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## Maternal anemia type during pregnancy is associated with anemia risk among offspring during infancy

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### Abstract

**BACKGROUND**—We evaluated the association between etiology of maternal anemia and iron status throughout infancy.

**METHODS**—Samples from a study designed to examine Praziquantel treatment during pregnancy were used (n=359). All women were infected with schistosomiasis and randomized to

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Praziquantel or placebo at 16±2 weeks gestation. Hemoglobin, serum ferritin (SF), soluble transferrin receptor (sTfR), hepcidin, C-reactive protein, and interleukin-6 were measured in maternal and infant blood. The relationship between both maternal Praziquantel treatment and etiology of anemia and infant iron status was evaluated.

**RESULTS**—Maternal iron deficiency anemia was associated with increased risk of infant anemia at six months of age. Infants of mothers with the lowest levels of circulating hepcidin during gestation, likely a marker for iron deficiency, had higher sTfR:SF levels and lower hemoglobin levels, particularly at twelve months of age. Maternal non-iron deficiency anemia (NIDA) did not impact infant anemia risk or iron status. Maternal treatment for schistosomiasis had no effect on infant hematologic status.

**CONCLUSIONS**—Maternal iron deficiency anemia was associated with an increased risk for anemia or iron deficiency during late infancy. We did not observe an association between maternal NIDA and increased risk for iron deficiency during infancy.

**Clinical Trial Registration**—

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## INTRODUCTION

Anemia affects an estimated 273 million children under the age of five years throughout the world, with a disproportionate burden in low and middle income countries (LMICs) (1). Maternal anemia during pregnancy has been associated with increased risk of maternal and fetal mortality, poor intrauterine growth, and impaired cognitive development in affected offspring (2). Importantly, in both human and animal models, short- and long-term neurodevelopmental deficits have been demonstrated to persist even after iron repletion (3–6). Inadequate body iron is thought to account for approximately 50% of anemia in women globally (iron-deficiency anemia: IDA). Additionally, non-iron deficiency anemia (NIDA), related to other micronutrient deficiencies and/or infections with resulting anemia of inflammation (AI), is also a major contributor to anemia in this population (1, 7).

Due to the importance of iron in neurocognitive development (3–6, 8), identifying risk factors for infant iron deficiency is critical. Though the fetus is thought to efficiently obtain iron *in utero*, many studies have demonstrated lower cord hemoglobin and iron levels among infants of anemic women, particularly women with severe anemia (9, 10). Maternal iron prophylaxis during gestation and newborn serum ferritin (SF) are both positive predictors of SF in infancy (11). Further, in a recently published study from The Philippines, we found that maternal IDA, but not NIDA (due largely to AI), influenced newborn iron endowment (12). Less is known about the longitudinal effect of maternal IDA and AI, both of which may continue to influence infant iron status. Specifically, the impact of both maternal IDA and AI on infant iron status may not become evident until after *in utero* stores are depleted, usually around four months of age among term breastfed infants (13), and decreased iron bioavailability may continue to limit iron bioavailability in breastmilk (10). No studies have examined the role of AI on longitudinal infant iron status. Given AI causes decreased iron bioavailability, we hypothesized that this process might impact infant iron status through decreased fetal and infant iron acquisition *in utero* and in breast milk.

Determining the etiology of maternal anemia and its impact on longitudinal infant iron status and anemia risk is important as interventions to reduce this risk differ significantly. In the context of AI, hepcidin production, driven primarily by the pro-inflammatory cytokine, interleukin (IL)-6 (14) leads to decreased iron absorption in the gut and iron sequestration in the reticuloendothelial system by causing the degradation of membrane-bound ferroportin (15). In the context of iron deficiency, hepcidin is typically suppressed to maintain ferroportin integrity, allowing increased iron absorption in the gut and release from macrophages (16). Maternal hepcidin has also been shown to be inversely related to iron bioavailability to the fetus (17).

In previous work conducted among children and non-pregnant adults in Leyte, The Philippines, we demonstrated that *Schistosoma japonicum* infection is related to anemia risk across all infection intensities (18, 19). Importantly, the predominant cause of anemia in this context is NIDA (20% overall prevalence), primarily due to AI, with a prevalence of IDA of 12% (18, 19). Though the etiology of NIDA is multi-factorial, in those studies we found that less than 1% of individuals with NIDA had a macrocytic anemia, making B12 or folate deficiency the primary cause of NIDA, unlikely. *S. japonicum* infection in this population is believed to lead to AI via chronic immune activation, both in response to adult worms and trapped eggs (20), and as a result of microbial translocation across the gut epithelium as eggs penetrate the gut wall from the largely sterile bloodstream (21).

The objectives of this study were to evaluate the impact of 1) the specific etiology of maternal anemia and 2) maternal treatment for *S. japonicum* on infant iron and anemia status, and 3) examine the relationship between measures of maternal and newborn iron status and longitudinal anemia risk in infancy.

## SUBJECTS AND METHODS

### Study Site and Population

Our study population has been described elsewhere (22). The study was conducted in Leyte, The Philippines where *S. japonicum* is endemic. Geo-helminth infections were also prevalent. Briefly, all women were positive for schistosomiasis during the first trimester of pregnancy and randomized to Praziquantel treatment or placebo at 12–16 weeks gestation. All of the women were provided with a daily multivitamin with iron (29 mg elemental Fe) dispensed at enrollment and the 22- and 32-week visits. At the 22- and 32-week visits, we captured reported compliance, and 100% of study participants reported taking their prenatal vitamins as reported for the trial (22).

Eligibility criteria included infection with *S. japonicum*, the presence of a singleton pregnancy in the second or third trimester, age 18 years or greater, and provision of informed consent. Of note, treatment did not impact birth weight, risk of low birth weight (LBW) or small for gestational age, but did impact maternal ferritin at 32 weeks gestation as reported (22).

### Stool Examination

Maternal parasite burden was determined by examination of three consecutive stool specimens. Each of the three stool specimens was examined in duplicate for *S. japonicum*, *Ascaris lumbricoides*, *Trichuris trichuria*, and hookworm by the Kato Katz method. Intensity of infection for each helminth was determined using World Health Organization (WHO) criteria (23).

### Blood Collection and processing

Venipuncture was performed at 32 weeks gestation and for the infant at six and twelve months of age. A cord blood sample was also obtained at delivery. A complete hemogram was obtained on a Horiba ABX hematology analyzer (Kyoto, Japan) for the infant samples, including mean corpuscular volume (MCV). SF and serum transferrin receptor (sTfR) were assessed using commercially available Enzyme-linked immunosorbent assays (ELISAs) (R&D systems, Minneapolis, MN). C-reactive protein (CRP) and IL-6 were analyzed using a multiplex bead-based platform (BioRad, Hercules, CA) and custom sandwich- or competitive-style ELISA assays (24). The reference ranges for sTfR and sTfR-SF Index ( $=sTfR/\log_{10}(SF)$ ) based on the Quantikine assay kits from R&D Systems were 12.16–27.25 nmol/L and 6.42–22.37 (using nmol/L for sTfR and ng/mL for SF), respectively (25). Hepcidin was measured using a commercially available ELISA kit (DRG Diagnostics, Marburg, Germany). The reference range (2.5 – 97.5% ile) among healthy non-pregnant subjects is 1.49–41.46). Participants with extremely low undetectable concentrations of biomarkers were assigned the lowest detectable concentrations.

### Assessment of newborn outcomes

Gestational age at birth was based on obstetric ultrasound at 8–12 weeks gestation. The newborn was weighed on a Tanita model BD-585 (Arlington Heights, MD) portable scale within 24 hours of birth. This digital scale measures birth weight with precision to ten grams.

### Anemia and Iron Status

Infant anemia was defined as hemoglobin concentration  $<11.0\text{g/dl}$  (26). Infant IDA was identified using ferritin concentration  $<12\mu\text{g/L}$  in the absence of inflammation (CRP  $>5\text{mg/L}$ ) or ferritin  $<30\mu\text{g/L}$  in the presence of inflammation (CRP  $>5\text{mg/L}$ ) (27). Infants who were anemic but not iron-deficient were regarded as having NIDA. Maternal anemia in the presence of ferritin  $<30\mu\text{g/L}$  was used to define maternal IDA, and anemia with ferritin level  $>30\mu\text{g/L}$  to define maternal NIDA (28). We used the sTfR-SF Index as the primary measure of infant iron status, given this ratio is less affected by inflammation (29) and has been shown to reflect bone marrow iron stores in children (30). Red cell macrocytosis was defined as an MCV  $>100\text{fl}$ .

### Statistical analysis

We examined the relationship between two primary predictors (maternal treatment status as Praziquantel versus placebo and the type of maternal anemia as no anemia, IDA, or NIDA) and three primary outcomes (infant iron status, hemoglobin, and anemia status). Analyses

were conducted using SAS 9.3 (SAS Institute, Cary, NC). Statistical significance was defined as  $P < 0.05$ . Concentrations of ferritin, IL-6, CRP, sTfR, and hepcidin, and the sTfR-SF Index were skewed and were, therefore, natural log-transformed for regression analyses. The following confounding and mediating covariates were evaluated for inclusion in regression models if they were related to the outcomes of interest in bivariate analyses with  $P < 0.2$ : newborn sex; LBW; preterm birth; birth length; maternal educational attainment; parity; socioeconomic status; maternal obesity; maternal alcohol consumption; maternal smoking; current breastfeeding at months one, six and twelve; infant nutritional status at six and twelve months; and maternal infection with *A. lumbricoides*, hookworm, or *T. trichiura* at 32 weeks gestation. Maternal age and treatment were included in all models. Log-binomial regression models were used to evaluate the association of maternal anemia type at 32 weeks' gestation with the Page 9 of 27 risk of infant anemia at six and twelve months. Generalized estimating equations models were used to evaluate the association of the change in hemoglobin from six to twelve months across maternal hepcidin at 32 weeks' gestation and cord blood hepcidin concentration, in tertiles. Linear regression models were used to evaluate the association of maternal and cord blood hepcidin tertiles with infants' hematologic biomarker concentrations at each time point.

### Ethical clearance and informed consent

The institutional review boards of Rhode Island Hospital and The Research Institute of Tropical Medicine approved this study. Written informed consent was obtained from each participant. All women were offered treatment for *S. japonicum* and geo-helminths following pregnancy and lactation, in accordance with Department of Health policy in the Philippines at the time of the study.

## RESULTS

Characteristics of the 359 mother-infant pairs (Figure 1) stratified by maternal treatment status are outlined in Table 1. The distribution of maternal biomarkers at 32 weeks' gestation is also presented in Table 2. The majority of infants were born at term, with a mean birth weight of 2.85kg. Geo-helminth infections were also prevalent. Hemoglobin and iron status at 6 and 12 months of age were similar among infants born to mothers who were treated with Praziquantel, compared to those the placebo group. At both six and twelve months of age, the prevalence of infant anemia was high (66% and 64%, respectively). At six months of age 45% of infants had IDA and this increased to 51% at 12 months of age. At six and twelve months of age, 21% and 13% of infants had NIDA, respectively (Table 1). Of note, only one infant at six months of age had an  $MCV < 100$  fl.

### Infant anemia status is associated with maternal anemia status during gestation

Infant anemia prevalence differed significantly by the type of maternal anemia during pregnancy (Figure 2). At six months of age, infants of mothers with IDA at 32 weeks' gestation had significantly higher anemia risk, compared to infants of mothers with NIDA ( $P < 0.006$ ). Interestingly, infants of women with NIDA had the lowest anemia prevalence at six months of age. Infant anemia prevalence at twelve months of age was no longer related to maternal anemia status ( $P = 0.78$ ). The prevalence of infant anemia did not differ

significantly between six and twelve months of age, and was relatively high across the entire cohort at both time points (Table 1, Figure 2).

### **Maternal hepcidin is associated with infant iron status**

Infants of mothers in the lowest tertile of 32-week hepcidin levels had a significantly higher median sTfR-SF Index at twelve months of age, reflecting decreasing iron status, compared to infants of the same age born to mothers with the highest levels of hepcidin (Figure 3a). Among mothers with the lowest levels of circulating hepcidin at 32 weeks' gestation, infants displayed a significant increase in the median sTfR-SF Index, from six to twelve months of age (Figure 3a). Interestingly, we observed no differences in iron status in six-month-old infants based on maternal hepcidin levels. Cord blood hepcidin concentration was not associated with the sTfR-SF Index at six or twelve months and the change in sTfR-SF Index from six to twelve months did not differ by cord blood hepcidin tertile (Figure 3b).

### **Maternal hepcidin is associated with infant hemoglobin levels**

There were no significant differences in infant hemoglobin concentrations at six months across maternal hepcidin tertiles. Infants from mothers in the lowest hepcidin tertile during gestation, however, had significantly lower hemoglobin concentration at twelve months of age ( $P<0.01$ ) compared to those with moderate hepcidin levels (Figure 4a). These infants also had a significant decrease in hemoglobin concentration from six to twelve months ( $P<0.05$ ), while there were no significant changes in hemoglobin concentration among infants in the other maternal hepcidin tertiles (Figure 4a). There was no association between cord blood hepcidin tertiles and infant hemoglobin concentration at six or twelve months of age (Figure 4b).

### **Infant anemia type and iron and inflammatory biomarkers**

The pro-inflammatory cytokine IL-6 was higher in infants with NIDA compared to infants with IDA ( $P=0.027$ ) at both ages and higher in infants with NIDA compared to infants without anemia ( $P=0.036$ ) at twelve months (Figure 5a and 5e). As expected, hepcidin concentrations were lower in infants with IDA at both six and twelve months compared to infants with NIDA ( $P<0.01$ ; Figure 5b and 5f), and lower in IDA compared to non-anemic children only at twelve months of age ( $P<0.05$ , Figure 5f). In addition, despite significantly higher IL-6 levels in NIDA infants compared to their nonanemic counterparts, hepcidin levels were not significantly different between these two groups at twelve months of age (Figure 5f). Conversely, sTfR concentrations were highest in IDA infants compared to either non-anemic or NIDA counterparts of the same age ( $P<0.01$ , Figure 5c and 5g). CRP levels were not significantly different based on infant anemia status at either time point (Figure 5d and 5h). Overall, while sTfR and sTfR-SF Index increased over time (6 months compared to 12 months:  $P<0.01$ ) and across all groups, ferritin ( $P=0.002$ ) and hepcidin decreased ( $P=0.001$ ), reflecting a downward trend in iron status in late infancy (data not shown). The concentrations of hemoglobin did not change significantly ( $P=0.68$ ) over time, likely reflecting the order in which these indices of iron status change.

## DISCUSSION

In this study we evaluated the influence of maternal anemia type and gestational treatment for schistosomiasis on infant iron status and anemia risk. In our previous studies in non-pregnant subjects, we have demonstrated that schistosomiasis is associated with AI (18, 19). Though treated mothers demonstrated improved iron stores, and their newborns displayed modest improvements in iron endowment (22), in the present analysis we were unable to identify an association between maternal treatment and iron status or anemia risk during later infancy. This may in part be due to prolonged pro-inflammatory response to dying worms and eggs following Praziquantel administration (31) and the improvement in hemoglobin in non-pregnant subjects after treatment does not occur for 3–6 months. (32). We did, however, identify a significant relationship between maternal IDA, but not NIDA, during pregnancy and infant anemia risk at six months of age, as well as maternal iron status biomarkers and infant anemia risk.

Understanding the influence of maternal anemia type on infant iron deficiency and anemia risk is critical to successfully preventing and treating anemia among young children (33). Improved survival in early infancy has been shown among neonates and infants of mothers who received iron supplementation in multiple LMICs, often in a dose-responsive fashion (34, 35). Given IDA and AI require different approaches to treatment, clarifying the extent to which maternal anemia type and iron status affects infant anemia risk is therefore critical.

We found that infants were more likely to be anemic if their mothers had IDA versus NIDA during pregnancy, despite the fact that all women were provided with pre-natal vitamins with iron. This association, however, is lost by twelve months of age, and overall rates of anemia in this infant population increase. It is possible that many of the infants at 12 months of age have pre-anemic iron deficiency, given demonstrated decreases in ferritin from 6 to 12 months of age without significant increase in anemia risk. Although the maternal-fetal dyad is adapted to maximize fetal iron uptake during gestation (36), infant iron stores may wane, particularly when breast milk is iron poor, by six months of age (37). The relatively high rates of iron deficiency in this pediatric population at both six and twelve months may also be reflective of the relative poor nutritional quality of complementary foods introduced during infancy in this population. Alterations in iron metabolism during pregnancy as seen in AI did not impact newborn iron status or anemia risk, suggesting this mechanism does not significantly impact iron-transfer (12).

Interestingly, we found that maternal hepcidin, but not newborn hepcidin, was related to infant iron status and hemoglobin. In addition, low maternal hepcidin was associated with a significant worsening of infant iron status from six to twelve months. Hepcidin is a key regulator of iron bio-availability, and is up-regulated in response to inflammatory cytokines, particularly IL-6, and down-regulated in the context of iron deficiency (14–16). It is likely that the relationship between low maternal hepcidin and increased risk for both infant iron deficiency and anemia is due to the fact that hepcidin is a marker for women with more significant iron deficiency, who may be less able to transfer sufficient iron to the fetus *in utero* and in breast milk to maintain stores at 12 months of age. This is further supported by the worsening iron status and hemoglobin from six to twelve months of age only among

infants whose mothers had the lowest hepcidin levels. This may also be partly explained by a shared maternal-infant environment with iron-poor solid foods as the infant transitions to the addition of complementary foods.

In addition, we found a high prevalence of NIDA at both six and twelve months of age. Hematologic and inflammatory biomarker dynamics may reflect the etiology of anemia among infants. The MCV, elevated in the context of megaloblastic anemias due to B12 and folate deficiency was only elevated in 0.3% of infants at six and twelve months of age, suggesting these micronutrient deficiencies were not a significant cause of NIDA. IL-6 concentrations at six and twelve months were higher among infants with NIDA, compared to IDA, and hepcidin was significantly higher among infants with NIDA compared to IDA at six and twelve months. Together, these findings suggest that NIDA in most of these infants is driven by AI.

Limitations to this study include a lack of standardized definitions to define the etiology of anemia during pregnancy and infancy. We used hemoglobin and ferritin to define maternal anemia types based on bone marrow studies (28) and the WHO definition for infant anemia type (27). Unfortunately, we did not capture timing of the introduction of solid foods, thus limiting our ability to make conclusive statements regarding the impact of complementary foods on iron status. In addition, we did not assess infants for hemoglobinopathies which may also cause NIDA, such that we cannot determine the contribution of hemoglobinopathies or other less common causes of NIDA. Finally, we do not have a measure of maternal iron status or hemoglobin at the time of delivery. Given the importance of third trimester iron transfer, a more proximate measure of maternal iron status peri-natally might have changed maternal classification with respect to iron status and anemia type. Serum iron was also not measured in this study, although quantitation of sTfR and ferritin have been shown to be excellent markers for iron status.

Iron status during infancy is likely influenced by both newborn iron endowment and the availability of iron during infancy in breast milk and complementary foods (38). Indeed, other groups have shown that exclusive breastfeeding improves iron status of infants in resource poor settings (39). Our finding that maternal IDA continues to influence infant iron deficiency and anemia during infancy supports the importance of maternal iron nutriture and the need for improved pre-conceptual maternal iron status, better compliance with pre-natal vitamins with iron, as well as continued provision of multi-vitamins with iron post-natally. The high prevalence of AI among infants highlights the high infection and inflammatory burden experienced by these infants and its contribution to anemia.

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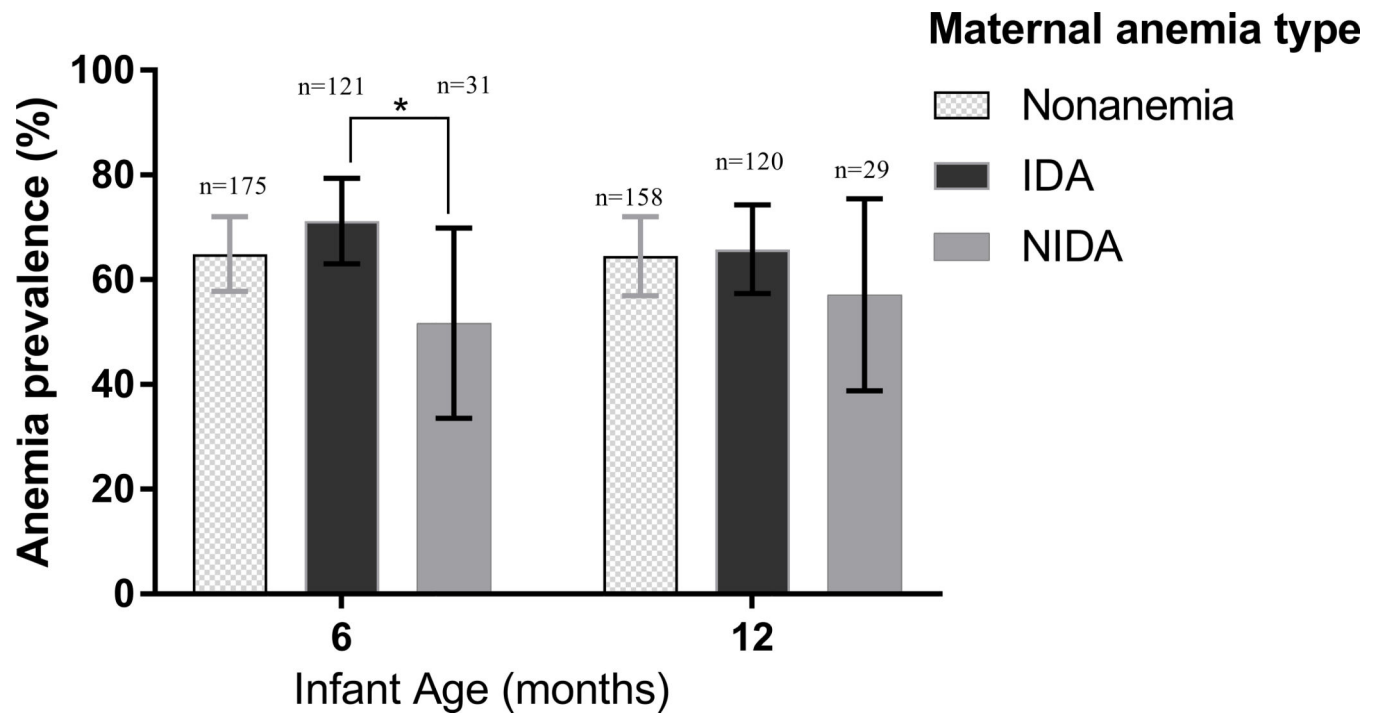
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**Please enter five bullet points answering the following questions**

1. What is the key message of your article?
2. What does it add to the existing literature?
3. What is the impact?
  - Maternal iron deficiency anemia during gestation was associated with increased risk of infant anemia.
  - Maternal non-iron deficiency anemia, mostly due to anemia of inflammation, did not impact infant iron status nor hemoglobin levels.
  - Infants of mothers with low levels of circulating hepcidin, likely a marker for iron deficiency, had lower iron and hemoglobin levels.
  - Newborn hepcidin was not associated with infant iron status nor hemoglobin.
  - Our findings further support the importance of maternal iron nutriture and the need for improved pre- conceptual maternal iron to impact infant iron acquisition both trans-placentally and through breast milk.

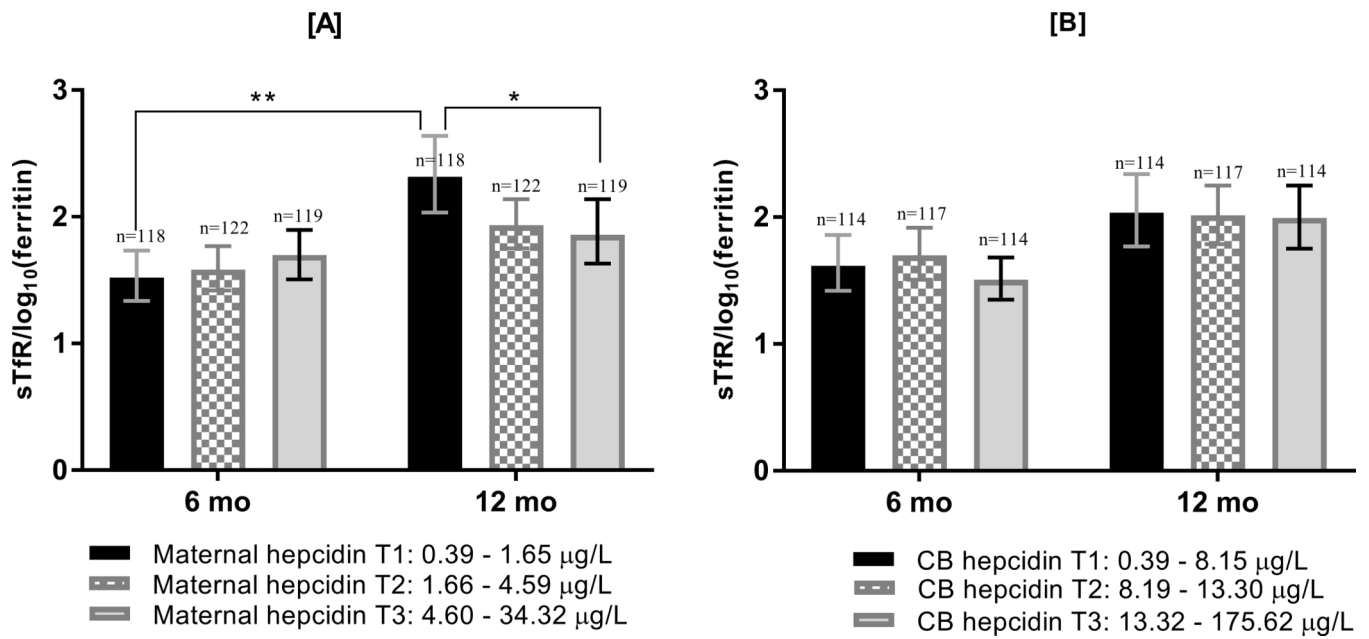


**Figure 1.**  
Study participants.



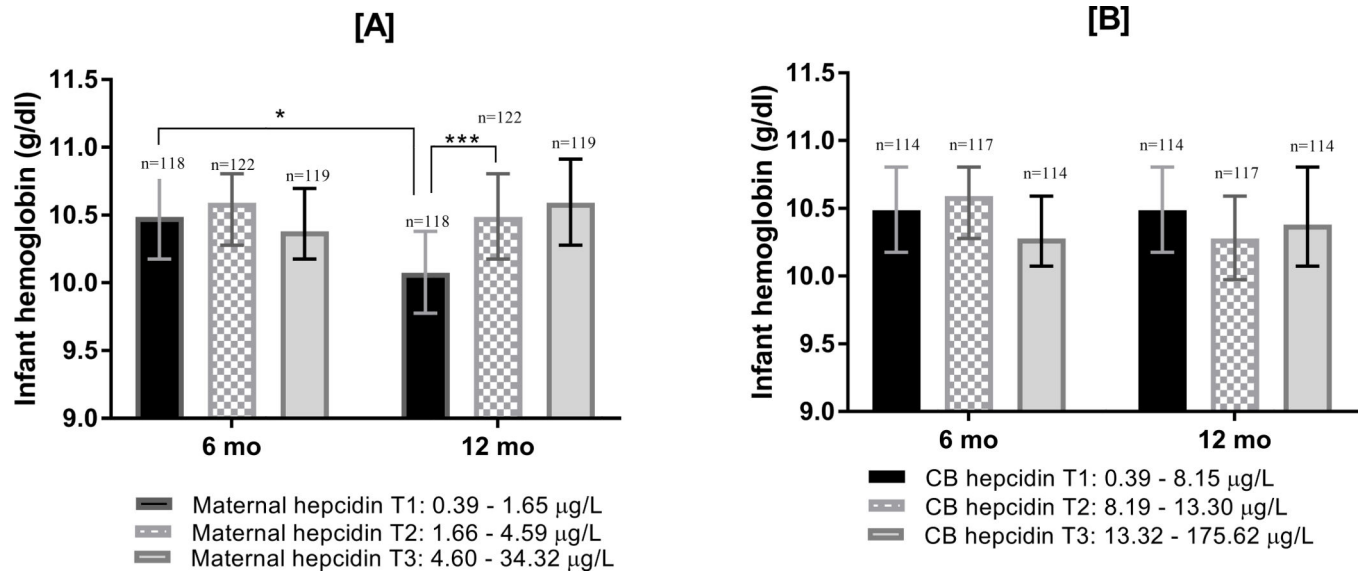
**Figure 2. Prevalence of infants' anemia at six months is highest in infants born to mothers who had iron deficiency anemia during gestation.**

Error bars represent 95% confidence intervals. \*  $P < 0.05$ .



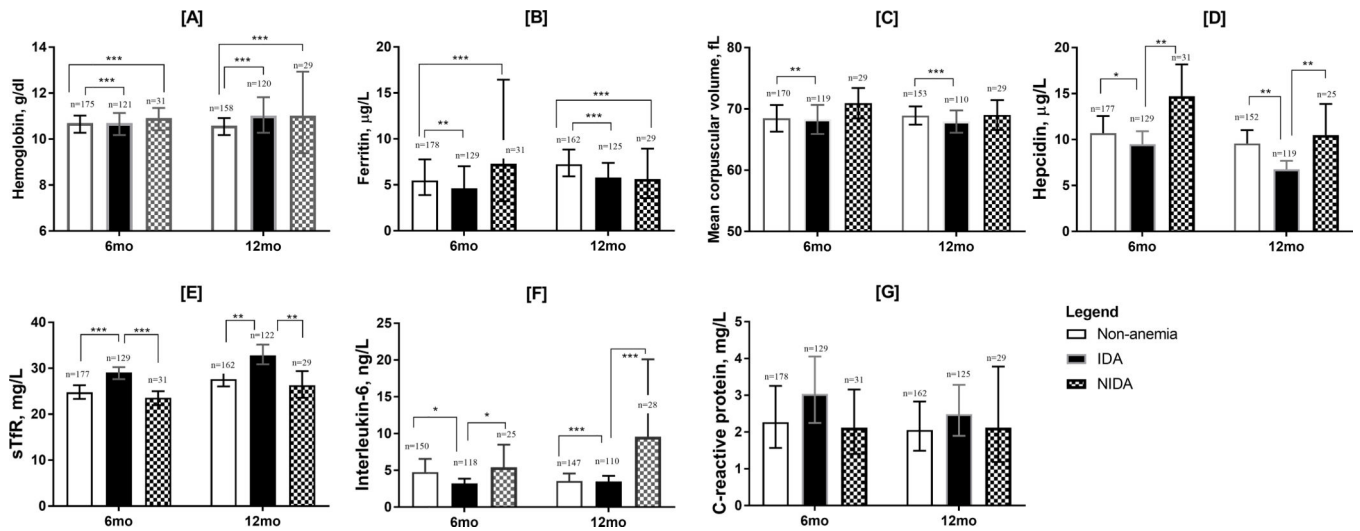
**Figure 3. Maternal hepcidin (a) but not newborn hepcidin (b) is related to infant iron status at twelve months of age.**

a) Infants of mothers with the lowest hepcidin during pregnancy displayed the highest sTfR-ferritin index, and thus the lowest iron status, at twelve months of age. Infants born to those mothers with hepcidin levels in the lowest tertile had significantly higher sTfR-F index values at twelve months compared to six months of age. b) Fetal hepcidin levels were not related to infant iron status at either six or twelve months of age. CB: cord blood, error bars represent the 95% confidence intervals. \*\*  $P < 0.01$ .



**Figure 4. Maternal circulating hepcidin (a) but not fetal hepcidin (b) is related to infant hemoglobin concentration at twelve months of age.**

a) Infants from mothers with the lowest hepcidin during pregnancy displayed the lowest hemoglobin levels at twelve months of age. Infants born to those mothers with hepcidin levels in the lowest tertile had significantly lower hemoglobin values at twelve months compared to six months of age. b) Newborn hepcidin levels were not related to infant hemoglobin at either six or twelve months of age. CB: cord blood, error bars represent the 95% confidence intervals. \*  $P < 0.05$ . \*\*\*  $P < 0.01$ .



**Figure 5. Hematologic and inflammatory biomarker concentrations are related to infants’ anemia type at six (a-d) and twelve (e-f) months of age.**

IL-6 and hepcidin are lower and sTfR is higher in six month old infants with IDA compared to others. This trend continues at twelve months of age. Further, IL-6 and hepcidin are higher and sTfR lower in six month old infants with NIDA compared to non-anemic infants at six and twelve months, respectively. Error bars represent 95% confidence intervals. Asterisk symbols, \*, \*\* and \*\*\* represent  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively and compare the concentration of each biomarker by anemia type.



**Table 1.**

Characteristics of the study population, stratified by maternal treatment status

	Praziquantel (n=179)	Placebo (n=180)	Total (n=359)
Maternal, 32 weeks' gestation, n=359			
Non-anemic, n (%)	99 (55)	92 (51)	191 (53)
IDA, n (%)	62 (35)	74 (41)	136 (38)
NIDA, n (%)	18 (10)	14 (8)	32 (9)
Hemoglobin, g/dl, median (IQR)	11 (10.2, 11.8)	11 (10, 11.9)	11 (10.1, 11.8)
Ferritin, µg/L, median (IQR)	14.7 (2.8, 28.4)	10.5 (2.8, 21.8)	12.1 (2.8, 25.3)
Hepcidin, µg/L, median (IQR)	3.4 (1.1, 5.5)	2.6 (1.0, 5.7)	3.0 (1.1, 5.6)
MCV, fL, median (IQR)	88 (84, 92)	88 (84, 92)	88 (84, 92)
MCV, >100fL, %	1 (1)	4 (2)	5 (1)
Infant, birth, n=359			
Male, n (%)	97 (54)	94 (52)	191 (53)
Birth weight (g); mean (95% CI)	2.85 (2.78 – 2.91)	2.85 (2.79 – 2.91)	2.85 (2.81 – 2.89)
Gestational Age (weeks); median (IQR)	39 (38 – 39)	38.7 (37.9 – 39.4)	38.7 (38.0, 39.4)
Infant, six months of age, n=333			
Breastfed/mixed, n (%)	152 (93)	139 (89)	323 (98)
Exclusively breastfed, n (%)	152 (93)	152 (91)	304 (92)
Non-anemic, n (%)	48 (30)	61 (38)	109 (34)
IDA, n (%)	91 (57)	75 (47)	166 (52)
NIDA, n (%)	20 (13)	24 (15)	44 (14)
Hemoglobin, g/dl, median (IQR)	10.4 (9.5, 11.2)	10.4 (9.6, 11.6)	10.4 (9.5, 11.3)
Ferritin, µg/L, median (IQR)	9.5 (1.6, 24.5)	10.0 (2.5, 28.5)	9.5 (1.8, 26.8)
Hepcidin, µg/L, median (IQR)	11.0 (5.6, 21.8)	10.6 (6.1, 19)	10.8 (6.0, 21.3)
sTfR, mg/L, median (IQR)	1.9 (1.6, 2.3)	1.8 (1.5, 2.3)	1.9 (1.6, 2.3)
CRP, mg/L median (IQR)	3.1 (0.9, 8.6)	2.1 (0.8, 6.9)	2.6 (0.8, 7.3)
sTfR:log(ferritin), median (IQR)	1.6 (1.2, 2.2)	1.5 (1.0, 2.2)	1.5 (1.1, 2.2)
MCV, fL, median	71 (67, 75)	71 (66, 75)	71 (66, 75)
MCV, >100fL, %	1 (1)	0 (0)	1 (0.3)
Infant, twelve months of age, n=312			
Breastfed/mixed, n (%)	141 (96)	148 (95)	289 (95)
Exclusively breastfed, n (%)	129 (88)	139 (89)	268 (88)
Non-anemic, n (%)	59 (40)	51 (33)	110 (36)
IDA, n (%)	80 (54)	95 (61)	175 (57)
NIDA, n (%)	10 (7)	10 (6)	20 (7)
Hemoglobin, g/dl, median (IQR)	10.5 (9.6, 11.5)	10.4 (9.6, 11.4)	10.4 (9.6, 11.4)
Ferritin, µg/L, median (IQR)	5.9 (1.6, 15.2)	5.7 (0.7, 16.1)	5.9 (1.1, 16.1)
Hepcidin, µg/L, median (IQR)	7.5 (5.2, 16.3)	6.9 (4.9, 11.3)	7.3 (4.9, 13.1)
sTfR, mg/L median (IQR)	2.1 (1.7, 2.7)	2.1 (1.8, 2.8)	2.1 (1.7, 2.7)
CRP, mg/L, median (IQR)	3.1 (0.8, 8.0)	1.5 (0.5, 6.1)	2.5 (0.7, 6.5)
sTfR:log(ferritin), median (IQR)	2.0 (1.4, 2.9)	1.9 (1.4, 3.0)	1.9 (1.4, 2.9)

	<b>Praziquantel (n=179)</b>	<b>Placebo (n=180)</b>	<b>Total (n=359)</b>
MCV, fL, median	69 (64, 75)	70 (64, 74)	70 (64, 74)
MCV, >100fL, %	0 (0)	0 (0)	0 (0)

The influence of Praziquantel on population characteristics was assessed using chi-square tests for categorical variables and Wilcoxon tests for continuous variables. None of the characteristics evaluated were significantly different by treatment status.

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**Table 2.**

Characteristics of mothers by anemia type at 32 wk of gestation

	<b>IDA (n=136)</b>	<b>NIDA (n=32)</b>	<b>No anemia (n=191)</b>
Maternal, 32 weeks' gestation			
Hemoglobin, g/dL	10.0 ± 0.1	10.1 ± 0.1	11.9 ± 0.1
Serum ferritin, µg/L	5.7 (0.7 – 16.4)	46.7 (37.7 – 72.3)	13.9 (11.6 – 15.5)
Serum hepcidin, µg/L	2.5 (0.9 – 4.9)	6.2 (3.4 – 10.5)	2.8 (1.1 – 5.6)
Serum CRP, mg/L	3.9 (2.0 – 8.1)	7.3 (2.2 – 21.7)	4.3 (1.9 – 8.4)
MCV, fL	86.5 (81.0 – 90.0)	89.5 (86.0 – 92.0)	89.0 (86.0 – 93.0)

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