Iron Homeostasis in Mother and Child during Placental Malaria Infection

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Abstract. In malaria-endemic areas, iron deficiency and placental *Plasmodium falciparum* infection commonly coexist. In primigravidae and their newborns, hepcidin and other iron parameters were evaluated in groups and classified according to placental *P. falciparum* and maternal anemia status. Mothers had relatively high hepcidin levels considering their low iron status. In cord blood, levels of hepcidin, hemoglobin, and other iron parameters were also similar for groups. We conclude that maternal hepcidin is not significantly altered as a function of placental infection and/or anemia. Importantly, fetal hemoglobin and iron status were also unaffected, regardless of the presence of placental infection or maternal anemia.

INTRODUCTION

Placental malaria is a significant public health problem, because it is consistently associated with maternal morbidity and poor birth outcome.^{1–3} Iron deficiency is also highly prevalent in malaria-endemic areas, and apart from *Plasmodium falciparum* infection, it is another well-known risk factor for maternal anemia and perinatal complications.^{4,5} Therefore, iron supplementation is considered a logical public health intervention. However, not all studies of iron supplementation in malaria-endemic regions have shown favorable results.^{6,7} The iron regulatory hormone hepcidin has been reported to inhibit the intestinal iron uptake and the release of iron from macrophages by degrading the sole cellular iron export protein ferroportin.^{8,9} Inflammatory stimuli, including those associated with *P. falciparum* infection, increase hepcidin production, resulting in low plasma iron levels and reduced bone marrow iron supply.^{10–12}

During blood-stage malaria infection, *P. falciparum*-infected erythrocytes can bind chondroitin sulphate A on syncytiotrophoblasts, leading to their accumulation in placental intervillous spaces. Syncytiotrophoblasts express iron-regulatory proteins, including ferroportin, which is responsible for iron release into the fetal circulation.¹³ In addition, animal studies revealed that hepcidin and iron levels in the fetal liver may also regulate maternal–fetal iron transport.^{14–16}

We aimed to study maternal and fetal hepcidin and other iron parameters to improve our understanding of human placental iron transport. Therefore, we retrospectively assessed plasma concentrations of these indices in primigravidae, as a function of placental *P. falciparum* infection and maternal anemia, and in corresponding cord-blood samples.

MATERIALS AND METHODS

Study population and design. Archived samples from a malaria in pregnancy study, carried out in an area of Gabon characterized by stable meso- to hyperendemic malaria transmission, were used for this study.¹⁷ Paired maternal and umbilical cord-blood samples were obtained at time

of parturition from pregnant women attending the Albert Schweitzer Hospital in Lambarene, Gabon from July 2000 to February 2004. Medical records of the mothers were examined to determine whether malaria episodes were detected and appropriately treated during pregnancy. Age at delivery, number of previous pregnancies, and the child's birth weight and length were recorded. Antenatal universal iron supplementation during pregnancy was local practice (following the WHO recommendation of 60 mg iron daily for all pregnant women); however, therapy compliance was not recorded.¹⁸

Samples from primigravidae without peripheral parasitemia (determined by microscopy) who gave birth to singleton live births and who had no signs or symptoms of systemic infection were included. Primigravid women were selected for this study, because they are more susceptible to *P. falciparum* infection than multigravidae and have a higher risk of adverse outcome.¹ Further sample selection was performed on the basis of placental infection status, defined as the presence of *P. falciparum* parasites in giemsa-stained placental blood impression smears, and on the presence or absence of maternal anemia, defined as a hemoglobin concentration below 10 g/dL. This selection procedure resulted in four groups: (1) no placental malaria and no anemia, (2) no placental malaria and anemia, (3) placental malaria and no anemia, and (4) placental malaria and anemia. Sample size was determined by the availability of paired maternal and umbilical cord samples that matched the selection criteria.

Informed consent, including approval for future measurements, was obtained from mothers before inclusion. Ethical clearance for the study was given by the Ethics Committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné.

Laboratory analysis. Giemsa-stained thick and thin smears of both maternal and cord-blood and impression smears of placental blood were assessed microscopically for the presence of *P. falciparum* parasites. Hemoglobin concentrations were determined by a standard hematology analyzer. Blood samples were stored at –80°C for further analysis. Plasma concentrations of iron, total iron-binding capacity (TIBC), ferritin, soluble transferrin receptor (sTfR), and C-reactive protein (CRP) were determined as described previously.¹⁰ Plasma hepcidin-25 measurements were performed in 2009 by a combination of weak cation-exchange chromatography and time-of-flight mass spectrometry (TOF MS). An internal standard (synthetic

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hepcidin-24; Peptide International Inc., Louisville, KY) was used for quantification (www.hepcidinanalysis.com).¹⁹ Peptide spectra were generated on a Microflex LT matrix-enhanced laser desorption/ionization TOF MS platform (Bruker Daltonics, Bremen, Germany). Plasma hepcidin-25 concentrations were expressed as nmol/L. The lower limit of detection of this method was 0.5 nmol/L; average coefficients of variation were 2.7% (intrarun) and 6.5% (interrun). The median reference level of plasma hepcidin-25 is 4.2 nmol/L, with a range of 0.5–13.9 nmol/L.²⁰

The reliability of the archived samples for the present study was assessed by mass profiling of eight paired cord and maternal samples. We found the peptide spectra to be similar to that of freshly frozen sera. More specifically, degradation products as observed in sera that had been inadequately stored for a prolonged period of time were absent throughout the peptide profile (i.e., at *m/z* 5,000–6,000 and *m/z* 7,000–8,500), providing confidence in the good quality of the samples.

Statistical analysis. Data are expressed as median values with interquartile range (IQR). Differences among groups were tested by Kruskal-Wallis tests. Differences between two groups were assessed by Mann-Whitney tests. Missing values were assumed to be at random. CRP concentrations < 5 mg/L were included in the analyses as CRP concentrations of 5 mg/L. Significance was defined as two-tailed ($P < 0.05$). The statistical software packages SPSS 16.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 4.00 (GraphPad Software Inc., San Diego, CA) were used for all data analyses.

RESULTS

Study group characteristics. A total number of 69 primigravidae were stratified in four groups according to placental *P. falciparum* infection status and hemoglobin level. The characteristics of the study population are shown in Table 1. Hemoglobin concentrations were 12.8 g/dL (IQR = 11.6–13.4) and 8.2 g/dL (IQR = 7.5–8.8) in non-anemic and anemic mothers, respectively. There were no differences in the four study groups in either white blood count or platelet numbers. CRP concentrations were elevated in most women, with the highest values in those with placental infections. The percentage

of low birth weight (i.e., a birth weight below 2.5 kg) was relatively high in all subgroups (range = 18.8–35.7%). Despite a trend for lower birth weight in the groups with anemia, there was no significant difference in birth weight among the groups.

Maternal hepcidin and iron parameters. Maternal plasma hepcidin concentrations were similar across the groups ($P = 0.70$; Figure 1). Moreover, no significant differences in plasma iron concentration, transferrin saturation, TIBC, and ferritin concentration were found across the groups (Table 1). However, serum ferritin concentrations were higher in the group of women with placenta malaria as a whole compared with women without placenta malaria. Marked differences were also observed for sTfR concentrations, and the highest and lowest values were in women without placental infection and with and without anemia, respectively.

Cord-blood parameters. Cord parameters were independent of hemoglobin and malaria status of the mother (Table 2). Concentrations of hemoglobin, hepcidin, and other iron status indices were similar across the four groups. CRP concentrations were not elevated in all groups, and there was neither anemia nor signs of iron deficiency (i.e., hypoferrremia, high TIBC, or low plasma ferritin concentrations).

DISCUSSION

In the current study, the effects of placental *P. falciparum* infection and anemia on hepcidin and iron parameters in mothers and their offspring were assessed, and some important findings are hereby reported. First, we show that primigravid women with or without placental infection and with or without anemia have relatively low ferritin concentrations, low plasma iron concentrations, and low transferrin saturation as well as high TIBC concentrations, suggesting some degree of iron deficiency. We refrained from assessing iron deficiency by previously used formulas (e.g., the sTfR to log ferritin ratio), because they have not been standardized or validated for our study population.²¹ In pregnancy, iron deficiency resulting from a physiologic increase in erythroid iron demand is indeed a common finding. Moreover, nutritional iron deficiency is also highly prevalent in malaria-endemic regions, even despite implementation of active iron supplementation programs.²²

TABLE 1
Clinical data and iron status indices in pregnant women

Characteristic	Placental malaria –		Placental malaria +		<i>P</i> †
	Anemia – (<i>N</i> = 21)*	Anemia + (<i>N</i> = 18)	Anemia – (<i>N</i> = 16)	Anemia + (<i>N</i> = 14)	
Age (years)	18.0 (17.0–19.0)	17.0 (16.0–19.3)	17.0 (16.0–18.0)	17.0 (16.8–19.3)	0.43
Hemoglobin (g/dL)	13.2 (11.9–13.8)	8.2 (7.6–8.5)	12.2 (11.2–13.2)‡	8.3 (6.6–9.3)	0.00
Cell counts (10 ⁹ /L)					
WBC	11.0 (9.2–18.0)	10.5 (9.2–13.0)	11.0 (9.2–13.8)	10.0 (7.6–12.0)	0.72
Platelets	185 (142–245)	207 (130–163)	152 (109–191)	190 (126–259)	0.21
CRP (mg/L)	12.0 (5.0–31.0)	17.5 (6.0–43.3)	25.0 (11.0–47.3)‡	32.0 (17.8–67.5)§	0.43
CRP < 10; <i>n</i> (%)	10 (47.6)	8 (44.4)‡	4 (25.0)§	2 (14.3)‡	0.005
Iron parameters					
Hepcidin (nmol/L)	4.2 (1.6–8.0)	2.8 (0.5–13.1)	4.2 (2.0–8.1)	3.2 (0.9–5.0)	0.70
Iron (μmol/L)	9.0 (8.0–12.5)	8.0 (4.8–15.3)	8.0 (6.0–12.0)	7.5 (4.8–13.8)	0.55
TIBC (μmol/L)	80.0 (73.5–89.5)	83.0 (73.3–102)	86.0 (73.0–104)	80.0 (59.5–85.3)	0.25
TS (%)	11.2 (7.9–18.3)	9.4 (5.9–20.5)	8.1 (5.9–16.5)	9.6 (6.6–19.4)	0.55
Ferritin (μg/L)	30.0 (16.0–45.0)	29.0 (11.0–147)	44.0 (19.3–121)	86.0 (20.5–220)	0.37
sTfR (mg/L)	1.4 (1.2–2.1)	2.3 (1.4–3.6)‡	2.0 (1.5–2.4)	1.6 (1.5–2.3)	0.09

WBC = white blood cell count; CRP = C-reactive protein; TIBC = total iron-binding capacity; TS = transferrin saturation; sTfR = soluble transferrin receptor.

*Data are presented as median with interquartile range. Anemia was defined as hemoglobin concentration below 10 g/dL.

†Values represent differences among the four groups by Kruskal-Wallis test.

‡ $P < 0.05$ compared with the group of mothers without placental malaria infection and without anemia (by Mann-Whitney test).

§ $P < 0.01$ compared with the group of mothers without placental malaria infection and without anemia (by Mann-Whitney test).

FIGURE 1. Plasma concentrations of hepcidin stratified for anemia and placental infection status. Data are depicted for maternal and cord-blood; the line represents the median value. □ = no placental malaria and no anemia; ○ = no placental malaria with anemia; ● = placental malaria and no anemia; ▼ = placental malaria with anemia.

Second, we found that maternal plasma hepcidin concentrations were not altered by placental infection and that hepcidin concentrations were relatively high considering the presence of iron deficiency. The expression of hepcidin is principally regulated by a combination of factors: hypoxia, increased erythropoietic activity, and iron deficiency suppress hepcidin expression, whereas inflammatory cytokines (especially interleukin-6) stimulate the production of hepcidin.²³ The relatively high hepcidin concentrations in the group of anemic mothers without placental malaria are, therefore, remarkable. The slightly elevated CRP values in some women in this group may be explained by the normal increase in CRP measurements in the third trimester and at parturition, and mild inflammation could also have contributed to these relatively high hepcidin levels.²⁴ It should be noted that only subjects with a parasite-negative peripheral blood smear were included in this study to diminish the potentially confounding effects of higher-grade systemic (inflammatory) infection. Parasitemia in pregnant women is associated with higher inflammatory markers together with higher urinary hepcidin concentrations.^{25,26} Indeed, very high hepcidin concentrations were measured in African children with clinical malaria, whereas slightly raised hepcidin levels were found in those with lower density parasitemia.^{10,11}

Third, we found a trend for lower sTfR concentrations in anemic women with placental infection compared with

mothers without infection. Because sTfR is a marker for erythropoietic activity, this may suggest that placental parasitemia is associated with reduced erythroid progenitor proliferation.²¹

The functional consequences of these findings are uncertain, although one might speculate that the effectiveness of iron supplementation in the study participants may be reduced by the relatively high hepcidin concentrations. However, iron retained in the erythrocyte precursor by hepcidin degradation of ferroportin may well compensate for this decreased iron supply and/or at least partly, protect against anemia.²⁷

Finally, we found no effect of placental infection or maternal anemia on cord-blood concentrations of hemoglobin, CRP, hepcidin, and other iron parameters. This is in agreement with a study conducted in Malawi that also found no significant effect of peripheral or placental *P. falciparum* infection on cord-blood concentrations of hemoglobin, CRP, and sTfR and only a slightly effect on ferritin.²⁸ Moreover, among Nigerian women, placental infection was strongly associated with maternal anemia but not with the mean hematocrit in their newborns.²⁹ Interestingly, although the clinical implications are still to be elucidated, placental hepcidin mRNA expression was increased in *P. falciparum*-infected placentas and was negatively correlated with birth weight, but it was not associated with maternal anemia.³⁰ In the present study, the relationship between low birth weight and placental infection was absent, which may be attributed to the low birth weights of newborns of non-diseased mothers.

Our study is limited by its retrospective character, the absence of long-term data, the lack of information on premature delivery, its relatively small sample size, and the usage of archived samples. Concerning the latter, both the normal MS profiles of all serum samples without signs of degradation products as well as the broad range in concentrations of the laboratory markers observed provided confidence in the reliability of the data obtained.

The study is, however, unique in the selection of primigravidae and cord-blood samples with either placental *P. falciparum* infection, anemia, or both in combination with the exclusion of peripheral parasitemia.

TABLE 2
Clinical data and cord-blood iron status indices in newborns

Characteristic	Placental malaria –		Placental malaria +		P†
	Anemia – (N = 21)*	Anemia + (N = 18)	Anemia – (N = 16)	Anemia + (N = 14)	
Birth weight (kg)	2.8 (2.5–3.0)	2.6 (2.4–2.8)	2.9 (2.6–3.2)	2.7 (2.1–3.0)	0.32
LBW‡; n (%)	4 (19.0)	5 (27.8)	3 (18.8)	5 (35.7)§	0.65
Birth length (cm)	50.0 (48.5–50.0)	49.0 (48.0–50.0)	50.0 (49.0–50.0)	48.0 (46.5–49.3)¶	0.09
Hemoglobin (g/dL)	15.1 (13.8–16.0)	14.2 (13.0–15.2)	13.9 (12.5–14.7)¶	14.6 (12.9–15.6)	0.21
Cell counts (10 ⁹ /L)					
WBC	12.8 (10.0–15.1)	11.6 (8.3–14.2)	12.4 (9.6–14.4)	10.1 (8.4–13.3)	0.94
Platelets	299 (246–364)	296 (202–367)	265 (218–319)	273 (231–301)	0.58
CRP < 5 mg/L; n (%)	20 (95.2)	16 (94.1)	15 (93.7)	14 (100.0)	0.79
Iron parameters					
Hepcidin (nmol/L)	4.5 (2.8–6.8)	5.5 (2.4–9.5)	3.7 (3.0–6.9)	4.4 (1.8–6.5)	0.33
Iron (μmol/L)	22.0 (19.5–28.0)	20.0 (14.0–27.0)	19.0 (18.0–21.0)¶	17.0 (13.0–22.0)¶	0.14
TIBC (μmol/L)	41.0 (37.0–46.5)	43.0 (36.0–52.5)	46.0 (35.8–47.8)	33.5 (59.5–85.3)	0.39
TS (%)	57.1 (45.5–70.9)	45.5 (32.7–59.4)	42.9 (35.1–60.0)¶	61.0 (39.0–69.6)	0.64
Ferritin (μg/L)	122 (97.0–290)	146 (84.0–217)	161 (131–224)	137 (89.3–193)	0.72
sTfR (mg/L)	2.3 (1.9–2.8)	2.3 (1.9–3.7)	2.4 (1.8–2.8)	1.8 (1.6–3.1)	0.44

LBW = low birth weight; WBC = white blood cell count; CRP = C-reactive protein; TIBC = total iron-binding capacity; TS = transferrin saturation; sTfR = soluble transferrin receptor.

* Data are presented as median with interquartile range. Anemia was defined as hemoglobin concentration below 10 g/dL.

† Values represent differences among the four groups by Kruskal-Wallis test.

‡ LBW was defined as a weight below 2.5 kg.

§ P < 0.05 compared with the group of mothers with anemia but without placental malaria (by Mann-Whitney test).

¶ P < 0.01 compared with the group of mothers without placental malaria infection and without anemia (by Mann-Whitney test).

In conclusion, neither placental infection nor maternal anemia was related to maternal and cord hepcidin concentrations and iron status. Our data also suggest that iron metabolism is biased to maintaining the fetal iron supply, even in the presence of maternal anemia and iron deficiency. Here, the fetus seems to be the winner in the maternal–fetal conflict, deriving its nutrients independently from maternal iron and placental malaria infection.³¹ Whether the effectiveness of iron supplementation of pregnant women in the third trimester is impaired by the moderately elevated hepcidin concentrations needs to be determined in future studies.

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