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Haptoglobin phenotypes and iron status in children living in a malaria endemic area of Kenyan coast

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ABSTRACT

Malaria infection may be affected by host genetic factors as well as nutritional status. Iron status and the phenotype of haptoglobin, a heme-binding acute phase reactant may be determinants of malaria parasitemia. A combination of cross sectional studies and longitudinal follow-up were used to describe the association between iron status, C-reactive protein, malaria infections and host genetic factors including; haptoglobin (Hp) phenotypes, in children below 9 years in a malaria endemic area in Coastal Kenya. The prevalence of 0.45 and 0.41, respectively for Hp 1-1 and Hp 2-1 phenotypes was significantly higher than 0.14 for Hp 2-2 phenotype (n = 162). Children with Hp 2-2 phenotype showed significantly higher iron storage compared to those with Hp 1-1 and Hp 2-1 phenotypes when children with malaria parasites and high C-reactive protein (>9 mg/L) were excluded from the analysis. There were no significant differences in malaria parasite densities among Hp phenotypes but children with Hp 2-2 had lower number of clinical malaria episodes (P = 0.045). Taken together, this study shows that the presence of malaria may complicate the interpretation of iron status in children based on their Hp-phenotypes. Further studies will be required to address possible interactions among the various genetic factors and iron status in a malaria endemic setting.

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1. Introduction

Iron status in man is influenced by both environmental and genetic factors. Molecular variation of haptoglobin (Hp), a plasma protein, is one of the genetic factors known to influence iron status (Langlois et al., 2000). Hp is a type II acute phase protein secreted by hepatocytes during an inflammatory reaction. In humans, Hp is characterized by a genetic polymorphism with three structurally different phenotypes (Hp 1-1, Hp 2-1, and Hp 2-2) which are the result of the expression of two different alleles; *Hp1* and *Hp2*, of the Hp gene located on chromosome 16q22 (Langlois and Delanghe, 1996). Hp is responsible for the removal of free hemoglobin (Hb) in the form of Hp–Hb complexes from the circulation with subsequent reduction in Hb loss through the kidney. Consequently, the kidney is protected against damage and iron recycling is permitted. Hp 2-2 is associated with higher serum iron and transferrin saturation levels as compared to other phenotypes reflecting a relative increase in the iron transport (Delanghe and Langlois, 2002). For example, Hp 2-2 male Caucasians have been characterized with larger iron stores as evidenced by higher serum ferritin levels compared to their Hp 1-1 and Hp 2-1 counterparts (Langlois et al., 2000).

Haptoglobin phenotypes differ in their biological activities and have been associated with resistance or susceptibility to infectious diseases (Langlois and Delanghe, 1996). Iron withholding is an important example of nutritional immunity playing a role in the defense against infectious diseases (Pradines et al., 2003; Weiss et al., 1994, reviewed in Gordeuk et al., 2001; Weinberg, 2009). Haptoglobin acts as a natural bacteriostatic agent by preventing the utilization of hemoglobin by pathogenic bacteria, which require iron for their growth. In malaria endemic areas, Hp 1-1 but not Hp 2-2 with disease severity (Elagib et al., 1998), perhaps through iron withholding related mechanisms. While the role of iron in malaria susceptibility remains controversial, there is evidence that iron deficiency protects against disease (reviewed in Oppenheimer, 2001). We previously observed an association between iron deficiency and protection against malaria infection in a cohort of children living on the coast of Kenya (Nyakeriga et al., 2004). Given the existing association between haptoglobin polymorphism and iron status or malaria susceptibility, there was need to investigate the interaction of these three conditions in our cohort of children. We subsequently investigated the effect of haptoglobin polymorphism on biochemical markers of iron and malaria infection.

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2. Methodology

2.1. Study population

The study was conducted in Ngerenya area of Kilifi District located in the north-coast of Kenya. The study population consisted of a cohort of children <9 years old, belonging to the Mgiriama ethno linguistic group. Malaria transmission in this area occurs throughout the year, although the majority of clinically evident infections occur following short and long rains that generally occur during the months of October–November and March–July respectively (Snow et al., 1997). Residents of Ngerenya experience an average of 10 malaria infective bites/person/year (Mbogo et al., 1993). Our cohort was under weekly surveillance for clinical malaria events between Nov 2001 and May 2003. In addition, we conducted six-monthly cross-sectional surveys in May and October, 2002. At each survey, routine data were collected regarding a range of clinical parameters including axillary temperature, clinical symptoms of fever and the presence of malaria parasites on a finger-prick blood film. In addition, a venous blood sample, collected during the survey in May, was used to determine markers of iron status (including serum iron, transferrin, ferritin and sTfR) and, total as well as malaria-specific immunoglobulins (total IgG and IgE, and malaria–specific immunoglobulin subclasses including IgG1, IgG2, IgG3, and IgG4) in plasma samples.

2.2. Laboratory assays

Plasma samples obtained in May 2002 were used for iron assays and immunoglobulin measurements. Ferritin and transferrin were measured by turbidimetry and plasma iron by Ferrozine®-based photometry using an automated analyzer (Hitachi 917, Hitachi Corp., Japan). Transferrin saturations (TFS; %) were calculated from the plasma iron and transferrin measurements by standard methods (Kasvosve et al., 2000; Langlois et al., 2000). We measured soluble transferrin receptor (sTfR) concentration by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (R&D Systems, Minneapolis). Plasma ferritin is an acute phase reactant and is unreliable in the presence of a range of infections and inflammatory conditions. We therefore measured C-reactive protein (CRP) (by turbidimetry) as an independent measure of inflammation in order to aid interpretation. The hemoglobin typing was done by agarose gel electrophoresis and anti-malarial antibodies were measured against crude parasite sonicate antigen using ELISA as previously described (Nyakeriga et al., 2004). The alpha-thalassaemia genotyping was done according to Chong and others (Chong et al., 2000).

The haptoglobin phenotype was determined using starch gel electrophoresis of hemoglobin-supplemented serum. Visualization of Hp–Hb complex bands was achieved by staining the gel with metal-enhanced peroxide reagents as described by Delanghe and others (Delanghe et al., 2000).

Blood films were stained with Giemsa and examined for malaria parasites by standard microscopy. Parasite densities were recorded as a ratio of parasites to white blood cells (read from thick smears), or to red blood cells (from thin smears) for heavier infections. Densities (parasites/μl whole blood) were then calculated assuming a white blood count of 8×10^3/μl or a red cell count of 5×10^6/μl.

2.3. Statistical analysis

Continuous data (CRP, markers of iron status, parasite density and age) were checked for normality and homogeneity of variance respectively by Kolmogorov–Smirnov and Levene tests. All variables, except for immunglobulins levels, failed to meet the assumptions of normality even after log and square-root transformation. Consequently, the effects of Hp phenotypes on these variables were determined using a non-parametric Kruskal-Wallis test. Chi square test was used to determine the association between Hp phenotypes and host gender, malaria prevalence, iron deficiency, and sickle cell and α-thalassaemia traits. Analysis of variance (ANOVA) was used to analyze the differences in immunoglobulin levels among Hp groups. The nutritional status was presented as height-for-age (HAZ), weight-for-age (WAZ) or as weight-for-height (WHZ) *z*-scores. Iron deficiency was defined according to low plasma ferritin in combination with low saturation transferrin as we previously described (Nyakeriga et al., 2004). Clinical malaria episode was defined as presence of blood smear parasite positive and an axillary temperature of >37 °C. All analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC). Statistical significance was set at P value $\leq$0.05.

The Kenya Medical Research Institute National Ethical Review Committee granted ethical permission for this study. Individual written informed consent was obtained from the parents of all study participants.

3. Results

The prevalence of 0.14 for Hp 2-2 phenotype was significantly lower than 0.45 and 0.41, respectively for Hp 1-1 and Hp 2-1 phenotypes ($n = 162$, $\chi = 27.44$, $df = 2$, $P < 0.0001$; Table 1). The observed Hp phenotype distribution was in agreement with the Hardy-Weinberg equilibrium.

The anthropometric measures (data not shown), age and proportion of parasite positive blood smears as well as the female to male ratio were comparable among the three phenotypes. The biochemical markers of iron status were comparable when children with blood smears parasite positive or with high CRP (>9 mg/L) were included in the analysis (data not shown). However, when these children (with inflammation or malaria parasites) were excluded from analysis there was a trend towards iron sufficiency in children with Hp 2-2 phenotype compared to those with other Hp phenotype, with transferrin and sTfR reaching statistical significance (Table 1). While remaining relatively higher in children with Hp 2-2 phenotype, differences in plasma ferritin, iron and transferrin saturation levels did not reach significance. Similarly, the proportion of children that were defined as being iron deficient was highest in children with Hp 1-1 phenotype and lowest in those with Hp 2-2 phenotypes, but the differences did not reach significance. Further analysis revealed no significant differences in the proportion of children with sickle cell trait or alpha-thalassaemia among the Hp phenotype, as shown in the table.

We further investigated the level of anti-malarial antibody levels (Fig. 1). There was no difference in the antibody levels among the Hp groups. Similarly, we compared the prevalence of clinical malaria episodes in the children by their haptoglobin phenotypes as summarized in Table 2. In comparison to children with Hp 1-1, and Hp 2-1, children with Hp 2-2 phenotype had lower incidences of clinical malaria. There was no child with more than one clinical malaria episode in the six months of follow-up preceding assessment of iron indices ($P = 0.045$).

4. Discussion

In this study we assessed the association of haptoglobin phenotypes, iron status and susceptibility/resistance to malaria infection. We have shown that there was a significant association between biochemical markers of iron and haptoglobin polymorphism. Compared to the other phenotypes Hp 2-2 demonstrated lower levels of plasma sTfR and higher plasma transferrin, raised ferritin but lower levels of plasma iron. Overall, these markers of iron indicated

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