a hospital-based labor and delivery visit, were obtained.

At baseline, 1,182 pregnant women were enrolled in the trial and of those 78 were lost-to-follow-up (~7%) before delivery. We limited the study to 1070 singleton births (n=34 multiple gestations). There were 160 individuals with missing hemoglobinopathy information and 5 women with exclusionary hemoglobinopathies (co-inherited or homozygous hemoglobinopathies) leaving us with analytic sample of 895 overall (**Figure 1).**

Hemoglobinopathy Assessment. At enrollment, 8mL of venous blood was collected and Hb genotypes were determined by alkaline electrophoresis on cellulose acetate (Helena laboratories, Beaumont, TX). Hemoglobinopathies were classified as sickle cell trait (SCT), hemoglobin C trait (HbAC), and no hemoglobinopathy (HbAA).

Maternal Anemia Assessment. Anemia was assessed at the first and second study visits and during labor through venous blood collection using automated hematology analyzer or colorimeter. Hemoglobin concentration was measured either with a haematology analyzer (Erma Laboratory, Japan) or Hemo_Control (EKF Diagnostics, Germany) colorimeter(16). Patients were considered to have low-hemoglobin anemia if their hemoglobin levels fell below the following standard WHO trimester-specific thresholds, which account for physiologic hemodilution during pregnancy: less than 110 g/L, 105 g/L, and 110 g/L for the first, second, and third trimesters of pregnancy, respectively.

Malaria Assessment Placental parasitemia at delivery captures both symptomatic and asymptomatic placental malarial cases, including those that appear negative on blood smears(17). After delivery, a biopsy of the placenta was collected. PCR was run on a blood sample from each biopsy. Samples without amplification were considered negative and a density of at least 5 parasites/μL was considered positive. A 2-parasite value was assigned if amplification was observed but was out of the lower range and will be considered negative. To assess and ensure quality of the PCR results, a negative control with no template DNA was run in all reactions and a quality control pilot program was used to evaluate the reliability of measurements(18).

Covariate Assessment. Gestational age was measured at each visit using last menstrual period, fundal height with manual palpitation, Ballard score, or a combination thereof. Iron deficiency was adjusted for inflammatory conditions and considered positive for serum ferritin values < 12 μg/L if C-reactive protein (CRP) was less than or equal to 5 mg/mL or between 12 and 70 μg/L if CRP concentration > 5 mg/mL(16). Additional details of data collection have been previously described(15).

Statistical Analysis

Hemoglobinopathy carrier status and maternal anemia at delivery. To assess both the total effect of carrier status on maternal anemia at delivery and the role of placental parasitemia, we took a natural direct effects approach(19). Data was weighted based on the conditional probability of having placental parasitemia, the mediator. We ran a logistic regression predicting the presence of placental parasitemia (**Table S3**) to estimate weights(20, 21). Then, we conducted two modified Poisson regressions with robust standard errors, one for each carrier state, SCT and HbAC, and calculated the risk ratio of anemia among carrier status as compared to normal hemoglobin status (HbAA)(22). A modified Poisson regression, which uses a sandwich error term to estimate relative risks, provides consistent and efficient results in context of small samples sizes where convergence is challenged in log-linear models and where the outcome is common(22). We adjusted for risk factors of the maternal anemia selected *a priori,* including gravidity, markers of socioeconomic status (literacy and electricity), season at enrollment (wet versus dry), trial arm, completion of IPTp, and iron deficiency at baseline. The total, natural direct effect, and indirect effect of each carrier trait were then estimated. Detailed equations are shown in the supplement. We calculated proportion mediated due by placental parasitemia as the ratio of the indirect effect to the total effect and calculated standard errors using the delta method.

Trends in maternal anemia across pregnancy. In a secondary analysis, a generalized estimating equation model was created to estimate the effect of carrier traits on anemia at any point during pregnancy. Total, direct, and indirect effects were calculated in parallel to the cross-sectional analysis. Confidence intervals were calculated using a quasi-Bayesian approximation with 1,000 simulations. All analysis was conducted in R. The effect estimates were calculated using the medflex package for the primary cross-sectional analysis and the gee package was used for the longitudinal analysis.

Treatment of missing data: With a total of 895 women with HbAA, SCT, or HbAC hemoglobin values, multiple imputation was used to handle missing data. A regression-based approach with chained equations with predictive mean matching for both baseline and delivery measurements was used through the *mice* R package. We conducted 100 multiple imputations (m=100) and included all possible predictors in the imputation model(23). In the longitudinal analysis, we imputed binary and continuous repeated measurements (including anemia, iron deficiency, hemoglobin levels, ferritin level) taken at every visit using a two-level logistic model and a linear two-level model, respectively(24). A complete case analysis was also completed for comparison.

Results

Participant Demographics At enrollment, patients were randomly assigned to 1 of the 3 IPTp treatments resulting in three treatment groups of equal size (**Table 1**). 19% of patients were primigravidae and at delivery, approximately 35% of patients had anemia. The mean gestational age at delivery was 39.6 weeks. Overall, characteristics were evenly distributed across hemoglobinopathies types. However, anemia at delivery was observed in 40% of SCT patients as compared to 34% of non-carrier patients. Among HbAC individuals, 37% had placental parasitemia as compared to 21% of HbAA individuals and 22% of SCT individuals. Illiteracy was also highest in the SCT group with 80% unable to read and lowest in the HbAC group with 65% unable to read.

Hemoglobinopathy carrier status and maternal anemia at delivery. SCT was associated with an approximately 20% increased risk for anemia at delivery (RR = 1.19, 95% CI 0.99, 1.45), after controlling for risk factors associated with anemia (**Table 2, Figure 2**). HbAC was not associated with maternal anemia (RR=0.98, 95% CI 0.68, 1.41).

The effect of hemoglobinopathy carrier traits on maternal anemia was not mediated by placental parasitemia (**Table 2, Figure 2**). There was no effect of placental parasitemia on maternal anemia after removing the direct effect of SCT on maternal anemia (RR = 1.00, 95% CI 0.98, 1.02). The direct effect estimate, which focuses solely on the uninterrupted pathway from carrier trait to anemia, found that SCT on maternal anemia is 1.19 (95% CI 0.99, 1.44) times higher as compared to that of non-carriers. The proportion mediated, which described the proportion of the total effect estimate that can be explained by the effect of SCT through placental parasitemia was 0.008 (95% CI -0.09, 0.10), suggesting that nearly all of the total effect is explained by the direct effect of SCT on anemia.

In line with the total effect of HbAC on maternal anemia, we did not observe any direct effect of HbAC (RR= 0.96, 95% CI 0.66, 1.38) nor an indirect effect (RR = 1.02, 95% CI 0.99, 1.06) on maternal anemia. Likewise, HbAC was not associated with placental parasitemia in a multivariate regression (RR = 1.35, 95% CI 0.87, 2.11) though there was a trend of high placental parasitemia with 37% of pregnant HbAC individuals having placental parasitemia as compared to 22% of HbAA individuals (**Table 1, Table S3**).

Trends in maternal anemia across pregnancy. We also examined the effect of hemoglobinopathy carrier status on anemia measured at any point during pregnancy (**Table 3**). The total and direct effects of SCT on maternal anemia were attenuated as compared to the analysis considering only anemia at delivery. Specifically, the risk of maternal anemia during pregnancy and delivery amongst SCT carriers was nearly identical to that amongst non-carriers (RR 0.99, 95% CI 0.94, 1.05) and the risk of anemia was not mediated by placental parasitemia (RR 0.99, 95% CI 0.99, 1.00) (**Table 3).**

Likewise, we did not observe a total effect (RR = 0.97, 95% CI 0.87, 1.07) or direct effect (RR = 0.96, 95% CI 0.87, 1.07) of HbAC on anemia at any point during pregnancy, which was unchanged after accounting for the mediated effect of HbAC on anemia through placental parasitemia (RR = 1.00, 95% CI 0.89, 1.02). (**Table 3).**

The complete case analysis showed similar results for both hemoglobin traits in the cross-sectional and longitudinal mediation analyses (**Table S4, S5**). However, in the complete case analysis of the mediator, the risk of placental parasitemia was 1.58 (1.11, 2.25) times higher among HbAC pregnant people as compared to HbAA pregnant people (**Table S6**).

Discussion

In an analysis of pregnant patients in Benin, we observed higher risk of maternal anemia at delivery among those with SCT as compared to those without any hemoglobinopathy, after adjusting for anemia risk factors. When considering the relationship between SCT and anemia at any point during pregnancy, associations attenuated. Furthermore, this association did not appear to be mediated by placental malaria and SCT was not associated with risk of placental malaria.

In contrast, HbAC was not associated with maternal anemia during delivery or at any time during pregnancy and this relationship is not altered after accounting for the placental malaria. While there was an overall trend of higher placental parasitemia between HbAC compared to HbAS, this was not significant. These findings suggest that hemoglobinopathy carrier traits have distinct relationships with malaria burden and hematological parameters during pregnancy and delivery.

Anemia during pregnancy has been associated with increased risk of postpartum hemorrhage, risk of maternal mortality, as well as adverse infant outcomes (2, 25, 26). While some studies have found that SCT increases risk of anemia in the third trimester and at delivery (as well as outside of pregnancy), others have found no association(8, 27-30). Imprecise outcome definitions, a failure to account for gestational age or hemodilution, uncontrolled confounding or low power may explain these wide range of results(27). Despite the mixed evidence, other clinical research findings suggest that these hematologic features have negative clinical implications, including substantial evidence of carriers' increased risk of renal disease, in particular chronic kidney disease (CKD), and increased risk of venous thrombosis.

In the context of Benin and other malaria endemic countries, SCT imparts a selective advantage vis-à-vis less severe malaria infections(31, 32). The *Plasmodium falciparum* parasite infects red blood cells (RBC) and induces sickling in HbAS erythrocytes, which reduces the RBC lifespan and alters cytoadhesion behavior associated with severe disease(33, 34). In contrast, the relationship between SCT and placental malaria is unclear. Placental malaria utilizes a placenta-specific ligand, VAR2CSA, to mediate placental-adhesion and sequesters within the placenta (in contrast to the spleen). Biomolecular studies show HbAS individuals have reduced antibody recognition of infected erythrocytes and diminished expression of parasite proteins that mediate placental adhesion, though placental malaria-specific antibody levels are similar between HbAS and HbAA individuals(35, 36). Several small observational studies

included data on HbAS and placental malaria and did not demonstrate association, though robust statistically testing or confounding adjustment was not undertaken (37-40). In line with previous research, we did not find that SCT was protective against placental malaria. However, this susceptibility to placental malaria does not appear to explain the increased risk of anemia in SCT carriers. Direct effect may be explained by the observation that the body can compensate for SCT cells' structural deficiencies, such as through higher rates of erythropoiesis, but under strong physiologic demands, like the end of pregnancy when there is an additional demand for erythropoiesis, the body may be unable to further compensate(35-37). Alternatively, experimental work has suggested that SCT allows for advantageous maintenance of an infection below detectable limits to continue to bolster immunity, which would be accompanied by a continuous lysis of infected erythrocytes (and potentially anemia)(38). The absence of significant interaction may be explained by the strong effect of malaria on anemia, in which the erythrocyte destruction by parasites may outweigh weaker effects of sickling-induced anemia.

The other hemoglobinopathy of interest, HbAC, is less researched than SCT(11). As such, there is little research to situate the null results with a possible positive trend between HbAC and slightly higher rates of placental parasitemia. Interestingly, molecular research has found that *Plasmodium falciparum*-infected HbAC red blood cells have an abnormally expressed placenta-specific ligand, VAR2CSA, which the immune system uses to recognize and destroy infected cells(39). While this modified VAR2CSA ligand may dampen the immune response to potential placental infection, it also likely provides the advantage of reduced cytoadherence, the process by which infected red blood cells invade deep tissues and evade the immune system, to placental cells(40). The reduced cytoadherence through the modified VAR2CSA is stronger in HbAC than HbAS and may explain a previously found association between HbAC and increased birthweight(41). Furthermore, in a separate paper, the reduction of HbAS iRBC surface recognition by HbAS plasmas was only seen after accounting for erythrocyte abnormalities, such as SCT, HbAC, G6PD and α-thalassemia, and the possibility of co-carrying these diseases(42). Relatedly, there is also evidence that there is impaired cytoadherence in infected SCT erythrocytes(43). HbAC and HbAS may have different mechanisms of preventing severe infection or relationships to other co-occurring erythrocyte abnormalities and therefore may have different clinical effects on maternal and neonatal health(31).

Our study has several strengths. We used a highly sensitive measure of placental parasitemia, PCR from placental biopsies. Placental malaria utilizes a placental-specific ligand to mediate placental adhesion, and the relationship

between carrier traits and placental malaria is less understand than with peripheral venous malaria. Extant descriptive studies have included data on this relationship but did rigorously test the association (41, 44-46). This study is also strengthened by limited confounding as few variables lead into the exposure, a genetic variant, in the racially homogenous population sampled in this study; this likely bolsters exchangeability of the total effect. Furthermore, our approach examines how other modifiable factors might contribute to clinical effect of blood variants. While genetic traits are fixed, modifiable mediators can be studied to inform interventions and understand causal pathways. Isolating the different causal pathways can better elucidate the underlying mechanisms that link genetic traits to important health outcomes.

Our study also has several limitations. Effect estimates derived from a mediation analysis are particularly susceptible to confounding as they have an additional assumption beyond the standard causal assumptions. Natural direct and indirect effects, as presented here, require control of confounders of the mediator (placental malaria) and outcome (maternal anemia) that are affected by exposure (carrier status). While it is possible that SCT impacts confounders between placental malaria and maternal anemia, such as health behaviors and social factors, genetic status does not appear to have a strong social implications in this setting. Nonetheless, this paper does not pose mechanistic explanations, but rather descriptive or natural mediating processes for hemoglobinopathy carriers as compared to those with no hemoglobinopathy traits. This study did not account for coinheritance of other haemoglobinopathies, such as alpha thalassemia, which is known to create negative epistasis with SCT(31) or G6PD deficiency. Coinheritance of blood traits is common, and various combinations may normalize or worsen red blood cell parameters leading to a large distribution of severity and subtypes of disease(30, 31). Future work that incorporates a more complete genomic analysis, earlier gestational age at enrollment, and in a larger sample size will likely improve the ability to further understand association between common hemoglobin variants and anemia throughout the course of pregnancy.

In this longitudinal study of pregnancy with a high prevalence of both hemoglobin variants and malaria burden, we observed evidence that pregnant participants with SCT have higher risk of maternal anemia at delivery in comparison to pregnant participants without hemoglobinopathies, and that this association was not mediated by placental parasitemia. Additionally, SCT does not appear to protect against placental parasitemia, as suggested by biomolecular studies (35, 36).These findings support the need to further study the impact of hemoglobin variants on

pregnancy outcomes. The importance of even mild elevations in risk is underscored by the prevalence of these variants, as small increases in individual relative risk can translate to large increases in population attributable risk where the prevalence of the exposure is high. Hemoglobin structural variants are common globally with an exceptionally high prevalence of SCT in Benin (15.9%)(12, 47). HbAC is less common but affects 6.4% of individuals in Benin and is the most common non-sickling hemoglobin variant in US, affecting 2 to 3% of African Americans(11, 48). These finding support the need for further research on the impact of hemoglobinopathy carrier traits on maternal pregnancy outcomes, considering the complex interplay of confounders in heterogeneous populations.

Tables, Figures, and Equations

Table 1: Distribution of participant characteristics, overall and by hemoglobinopathy.

	Overall N = 895	HbAA 656 (73.3%)	SCT 185 (20.7%)	HbAC 54 (6.0%)
Characteristics				
Maternal age at enrollment in years [Mean (SD)]	25.8 (5.58)	25.6 (5.49)	25.9 (5.55)	27.5 (6.49)
Missing **[n (%)]**	1 (0.1%)	1 (0.2%)	0 (0%)	0 (0%)
Participant can read [n (%)]				
No	677 (75.6%)	493 (75.2%)	149 (80.5%)	35 (64.8%)
Yes	217 (24.2%)	162 (24.7%)	36 (19.5%)	19 (35.2%)
Missing	1 (0.1%)	1 (0.2%)	0 (0%)	0 (0%)
Participant home has electricity [n (%)]				
No	517 (57.8%)	384 (58.5%)	105 (56.8%)	28 (51.9%)
Yes	377 (42.1%)	271 (41.3%)	80 (43.2%)	26 (48.1%)
Missing	1 (0.1%)	1 (0.2%)	0 (0%)	0 (0%)
Primigravida [n (%)]				
Multigravida	728 (81.3%)	529 (80.6%)	154 (83.2%)	45 (83.3%)
Primigravidae	166 (18.5%)	126 (19.2%)	31 (16.8%)	9 (16.7%)
Missing	1 (0.1%)	1 (0.2%)	0 (0%)	0 (0%)
Placental parasitemia at delivery (PCR)[b] [n (%)]				
No Parasites	443 (49.5%)	330 (50.3%)	90 (48.6%)	23 (42.6%)
Any Parasites	195 (21.8%)	135 (20.6%)	40 (21.6%)	20 (37.0%)
Missing	257 (28.7%)	191 (29.1%)	55 (29.7%)	11 (20.4%)
Anemia at delivery[a] [n (%)]				
No	485 (54.2%)	360 (54.9%)	95 (51.4%)	30 (55.6%)
Yes	316 (35.3%)	224 (34.1%)	74 (40.0%)	18 (33.3%)
Missing	94 (10.5%)	72 (11.0%)	16 (8.6%)	6 (11.1%)
Gestational age at birth in weeks [Mean (SD)]	39.6 (2.44)	39.5 (2.61)	39.6 (1.89)	40.1 (1.96)
Missing [n (%)]	39 (4.4%)	31 (4.7%)	7 (3.8%)	1 (1.9%)
Iron deficiency at enrollment (<11 ug/L, CRP adjusted) [n (%)]				
No	611 (68.3%)	450 (68.6%)	127 (68.6%)	34 (63.0%)
Yes	284 (31.7%)	206 (31.4%)	58 (31.4%)	20 (37.0%)
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Trial Arm [% (n)]				
Mefloquine full dose	300 (33.5%)	222 (33.8%)	58 (31.4%)	20 (37.0%)
Mefloquine split dose	289 (32.3%)	200 (30.5%)	67 (36.2%)	22 (40.7%)
Sulfadoxine-pyrimethamine	305 (34.1%)	233 (35.5%)	60 (32.4%)	12 (22.2%)
Missing	1 (0.1%)	1 (0.2%)	0 (0%)	0 (0%)
Incomplete Treatment [% (n)] Missed or Refused Second Dose				
No	871 (97.3%)	633 (96.5%)	185 (100%)	53 (98.1%)
Yes	24 (2.7%)	23 (3.5%)	0 (0%)	1 (1.9%)
Missing	0 (0%)	0 (0%)	0 (0%)	0% (0)
Season at first visit [% (n)]				
Dry Season	524 (58.5%)	381 (58.1%)	108 (58.4%)	35 (64.8%)
Wet Season	370 (41.3%)	274 (41.8%)	77 (41.6%)	19 (35.2%)
Missing	1 (0.1%)	1 (0.2%)	0 (0%)	0 (0%)
Number of positive blood smears during pregnancy and delivery [Mean (Min, Max)]	0.40 (0, 4)	0.40 (0, 3)	0.37 (0, 4)	0.37 (0, 2)

[a]Defined as < 110 g/L or 105 g/L conditional on gestational age; [b]Defined as any non-zero value; Abbreviations: HbAA = no hemoglobinopathy; SCT = Sickle Cell Trait; HbAC = Hemoglobin C Trait

Table 2: Direct and mediated associations of hemoglobinopathy carrier states with maternal anemia at delivery

	SCT[a] N=841 RR (95% CI)	HbAC[a] N=710 RR (95% CI)
Total effect estimate[a]	1.19* (0.99, 1.45)	0.98 (0.68, 1.41)
Direct effect estimate[a]	1.19* (0.99, 1.44)	0.96 (0.66, 1.38)
Indirect effect estimate[a]	1.00 (0.99, 1.02)	1.02 (0.99, 1.06)
Proportion mediated (through malaria)	0.008 (-0.08, 0.10)	-- [b]

∗p<0.1, **p<0.05, ***p<0.01

*[a]Effects calculated using betas and robust standard errors from multivariate results (**Table S1**) using decomposition equations (**Equation Set 3**). Adjustment set for models includes primigravida, ability to read, electricity at home, season (wet versus dry), trial arm, incomplete dose, iron deficiency at enrollment, and placental parasitemia at delivery. Total effect: the effect of hemoglobinopathy status on maternal anemia at delivery. Natural direct effect: the effect of hemoglobinopathy status on maternal anemia on delivery, blocking the effect of hemoglobinopathy status on placental parasitemia. Natural indirect effect: the effect of placental parasitemia set to its expected value under each hemoglobinopathy status on maternal anemia and fixing hemoglobinopathy status to no hemoglobinopathy (HbAA). Proportion mediated: proportion of total effect mediated by placental parasitemia. [b]Proportion mediated not shown when effect estimates are on opposite sides of the null.*

Table 3: Direct and mediated effects of hemoglobinopathy carrier states on maternal anemia at any point during pregnancy and delivery

	SCT N = 841 (2523 visits) RR (95% CI)	HbAC N = 710 (2130 visits) RR (95% CI)
Total effect estimate[a]	0.99 (0.94, 1.05)	0.97 (0.87, 1.07)
Direct effect estimate[a]	0.99 (0.94, 1.05)	0.96 (0.87, 1.07)
Indirect effect estimate[a]	0.99 (0.99, 1.00)	1.00 (0.89, 1.02)
Proportion mediated (through malaria)	0.004 (-0.001, 0.02)	-- [b]

∗p<0.1, **p<0.05, ***p<0.01

*[a]Effects calculated using betas and robust standard errors from multivariate GEE Poisson models with probability weighting described in using decomposition equations (**Equation Set 1, 2, and 3**). [b]Indirect effects were calculated using betas from multivariate GEE Poisson models and confidence intervals were estimated using a quasi-Bayesian approximation. Adjustment set for models includes gestational age, primigravida, ability to read, electricity at home, season (wet versus dry), trial arm, iron deficiency at enrollment, and placental parasitemia at delivery and controlled for correlation between visits of the same participant. Total effect: the effect of hemoglobinopathy status on maternal anemia during pregnancy or delivery. Direct effect: the effect of hemoglobinopathy status on maternal anemia during pregnancy or delivery, fixing placental parasitemia value to zero. Indirect effect: the difference between total and direct effect, capturing the effect of placental parasitemia through hemoglobinopathy status on maternal anemia. Proportion mediated: proportion of total effect mediated by placental parasitemia. [b]Proportion mediated not shown when effect estimates are on opposite sides of the null.*

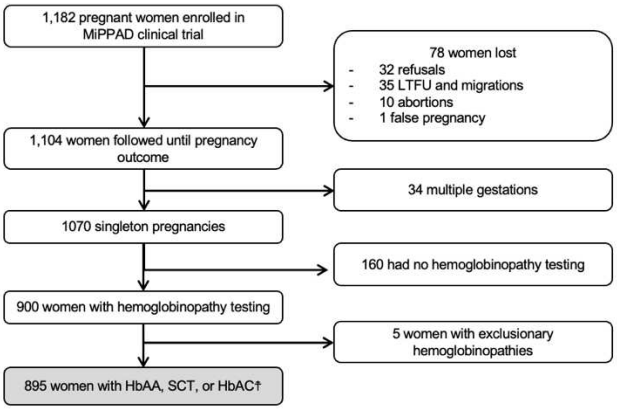

Figure 1. Sample Size Flow Chart *†Sample used in sensitivity analysis with multiple imputation.*

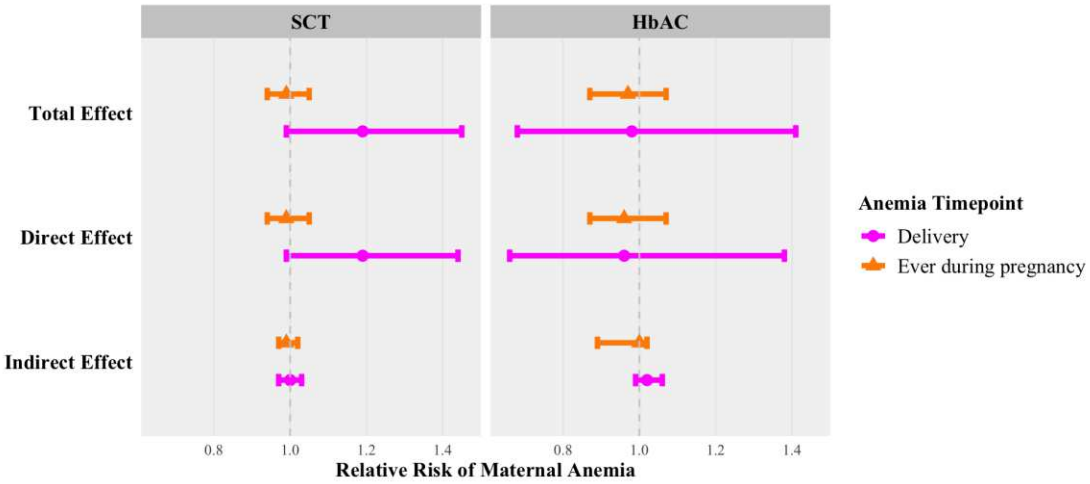

Figure 2. Total, direct, and indirect relative risks and 95% confidence intervals (CI) for the association of hemoglobinopathy carrier traits (SCT, HbAC) with maternal anemia at multiple pregnancy timepoints. *Relative risk represents the difference in risk of maternal anemia comparing hemoglobinopathy trait to no hemoglobinopathy trait. Total effect: the effect of hemoglobinopathy status on maternal anemia during pregnancy or delivery. Direct effect: the effect of hemoglobinopathy status on maternal anemia during pregnancy or delivery, fixing placental parasitemia value to zero. Indirect effect: the difference between total and direct effect, capturing the effect of placental parasitemia through hemoglobinopathy status on maternal anemia.*

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