Iron Deficiency and Malaria among Children Living on the Coast of Kenya

Alice M. Nyakeriga,[1,2,3] Marita Troye-Blomberg,[3] Jeffrey R. Dorfman,[1] Neal D. Alexander,[5] Rune Bäck,[4] Moses Kortok,[1] Alex K. Chemtai,[2] Kevin Marsh,[1] and Thomas N. Williams[1]

[1]Kenya Medical Research Institute/Wellcome Trust Programme, Centre for Geographic Medicine Research, Coast, Kilifi District Hospital, Kilifi, and [2]Faculty of Health Sciences, Moi University, Eldoret, Kenya; [3]Department of Immunology, Wenner-Gren Institute, Stockholm University, and [4]Division of Clinical Chemistry, Huddinge University Hospital, Stockholm, Sweden; [5]Infectious Disease Epidemiology Group, London School of Hygiene and Tropical Medicine, London, United Kingdom

Both iron deficiency and malaria are common in much of sub-Saharan Africa, and the interaction between these conditions is complex. To investigate the association between nutritional iron status, immunoglobulins, and clinical *Plasmodium falciparum* malaria, we determined the incidence of malaria in a cohort of children between the ages of 8 months and 8 years who were living on the Kenyan coast. Biochemical iron status and malaria-specific immune responses were determined during 2 cross-sectional surveys. We found that the incidence of clinical malaria was significantly lower among iron-deficient children (incidence-rate ratio [IRR], 0.70; 95% confidence interval [CI], 0.51–0.99; $P<.05$), that the incidence of malaria was significantly associated with plasma ferritin concentration (IRR for log ferritin concentration, 1.48; 95% CI, 1.01–2.17; $P<.05$), and that iron status was strongly associated with a range of malaria-specific immunoglobulins. We conclude that iron deficiency was associated with protection from mild clinical malaria in our cohort of children in coastal Kenya and discuss possible mechanisms for this protection.

Both iron deficiency and malaria are common in much of sub-Saharan Africa, and the interaction between these conditions is complex. The literature regarding the relationship between them is dominated by studies in which iron has been given therapeutically, either as prophylaxis or for the treatment of iron deficiency. Although early studies of this kind have suggested that iron supplementation might be associated with an increased incidence of clinical malaria [1–3], this suggestion had not been confirmed in studies conducted more recently [4–7]. Nevertheless, a recent meta-analysis has shown that the evidence still favors a small but significantly increased risk of malaria after iron supplementation [8]. Relatively little is known about the converse association—that is, the incidence of malaria among iron-deficient children. Although studies of animals have suggested that iron deficiency may be associated with protection from malaria [9], few data are available for humans. We therefore conducted an observational study in which we investigated the relationship between biochemical iron status, immunoglobulin concentrations, and the incidence of clinical malaria among children living on the coast of Kenya, with the dual aims of gaining further insight into the interaction between iron and malaria and of informing the design of future studies investigating the benefits and risks of iron supplementation in our community.

Received 29 December 2003; accepted 10 February 2004; electronically published 2 July 2004.

Financial support: United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases; Swedish Agency (Sida/Sarec) for Research Cooperation with Developing Countries; Wellcome Trust.

Reprints or correspondence: Alice Nyakeriga, KEMRI/Wellcome Trust Programme, Centre for Geographic Medicine Research, Coast, PO Box 230, Kilifi, Kenya (nyakeriga@imun.su.se).

The Journal of Infectious Diseases 2004; 190:439–47

© 2004 by the Infectious Diseases Society of America. All rights reserved.

0022-1899/2004/19003-0003$15.00

MATERIALS AND METHODS

Study area. The study was conducted in the Ngerenya area in Kilifi District on the Kenyan coast. Malaria transmission in this area occurs throughout the year, although the majority of clinically evident infections occur after long and short periods of rainfall that, in

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018

general, occur during the months of October–November and March–July, respectively [10]. Residents of Ngerenya experience an average of 10 infective bites/person/year [11]. The population of this rural farming community is predominantly of the Mgiriama ethnolinguistic group, which is a subgroup of the Mijikenda of the northern Kenyan coast.

Study design. The current study was nested within an ongoing, rolling, cohort study evaluating the history and acquisition of natural immunity to malaria that has been described in detail elsewhere [12]. Children residing in randomly selected households were recruited, at birth, to this study and remained under surveillance until their eighth birthday. Selection of households for inclusion in the study was based on a random-cluster sample of all the households identified within the prescribed study area during a prestudy census in April 1998 [12]. The present study was conducted during an 18-month period, between November 2001 and May 2003, and involved 240 children between the ages of 8 months and 8 years. Data on morbidity due to malaria were collected throughout the period, while 2 cross-sectional surveys were conducted at 6 and 12 months after the study began, in May and October 2002 (figure 1). At each survey, data were collected regarding a range of clinical parameters, together with a sample of venous blood. In addition, all children were weighed using Seca model 835 digital scales (CMS instruments). For children >1 year old, height was measured using a Leicester height measure (CMS Instruments); for younger children, length was measured using a length board (Appropriate Health Resources and Technology Action Group design, as modified by Nicoll and Ulijaszec [13]).

Morbidity surveillance. Cohort children were visited weekly. At each visit, a standard morbidity questionnaire was completed that detailed each child's state of health, any clinical symptoms, and any recent medications. Axillary temperature was measured at each visit by use of a digital thermometer. Thick and thin blood smears were prepared for all children with either a history of fever during the preceding 48 h or an axillary temperature >37.5°C. Children whose slides were positive for malaria parasites were treated according to standard Government of Kenya guidelines. First-line treatment was with sulphadoxine/pyrimethamine (Falcidin; Cosmos). In the event of treatment failure, second-line treatment was with amodiaquine. Children born in the study households during the study period were recruited at birth and entered the current study at 8 months of age. Children left the study if their parents withdrew informed consent, if they moved from the study area for >2 months, or if they died.

Laboratory procedures. Blood films were stained with Giemsa and examined for malaria parasites by standard methods. Parasite densities were recorded as a ratio of parasites to white blood cells (WBCs) (from thick smears) or to red blood cells (RBCs) (from thin smears) for heavier infections. Densities (parasites per microliter of whole blood) then were calculated on the basis of a WBC count of 8×10^3 cells/μL or an RBC count of 5×10^6 cells/μL. Hemoglobin typing (for sickle-cell trait) was conducted by cellulose-acetate electrophoresis. Routine hematological assessments were not part of the study.

Assessment of biochemical iron status. Venous blood was collected into heparin, and the plasma was separated by centrifugation. Plasma then was frozen immediately and stored at −80°C, pending further processing. All subsequent investigations were conducted using plasma. Concentrations of C-reactive protein (CRP), ferritin, and transferrin were measured by turbidimetry, and plasma iron concentration was measured by Ferrozine-based photometry using an automated analyzer (Hitachi 917, Hitachi Corp.). Transferrin saturations (TFSs; in percentage) were calculated from the plasma iron and transferrin measurements, by use of standard methods [14].

Definitions of biochemical iron status. Iron deficiency was defined as plasma ferritin concentration <12 μg/mL, in association with TFS <10%, as described elsewhere [15]. Children with plasma ferritin concentration $\geqslant$12 μg/mL and TFS $\geqslant$10%

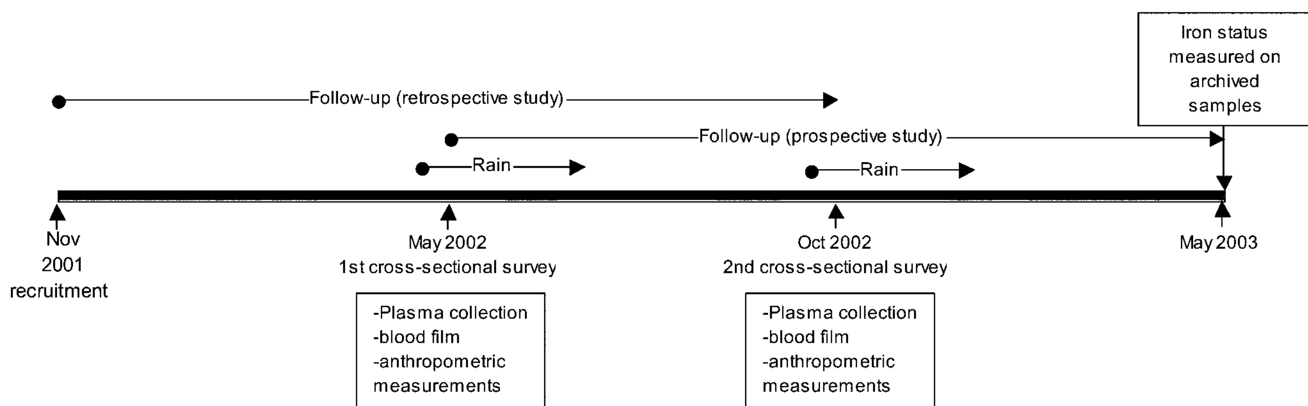

Figure 1. Study schedule

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018

were classified as iron replete if no evidence of current malarial infection or inflammation was found (i.e., a blood film negative for malaria and CRP concentration <10 mg/L). Inflammation and current malarial infections have unpredictable effects on markers of iron status; thus, reliable measurement of iron in subjects with either condition is not possible [16]. Children who did not meet the criteria for iron sufficiency or iron depletion, either because they had evidence of inflammation or malaria at the time of venesection or because the results of ferritin and TFS assays were discordant, were not classified and were excluded from analyses exploring associations between category of iron status and clinical disease. All biochemical assays were conducted on archived samples at the end of the study, at which time children found to be iron deficient were offered standard therapy based on locally available guidelines (20 mg of ferrous sulfate/kg/day for 30 days and 100 mg of mebendazole twice daily for 3 days).

Immunoglobulin measurement. Immunoglobulin assays were done by ELISA in 96-well plates (Costar), as described elsewhere [17]. In brief, for the measurement of total IgE, plates were precoated with affinity-purified goat anti–human IgE (Vector Laboratories), and, for the measurement of malaria-specific immunoglobulins, plates were precoated with crude malaria schizont extract prepared from *Plasmodium falciparum*–infected RBCs enriched with mature schizont extracts on percoll gradients [18]. Plates then were blocked with 1% bovine serum albumin and incubated with fixed volumes of prediluted plasma, and bound antibodies were detected by subsequent incubation with monoclonal antibodies (MAbs) with specificities for the appropriate immunoglobulin class or subclass. Total antimalarial IgG and antimalarial IgG subclasses were detected using commercially available biotinylated mouse anti–human MAbs. Total IgG was detected by use of clone G18-145, IgG1 by clone G17-1, IgG2 by clone G18-21, IgG3 by clone G18-3, and IgG4 by clone JDC-14, all of which were supplied by Pharmingen. IgE was detected using a biotinylated goat anti–human IgE (Vector Laboratories). Plates were washed extensively between incubations, by use of an automated plate washer (Skatron Instruments). Alkaline phosphatase–conjugated streptavidin (Mabtech) was added, followed by the enzyme substrate *o*-phenylene diamine (Sigma). Optical densities were read at 405 nm. Concentrations of total IgE (in nanograms per milliliter) were determined by reference to a standard curve derived using serial dilutions of purified myeloma IgE (National Institute for Biological Standards and Control). Since no standards are available for the quantification of malaria-specific antibodies, all other antibody concentrations were recorded in arbitrary units per milliliter, on the basis of the optical density readings, with reference to curves derived from serial dilutions of a control standard. All assays were conduced in duplicate. Antibody measurements from each survey were batch processed within 2 months of blood collection.

Data processing and statistical analysis. All data were checked for consistency before double entry into a computer database (FoxPro, version 6.0; Microsoft). After further checks of range and consistency, the duplicate databases were merged and revalidated, and any errors were corrected by reference to the primary data. Data were analyzed using STATA, version 8 (Timberlake). For the purposes of this study, malaria was defined according to 2 alternative definitions. "Malaria definition 1" was indicated by either a measured fever (axillary temperature >37.5°C) at the time slides were prepared or a clinical history of fever, within the preceding 48 h, in conjunction with a slide positive for blood-stage asexual *P. falciparum* parasites at any density. "Malaria definition 2" was indicated by a measured fever in conjunction with *P. falciparum* parasites at any density for children <1 year old or at a density >2500 parasites/μL for children $\geqslant$1 year old. Malaria definition 2, derived by means of multiple logistic regression methods described elsewhere [19], is associated with both a sensitivity and specificity of >80% in the diagnosis of clinical malaria in this area [12]. *Z* scores for the anthropometric parameters weight for age, height for age, and weight for height were calculated using Epi-Info, version 6.0 (Centers for Disease Control and Prevention). Biological data that did not have a normal distribution were log transformed prior to further analysis. These parameters included all biochemical markers of iron status and all immunoglobulin values. Biological data from iron-replete and iron-deficient children were compared separately for each survey by use of unpaired Student's *t* tests, with the exception of proportion data, which were compared by use of Fisher's exact test. We used Poisson regression analysis to investigate the association between a range of biological parameters and the subsequent incidence of clinical *P. falciparum* malaria during the follow-up period. For the purpose of this analysis, children retained their biological status (for the iron and immunoglobulin variables) throughout the 6-month period following a cross-sectional survey. Their status was refreshed at the follow-up survey if they attended, or they were dropped from further analysis if absent. New children joined the study if they attended the second survey for the first time. Children were considered not at risk and were dropped from both numerator and denominator populations for 21 days after receiving treatment with an antimalarial drug. Confidence intervals (CIs) and *P* values were inflated by means of the "sandwich" estimator, as described by Armitage et al. [20].

We conducted a second analysis to investigate whether the plasma concentrations of the biochemical markers of interest were related to the incidence of malaria during the period before each cross-sectional survey. For this analysis, each child was ascribed their biochemical data for each survey for the

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018

entire 6-month period prior to the survey. We then used Poisson regression models to investigate the association between malaria incidence and the biochemical parameters of interest. Because active malarial infection and inflammation influence both plasma ferritin and malaria-specific immunoglobulin concentrations, data for all children with slides positive for malaria or a CRP concentration ⩾10 mg/L at the time of cross-sectional survey were excluded from this analysis.

Ethical permission for this study was granted by the National Ethical Review Committee of the Kenya Medical Research Institute (KEMRI), in Nairobi. Individual written informed consent was obtained from the parents of all study participants.

RESULTS

At the initial survey in May 2002, 78 (33.3%) of 234 study participants were iron deficient, and 95 (41%) of 234 were iron replete, as determined on the basis of the definitions used. Seven children (3%) had increased concentrations (⩾10 mg/L) of CRP. The remaining children (54 [23%] of 234) could not be classified as either iron deficient or iron replete, because neither definition was fully satisfied. At the subsequent survey in October 2002, 91 (38%) of 240 children were iron deficient, 104 (43%) were iron replete, 7 (3%) had increased CRP concentration, and 38 (16%) could not be classified. Of the 204 children for whom data on iron status were complete at both surveys, 57 (28%) remained iron replete at both time points, and 49 (24%) remained iron deficient. Eight children (4%) who were iron replete at the time of the first survey had become iron deficient at the second survey, whereas 12 (6%) children who were iron deficient at the first survey subsequently recovered and became iron replete. The remaining children could not be classified.

The characteristics of the iron-replete and iron-deficient children at the time of the 2 cross-sectional surveys in May and October 2002 are summarized in table 1. No significant differences in anthropometric characteristics or sex ratios were observed between the 2 groups at either survey, although children in the iron-deficient group were significantly younger at both surveys.

A comparison of immunoglobulin concentrations between the 2 groups is presented in table 2. The concentrations of malaria-specific IgG2, IgG4, and IgE and of total IgE were significantly lower in iron-deficient children than in iron-replete children at both surveys. In addition, the concentration of malaria-specific total IgG was significantly lower in iron-deficient children at the first survey, although the differences seen at the second survey did not reach significance. To further investigate the relationship between iron status and the concentrations of the various immunoglobulins measured at the cross-sectional surveys, we examined the effect of a change in iron status on immunoglobulin concentration in children for whom data were available from both surveys. In general, a change in iron status from iron replete to iron deficient was associated with a fall in rank of the concentration of all immunoglobulins measured, and a change from iron deficient to iron replete was associated with a rise in rank of immunoglobulin concentration. Nevertheless, these differences only attained significance in the case of total malaria-specific IgG and IgG2 ($P = .01$ and $P = .002$, respectively [Kruskal-Wallis test]), possibly because of the small number of children for whom iron status changed over the 6-month period (data not shown). Regression analysis investigating the association between the concentrations of each immunoglobulin and the incidence of malaria during the preceding 6-month period showed strong positive associations for both total malaria-specific IgG and all subclasses of malaria-specific IgG (table 3), supporting the assertion that both assays indicate markers of malaria exposure. However, malaria-specific IgE concentration was associated with subsequent incidence of malaria but did not associate with ex-

Table 1. Clinical characteristics of iron-deficient and iron-replete children.

	May 2002			October 2002		
Characteristic	Iron-replete children ($n = 95$)	Iron-deficient children ($n = 78$)	P	Iron-replete children ($n = 104$)	Iron-deficient children ($n = 91$)	P
Age, years	4.57 (1.71)	3.65 (2.08)	<.01	5.01 (1.81)	4.22 (2.13)	<.01
Log ferritin concentration, μg/mL	1.43 (0.27)	0.78 (0.19)	<.01	1.38 (0.22)	0.77 (0.16)	<.01
Transferrin saturation, %	17.61 (5.35)	5.28 (2.40)	<.01	17.70 (7.17)	5.27 (2.24)	<.01
CRP concentration, mg/L	1.22 (2.44)	1.90 (4.66)	.25	1.41 (2.20)	1.41 (3.85)	.88
WAZ	−1.25 (0.98)	−1.45 (0.97)	.29	−1.44 (0.95)	−1.44 (0.82)	.99
HAZ	−1.58 (0.92)	−1.67 (1.11)	.43	−1.85 (1.03)	−1.77 (1.05)	.62
WHZ	−0.38 (0.98)	−0.56 (0.89)	.47	−0.38 (0.90)	−0.47 (0.84)	.49
Sex ratio[a]	0.72	0.70	1.00	0.65	0.82	.47

NOTE. Data are mean (SD), except where indicated otherwise. Statistical comparisons were done by use of unpaired Student's *t* test (2-sided). See Materials and Methods for definitions of the iron-replete and iron-deficient classifications. CRP, C-reactive protein; HAZ, height-for-age *z* score; WAZ, weight-for-age *z* score; WHZ, weight-for-height *z* score.

[a] Ratio of female:male children. Significance was determined by use of Fisher's exact test.

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018

Table 2. Immunoglobulin concentrations in iron-replete and iron-deficient children.

Immunoglobulin	Concentration, May 2002[a]			Concentration, October 2002[a]		
	Iron-replete children (n = 95)	Iron-deficient children (n = 78)	P	Iron-replete children (n = 104)	Iron-deficient children (n = 91)	P
Malaria specific, AU/mL						
Total IgG	3.74 (0.42)	3.56 (0.45)	<.01	3.95 (0.42)	3.92 (0.44)	.55
IgG1	3.46 (0.55)	3.39 (0.55)	.39	2.91 (0.74)	2.86 (0.79)	.67
IgG2	1.54 (0.62)	1.15 (0.70)	<.001	1.46 (0.65)	1.15 (0.65)	.001
IgG3	2.08 (0.52)	2.02 (0.52)	.46	3.39 (0.47)	3.36 (0.44)	.80
IgG4	2.27 (0.62)	2.06 (0.62)	.03	2.00 (0.69)	1.78 (0.56)	.03
IgE	2.69 (0.42)	2.51 (0.37)	<.01	3.60 (0.41)	3.43 (0.40)	<.01
Total IgE, ng/mL	2.86 (0.58)	2.44 (0.65)	<.001	2.41 (0.45)	2.16 (0.42)	<.001

NOTE. Statistical comparisons were done by use of unpaired Student's t test (2-sided). AU, arbitrary units.

[a] Data were log transformed and are given as mean (SD).

posure to malaria in the preceding season, implying that elevated concentrations of this immunoglobulin could be indicative of susceptibility to disease.

The incidence of clinical malaria among iron-replete and iron-deficient children during the 12-month follow-up period is summarized in table 4. A trend was seen toward a lower incidence of clinical malaria (both definitions 1 and 2) among iron-deficient children, although incidence did not reach significance for either definition. On further analysis by Poisson regression, adjustment for the confounding variables age (fitted as a categorical variable, in 1-year categories), sickle hemoglobin status (hemoglobin AA or hemoglobin AS), season (categorized into 12-week blocks), and ethnic group—all of which were found to be independently associated with malaria when univariate analysis was done—confirmed this effect of iron deficiency to be significant, at least in the case of malaria definition 1 (table 4). Furthermore, in a separate regression analysis that excluded data for children with an increased CRP concentration at the time of ferritin measurement, children with a blood film positive for malaria, and children who had experienced an episode of *P. falciparum* malaria during the preceding 21 days, we found a significant association between log ferritin concentration and malaria incidence. This was true both before and after adjustment for confounding variables: in regression analysis of malaria definition 1 against log ferritin concentration, the adjusted incidence-rate ratio (IRR) was 1.48 (95% CI, 1.01–2.17; $P < .05$). A similar trend was seen for malaria definition 2, although it did not reach statistical significance (IRR, 1.37; 95% CI, 0.88–2.14; $P = .18$). Although previous studies have shown that younger, non–malaria-immune infants show an

Table 3. Association between concentrations of plasma immunoglobulins and the incidence of clinical malaria in the study cohort.

Immunoglobulin	Retrospective study[a]				Prospective study,[b] malaria definition 1	
	Malaria definition 1		Malaria definition 2			
	IRR (95% CI)[c]	P	IRR (95% CI)[c]	P	IRR (95% CI)[c]	P
IgG	2.90 (2.10–3.99)	<.0001	3.70 (2.45–5.57)	<.0001	1.26 (0.90–1.77)	.17
IgG1	2.20 (1.73–2.73)	<.0001	2.15 (1.55–2.99)	<.0001	1.04 (0.85–1.27)	.69
IgG2	1.94 (1.52–2.47)	<.0001	1.87 (1.29–2.69)	<.001	1.23 (1.00–1.52)	.05
IgG3	1.20 (1.03–1.40)	.02	1.35 (1.07–1.70)	.01	1.35 (1.05–1.73)	.02
IgG4	1.84 (1.44–2.34)	<.001	1.78 (1.18–2.68)	<.01	1.15 (0.94–1.40)	.17
Total IgE	1.32 (0.99–1.76)	.06	1.31 (0.82–2.10)	.25	1.07 (0.80–1.43)	.67
Malaria-specific IgE	0.81 (0.62–1.06)	.12	1.18 (0.81–1.73)	.38	1.60 (1.14–2.25)	<.01

NOTE. Log-transformed data for immunoglobulin concentrations were used in the analyses. See Materials and Methods for descriptions of malaria definitions 1 and 2. CI, confidence interval; IRR, incidence-rate ratio.

[a] The 6-month period preceding immunoglobulin assessment.

[b] The 6-month period following immunoglobulin assessment. No significant associations were found between the concentration of any individual immunoglobulin and the incidence of malaria definition 2, with the exception of malaria-specific IgE (IRR, 1.58; 95% CI, 1.09–2.27; $P < .02$).

[c] IRRs were determined by Poisson regression. IRRs for the prospective study were adjusted for the confounding variables age (fitted as a categorical variable, in 1-year categories), season (categorized into 12-week blocks), and ethnic group. Unadjusted IRRs are reported for the retrospective analysis. 95% CIs were inflated for within-person clustering of episodes by use of the "sandwich" estimator, as described by Armitage et al. [20].

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018

Table 4. Incidence of *Plasmodium falciparum* malaria among iron-replete and iron-deficient children during 12 months of follow-up.

Variable	Iron-replete children (n = 154)	Iron-deficient children (n = 125)
No. of child weeks of observation	4545	3788
Malaria definition 1		
No. of episodes	119	73
Annual incidence	1.36	1.0
Adjusted IRR (95% CI)[a]	1.00	0.70 (0.51–0.99)[b]
Malaria definition 2		
No. of episodes	76	50
Annual incidence	0.87	0.69
Adjusted IRR (95% CI)[a]	1.00	0.83 (0.57–1.24)[c]

NOTE. See Materials and Methods for descriptions of malaria definitions 1 and 2. CI, confidence interval; IRR, incidence-rate ratio.

[a] IRRs adjusted for malaria in iron-deficient children, compared with that in iron-replete children. 95% CIs were calculated by Poisson regression and were inflated for within-person clustering of episodes by use of the "sandwich" estimator, as described by Armitage et al. [20].

[b] $P < .05$ (by Poisson regression).

[c] $P = .36$ (by Poisson regression).

advantage in having low iron concentrations [21], this is not the case for older, semi-immune children. We therefore investigated the possibility that age could be acting as an effect modifier in our study, through age-stratified analyses and the fitting of interaction terms. No evidence was found for a role for age as an effect modifier.

Despite the evidence that iron deficiency was protective against clinical malaria, we found no statistical difference in parasite densities during incident malarial infections in iron-replete and iron-deficient children after assessment: for iron-replete children, the geometric mean parasite density during infection was 13,712 parasites/μL (95% reference range, 9031–20,818 parasites/μL), compared with 12,142 parasites/μL (95% reference range, 7350–20,059 parasites/μL) for iron-deficient children ($P = .72$). Furthermore, we found no evidence for an association between iron deficiency (either as a categorical variable or based on log ferritin concentration) and the incidence of nonmalarial fever or morbidity from all diseases, including malaria (data not shown).

DISCUSSION

A protective effect of iron deficiency against malarial infection in humans has been suspected for many years and has been supported by studies of animals [22]. Nevertheless, remarkably few observational studies have assessed the incidence of malaria in cohorts of children whose iron status has been categorized on the basis of biochemical parameters, possibly because of the logistic and ethical considerations associated with such studies.

We investigated the association between iron status and malaria through a number of different approaches and concluded that iron deficiency was associated with a small but significant degree of protection from episodes of clinical malaria in our cohort of young children living on the Kenyan coast. First, the incidence of malaria during prospective surveillance was lower among iron-deficient children than among iron-replete children. Although this effect was strongest for malaria definition 1, trends were consistent for both malaria definitions. Furthermore, evidence of a protective effect of iron deficiency was supported by the significant positive association between log ferritin concentration, obtained at the cross-sectional surveys, and the incidence of malaria in the subsequent 6-month period: since plasma ferritin concentrations correlate positively with iron stores in the body [23, 24], this observation implies that the incidence of malaria decreased with decreasing levels of stored iron. Finally, the concentration of plasma immunoglobulins with specificity for malaria schizont extract was consistently lower in iron-deficient children than in iron-replete children, at the cross-sectional surveys (table 2), and, in general, parallel changes in biochemical iron status and immunoglobulin concentration were observed. Two explanations for these observations are possible. First, the immunoglobulin concentrations simply reflected exposure: iron-deficient children had lower immunoglobulin concentrations because they developed malaria less often during the period preceding assessment. This interpretation of the data is supported by the strong positive association between the concentrations of most immunoglobulins and the incidence of malaria during the period preceding assessment (table 3), suggesting that, as expected, malarial infection resulted in an increase in the concentrations of the immunoglobulins we measured. However, we cannot exclude the reverse explanation: iron deficiency results in a specific defect in immunoglobulin production that is the cause of protection from malaria.

A number of studies have found an association between low concentrations of malaria-specific IgG2 antibodies and protection from malaria [25, 26]. Consistent with these findings, we found that the concentration of malaria-specific IgG2 antibodies was significantly lower in iron-deficient children, among whom the incidence of disease was lowest. Similarly, we found that the concentration of malaria-specific IgE was significantly lower in iron-deficient children and also was associated with the subsequent incidence of malaria. It is possible, therefore, that iron deficiency protects against malaria through an immune mechanism involving a specific defect in the production of particular immunoglobulin subclasses. Nonetheless, our data do not support this position strongly. Although we observed a trend toward an association between low malaria-specific IgG2 and IgG4 concentrations and subsequent malaria episodes, this trend was barely significant and

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018

much weaker than the retrospective association between malaria-specific antibodies and all IgG subclasses (table 3). Furthermore, studies do not suggest that iron deficiency results in defective immunoglobulin production in humans. In recent years, the relationship between iron and immunity has been studied extensively (reviewed by Peto and Hershko [27]). Although specific defects of cell-mediated immunity have been described in iron-deficient subjects in a number of studies [15, 28–30], little evidence has been found of a systemic defect in immunoglobulin production in iron-deficient subjects. Although a number of alternative mechanisms have been proposed whereby iron deficiency might result in protection from malaria, including a limiting effect on parasite nutrition [31–33], further studies will be necessary to determine which mechanisms, if any, are relevant to humans.

Our conclusion that iron deficiency is associated with protection from clinical malaria is predicated on the assumption that our classification of children as iron deficient or iron replete was correct. Biochemical markers of iron status can be unreliable in tropical communities. For example, plasma ferritin is an acute-phase protein and, therefore, is elevated in acute infections and in malaria [16, 34]. Therefore, potential misclassification is the main concern regarding the use of ferritin concentration in the diagnosis of iron deficiency in tropical countries: children who are iron deficient may be categorized as iron replete if their plasma ferritin concentration is falsely high because of concomitant malaria or inflammation. Our classification of iron status included plasma ferritin concentration: we therefore were anxious to exclude any possibility of confounding that might result from the interaction between malaria and ferritin. As a result, in our regression analysis of malaria against iron status, we excluded data from children with evidence of inflammation (CRP concentration $\geqslant$10 mg/L), children with a blood film positive for malaria at the time of assessment, and children who had experienced an episode of malaria during the 21 days prior to assessment. We also excluded children whose results for TFS and ferritin concentration were discordant. Consequently, we excluded ~30% of the children from the analysis, because we could not categorize their iron status with confidence. We believe that, by using these strict exclusion criteria, we minimized the possibility of confounding through misclassification. Nevertheless, our results could be explained by other confounding factors of which we were unaware. One potential group of confounding factors are the hemoglobinopathies, all of which are thought to provide protection from malaria. Although we adjusted our analyses for the effect of sickle-cell trait, we did not have current data on the thalassemia status of the children in our cohort. Although β-thalassemia is not known to occur in this population, α-thalassemia is thought to occur at a frequency of ~40% [35]. Nevertheless, we believe that our findings are unlikely to have been caused by confounding from this condition: available data suggest that α-thalassemia is associated with increased stores of iron [36]. Consequently, if thalassemia was acting as a confounder, we would have expected an over-representation of affected children in the iron-replete group, which would have tended to mask the effect we observed. Further data on the interaction between thalassemia and iron status in African populations are urgently needed.

Two recent meta-analyses of placebo-controlled trials of iron supplementation in humans both suggest a small increase in the risk of malaria after iron supplementation, as evidenced by a range of different markers, including episodes of clinical malaria, diarrhea, and spleen enlargement and by end-of-trial cross-sectional surveys [8, 37]. How our observation that iron deficiency protects children against malaria relates to the distinctly different conclusion that iron supplementation leads to an increased risk of malaria raises an important biological question: if iron deficiency protects against malaria, why should supplementation result in susceptibility? A number of mechanisms seem plausible. First, the explanation might lie in immunological naïveté: a period of relative protection from malaria might result in a failure to develop immunity to the disease. Second, iron supplementation could result in susceptibility due to a direct effect of iron itself: the most dramatic associations between iron supplementation and malaria susceptibility have occurred when iron was given by intramuscular injection [21], which raises the question of whether iron itself or the method or formulation of supplementation is the critical factor. Finally, it is possible that the physiological effects of iron supplementation, rather than iron itself, could predispose children to malaria. For example, in most iron-deficient patients, supplementation is followed by an increased production of reticulocytes, which, in turn, may be more susceptible to infection [38]; alternatively, iron supplementation might result in modulation of the host immune response [39]. Whatever the cause of this reversal, our data suggest an important dilemma for policymakers in malaria-endemic communities: what is the balance between the risks and the benefits of iron supplementation?

Nutritional iron deficiency is recognized as a global health problem. Its prevention is associated with a number of potential health benefits, including prevention of anemia and protection against neurocognitive impairment. Accordingly, the International Nutritional Anemia Consultative Group produced a recent consensus statement [40] based on an extensive review of both published and unpublished literature. On the basis of the evidence available, they concluded that the hematological improvements associated with iron supplementation are large and are likely to confer substantial health benefits. Although they acknowledge that "clinically important risk elevations [for malaria] are not ruled out" [40, p. 2] by the available data, in view of the balance of current

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018

evidence in favor of benefit over risk they strongly advocate the active promotion of iron-supplementation programs in malaria-endemic areas, in conjunction with continued vigilance in the monitoring of such programs for adverse effects. We suggest that such monitoring is not a trivial exercise: subtle effects on the incidence of malaria, severe malaria, and other infections will be identified only through large, well-conducted, placebo-controlled trials designed specifically to address this issue. To date, none of the studies conducted have been designed or have the power to address this question. Although the conclusion that high rates of iron deficiency are bad for the health of communities seems intuitively obvious [37], widespread support for iron-supplementation programs will only be embraced in malaria-endemic communities once the risk-benefit question has finally been answered.

Acknowledgments

We thank Hedvig Perlmann, Margreta Hargstedt, and Brett Lowe for their technical support. We acknowledge an anonymous reviewer for providing helpful comments. This article is published with the permission of the director of KEMRI.

References

1. Murray MJ, Murray NJ, Murray AB, Murray MB. Refeeding-malaria and hyperferraemia. Lancet **1975**; 1:653–4.
2. Oppenheimer SJ, Gibson FD, Macfarlane SB, et al. Iron supplementation increases prevalence and effects of malaria: report on clinical studies in Papua New Guinea. Trans R Soc Trop Med Hyg **1986**; 80:603–12.
3. Smith AW, Hendrickse RG, Harrison C, Hayes RJ, Greenwood BM. The effects on malaria of treatment of iron-deficiency anaemia with oral iron in Gambian children. Ann Trop Paediatr **1989**; 9:17–23.
4. Menendez C, Kahigwa E, Hirt R, et al. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. Lancet **1997**; 350:844–50.
5. Verhoef H, West CE, Nzyuko SM, et al. Intermittent administration of iron and sulfadoxine-pyrimethamine to control anaemia in Kenyan children: a randomised controlled trial. Lancet **2002**; 360:908–14.
6. Berger J, Dyck JL, Galan P, et al. Effect of daily iron supplementation on iron status, cell-mediated immunity, and incidence of infections in 6–36 month old Togolese children. Eur J Clin Nutr **2000**; 54:29–35.
7. Desai MR, Mei JV, Kariuki SK, et al. Randomized, controlled trial of daily iron supplementation and intermittent sulfadoxine-pyrimethamine for the treatment of mild childhood anemia in western Kenya. J Infect Dis **2003**; 187:658–66.
8. Shankar AH. Nutritional modulation of malaria morbidity and mortality. J Infect Dis **2000**; 182(Suppl 1):S37–53.
9. Dhur A, Galan P, Hercberg S. Iron status, immune capacity and resistance to infections. Comp Biochem Physiol A **1989**; 94:11–9.
10. Snow RW, Omumbo JA, Lowe B, et al. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. Lancet **1997**; 349:1650–4.
11. Mbogo CN, Snow RW, Kabiru EW, et al. Low-level *Plasmodium falciparum* transmission and the incidence of severe malaria infections on the Kenyan coast. Am J Trop Med Hyg **1993**; 49:245–53.
12. Mwangi TW. Clinical epidemiology of malaria under differing levels of transmission [thesis]. Oxford: Open University, **2003**.
13. Nicoll A, Ulijaszek S. Modifications of the AHRTAG child length measurer. Trop Doct **1987**; 17:129–31.
14. Kasvosve I, Delanghe JR, Gomo ZA, et al. Transferrin polymorphism influences iron status in blacks. Clin Chem **2000**; 46:1535–9.
15. Galan P, Thibault H, Preziosi P, Hercberg S. Interleukin 2 production in iron-deficient children. Biol Trace Elem Res **1992**; 32:421–6.
16. Phillips RE, Looareesuwan S, Warrell DA, et al. The importance of anaemia in cerebral and uncomplicated falciparum malaria: role of complications, dyserythropoiesis and iron sequestration. Q J Med **1986**; 58:305–23.
17. Perlmann H, Helmby H, Hagstedt M, et al. IgE elevation and IgE antimalarial antibodies in *Plasmodium falciparum* malaria: association of high IgE levels with cerebral malaria. Clin Exp Immunol **1994**; 97:284–92.
18. Troye-Blomberg M, Perlmann H, Patarroyo ME, Perlmann P. Regulation of the immune response in *Plasmodium falciparum* malaria. II. Antigen specific proliferative responses in vitro. Clin Exp Immunol **1983**; 53:345–53.
19. Smith T, Schellenberg JA, Hayes R. Attributable fraction estimates and case definitions for malaria in endemic areas. Stat Med **1994**; 13:2345–58.
20. Armitage P, Berry G, Matthews JNS. Using STATA's robust cluster command as appropriate. In: Armitage P, Berry G, Matthews JNS. Statistical methods in medical research. 4th ed. Oxford: Blackwell Scientific Publications, **2001**:378–454.
21. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr **2001**; 131:616S–35S.
22. Harvey PW, Bell RG, Nesheim MC. Iron deficiency protects inbred mice against infection with *Plasmodium chabaudi*. Infect Immun **1985**; 50:932–4.
23. Ances IG, Granados J, Baltazar M. Serum ferritin as an early determinant of decreased iron stores in pregnant women. South Med J **1979**; 72:591–2, 604.
24. Jacob RA, Sandstead HH, Klevay LM, Johnson LK. Utility of serum ferritin as a measure of iron deficiency in normal males undergoing repetitive phlebotomy. Blood **1980**; 56:786–91.
25. Bouharoun-Tayoun H, Druilhe P. *Plasmodium falciparum* malaria: evidence for an isotype imbalance which may be responsible for delayed acquisition of protective immunity. Infect Immun **1992**; 60:1473–81.
26. Ndungu FM, Bull PC, Ross A, Lowe BS, Kabiru E, Marsh K. Naturally acquired immunoglobulin (Ig)G subclass antibodies to crude asexual *Plasmodium falciparum* lysates: evidence for association with protection for IgG1 and disease for IgG2. Parasite Immunol **2002**; 24:77–82.
27. Peto TE, Hershko C. Iron and infection. Baillieres Clin Haematol **1989**; 2:435–58.
28. Bagchi K, Mohanram M, Reddy V. Humoral immune response in children with iron-deficiency anaemia. Br Med J **1980**; 280:1249–51.
29. Krantman HJ, Young SR, Ank BJ, O'Donnell CM, Rachelefsky GS, Stiehm ER. Immune function in pure iron deficiency. Am J Dis Child **1982**; 136:840–4.
30. Jarvis JH, Jacobs A. Morphological abnormalities in lymphocyte mitochondria associated with iron-deficiency anaemia. J Clin Pathol **1974**; 27:973–9.
31. Raventos-Suarez C, Pollack S, Nagel RL. *Plasmodium falciparum:* inhibition of in vitro growth by desferrioxamine. Am J Trop Med Hyg **1982**; 31:919–22.
32. Fritsch G, Sawatzki G, Treumer J, Jung A, Spira DT. *Plasmodium falciparum:* inhibition in vitro with lactoferrin, desferriferrithiocin, and desferricrocin. Exp Parasitol **1987**; 63:1–9.
33. Fritsch G, Treumer J, Spira DT, Jung A. *Plasmodium vinckei:* suppression of mouse infections with desferrioxamine B. Exp Parasitol **1985**;60: 171–4.
34. Adelekan DA, Thurnham DI. Plasma ferritin concentrations in anemic children: relative importance of malaria, riboflavin deficiency, and other infections. Am J Clin Nutr **1990**; 51:453–6.
35. Yates SNR. Human genetic diversity and selection by malaria. Oxford: Institute of Molecular Medicine, **1995**.

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018

36. Rees DC, Williams TN, Maitland K, Clegg JB, Weatherall DJ. Alpha thalassaemia is associated with increased soluble transferrin receptor levels. Br J Haematol **1998**; 103:365–9.
37. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. BMJ **2002**; 325:1142–52.
38. Pasvol G, Weatherall D, Wilson RJM. The increased susceptibility of young red cells to invasion by the malaria parasite *Plasmodium falciparum*. Br J Haematol **1980**; 45:285–95.
39. Weiss G, Wachter H, Fuchs D. Linkage of cell-mediated immunity to iron metabolism. Immunol Today **1995**; 16:495–500.
40. International Nutritional Anemia Consultative Group. Consensus statement: safety of iron supplementation programs in malaria-endemic regions. Washington, DC: International Life Sciences Institute's Human Nutrition Institute, **1998**:1–6.

Downloaded from https://academic.oup.com/jid/article-abstract/190/3/439/937757
by Moi University user
on 16 July 2018