Malaria and Iron Load at the First Antenatal Visit in the Rural South Kivu, Democratic Republic of the Congo: Is Iron Supplementation Safe or Could It Be Harmful

Esto Bahizire,[1,2,3]* Umberto D'Alessandro,[4,5] Michèle Dramaix,[1] Nicolas Dauby,[6] Fabrice Bahizire,[3] Kanigula Mubagwa,[7] and Philippe Donnen[8]

[1]*Center of Research in Epidemiology, Biostatistics and Clinical Research, Université Libre de Bruxelles, Brussels, Belgium;* [2]*Centre de Recherche en Sciences Naturelles de Lwiro, Bukavu, DR Congo;* [3]*Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya;* [4]*Medical Research Council Unit The Gambia, Fajara, The Gambia;* [5]*London School of Hygiene and Tropical Medicine, London, United Kingdom;* [6]*Department of Infectious Diseases, Centre Hospitalier Universitaire Saint-Pierre, Brussels, Belgium;* [7]*Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium;* [8]*Center of Research in Health Policy and Systems-International Health, Université Libre de Bruxelles, Brussels, Belgium*

* Address correspondence to Esto Bahizire, Center of Research in Epidemiology, Biostatistics and Clinical Research, Université Libre de Bruxelles, Brussels, Belgium, Centre de Recherche en Sciences Naturelles de Lwiro, Bukavu, DR Congo. E-mail: ebahizir@ulb.ac.be

Abstract.

We investigated the relationship between malaria infection and iron status in 531 pregnant women in South Kivu, Democratic Republic of the Congo. Sociodemographic data, information on morbidity, and clinical data were collected. A blood sample was collected at the first antenatal visit to diagnose malaria and measure serum ferritin (SF), soluble transferrin receptor, C-reactive protein, and α1-acid-glycoprotein. Malaria prevalence was 7.5%. Median (interquartile range) SF (adjusted for inflammation) was significantly higher in malaria-infected (82.9 μg/L [56.3–130.4]) than in non-infected (39.8 μg/L [23.6–60.8]) women ($P < 0.001$). Similarly, estimated mean body iron stores was higher in malaria-infected women ($P < 0.001$). Malaria was significantly and independently associated with high levels of SF. Efforts to improve malaria prevention while correcting iron deficiency and anemia during pregnancy are warranted.

In sub-Saharan Africa, pregnancy-associated malaria (PAM) and anemia are issues of public health concern.[1,2] The etiology of anemia is multifactorial, but common causes include nutritional deficiencies (e.g., iron deficiency, ID), infections such as malaria and soil transmitted helminths, hemoglobinopathies, and other genetic disorders.[2] According to World Health Organization (WHO), about 50% of all anemia cases may be due to ID. Hence, daily iron supplementation (IS) during pregnancy has been recommended to reduce the burden of anemia.[3] This policy is being implemented in most sub-Saharan African countries, including the Democratic Republic of Congo (DRC). However, recent data from South Kivu, in the eastern part of DRC, show a lower-than-expected prevalence of ID[4,5] and further raise questions on the possible negative outcome of IS on malaria. In South Kivu, prevalence of ID was 5.4% among nonpregnant women[5] and 7.6% among pregnant women.[6] Yet, in this same province, malaria remains common among pregnant women.[7]

There might be an interaction between iron load and malaria, but it remains unclear whether high iron loads predispose to malaria and whether iron depletion is protective.

Studies in animal models have reported that placental iron overload in mice infected by *Plasmodium berghei* was associated with poor outcomes.[8] In humans, among Beninese women, high iron levels increased the risk of PAM, including high *Plasmodium falciparum* density.[9] Similarly, IS in Tanzanian preschool children was associated with increased morbidity and mortality due to infectious diseases, including malaria.[10] In another study, ID among pregnant women was reported to protect against placental malaria.[11] A meta-analysis concluded that IS does not increase the risk of *falciparum* malaria, although ID had a protective effect.[12]

The present report is based on a study carried out to evaluate the relationship between iron load and PAM at the first antenatal visit before starting IS and intermittent preventive treatment (IPT) in South Kivu.

This is a secondary analysis of data collected during a cross-sectional study conducted between December 2013 and March 2014 among pregnant women in the second trimester attending their first antenatal visit. The study was conducted in Miti-Murhesa, a rural health zone from South Kivu. Sampling methods are published elsewhere.[6]

A thick blood smear stained with 3% Giemsa for 30 minutes was used to diagnose malaria and a thin blood smear for species identification, according to WHO guidelines. Serum ferritin (SF), soluble transferrin receptor (sTfR), C-reactive protein (CRP), and α1-acid-glycoprotein (AGP) were determined by a sandwich enzyme-linked immunosorbent assay technique on a venous blood sample.[13]

Ferritin is an acute phase protein known to increase during infection/inflammation. Therefore, SF was adjusted according to an individual's inflammation status, defined using CRP > 5 mg/L and/or AGP > 1 g/L as cutoffs. Adjustment was done according to correction factors suggested by Thurnham et al.[14]: ferritin concentration multiplied by 0.77, 0.53, and 0.75 during incubation (only CRP increased), early convalescence (both CRP and AGP increased), and late convalescence (only AGP increased), respectively. ID was defined as adjusted SF < 15 μg/L. Body iron store (BIS, mg/kg) was calculated by using the Cook's formula[15]: $BIS = -[\log_{10}(sTfR * 1{,}000/ferritin) - 2.8229]/0.1207$. Based on BIS, ID was defined as BIS < 0 mg/kg.

Anemia was defined as altitude-adjusted hemoglobin (Hb) < 110 g/L and severe anemia as Hb < 70 g/L.

Statistical analyses were performed with STATA for Mac, version 12.1 (StataCorp, College Station, TX). The Mann–Whitney Wilcoxon and the T tests were used to compare medians or means, respectively, between two groups. Pearson's χ^2, Fisher's exact test, odd ratios, and 95% confidence intervals (CI) were used to test for associations between malaria and others variables. The logistic regression model was used to examine the independent association between malaria and different factors. For all analyses, the statistical significance level was fixed at < 0.05.

The study was approved by the local Institutional Ethics Committee of the Catholic University of Bukavu and was authorized by the Ministry of Health office in the South Kivu Province. All participants gave an informed consent. Data from human immunodeficiency virus–positive women were excluded and patients managed according to the national guidelines.

Five-hundred-thirty-one pregnant women, with a median (interquartile range, IQR) age of 25.5 (21.1–31.3) years and between 12 and 24 weeks of gestational age, were recruited. Characteristics of the women and others results are reported elsewhere.[6] More than a quarter

of the study population were primigravidae and half were in their fourth or higher pregnancy. Nearly 38% (199/530) and 16.5% (86/522) reported fever and antimalarial treatment, respectively, within the previous 3 months, whereas 63.8% (336/527) reported sleeping under insecticide-treated mosquito net (ITN) the prior night. The prevalence of anemia was 17.6%, including 0.2% severe anemia (N = 529).

Overall malaria prevalence was 7.5% (95% CI: 5.4–10.1), with parasite density ranging between 160 and 48,000 parasites/μL. The large majority of infections (38/40) were due to *P. falciparum*, with two due to *Plasmodium malariae* (320 and 360 parasites/μL). SF, sTfR, AGP, CRP, and malaria status were available for 484 women. Median (IQR) ferritin (μg/L) was 41.4 (24.6–64.6). Prevalence (95% CI) of inflammation was 23.9% (20.2–28.0). ID prevalence based on ferritin was 7.6% (5.4–10.4). Using BIS, ID was 2.9% (N = 485). No woman with ID was infected by *P. falciparum* parasite, whereas in those without ID (N = 470) 7.4% were malaria infected.

Adjusted median SF (IQR) was significantly higher in malaria-infected [82.9 μg/L (56.3–130.4)] than non-infected [39.8 μg/L (23.6–60.8)] women; $P < 0.001$ (Figure 1). Similarly, mean (standard deviation) BIS (mg/kg) was significantly higher in malaria-infected [7.8 (3.3); N = 35] than in non-infected women [5.7 (2.9); N = 449; $P < 0.001$]. Among the 35 malaria-infected women with SF data, only one of them (infected with *P. malariae*) was iron deficient; *P. falciparum* was diagnosed in women with normal to high levels of SF.

Malaria-infection was significantly and independently associated with not sleeping under ITN the prior night and with high ferritin level (Table 1).

These results show that pregnant women with high SF had a significantly higher probability of being malaria-infected. However the direction of malaria-iron store interaction remains unclear. It is unclear whether high iron loads favor malaria infection or whether malaria causes high SF and BIS. In the first scenario, malaria parasites would benefit from iron-replete conditions to access iron for their metabolism and growth. In the second scenario, malaria would increase ferritin concentrations as it does for others acute phase proteins. Our data are consistent with either scenario. Inflammation was found in almost all infected women and ferritin levels were corrected for CRP/AGP levels. To our knowledge, there are no validated correction factors to adjust ferritin according to the presence of malaria, but data from population studies by others suggest that there is no difference in the prevalence of iron store depletion when correcting for CRP + AGP + malaria versus when correcting for CRP + AGP.[16] This may indicate that correcting for inflammation using both CRP and AGP as was done in our study is sufficient for correcting malaria effect. However, the correction factors might be insufficient to correct for the effect of malaria beyond inflammation if malaria were to induce a depletion of iron stores. The definitive way to ascertain the causality relationship between iron stores and malaria is to carry out prospective studies in which the incidence of malaria is measured in groups of low and high ferritin and/or BIS, but based on available information by other studies, we suggest that the higher corrected ferritin levels among malaria infected women were likely not a consequence of, but reflected replete iron stores predisposing to malaria.

If high iron loads favor malaria, then the next question is whether IS without effective malaria prevention during pregnancy might be harmful especially in iron-replete population. In South Kivu, ID does not seem to be common among women, regardless of pregnancy.[5,6] A similar low ID prevalence was also found in preschool children in the same area.[4,5] As mentioned previously, elsewhere IS has been associated with a higher risk of malaria during pregnancy[9] and a higher risk of morbidity and mortality due to infectious diseases, including malaria, among preschool children.[10] According to published data from others settings, ID

may protect against PAM.[9,11,12] Therefore, IS in iron-replete women may increase the risk of malaria-infection and consequently malaria-related anemia. The underlying mechanisms are not clearly understood, but they may be related to the use of iron by malaria parasites for their growth and replication and to the impairment of host immunity.[17]

Coverage of preventive interventions against malaria among pregnant women remains low in many countries. In 20 African countries, IPT coverage (≥ 3 doses of sulfadoxine–pyrimethamine, SP) was 31% (95% uncertainty interval: 29–32%) in 2015.[1] This is also the case in South Kivu, our study area, where according to the Ministry of Health, in 2016, only 11% of pregnant women received three doses of SP, 55% two doses, and only 60% of pregnant women were using ITN. In addition to this low level of prevention, the spread of SP resistance is another concern in the prevention of PAM.[18]

In South Kivu, 77.7% of pregnant women were supplemented in 2016 with daily iron (Ministry of Health). Unfortunately, we are unable to comment on the compliance. Ideally, IS should be given only to iron-deficient women. However, there are no field-deployable tests able to identify them. It has, therefore, been suggested that IS should be given along with effective preventive measures to reduce the risk of "iron-induced" malaria.[19] WHO antenatal care guidelines include the option of decreasing the iron dose according to the burden of anemia and ID (from 30–60 mg/day to 120 mg/week).[20] In places such as South Kivu, where ID is uncommon, a low iron dose might be safer.

Limitations of our study include the cross-sectional design, the use of microscopy (instead of molecular techniques) to diagnose malaria, which could have underestimated its prevalence as submicroscopic-malaria is common during pregnancy, hence resulting in the relatively small number of parasitized-pregnant women, and the use of ferritin correction factors and a BIS formula determined from undiseased groups. Based on our findings, large-scale longitudinal studies to measure the incidence of malaria in groups of low versus high iron stores and efforts to improve prevention against malaria, ID, and anemia during pregnancy are warranted in South Kivu.

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Authors' addresses: Esto Bahizire, Center of Research in Epidemiology, Biostatistics and Clinical Research, Université Libre de Bruxelles, Brussels, Belgium, Centre de Recherche en Sciences Naturelles de Lwiro, Bukavu, DR Congo, and Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya, E-mail: esto.bahizire@gmail.com. Umberto D'Alessandro, Medical Research Council Unit The Gambia, Fajara, Banjul, The Gambia, and London School of Hygiene and Tropical Medicine, London, United Kingdom, E-mail: udalessandro@mrc.gm. Michèle Dramaix, Center of Research in Epidemiology, Biostatistics and Clinical Research, Université Libre de Bruxelles, Brussels, Belgium, E-mail: michele.dramaix@ulb.ac.be. Nicolas Dauby, Department of Infectious Diseases, Centre Hospitalier Universitaire Saint-Pierre, Brussels, Belgium, E-mail: nicolas_dauby@stpierre-bru.be. Fabrice Bahizire, Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya, E-mail: fabricebahizire89@gmail.com. Kanigula Mubagwa, Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium, E-mail: kanigula.mubagwa@med.kuleuven.ac.be. Philippe Donnen, Center of Research in Health Policy and Systems-International Health, Université Libre de Bruxelles, Brussels, Belgium, E-mail: pdonnen@ulb.ac.be.

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FIGURE 1. Median of ferritin adjusted for inflammation among women without and with malaria. Mann–Whitney Wilcoxon $P < 0.001$.

TABLE 1

Association between malaria, ITN, iron load, and inflammation in pregnant women

					Logistic model, N = 480; 35 cases of malaria	
Variables	*n*	% Malaria	OR (95% CI)	*P*	aOR (95% CI)	*P*
Age (years)	–	–	–	0.798	–	–
≤ 20	95	8.4	1.52 (0.44–5.25)	–	–	–
> 20–35	364	7.7	1.38 (0.47–4.05)	–	–	–
> 35	70	5.7	1	–	–	–
Number of pregnancy	–	–	–	0.431	–	–
1	126	5.6	1	–	–	–
2–3	112	9.8	1.85 (0.69–4.95)	–	–	–
≥ 4	246	6.9	1.26 (0.51–3.13)	–	–	–
Sleeping under ITN the prior night	–	–	–	0.061	–	0.024
Yes	335	6.0	1	–	1	–
No	191	10.5	1.85 (0.92–3.72)	–	2.24 (1.12–4.51)	–
Inflammation*	–	–	–	< 0.001	–	–
Yes	116	20.7	8.47 (3.80–19.77)	–	–	–
No	368	3.0	1	–	–	–
Ferritin (ug/L, adjusted for inflammation)*	–	–	–	0.005†	–	0.021

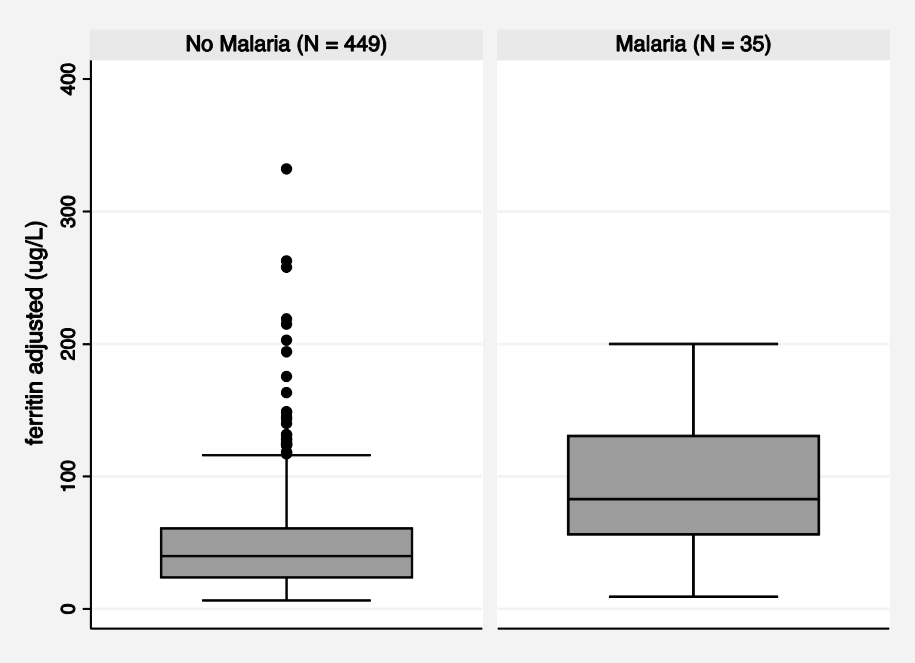
< 15	37	2.7	3.56 (0.31–41.39)	–	1.57 (0.14–17.77)	–
15 to ≤ 30	117	1.7	1	–	1	–
> 30	330	9.7	6.23 (1.47–26.38)	–	6.25 (1.47–26.57)	–

aOR = adjusted odds ratio; CI = confidence interval; ITN = insecticide-treated mosquito net; OR = odds ratio. The following variables have been removed by the stepwise backward procedure: age, level of education, employment, marital status, number of pregnancy, fever within the last 3 months, anti-malarial treatment within the last 3 months, mid-upper arm circumference, retinol binding protein, and serum albumin. Note: ferritin was already adjusted for inflammation.[14]

* Inflammation: C-reactive protein > 5 mg/L and/or α1-acid-glycoprotein > 1 g/L.

† Fisher exact.

Figure 1


No Malaria (N = 449)
Malaria (N = 35)
ferritin adjusted (ug/L)
0
100
200
300
400