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Research Article

Malaria and Age Variably but Critically Control Hepcidin Throughout Childhood in Kenya

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ABSTRACT

Both iron deficiency (ID) and malaria are common among African children. Studies show that the iron-regulatory hormone hepcidin is induced by malaria, but few studies have investigated this relationship longitudinally. We measured hepcidin concentrations, markers of iron status, and antibodies to malaria antigens during two cross-sectional surveys within a cohort of 324 Kenyan children ≤8 years old who were under intensive surveillance for malaria and other febrile illnesses. Hepcidin concentrations were the highest in the youngest, and female infants, declined rapidly in infancy and more gradually thereafter. Asymptomatic malaria and malaria antibody titres were positively associated with hepcidin concentrations. Recent episodes of febrile malaria were associated with high hepcidin concentrations that fell over time. Hepcidin concentrations were not associated with the subsequent risk of either malaria or other febrile illnesses. Given that iron absorption is impaired by hepcidin, our data suggest that asymptomatic and febrile malaria contribute to the high burden of ID seen in African children. Further, the effectiveness of iron supplementation may be sub-optimal in the presence of asymptomatic malaria. Thus, strategies to prevent and eliminate malaria may have the added benefit of addressing an important cause of ID for African children.

1. Introduction

Malaria and iron deficiency (ID) are major public health problems for children living in sub-Saharan Africa. Malaria caused an estimated 437,000 deaths in young African children in 2013 (WHO, 2014) and >70% of children have asymptomatic malaria in some malaria-endemic areas (Houngbedji et al., 2015), while ID is thought to impair cognitive development (Black et al., 2011) and is the leading cause of years lived with disability in sub-Saharan Africa (Vos et al., 2012). Hepcidin, the iron-regulatory hormone, may provide a critical link between malaria and ID. Hepcidin controls the absorption and distribution of iron (Ganz, 2013) and is thought to play a role in the innate immune response by restricting iron availability for pathogen growth (Ganz, 2009; Drakesmith and Prentice, 2012). The synthesis of hepcidin is regulated by diverse, often competing, physiological processes, including iron stores, inflammation and erythropoietic drive (Ganz, 2011; Atkinson et al., 2014). Malaria also alters hepcidin concentrations. Febrile malaria is associated with increased plasma concentrations (Howard et al., 2007; de Mast et al., 2009; Casals-Pascual et al., 2012; Ayoya et al., 2009), while severe and complicated malaria is associated with reduced plasma levels in African children (Casals-Pascual et al., 2012; Burte et al., 2013). Asymptomatic malaria also increased plasma levels in Indonesian school-age children (de Mast et al., 2010). In turn, we hypothesized that hepcidin may mediate the risk of malaria and other infections by restricting iron availability (Ganz, 2009; Drakesmith and Prentice, 2012). Intriguing data from mouse models suggest that hepcidin may play a critical role in host defence against

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malaria (Wang et al., 2011), malaria superinfection (Portugal et al., 2011), and bacterial infection (Arezes et al., 2015), but how this may work in children is not known. In the current study, our objectives were to assess the effect of a range of factors including age, gender and malaria on hepcidin concentrations and in turn to assess the effect of hepcidin concentrations on subsequent infectious risk in a longitudinal surveillance study of Kenyan children intensively monitored for malaria and other febrile illnesses.

2. Materials and Methods

2.1. Ethics Statement

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

2.2. Participants and Procedures

The current study was nested within an ongoing, longitudinal cohort study evaluating the history and acquisition of natural immunity to malaria in children living in Kilifi District on the Kenyan coast (Mwangi et al., 2005). The current study involving 324 children was conducted during an 18-month period between November 2001 and May 2003 and included all children <8 years of age within the Ngerenya study area (Fig. 1). Participants were monitored for malaria and other diseases by weekly active surveillance as previously described (Mwangi et al., 2005). Two cross-sectional surveys were conducted at 6 and 12 months after the start of the study during which venous blood samples were collected. Children exited the study if informed consent was withdrawn or if they moved out of the study area for a period of >2 months.

2.3. Laboratory Procedures

Plasmodium falciparum parasitaemia was determined as previously described (Nyakeriga et al., 2004). Haemoglobin typing (HbA and HbS) was by electrophoresis (Helena Laboratories, Beaumont, TX) while α-thalassemia genotyping was by PCR (Chong et al., 2000). Plasma concentrations of ferritin, soluble transferrin receptor (sTfR) and C-reactive protein (CRP) were determined as previously described (Atkinson et al., 2014; Nyakeriga et al., 2004). IgG antibodies against whole *P. falciparum* schizont extract and against the 3D7 allele of apical membrane antigen 1 (AMA1) and merozoite surface protein 2 (MSP2) were assayed by enzyme linked immunosorbent assay (ELISA) (Mugyenyi et al., 2013).

Plasma hepcidin was quantified by competitive ELISA (Hepcidin-25 (human) EIA Kit, Bachem) (Atkinson et al., 2014). Standards and samples were analyzed in duplicate or triplicate. Samples giving readings outside the standard linear region were repeated at appropriate dilutions. Readings with coefficient of variation >10% were repeated. The lower limit of detection (LOD) of hepcidin was estimated at 0.08 ng/ml based on the hepcidin value corresponding to 3 standard deviations below the mean no hepcidin blank optical density at 450 nm; undiluted samples giving reading of <LOD were reported as LOD/2 = 0.04 ng/ml.

2.4. Case Definitions

Clinical malaria was defined as a fever (axillary temperature ≥37.5 °C) in conjunction with a positive blood smear for *P. falciparum* parasites at any density for children age <1 year or at a density of >2500 parasites/μl for children age ≥1 year (Mwangi et al., 2005). Asymptomatic malaria was defined during cross-sectional surveys as smear positive *P. falciparum* malaria in the absence of fever or other symptoms of clinical illness, while non-malarial fever was defined as a fever in conjunction with a negative malaria blood smear. Inflammation was defined as plasma CRP concentration of ≥5 mg/l (WHO, CDC, 2007). ID was defined as a ferritin concentration of <12 μg/l, or <30 μg/l in the presence of inflammation respectively (Atkinson et al., 2014; WHO/UNICEF/UNU, 2001). The ferritin index, a measure of bone marrow iron depletion, was defined as soluble transferrin receptor/log ferritin (Punnonen et al., 1997).

2.5. Statistical Analyses

All analyses were conducted using STATA v.12.0 (StataCorp. College Station, TX). Associations between hepcidin concentration (or other variables such as iron status) and independent parameters were evaluated using generalized estimating equation (GEE)-based linear regression models that included an exchangeable correlation structure and a robust variance estimator to account for correlation between measurements at two time points from the same child. Analyses were age-adjusted as appropriate. We did not restrict fitting independent parameters, such as age, to linear effects. We allowed for nonlinear effects by fitting and significance testing multivariable fractional polynomials with use of the Royston and Altman algorithm entering hepcidin concentration and other variables simultaneously in the model. This allowed the model to optimize the model fit using power and log functions to approximate the shape of the relationship of the parameter with hepcidin (Royston and Altman, 1994). The association between hepcidin concentration and the subsequent risk of clinical malaria or non-malarial fever was evaluated using Cox proportional hazards analysis during the 6-month period of monitoring after each cross-sectional survey. Therefore, each of the 324 children could contribute up to 2 periods of observation and the sandwich estimator was used to cluster analysis by individual (Armitage et al., 2001).

Multivariable models included covariates with a significance of $p \le 0{\cdot}1$ in univariable models. We used $p < 0{\cdot}05$ to interpret the findings in the final multivariable model. For clinical malaria hazards ratios were adjusted for age, ethnicity, sickle cell trait and period of monitoring and

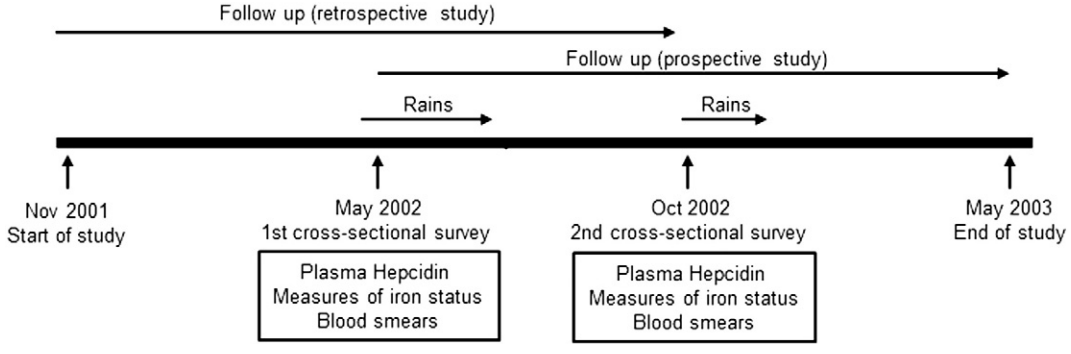

Fig. 1. Study construction. A total of 324 children were recruited to the study; 245 contributed data to both the May and October surveys, 48 to the May survey only and 31 to the October survey only.