Transactions of the Royal Society of Tropical Medicine and Hygiene 104 (2010) 713–719

Contents lists available at ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Influence of haemoglobins S and C on predominantly asymptomatic *Plasmodium* infections in northern Ghana

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ARTICLE INFO

Article history:
Received 10 February 2010
Received in revised form 2 August 2010
Accepted 2 August 2010

Keywords:
malaria
sickle
haemoglobin C
Plasmodium malariae
Ghana
dry season

ABSTRACT

The haemoglobin (Hb) variants HbS and HbC protect against severe malaria. Yet, the influence particularly of HbC on asymptomatic or mild *Plasmodium* infection is not well established.

In a dry season cross-sectional survey among 2108 children aged 0.5-9 years in the Northern Region of Ghana, *Plasmodium* species and density, as well as Hb, were analysed with respect to Hb genotypes. HbAC occurred in 19.7% and HbAS in 7.4% (HbSC, 0.8%; HbCC, 0.8%; HbSS, 0.3%). Overall, 56% of the children had microscopically visible parasitaemia. By PCR, *P. falciparum, P. malariae,* and *P. ovale* were present in 74.5%, 9.7%, and 5.5%, respectively. Febrile parasitaemia was rare (2.8%) but anaemia (Hb < 11 g/dL) frequent (59.3%). Children with HbAA and HbAC showed virtually identical malariometric parameters. In contrast, children with HbAS had significantly less parasitaemia, lower parasite densities, and a higher proportion of submicroscopic *P. falciparum* infection. Remarkably, in children with HbCC, *P. malariae* infection occurred in 37.5% (adjusted odds ratio (aOR), 5.8; 95% CI, 1.8-18.8) and *P. ovale* in 18.8% (aOR, 3.61; 95% CI, 0.97-13.5).

In this population with predominantly asymptomatic *Plasmodium* infection, HbAC shows no discernible effect on malaria-related parameters. Homozygous HbC, in contrast, confers an increased risk of *P. malariae* infection which conceivably may modulate falciparum malaria.

1. Introduction

The high prevalence of the sickle cell trait (haemoglobin (Hb) AS) in malaria-endemic regions and vast epidemiological evidence support the hypothesis that HbAS is under evolutionary selection because of the protection it confers against malaria.[1] Such protection may range from infection per se up to death due to severe disease. In areas of intense and stable transmission, the majority of *Plasmodium falciparum* infections are seen in asymptomatic children, fewer cause mild disease, and only 1-2% of malaria cases are considered to result in life-threatening, severe and complicated malaria.[2] In such and other areas, HbAS has been shown to provide relative resistance against all-cause mortality, severe malaria, uncomplicated malaria, and high density parasitaemia; in general, protection appears to increase with increasing disease severity.[3–6] The picture is less clear with respect to the bulk of infections, i.e.,

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doi:10.1016/j.trstmh.2010.08.001

asymptomatic parasitaemia, and regarding parasite density in such episodes.[1,3–5,7,8]

As in HbS, an amino-acid substitution ($\alpha_2\beta_2^{Glu6\rightarrow Lys}$) in adult HbA forms the underlying disorder of HbC. This trait occurs in polymorphic frequencies almost exclusively in the northern savannahs of West Africa. Case-control studies have revealed that HbAC and even more so HbCC protect against clinical malaria in Burkina Faso and against severe malaria in Mali and Ghana.[4,6,9,10] However, effects on the mere prevalence of *P. falciparum* infection and on parasite densities are generally not seen.[4,11,12] One longitudinal study from Burkina Faso reported negative associations of HbC (heterozygous and homozygous combined) with mild malaria and with parasite density in these episodes.[13]

The bottom of the malaria pyramid is formed by asymptomatic infections which influence the incidence of clinical malaria. Asymptomatic infections decreased the prospective risk of subsequent malaria in Senegal and Mali[14,15] but increased it in Uganda.[16] Unraveling potential effects of the haemoglobinopathies already at this level may contribute to a better understanding of malaria epidemiology, of the mechanism of protection, and of the increasingly realized interaction of the Hb variants upon reappearance of parasitaemia or clinical malaria after antimalarial interventions.[15,17]

In the hyperendemic Northern Region of Ghana, we therefore examined the influence of HbC and HbS on malariometric indices among more than 2000 predominately asymptomatic children including the PCR-based ascertainment of submicroscopic infections and *Plasmodium* species.

2. Material and methods

The cross-sectional study was performed between January and April 2002 (i.e., in the dry season) in the Northern Region of Ghana. Malaria in the region is hyperendemic with perennial transmission and modest seasonal variation.[18] At the time of study conduct, malaria control relied largely on treatment with chloroquine (CQ), despite widespread drug resistance.[19] Bed nets were not commonly used.[20] More than half of the children with severe malaria admitted to the regional hospital show severe anaemia.[21]

Recruitment procedures have been described in detail elsewhere.[18] Briefly, ≥70 children aged six months to nine years were randomly recruited in 30 communities or census units in the regional capital Tamale and the surrounding districts following a two-stage sampling strategy with probability proportional to population size.

Age, sex, and axillary temperature of every child were documented, and a venous blood sample collected into ethylendiamintetraacetat (EDTA). Fever was characterized as an axillary temperature ≥37.5 °C. Spleen size was palpated, and a Hackett score of ≥2 (palpable below costal margin) considered a robust indicator of enlargement. Hb concentrations were measured by a HemoCue photometer (HemoCue AB, Ångelholm, Sweden), and anaemia was defined as Hb <11 g/dL. Malaria parasites were counted per 200 white blood cells (WBCs) on Giemsa-stained thick blood films, and parasite density was calculated assuming a mean WBC count of 8000/μL. Herein, 'parasitaemia' refers to a positive result on expert microscopy. Malaria was defined as any parasitaemia plus fever. Children with mild malaria and all children with >5000 parasites/μL were treated with sulfadoxine-pyrimethamine. Patients with severe malaria[21] were transferred to the next hospital for treatment. Other diseases were treated according to Ghana Health Service regulations. Full blood aliquots were stabilized (AS1, Quiagen, Washington, DC, USA), and DNA was extracted by commercial kits (Qiamp blood kit; Qiagen). *Plasmodium* species and submicroscopic infections were ascertained by nested PCR assays including negative and positive controls.[22] Hb genotypes were identified by restriction fragment-length polymorphisms of PCR-generated amplicons.[10] CQ blood concentrations were measured by enzyme-linked immunosorbent assay, with a detection limit of 31 nmol/L.[23]

In this secondary analysis of epidemiological data, malariologic and haematological parameters were compared between groups defined by their haemoglobin genotype. Parasite densities were normalized by log10 transformation, and geometric mean parasite densities (GMPDs) and 95% confidence intervals (CIs) were calculated. Continuous variables were compared between groups by Student's t test, analysis of variance (ANOVA), or Mann-Whitney U test, and proportions by χ^2 test or Fisher's exact test as applicable. Odds ratios (ORs) and 95% CIs were computed. Factors independently associated with *Plasmodium* infection were identified by logistic regression models.

3. Results

The mean age of the 2108 children (1077 girls, 1031 boys) was 49 months (range, 6-108 months); 60.1% (1266) were under the age of five years. Overall, 29.1% (614), 25.2% (531), and 45.7% (963) of the children lived in communities or census units with populations of <1000, 1000-10 000, and >10 000, respectively; 754 (35.8%) originated from the district of Tamale.

3.1. Malariometric parameters

Microscopically visible parasitaemia was present in 55.6% (1171) of the children, at overall low parasite density (GMPD, 505/μL; 95%CI, 459-555/μL). By PCR, *P. falciparum, P. malariae* and *P. ovale* occurred in 74.5% (1570), 9.7% (205), and 5.5% (115), respectively. Of these infections, 26.1% (409/1570), 21.5% (44/205) and 27.0% (31/115), respectively, were positive by PCR only, i.e., submicroscopic. Co-infection with *P. falciparum* was observed in 92.2% (189/205) of *P. malariae* infections and in 91.3% (105/115) of the *P. ovale* infections. Fever was present in 5.8% (122/2098) and febrile parasitaemia in only 2.8% (58/2098) of the children; 45.6% (956/2098) showed an enlarged spleen (Hackett score 2). For 10 children, data on body temperature were either not reliable, not written down or not measured. With age, parasitaemia and PCR-detected infections with all *Plasmodium* species increased in prevalence (each, $P < 0.001$) whereas a trend only was seen for GMPD by microscopy ($P = 0.1$; Figure 1).