Decline in childhood iron deficiency after interruption of malaria transmission in highland Kenya

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ABSTRACT

Background: Achieving optimal iron status in children in malaria-endemic areas may increase the risk of malaria. Malaria itself may contribute to iron deficiency, but the impact of an interruption in malaria transmission on the prevalence of iron deficiency is unknown.

Objectives: We aimed to determine whether 1) iron status improved in children living in 2 Kenyan villages with a documented cessation of malaria transmission and 2) changes in iron status correlated with changes in hemoglobin.

Design: We measured iron [hemoglobin, ferritin, soluble transferrin receptor (sTfR) and inflammatory [C-reactive protein (CRP)] markers in paired plasma samples from 190 children aged 4–59 mo at the beginning (May 2007) and end (July 2008) of a documented 12-mo period of interruption in malaria transmission in 2 highland areas in Kenya with unstable malaria transmission and ongoing malaria surveillance.

Results: Between May 2007 and July 2008, mean (±SD) hemoglobin increased from 10.8 ± 1.6 to 11.6 ± 1.6 g/dL. Median (25th, 75th percentile) ferritin increased from 17.0 (9.7, 25.6) to 22.6 (13.4, 34.7) µg/L (P < 0.001), whereas median sTfR decreased from 32.4 (26.3, 43.2) to 27.7 (22.1, 36.0) nmol/L (P < 0.001). Median CRP was low (<1 mg/L in both years) and did not change significantly. Iron deficiency prevalence (ferritin <12 µg/L, or <30 µg/L if CRP ≥10 mg/L) decreased from 35.9% (95% CI: 28.9%, 43.0%) to 24.9% (18.5%, 31.2%) (P = 0.005). The prevalence of iron deficiency with anemia (hemoglobin <11.0 g/dL) declined from 27.2% (20.7%, 33.8%) to 12.2% (7.4%, 17.1%) (P < 0.001). Improvement in iron status correlated with increase in hemoglobin and was greater than explained by physiologic changes expected with age.

Conclusions: In this area of unstable malaria transmission, the prevalence of iron deficiency in children decreased significantly after the interruption of malaria transmission and was correlated with an increase in hemoglobin. These findings suggest that malaria elimination strategies themselves may be an effective way to address iron deficiency in malaria-endemic areas. Am J Clin Nutr doi: 10.3945/ajcn.114.087114.

INTRODUCTION

Elucidating the complex relation between iron status and malaria infection in young children has been a global health priority since 2006 when a large randomized controlled trial on malaria-endemic Pemba Island, Tanzania, found that daily prophylactic iron supplementation significantly increased the risk of hospitalization and death in preschool-aged children (1). Subsequent studies similarly underscored a potentially dangerous interaction between malaria and iron supplements—or replete iron status—and harmful consequences. A large prospective study in a malaria-endemic area of Tanzania found that dietary iron deficiency in young children provided protection against both the frequency and severity of subsequent malaria episodes (2), whereas a recent study in Ghana found that children receiving an iron-fortified micronutrient powder were not at greater risk of incident malaria but did tend to be hospitalized more frequently than children consuming sprinkles without iron (3). Thus, nearly a decade after the Pemba trial, determining how to achieve adequate iron status while not worsening malaria infection or causing other morbidity remains an unanswered public health question with ramifications for the immediate health and long-term neurobehavioral development of tens of millions of children worldwide.

Alleviating malaria burden has been proposed as a necessary first step in reducing the unacceptably high prevalence of iron deficiency and anemia among children in low-income, malaria-endemic countries (4). Indeed, several studies have found that reducing malaria burden, whether with antimalarial treatment, chemoprevention, or insecticide-treated bed nets, significantly increased hemoglobin concentrations and reduced the prevalence of anemia in children (5–11). The mechanism of this anemia has not been explored, but malaria can cause anemia through a variety of mechanisms, many of which alter iron homeostasis. Malaria-induced inflammation that blunts bone marrow activity also traps recycled iron in macrophages and absorbs iron in enterocytes, leading to a functional iron deficiency, whereas repeated hemolytic episodes and accompanying urinary blood
loss can lead to loss of body iron (12, 13). The fact that the pathophysiologic mechanism of malaria-related anemia affects iron absorption, distribution, and loss thus raises the question of whether iron deficiency might in fact be an intermediary between findings of reduced malaria transmission and higher hemoglobin concentrations in a population. Such a finding would be significant because it would suggest that malaria-control strategies themselves may play an important role in improvement of iron status in malaria-endemic areas, reducing the need for potentially dangerous iron supplements.

We previously showed that after a yearlong interruption of malaria transmission in 2 highland sites in Kenya that was brought about by widespread indoor residual insecticide spraying and introduction of artemisinin combination therapy as first-line therapy for malaria, children ≤5 y of age experienced a significant increase in hemoglobin (14). In the present study, we sought to determine whether iron status also improved in these children after the period of interruption in malaria transmission compared with before the interruption and whether improved iron status contributed to the observed decline in anemia.

SUBJECTS AND METHODS

Study population and ongoing malaria surveillance

Samples for the current study were taken from an ongoing study of malaria epidemiology conducted since 2003 in the 2 Kenya highland areas (elevation: 1829–2132 m) of Kapsisiywa (2007 population: 3787) and Kipsamoite (2007 population: 4180), both characterized by low-level, unstable, seasonal malaria transmission. The larger study includes climate and geospatial analyses that have been described previously in detail (14–16), as well as a collection of demographic information from all consenting households beginning April 2003 and continuing every 4–6 mo to the present. Also as part of the larger study, malaria surveillance has been conducted in both villages since 2003 and includes passive surveillance with the use of microscopy and polymerase chain reaction testing for all symptomatic individuals in local Ministry of Health dispensaries (14, 16).

In March and April 2007, the Ministry of Health implemented widespread indoor residual insecticide spraying in the area. This spraying followed introduction of coartemether as the first-line treatment of uncomplicated malaria in late 2006 and early 2007. After these changes, a 12-mo period of interrupted transmission was recorded from April 2007 to March 2008 (16).

Two separate sitewide household surveys were conducted in May 2007 (ie, approximately at the beginning of this period in transmission interruption and again 14 mo later, in July 2008) (Figure 1). During the 2007 survey, venous blood was collected from all consenting asymptomatic individuals (n = 5733) who resided in the 2 sites. In the 2008 survey, venous blood was again drawn from a randomly selected subset of approximately one-third of the individuals sampled in 2007 (n = 1697). One hundred ninety of these individuals who had blood drawn at both times and who were ≤5 y in 2007 were randomly selected for iron study testing for the present study. At each stage, random selection was performed by a computer-generated algorithm in FileMaker Pro (Filemaker Inc). The only exclusion criterion for survey participation was lack of permanent residency at the study site.

As shown in Figure 1, clinical malaria cases were present at the site until 2 mo before the first blood collection, but there were no microscopy-confirmed cases of malaria in the entire population during the period of interruption. The reported use of insecticide-treated nets among children ≤5 y at each site was ~15%.

Ethics

Written informed consent for demographic information was obtained from household heads. For individual blood sampling, consent was obtained from the study participant’s parent or guardian. Ethical approval was obtained from the Kenya Medical Research Institute National Ethical Review Committee and the institutional review board for human studies at the University of Minnesota.

Iron and inflammatory marker assessment and cutoff definitions

Hemoglobin was measured photometrically during the household surveys by using finger-prick blood samples (HemoControl; EKF Diagnostics) as part of the larger parent study. Per WHO recommendations, hemoglobin concentrations were adjusted by −0.8 g/dL for altitude (17). Anemia was diagnosed when adjusted hemoglobin was <11.0 g/dL in children <5 y of age (17). Venous blood samples collected in May 2007 and July 2008 were centrifuged, and plasma was separated and stored at −80°C until shipment on dry ice to the University of Minnesota, where all plasma samples were stored at −80°C until January 2011–February 2012 when iron markers assays were conducted.

Any child found to be moderately anemic (hemoglobin <8 g/dL) during the time of blood collection was offered transport to the local health center for free evaluation and treatment, including iron and anthelmintic tablets. However, most caregivers (>85%) declined evaluation and treatment because they said that the child was not ill and thus they did not want to take the time to go to the health center.

An ELISA was used to measure ferritin (Ramco Laboratories) and soluble transferrin receptor (sTfR; R&D Systems) in stored plasma samples. Plasma C-reactive protein (CRP) was measured by Luminex Immunoassay (Milleplex MAP kit, CVD panel 2; Millipore Corporation). Of the samples, ~20–40% were randomly selected and assayed in duplicate for each of these assays. The kits used for CRP, sTfR, and ferritin report intra- and inter-assay CVs of up to 17.5%, 7.1%, and 9.6%, respectively. Median interassay CVs for duplicate measurements of CRP, sTfR, and ferritin among participant samples were 21.1%, 10.0%, and 21.4%, respectively. For samples assayed in duplicate, the average value was used in the final data analysis. A child was considered to have iron deficiency if plasma ferritin was <12 µg/L or if plasma ferritin was <30 µg/L when CRP was ≥10 mg/L (18–20).

Plasmodium falciparum antigen serologic testing

To assess malaria exposure, serologic responses to Plasmodium falciparum apical membrane antigen-1 (AMA-1) were measured. Antibody concentrations to AMA-1 were determined by using a multiplex cytometric bead assay. Development and validation of this assay are described in detail previously (21). Briefly, microspheres were coupled to Escherichia coli expressed recombinant AMA-1 protein (3D7). Coated beads were added to prewetted microtiter plates (MABVN 1250; Millipore Corporation), incubated with plasma, and then washed. Beads were then incubated with goat anti-human IgG (γ-chain specific) F(ab)’2 fragment-R-phycocerythrin (P8047; Sigma-Aldrich). The plates were washed and beads were analyzed on a BioPlex™ 200 machine (Bio-Rad Laboratories) and reported in units of median fluorescence intensity (MFI). The CV of the assay was 10%, with 10% of samples analyzed in duplicate.

Antibody concentrations for AMA-1 are expressed in arbitrary units (AU), which were calculated by dividing the MFI generated by the test sample by the mean MFI plus 3 SDs generated by samples from 9 North Americans never exposed to malaria (21, 22). Study participants with AU values >1.0 were considered antibody responders (21, 22). A decline in AMA-1 reflects declining exposure to malaria (23).

Statistical analysis

Hemoglobin concentrations (g/dL) and anemia prevalence between May 2007 and July 2008 were compared by using paired t tests and 2-tailed McNemar exact tests, respectively. CRP, ferritin, and sTfR values were compared between 2007 and 2008 with a paired t test by using log_{10}-transformed values. The prevalence of iron deficiency between 2007 and 2008 was compared by using a 2-tailed McNemar exact test. Linear regression models analyzing the change in hemoglobin between 2007 and 2008 were used to examine the relation between anemia and iron deficiency. Predictive factors included log change in ferritin concentration or the log change in sTfR as well as age, village location, and sex. Serologic reactivity to AMA-1 between 2007 and 2008 was compared by using 2-tailed McNemar exact tests.

Because iron status improves with age in children <5 y, we evaluated the physiologic increase in ferritin and decline in sTfR with that which would be expected with increasing age by creating five 1-y age categories at the time of the May 2007 collection: <12, 12 to <24, 24 to <36, 36 to <48, and 48 to <60 mo. The mean of log-transformed ferritin and sTfR values from the May 2007 collection was calculated for each of these 5 age groups. The expected change in these indicators with a 1-y increase in age was calculated as the difference in log-transformed ferritin and sTfR values between one age group and the age group that was 1 y older. The expected change with a 1-y increase in age was then compared with the actual mean change in log-transformed ferritin or sTfR values in the children from May 2007 to July 2008.

RESULTS

Study population

Of the 190 children <5 y who were randomly selected for iron study testing, there were sufficient plasma samples in both 2007 and 2008 to measure CRP in 187 children, sTfR in 188 children, ferritin in 181 children, and AMA-1 in all 190 children. Mean (±SD) ages for participants in this study were 2.8 ± 1.3 y (range: 4 mo–5 y) in 2007 and 4.0 ± 1.3 y (1.4 y–6 mo) in 2008.

Iron status indicators

As in the previously described full study cohort (14), this subcohort of children <5 y of age showed a significant increase in mean hemoglobin concentration and a decline in the prevalence of anemia (P < 0.001; Table 1). Iron status indicators also changed significantly. Between 2007 and 2008, median serum ferritin concentrations increased significantly from 17.0 to 22.6 µg/L. Median sTfR concentrations decreased significantly in this time from 32.4 to 27.7 nmol/L (Table 1 and Figure 2). Median plasma ferritin and sTfR did not differ significantly by sex in either the 2007 or 2008 survey (data not shown). Median CRP, a marker of acute inflammation, was low and did not differ significantly between 2007 and 2008 (Table 1).

Abbreviations used: AMA-1, apical membrane antigen-1; AU, arbitrary unit; CRP, C-reactive protein; MFI, median fluorescence intensity; sTfR, soluble transferrin receptor.
In 2007, 35.9% (95% CI: 28.9%, 43.0%) of children were iron deficient, a prevalence that decreased to 24.9% (95% CI: 18.5%, 31.2%) in 2008 ($P = 0.005$). Inflammation at the time of either of the samplings was uncommon. Among children who met the criteria for iron deficiency, only 4 of 65 in 2007 and 5 of 45 in 2008 had a CRP value $\geq 10$ mg/L.

Linear regression was used to evaluate the relation between change in hemoglobin and the change in ferritin or the change in sTfR after adjustment for age, sex, and study site (Kipsamoite compared with Kapsisiywa). Increases in hemoglobin correlated positively with log changes in ferritin ($b = 0.35; 95\% \text{ CI}: 0.04, 0.66; P = 0.03$) and negatively with log changes in sTfR ($b = -1.27; 95\% \text{ CI}: -1.85, -0.68; P < 0.001$).

A comparison of the expected change in log-transformed ferritin and sTfR with the observed change with age (Tables 2 and 3) showed that, for both markers, iron status improved more than what would be expected by age alone. In all age groups <48 mo of age, the measured increase in ferritin was in excess of what was predicted by age and sTfR values declined more than what was predicted by age. The values could not be directly compared statistically because one represents the change over the course of a year in a paired cohort and the other is the average change between children of different ages in a cross-sectional analysis.

### TABLE 1
Iron status indicators in children <5 y of age in a highland area of Kenya in May 2007 (start of interruption of malaria transmission) and after 14 mo of interrupted malaria transmission (July 2008)*

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>2007</th>
<th>2008</th>
<th>$P^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hemoglobin ($g/dL$)</td>
<td>Hemoglobin &lt;11.0g/dL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>187</td>
<td>10.8 ± 1.6$^a$</td>
<td>11.6 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L) (%)</td>
<td>187</td>
<td>0.64 (0.23, 2.50)$^a$</td>
<td>0.70 (0.23, 3.48)</td>
<td>0.43</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>187</td>
<td>17.0 (9.7, 25.6)</td>
<td>22.6 (13.4, 34.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sTfR (nmol/L)</td>
<td>188</td>
<td>32.4 (26.3, 43.2)</td>
<td>27.7 (22.1, 36.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iron deficiency (%)</td>
<td>181</td>
<td>35.9</td>
<td>24.9</td>
<td>0.005</td>
</tr>
<tr>
<td>Iron deficiency anemia (%)</td>
<td>180</td>
<td>27.2</td>
<td>12.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* CRP, C-reactive protein; sTfR, soluble transferrin receptor.
  Derived by using paired Wilcoxon rank sign tests for continuous variables and McNemar tests for prevalence comparisons.
  Adjusted for altitude according to WHO (17).
  Mean ± SD (all such values).
  Median; IQR in parentheses (all such values).
  Defined as ferritin $\leq 12 \mu g/L$ or $<30 \mu g/L$ if CRP is $>10$ mg/L.
  Iron deficiency and hemoglobin $<11.0g/dL$.

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**Serologic evidence of malaria exposure**

Serologic studies showed that antibodies to AMA-1 were present in 49.5% of children in 2007 and in 40.4% of children in 2008 ($P = 0.03$). From 2007 to 2008, there was a significant decrease in median (quartile 1, quartile 3) AMA-1 antibody concentrations [2007: 0.99 (0.52, 2.80) AU; 2008: 0.81 (0.45, 1.66) AU; $P < 0.001$].

**FIGURE 2.** Box and whisker plots of plasma ferritin (A) and plasma sTfR (B) values in May 2007 and July 2008. The middle lines represent median values, lower and upper box lines represent 25th and 75th percentiles, the ends of the “whiskers” represent the 95th percentile, and additional dots show outlier data points. The 2007 and 2008 values for plasma ferritin and sTfR were compared by using a paired Wilcoxon rank sign test. sTfR, soluble transferrin receptor.
Multiple mechanisms could explain the decline in iron deficiency that followed interruption of malaria. There is considerable evidence in mice and humans that during malaria infection, even asymptomatic infection, the body develops a functional iron deficiency via hepcidin-mediated sequestration of iron in macrophages and decreased iron absorption from the gut (24–27). Although we observed very little inflammation in children in the present study in either the 2007 or 2008 survey, isotope studies in anemic children with uncomplicated malaria show that the changes in iron markers associated with malaria infection may persist for ≥4 wk after successful malaria treatment (28). Accordingly, the significant increase in ferritin concentration between the 2 surveys could reflect restitution or normalization of iron status after inflammation-induced changes in this indicator. As part of this reestablishment of iron homeostasis, improved iron absorption in the gut after resolution of inflammation could increase body iron, contributing to the observed increase in ferritin.

Another possible explanation for the observed decline iron deficiency may be restitution of iron status after malaria-associated hemolysis. Although much of the hemoglobin from red blood cells hemolyzed during an acute malaria infection is complexed with haptoglobin and sequestered in macrophages, symptomatic malaria infection can also cause urinary iron loss secondary to hemolysis (12, 13, 29). With the cessation of hemolysis, hemoglobin would likely increase first, followed by replenishment of iron stores, reflected by increasing ferritin concentrations. Frequent venous sampling, beyond the scope of this study, would be necessary to capture and quantify all inflammatory and hemolytic events given the transience of many biomarker elevations including hepcidin.

In areas of high and moderate malaria transmission, previous studies have documented that reducing malaria transmission leads to a reduction in the prevalence of anemia (30–32). However, few studies assessed the effects of interventions to decrease malaria in areas of low malaria transmission, and even in areas of higher transmission the mechanisms of how malaria reduction led to a decrease in anemia were not assessed. Reductions in malaria-related hemolysis and suppression of erythropoiesis may also play a role, but the present study argues for a role for reduction in iron deficiency with reduction in malaria, something not previously postulated as an identified factor in reducing anemia in areas with malaria control or prevention measures.

A systematic evaluation of the impact of malaria infection on iron status over time is difficult in any setting because of the confounding influences on iron status including nutritional status, age, and helminth infection as well as the difficulty in estimating the degree of malaria exposure. This study design presented here has these same challenges but is uniquely suited to address this question for 2 reasons. First, the degree of malaria exposure is well defined with both active and passive case detection methods as well as supporting serologic evidence. Second, because of the discrete geographic area of these 2 sites, we can more completely assess other factors that affect iron status both through partnerships with health community leaders and through direct study activities. In these 2 communities there were no other changes that one would expect to cause a decrease in iron deficiency.

During the study period, there were no atypical aberrations in rainfall or temperature; no major changes in crop type, distribution, or output; and no Ministry or other health interventions of note other than the spraying campaigns and introduction of artemether-lumefantrine as first-line antimalarial therapy just before the start of the study. These highland sites have low levels of helminth infection (<1% stool helminth infection in samples from 2010; CC John, unpublished observations), and no major helminth treatment campaigns were conducted during this time frame. By serologic evidence, in 2007 at least 49% of the children had previous exposure to malaria, and the significant decrease in antibody concentrations between 2007 and 2008 provides additional evidence of decreased exposure to *P. falciparum* during the period of study. The data showing extremely low or absent malaria transmission, in the absence of evidence of any other clear mechanism for the change in iron deficiency and hemoglobin concentrations, provide strong epidemiologic evidence that a reduction in malaria transmission played a role in the reduction in iron deficiency. However, a randomized clinical trial would be required for definitive proof of causation.

The present study suggests that a substantial proportion of anemia may be attributable to malaria, even in areas of low malaria transmission, and that malaria-related anemia may be mediated in part by iron deficiency. We continue to seek ways in which iron status can be safely optimized in the setting of ongoing malaria transmission. This work is vital for many areas in which there are still high levels of malaria transmission. However, in areas of low-level transmission, the study data suggest...
that malaria control initiatives and work toward malaria elimi-
nation may play a role in addressing iron deficiency. The study
findings thus provide an additional important public health basis
for working toward malaria elimination and eradication.

We thank Jim Hodges at the University of Minnesota, Division of Biosta-
tistics, for advice on statistical analysis and Gregory Park at the University of
Minnesota, Division of Global Pediatrics, for laboratory advice. We also
thank the study subjects for participation in this study. This study was pub-
lished with the permission of the Director of the Kenya Medical Research
Institute.

The authors’ responsibilities were as follows—AEPF, CCJ, and SEC:
designed the research; AEPF, CCJ, BNO, GAA, and JMV: conducted the
research; AEPF, CCJ, and SEC: wrote the manuscript; and AEPF: performed
the statistical analysis and has primary responsibility for the final con-
tent of the manuscript. None of the authors had a conflict of interest to disclose.

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