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Iron Supplementation among Iron-Replete and Non-Anemic Pregnant Women: A Randomized Placebo-Controlled Trial in Tanzania

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Abstract

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Competing interest

None of the authors have any conflict of interest.

Authors and contributors

The paper was drafted by AE and WF with contributions from all authors. WF and ZP designed the trial. AE, NG, IA, SA, RM, LM, DR, and WF participated in field implementation. AE, NG, DS, and WF contributed to statistical analyses. All authors contributed to the development of and approved the final version of the manuscript. WF had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

This trial was registered “Prenatal Iron Supplements: Safety and Efficacy in Tanzania” (NCT01119612; <http://clinicaltrials.gov/show/NCT01119612>).

Importance—Anemia is common in pregnancy, and increases the risks of adverse pregnancy outcomes. Iron deficiency is a leading cause of anemia in sub-Saharan Africa and iron supplementation is the standard of care during pregnancy; however, recent trials among children have raised concerns regarding the safety of iron supplementation in malaria-endemic regions. There is limited evidence about the safety of iron supplementation during pregnancy in these areas.

Objective—To evaluate the safety and efficacy of iron supplementation in pregnancy in a malaria-endemic region.

Design—We conducted a double-blind placebo-controlled clinical trial among pregnant women from 2010–2012.

Setting—Pregnant women presenting for antenatal care in Dar es Salaam, Tanzania.

Participants—Iron-replete non-anemic women were eligible if they were HIV-uninfected, primigravidae or secundigravidae, and at or before 27 weeks of gestation. Screening of 21,316 women continued until the target enrollment of 1500 was reached.

Intervention—Participants were randomized to receive either 60 mg of iron or placebo, returning every four weeks for standard prenatal care including malaria screening, prophylaxis with sulfadoxine/pyrimethamine, and treatment, as needed.

Main outcomes—The primary outcomes were placental malaria, maternal hemoglobin at delivery, and birth weight.

Results—Maternal characteristics were similar at baseline in iron and placebo groups, and >90% used malaria control measures. The risk of placental malaria was not increased by maternal iron supplementation (relative risk (RR), 1.04; 95% CI, 0.63–1.71), nor did iron supplementation significantly affect birth weight (P=0.89). Iron significantly improved hemoglobin and iron status at delivery (both P<0.001). Iron supplementation reduced the risk of anemia at delivery by 40% (95% CI, 29–49%) and the risk of iron deficient anemia at delivery by 66% (95% CI, 38–81%).

Conclusions and Relevance—Prenatal iron supplementation among iron replete non-anemic women was not associated with an increased risk of placental malaria or other adverse events. Supplemented participants had improved hematologic and iron status at delivery compared to the placebo group. These findings provide strong support for continued administration of iron during pregnancy in malaria-endemic regions where good malaria control is present.

Introduction

Anemia affects a quarter of the global population, and the prevalence is higher among women and children, especially those living in sub-Saharan Africa (SSA).¹ Anemia is common in pregnancy, where the effects on obstetric and perinatal risks appear to depend on the degree and gestational timing of anemia.² Iron deficiency, the most widespread nutritional problem in the world, is also the leading cause of anemia during pregnancy. Prenatal iron supplementation is standard of care in most countries,³ where the goal is to reduce anemia during pregnancy and influence the iron endowment of the fetus and neonate.² Current World Health Organization (WHO) guidelines recommend iron supplementation for pregnant women, women of childbearing age, and children under two

years of age in areas with high prevalence of anemia (20–40%).^{4–6} However, in areas of high malaria burden, iron supplementation may carry a reciprocal effect of increasing the supply of iron to host pathogens, thereby increasing risk for malaria and other perinatal infections.⁷ In particular, findings from a trial in Pemba, Tanzania raised concerns that in areas of high malarial burden, iron supplemented children were more likely to be hospitalized or die.⁸ Due to these and other findings, reviewed in⁹, the WHO recommends that caution should be exercised in iron supplementation of children in areas of high malaria burden and that only children with anemia or at high risk of iron deficiency be targeted for possible supplementation.^{10,11}

The safety of routine prenatal iron supplementation in malaria-endemic regions has not been rigorously assessed. Malaria in pregnancy remains a major public health issue in SSA. Annually, an estimated 25 million women in SSA are at risk for malaria infection during pregnancy, a quarter of which show evidence of infection at delivery.^{12,13} Studies on iron deficiency in pregnancy have suggested that the risk of placental malaria may be decreased in iron deficient relative to iron replete women.^{14,15} In particular, *Plasmodium falciparum* infection is more frequent and of higher parasite density in pregnant than non-pregnant women¹⁶ and is associated with increased risks of maternal anemia, maternal death, prematurity, stillbirth and low birth weight (LBW, reviewed in¹³). Additionally, the risk of all-cause anemia is estimated to be approximately three-fold higher among infants born to mothers with placental malaria infection.^{17–19}

We conducted a randomized double-blind placebo-controlled trial to determine the safety and efficacy of prenatal iron supplements among non-anemic, iron replete women in Tanzania. Women who were severely anemic or iron deficient were provided with iron supplementation per Tanzanian guidelines for standard of care and not enrolled in the trial.

Methods

The trial was carried out in Dar es Salaam, Tanzania. Though malaria remains endemic in Tanzania, infection prevalence in Dar es Salaam is currently in a low risk category (*PPR*₂₋₁₀ 5%).²⁰ Recent estimates of malaria incidence and mortality show steady declines since a peak in 2003, where the decrease in Tanzania is among the highest in SSA at 7–8%.²¹ Nevertheless, in the absence of malaria interventions, risk for at least one placental infection during pregnancy in the Dar es Salaam area is 40–50% in primi- and secundigravida.²²

Pregnant women presenting at the Amtullabai Karimjee, Sinza and Magomeni antenatal clinics (ANCs) between September 2010 and October 2012 were invited to participate in the trial. Eligible participants were HIV-uninfected primigravidae or secundigravidae women that were at or before 27 weeks of gestational age at the time of screening, not severely anemic (hemoglobin (Hb) >8.5 g/dL) not iron deficient (serum ferritin >12 µg/L), and intended to stay in Dar es Salaam until delivery and for at least six weeks thereafter. Recruitment continued until the target sample size of 1500 participants was reached.

Pregnant women presenting at the ANCs were screened for HIV, anemia and low ferritin. ANC staff provided HIV testing, pre- and post-test counseling, and, in the event of

ineligibility for the study, standard prenatal care services including antiretroviral therapy and iron supplementation, as needed. Women who then consented to participation were randomized on the same day of their presentation. Enrolled mothers were administered a background questionnaire, a food frequency questionnaire and a full clinical examination.

Participants were individually randomized in equal numbers to receive a daily oral dose of 60 mg elemental iron (as ferrous sulfate) or placebo from the time of enrollment until delivery. The active and placebo tablets and packaging were indistinguishable from one another. Allocation was performed according to a computer-generated randomization sequence using blocks of size 20 created by a scientist not involved in data collection; study clinics were issued pre-labeled regimen bottles according to this sequence. At enrollment, each participant was assigned to the next numbered bottle of regimen at that site. At each subsequent visit, study supplements were dispensed in identical bottles labeled with the participant's study identification number prepared by study pharmacists who had no contact with the participants. The dose of 60 mg iron is the WHO-recommended dose for universal supplementation during pregnancy. Participants were instructed by clinical staff to consume the supplement with a meal. Participants were given folic acid daily per Tanzanian standard of care.

Each woman attended one of the three study clinics monthly until delivery and was provided with standard prenatal care, including intermittent preventive treatment in pregnancy malaria prophylaxis using SP (IPTp-SP; 1500 mg sulfadoxine, 75 mg pyrimethamine), given in the second and third trimesters. Participants were tested by peripheral blood smear for malaria parasites as needed and incident malaria cases were managed according to Tanzanian MOH guidelines. Vouchers for bednets were issued through a governmental program at all prenatal clinics. At each study visit, participants were administered a health questionnaire, given an obstetric examination and provided a monthly supply of study regimen. Study staff collected used regimen bottles at each visit and counted remaining pills.

On-call study midwives attended participants at delivery, collected, examined and sampled placentas, obtained blood samples and scheduled post-natal appointments. At the 6-week post-partum clinic visit, study staff ascertained survival status and morbidity and conducted anthropometric measurements and a physical examination of mother and child.

At screening, women were provided with HIV testing using two rapid assays (Alere Determine and Uni-Gold HIV-1/2), with confirmation of discrepant results using ELISA (Enzygnost HIV Integral II, Germany) at the Muhimbili University of Health and Allied Sciences (MUHAS) research laboratory. Screening values were obtained during recruitment for hemoglobin (Hemocue AB, Hb 201, Sweden) and ferritin (colloidal gold rapid assay, Glory Science Co., Ltd and Victory Medicine Inc., NY). Women with Hb ≥ 11 g/dL and ferritin results >20 μ g/L were eligible for randomization, whereas those with ferritin results in the 10–20 μ g/L range required confirmation by immunoturbidimetric assay using the Cobas Integra 400 plus (Roche Diagnostics, IN) in the research laboratory. A peripheral venous blood sample was taken from all women at enrollment, 20 weeks, 30 weeks, delivery, and 6 weeks post-partum. At enrollment and delivery, blood was tested at the

research laboratory for a complete blood count (CBC, AcT5 Diff AL (Beckman Coulter, FL)), serum ferritin and C-reactive protein (CRP, Cobas Integra 400 plus).

Placental malaria was evaluated using histopathologic examination and by PCR. The fresh placenta was sampled²³ and the tissue divided for use in histopathologic examination (microscopic infection) as well as for nucleic acid studies (submicroscopic infection). For placental histopathology, tissue was formalin-fixed, embedded, sectioned, and stained and examined by light microscopy and under polarized light for the presence of malaria pigment and parasitized erythrocytes; infections were classified by a placental histopathologist (DR).²⁴ A subset of 100 slides was submitted for external confirmation of diagnoses. To prepare placental tissue for nucleic acid studies, tissue was stabilized in RNAlater® (Qiagen, Germany) and homogenized, and genomic DNA was extracted using DNeasy® (Qiagen, Germany). Taqman® qRT-PCR was used for amplification using published primer and probe sets (*P. falciparum*-specific²⁵ and general *Plasmodium* (18S rRNA genes²⁶)). Tissue was tested for PCR inhibitors, and positive and negative controls were included on each plate for quality assurance.

Of 21,316 women screened for eligibility, 17,891 (84%) were excluded for not meeting the eligibility criteria (including being multigravida (n=7459), intending to deliver out of Dar es Salaam (n=3920), not providing blood sample to assess eligibility (n=2280), iron deficient (n=1762), advanced gestational age (n=1206), age less than 18 years (n=585), HIV positive (n=561), severe anemia (n=69), or high iron stores (n=49)), or for not returning to the study sites for confirmatory laboratory results when needed or declining to participate (n=1925) (Figure 1). Of 1500 randomized participants, delivery outcomes were obtained for 1469 (98%). Of these, it was not possible to collect placentas from 342 women who had early fetal loss (n=59), delivered at a non-study hospital (n=167), delivered outside of Dar es Salaam (n=98), or withdrew from the study (n=18). Of the remaining 1127 women, 1003 placenta samples were obtained (88%), and 124 women for whom collection was expected, this was missed.

The primary outcomes were prevalence of placental malaria (by histopathology or PCR), maternal hemoglobin at delivery, and infant birth weight. Secondary outcomes included prevalence of maternal anemia (Hb <11 g/dL) at delivery, LBW (< 2500 g), very LBW (< 2000 g), small for gestational age (< 10th percentile for gestational age, based on the Alexander growth standard²⁷ and the INTERGROWTH standard²⁸), and placental weight. Maternal hospitalizations during pregnancy, adverse perinatal outcomes such as maternal death, fetal loss, preterm birth (<37 weeks gestational age), and neonatal death, and maternal ferritin and iron deficiency (ferritin <12 µg/L) at delivery were also assessed. Finally, iron deficiency in the presence (ferritin <70 µg/L and CRP >8.2 µg/mL) or absence of inflammation (ferritin <30 µg/L and CRP > 8.2 µg/mL) at delivery was assessed using published cutoffs.²⁹

The sample size of 1500 was determined to provide at least 80% power at a 5% significance level to detect a 35% or higher effect of the intervention on placental malaria at a background rate of 20%, assuming 10% loss to follow up. Analyses followed the intention-to-treat principle and included all randomized participants. Differences in baseline measures

and outcomes between the two treatment arms were assessed with χ^2 tests of independence or Fisher's exact tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. Differences between treatment arms in binary outcomes measured repeatedly on participants were assessed using log-binomial models with an exchangeable correlation structure.³⁰ Twin pregnancies (n=28 pairs) were analyzed as a single outcome, where the final birth weight used was the average of the two twin birth weights. If the delivery outcome was stillbirth for either of the twins, the pregnancy was considered to have been a stillbirth. All P-values were two-sided. SAS version 9.3 (SAS Institute Inc., Cary, NC) was used for all analyses.

The Harvard School of Public Health Human Subjects Committee, the MUHAS Senate Research and Publications Committee, and Tanzania's National Institute for Medical Research granted institutional review board approval. The Tanzania Food and Drug Authority approved the use of the study regimens. Written informed consent was obtained from all women for their participation in the trial. Study progress was monitored by a Data Safety Monitoring Board (DSMB) annually or more frequently as determined by the DSMB. Interim analyses were conducted using an efficacy stopping rule for unblinding of $p < 0.001$ for primary endpoints and a safety rule for unblinding of $p < 0.05$ and possible further action at the discretion of the DSMB. The trial was registered at clinicaltrials.gov (identifier NCT01119612).

Results

From September 2010 to October 2012, 1500 participants were randomized into iron or placebo groups and followed monthly during the prenatal period, at delivery and at six weeks post-partum. Trial participation ended at the conclusion of the six weeks post-partum visit. There were no significant baseline differences between women in the iron and placebo groups for sociodemographic characteristics, use of anti-malarials, or nutritional status variables (Table 1, all $P > 0.05$). The mean gestational age at randomization was 18.2 weeks in both groups ($P = 0.96$). The mean (median) time between randomization and delivery was 148 (148) days ($P = 0.33$), and the time between randomization and six week post-partum visit was 305 (237) days ($P = 0.82$). The average compliance rate (number of tablets absent from returned regimen bottles divided by the number of days the participant had the bottle) was 77% (median=84%). The compliance rate was not significantly different between the groups (iron group = 76% (84%); placebo = 78% (85%); $P = 0.67$).

The overall prevalence of placental malaria was 6.6%; the risk of placental malaria was not different between the groups (relative risk (RR), 1.03; 95% confidence interval (CI), 0.65–1.65), nor was there increased risk for microscopic (RR, 1.14; 95% CI, 0.49–2.65) or submicroscopic (RR, 1.12; 95% CI, 0.65–1.92) placental malaria (Table 2). All infections detected by PCR and microscopy were *P. falciparum*.

Placental weight was not significantly different between the iron (458 ± 140 g) and the placebo groups (456 ± 144 g, $P = 0.97$). Risk of maternal death was not significantly affected by iron supplementation (RR, 0.67; 95% CI, 0.11–3.98). Although there were marginally fewer maternal hospitalizations in the iron group compared to the placebo group (RR, 0.57;

95% CI, 0.32–1.02), no single type of hospitalization was significantly associated with iron supplementation (data not shown). Women were more frequently hospitalized for reasons other than malaria; pregnancy complications accounted for 50% (n=10) of the total hospitalizations in the iron group and 63% (n=23) in the placebo group. Reasons for hospitalizations other than pregnancy complications or malaria accounted for 40% (n=8) in the iron group and 17% (n=6) in the placebo group.

Mean hemoglobin (P=0.98) and ferritin (P=1.00) at randomization did not differ between the groups (Table 3). Hemoglobin at delivery was significantly higher in the iron group (11.8 ± 2.0 g/dL) than the placebo group (10.9 ± 1.9 g/dL, $P<0.001$); women in the iron group improved their hemoglobin status at delivery by 0.13 ± 2.2 g/dL, whereas women in the placebo group showed a decreased hemoglobin by -0.7 ± 2.0 g/dL ($P<0.001$). Iron supplementation significantly decreased the risk of anemia at delivery by 40% (RR, 0.60; 95% CI, 0.51–0.71), but not severe anemia (RR, 0.68; 95% CI, 0.41–1.14). Serum ferritin at delivery was significantly higher in the iron treatment group ($P<0.001$). The mean increase in ferritin was significantly greater in the iron group (41.3 ± 295.6 µg/L) compared to the placebo group (11.3 ± 92.1 µg/L, $P<0.001$). The risk of maternal iron deficiency was decreased by 52% (RR, 0.48; 95% CI, 0.32–0.70) and the risk of iron deficiency anemia at delivery was decreased by 66% (RR, 0.34; 95% CI, 0.19–0.62). After adjusting for inflammation, iron reduced the risk of maternal iron deficiency at delivery by 37% and the risk of iron deficiency anemia by 59% (Table 3).

Iron supplementation did not significantly affect the risk of fetal loss (RR, 1.26; 95% CI, 0.86–1.92) or child mortality in the first six weeks after delivery (RR, 1.28; 95% CI, 0.67–2.45) (Table 4). Among live births, iron supplements did not have an effect on birth weight, risk of LBW or very LBW, nor were there any significant effects observed for gestational age at delivery, preterm births or very preterm births in the iron group compared to the placebo group. There was no significant difference between the two groups for having LBW in a preterm delivery, nor in a term delivery. No significant difference was observed for small for gestational age using either birth standard.

Discussion

In this randomized, placebo-controlled clinical trial, no evidence of harm was identified from iron supplementation of pregnant women who were iron-replete and non-anemic at initiation of antenatal care in a malaria-endemic area. Iron supplementation was not significantly associated with the primary outcomes of placental malaria or birth weight, whereas the supplemented group had significantly reduced risks of anemia and iron deficiency compared to the placebo group.

The safety of iron supplementation in malaria-endemic settings has been called into question due to concerns that increased intake of iron may increase malaria-associated morbidity and mortality. A large randomized trial of iron supplemented children in Pemba, Tanzania identified an excess of all-cause and malaria-related serious adverse events in children who received iron compared to those who did not, resulting in a premature halt of the study due to safety concerns.⁸ Both the human host and the malaria parasite require iron as an essential

nutrient; however, the exact relationship between iron and malaria is complex and not well understood. In the presence of infection, the host response has developed to reallocate iron stores away from invading pathogens by reducing the availability of serum iron. Malaria induces disruptions in host iron distribution and utilization through mechanisms involving the erythrocyte (hemolysis and erythropoiesis), iron metabolism (ferritin recycling by the macrophage and dietary iron absorption), and anemia; however, the mechanisms by which the parasite acquires and utilizes iron remains unclear.^{31,32}

Evidence from previous studies indicate there is apparent concern for iron supplements among individuals who are iron-replete with expected benefits among those who are iron deficient. A recent trial on iron fortification among children in Ghana showed that the incidence of malaria was lower in the iron group compared to the no-iron group; in subgroup analyses, iron was protective for children with iron deficiency anemia at baseline, but not for non-anemic iron-replete subjects.³³ Similarly, subgroup analyses in the Pemba trial showed that iron supplementation was apparently beneficial among children with iron deficiency anemia at baseline but no effect was seen in the non-anemic iron-replete group.⁸ The plausibility of risk has been extended to pregnancy; however there have been relatively few iron supplementation studies and none have evaluated the risk among baseline iron-replete, non-anemic women. Few of the many observational studies on iron deficiency and malaria have found that the risk of placental malaria is increased among iron-replete women.^{14,15} Moreover, observational studies inadequately address the question of safety of universal iron supplementation in pregnancy where malaria is endemic.

In our trial among pregnant women who were iron-replete and non-anemic at baseline, we noted no evidence of increased risk of malaria, maternal hospitalizations, or other serious adverse events associated with iron supplementation. A systematic review demonstrated that risk associated with iron supplementation in children was increased in trials where malaria control interventions and treatment were not available, such as the Pemba trial.³⁴ Regular use of malaria interventions at home had wide coverage in our study participants (>90%) and all women enrolled in our study were given IPTp-SP at the second and third trimesters. This high coverage of malaria prevention measures, coupled with the non-anemic, iron-replete baseline status of our participants, are likely to be important factors that explain the difference in findings between our trial and the Pemba study. At the same time, the low overall risk of malaria in the present trial resulting from successful malaria control reduced the statistical power to address one of the primary aims of the trial: the effect of iron supplementation on placental malaria risk. There remains the question as to the degree of malaria control required to offset the risks of supplementation identified in studies with poor or no malaria control.

Another important consideration in prenatal supplementation programs is the potential benefits on hematologic status. In our trial, we demonstrated that iron supplemented women had improved hematologic and iron status from baseline to delivery. Our relative risk estimates for maternal anemia, iron deficiency, and iron deficiency anemia at delivery are consistent with published results, including those presented in a recent comprehensive meta-analysis.³⁵ Measures associated with iron deficiency, including hemoglobin and serum ferritin concentrations, decrease during pregnancy, even in women with adequate iron stores.

The physiologic requirements for iron in pregnancy are substantial and are unlikely to be met even under optimum diet,³⁶ indicating that even women who are iron-replete early in pregnancy require supplementation to maintain a non-anemic iron-replete status.

Among the secondary outcomes evaluated in this study, iron supplementation was not associated with LBW, preterm birth, SGA deliveries, or other adverse perinatal outcomes. Two meta-analyses identified a decreased risk for LBW among iron supplemented women but did not identify risks for our other secondary birth outcomes, confirming the results of an updated Cochrane review.^{35,37,38} While these meta-analyses provide support for iron supplementation in the overall reduction of LBW, the benefit in non-anemic iron-replete women is less known. Among studies of women who are non-anemic at initiation of supplementation,^{39–47} only one study⁴⁷ demonstrated a significantly protective effect for iron on LBW, underscoring the multifactorial etiology of poor fetal growth.

Conclusion

Our trial has clearly demonstrated lack of harm from iron supplementation during pregnancy in iron-replete non-anemic women when good malaria control is present. Further, potential benefits associated with the improved iron and hematologic status as a result of iron supplementation in this trial provide strong support for continuing prenatal supplementation. Since anemia during pregnancy is associated with adverse outcomes for both mother and child, we therefore recommend continuation of iron supplementation of all pregnant women as standard of care in this, and similar environments with adequate concomitant malaria control.

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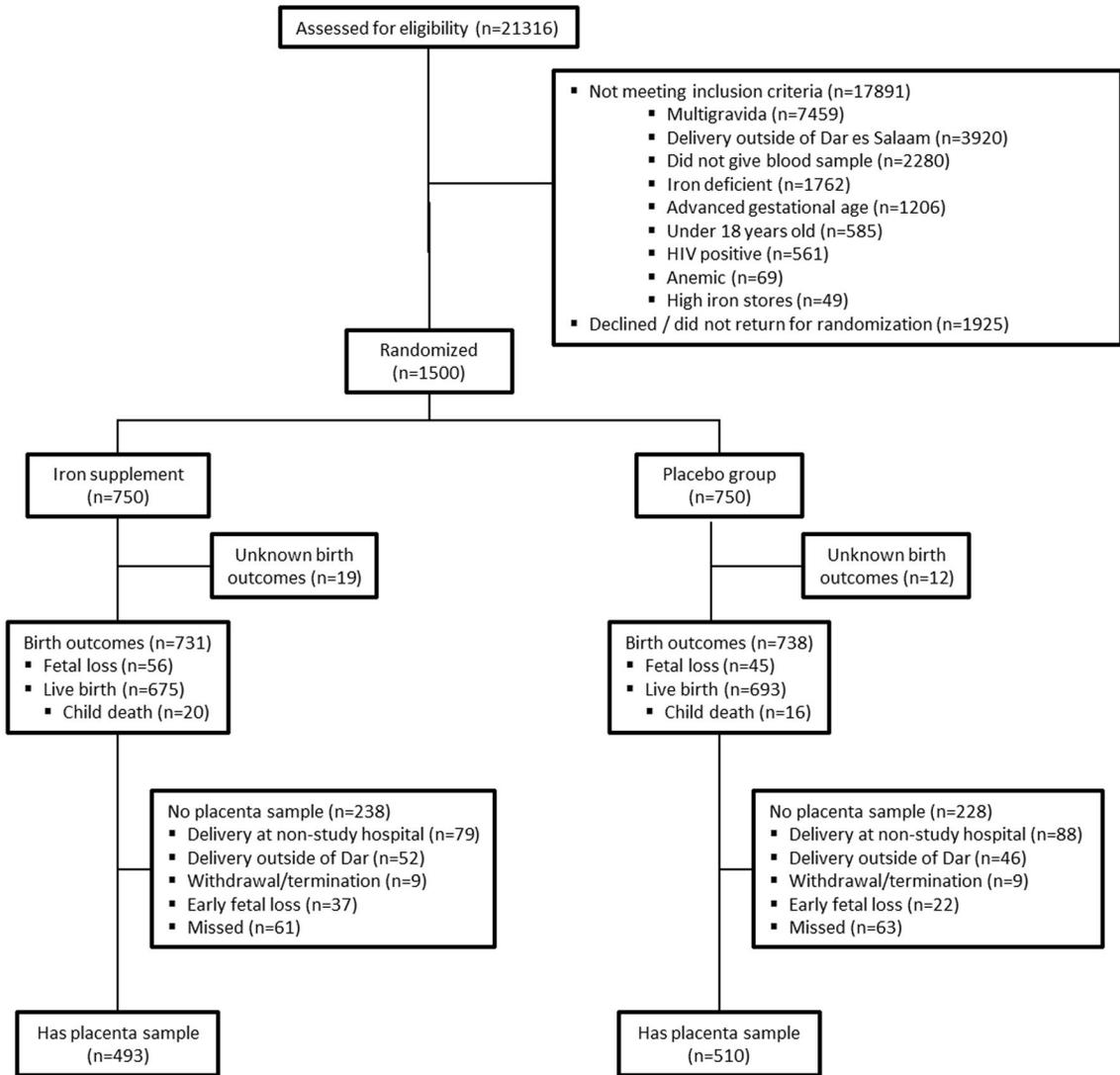


Figure 1.
Study Enrollment, Randomization, and Pregnancy Outcomes.

Table 1Participant characteristics at baseline. ^{a,b}

Characteristic	Iron (n=750)	Placebo (n=750)
Age (years)	23.7 (4.1)	24.1 (4.2)
Primigravida	60%	55%
Gestational age at randomization (weeks)	18.2 (4.3)	18.2 (4.4)
Education		
0–4 years	2%	2%
5–7 years	55%	54%
8–11 years	29%	28%
12 years	14%	17%
Marital status		
Married/Cohabiting	80%	81%
Other	20%	19%
Employment status		
Skilled	19%	20%
Unskilled or informal	30%	30%
Housewife/unemployed	48%	46%
Other	3%	4%
Housing type		
Own	19%	19%
Rent	71%	71%
Other	10%	10%
Roof		
Metal	98%	98%
Other	2%	2%
Floor		
Concrete	93%	94%
Tile	6%	5%
Dirt or wood	1%	1%
Malaria interventions		
Uses spray, coils or bednet nightly	91%	93%
Has bednet	89%	89%
Regularly uses bednet	86%	87%
Body mass index (kg/m²) at enrollment	24.5 (4.4)	24.6 (4.7)
Body mass index category (kg/m²) at enrollment		
< 18.5	4%	5%
18.5 – 24.9	58%	56%
25 +	37%	40%
Baseline MUAC (mm)	26.5 (3.6)	26.5 (3.6)

^aThere were no significant differences between the iron and placebo group ($P > 0.05$ in all cases).

^bMean (SD) for continuous variables.

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Table 2

Effect of iron supplementation versus placebo on maternal outcomes.

Outcome	Iron ^b	N	Placebo	N	Relative Risk (95% CI)	P Value
Placental malaria^a						
Microscopic – no. (%)	11 (2)	471	10 (2)	487	1.14 (0.49, 2.65)	0.77
Submicroscopic – no. (%)	26 (5)	486	24 (5)	501	1.12 (0.65, 1.92)	0.69
Any placental malaria – no. (%)	33 (7)	493	33 (6)	510	1.03 (0.65, 1.65)	0.89
Placental weight (g)	458 (140)	443	456 (144)	467		0.97
Maternal death – no. (%)	2 (0)	750	3 (0)	750	0.67 (0.11, 3.98)	1.00
Maternal hospitalizations – no. (%)	20 (0.4)	4380	36 (0.8)	4474	0.57 (0.32, 1.02)	0.06
Malaria – no. (%)	2 (0)	4380	7 (0.2)	4474	0.29 (0.06, 1.40)	0.12
Other – no. (%)	18 (0.4)	4380	29 (0.1)	4474	0.60 (0.33, 1.11)	0.10

^aMicroscopic infections were detected by placental histopathology. Submicroscopic infections were detected by qPCR on maternal placental tissue. Five participants (iron n=4, placebo n=1) had both microscopic and submicroscopic infections.

^bMeans (SD) for continuous variables.

Table 3
Effect of iron supplementation versus placebo on maternal hematologic and iron status.

Outcome	Iron ^a	N	Placebo	N	Relative Risk (95% CI)	P Value
Hemoglobin at randomization (g/dL)	11.7 (1.4)	738	11.7 (1.3)	741		0.98
Hemoglobin at delivery (g/dL)	11.8 (2.0)	481	10.9 (1.9)	501		<0.001
Mean change in hemoglobin (g/dL)	0.13 (2.2)	473	-0.7 (2.0)	495		<0.001
Anemia at delivery ^b – no. (%)	144 (30)	481	248 (50)	501	0.60 (0.51,0.71)	<0.001
Severe maternal anemia at delivery ^c – no. (%)	23 (5)	481	35 (7)	501	0.68 (0.41,1.14)	0.14
Ferritin at randomization (µg/L)	40.9 (33.3)	741	40.7 (31.4)	742		1.00
Ferritin at delivery (µg/L)	92.5 (171.1)	483	54.1 (90.3)	509		<0.001
Mean change in ferritin (µg/L)	41.3 (295.6)	476	11.3 (92.1)	503		<0.001
Iron deficiency at delivery ^d – no. (%)	33 (7)	483	73 (14)	509	0.48 (0.32,0.70)	0.001
Iron deficiency at delivery (CRP-adjusted) ^e – no. (%)	227 (47)	480	379 (75)	503	0.63 (0.56,0.70)	<0.001
Iron deficiency anemia at delivery ^{b,d} – no. (%)	14 (3)	464	43 (9)	491	0.34 (0.19,0.62)	<0.001
Iron deficiency anemia at delivery (CRP-adjusted) ^{b,e} – no. (%)	74 (16)	461	191 (39)	485	0.41 (0.32,0.52)	<0.001

^aMeans (SD) for continuous variables.

^bHemoglobin < 11 g/dL

^cHemoglobin < 8.5 g/dL

^dSerum ferritin < 12 µg/L

^eSerum ferritin < 70 µg/L and CRP > 8.2 µg/mL or serum ferritin < 30 µg/L and CRP > 8.2 µg/mL

Table 4

Effect of iron supplementation versus placebo on perinatal outcomes.^a

Outcome	Iron	N	Placebo	N	Relative Risk (95% CI)	P Value
Fetal loss ^b no. (%)	56 (7.7)	731	45 (6.1)	738	1.26 (0.86, 1.92)	0.24
Infant mortality at 6 weeks no. (%) ^c	20 (3.0)	675	16 (2.3)	692	1.28 (0.67, 2.45)	0.45
Birthweight	3155 (545) ^d	672	3137 (519)	691		0.89
< 2500 g – no. (%)	45 (6.7)	672	51 (7.4)	691	0.91 (0.62, 1.34)	0.62
< 2000 g – no. (%)	15 (2.2)	672	14 (2.0)	691	1.10 (0.54, 2.26)	0.79
Gestational age at delivery – weeks	39.3 (2.5)	665	39.2 (2.7)	685		0.37
Preterm birth – no. (%) ^b						
< 37 weeks	100 (15.0)	665	113 (16.5)	685	0.91 (0.71, 1.17)	0.46
< 34 weeks	21 (3.2)	665	27 (3.9)	685	0.80 (0.46, 1.40)	0.44
Low birthweight and preterm birth – no. (%) ^{d,e}	27 (4.1)	665	21 (3.1)	685	1.32 (0.76, 2.32)	0.32
Low birthweight and term birth – no. (%) ^{d,e}	18 (2.7)	665	30 (4.4)	685	0.62 (0.35, 1.10)	0.10
Small for gestational age – no. (%) ^{d,f}	121 (18.0)	672	125 (18.1)	691	1.00 (0.79, 1.25)	0.97
Small for gestational age (INTERGROWTH) ^g	101 (15.8)	641	105 (16.2)	648	0.97 (0.76, 1.25)	0.83

^aMean (SD) for continuous variables.

^bStillbirth and miscarriage

^cLive birth only

^dRestricted to births at 21 to 44 weeks gestation.

^eLow birthweight defined as < 2500 g.

^fSmall for gestational age was defined as birth weight below the 10th percentile for gestational age using the Alexander standard²⁷.

^gSmall for gestational age was defined as birth weight below the 10th percentile for gestational age using the INTERGROWTH standard²⁸, restricting to births 30 to 42 weeks gestation.