

Title: Lack of mortality in 22 children with sickle cell anemia and severe malarial anemia

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Abbreviation table

Abbreviation	Full term
CC	Community Control
CM	Cerebral Malaria
DNA	De-oxyribonucleic Acid
SCA	Sickle cell anemia
SMA	Severe Malarial Anemia

Abstract

Retrospective studies suggest that high mortality in children with sickle cell anemia (SCA) and severe malaria. We assessed mortality in Ugandan children with severe malarial anemia (SMA, n= 232) or cerebral malaria (CM, n= 267) by HbS genotype. Admission and 2-year follow-up mortality did not differ among children with SMA who had HbSS vs HbAA (admission, 0/22, 0%, vs. 1/208, 0.5%; follow-up, 1/22, 4.5%; 7/207, 3.4%, respectively, all $P>0.6$). The single child with CM and HbSS survived. The study findings highlight the need for large prospective studies of malaria-related mortality in children with SCA.

Introduction

Sickle cell hemoglobin (HbS) is the most common pathological hemoglobin variant worldwide (1). Heterozygotes (HbAS) are usually asymptomatic and protected against malaria (5,6), whereas homozygotes (HbSS, sickle cell anemia, SCA) are prone to end organ damage (2-4) and, in many parts of sub-Saharan Africa, early death (2). Children with HbSS may not have an increased risk of developing malaria, but two studies with small numbers of children with SCA and severe malaria (n=5 and n=21, respectively) suggest they have a high mortality rate with severe malaria (7,8). To further address the question of mortality with severe malaria in children with SCA, we compared risk of inpatient and follow-up mortality according to HbS genotype in a prospectively enrolled cohort of children with severe malaria (cerebral malaria [CM] or severe malarial anemia [SMA]) and healthy community controls.

Methods and Results

Study participants. The study was carried out at Mulago National Referral Hospital in Kampala, Uganda (9). All participants were enrolled in a study assessing neurodevelopmental impairment in severe malaria, conducted from November 2008 to December 2013. Details of the methods of the primary study including inclusion criteria and study enrollment are described elsewhere (10). Briefly, children 18 months – 12 years of age were enrolled. CM was defined as a child with unarousable coma and *Plasmodium falciparum* parasitemia on blood smear. SMA was defined a child with a hemoglobin level ≤ 5 g/dL and *P. falciparum* parasitemia on blood smear. Community

children were healthy children from the neighborhoods of children with severe malaria children. Children with known chronic disease, including known SCA, were excluded.

Clinical management. Children with CM or SMA were managed according to Ugandan Ministry of Health treatment guidelines for malaria, which at time of study included intravenous quinine treatment followed by oral quinine. All children with SMA received a blood transfusion (20ml/kg of whole blood or 10ml/kg of packed red blood cells), usually within 2 hours of admission.

Follow up. Study participants were followed up for 2 years after discharge from hospital to assess for illness, including malaria, readmissions and deaths.

HbS testing: Genomic DNA was isolated from whole blood samples for SMA & CM patients or filter papers for CCs using the DNeasy Blood and tissue kit (Qiagen, Valencia, CA). The beta hemoglobin region of interest was amplified using specific primers. Children with HbSS were referred to the Mulago Hospital Sickle Cell Clinic, and HbSS was confirmed by hemoglobin electrophoresis.

Statistical analysis. Analysis was done in STATA 12 (Stata Corporation). Proportions were compared with χ^2 analysis and mean or median values with Student's t-test or the Wilcoxon rank-sum test, respectively. Incidence rates were compared between groups by negative binomial regression.

Ethical review. Written informed consent was obtained from parents or guardians of study participants. Institutional Review Boards for human studies at Makerere University and the University of Minnesota granted ethical approval for the study.

Admission findings. 267 children with CM, 232 children with SMA and 216 CC were enrolled and had samples available for HbS genotyping. HbAS was more frequent in CC (41, 19.0%) than in SMA (2, 0.9%) or CM (2, 0.8%), confirming the protective effect of HbAS against severe malaria. HbAS as compared to HbAA reduced the risk of severe malaria (SMA or CM) by 96% (odds ratio, [OR], 0.04, 95% confidence interval (CI), 0.01, 0.10). HbSS was more frequent in SMA (22, 9.5%) than CM (1, 0.4%) and was not present in CC. HbSS as compared to HbAA increased risk of SMA 28-fold (OR 27.9, 95% CI, 3.7, 208.7).

Among children with SMA, children with HbSS were older and had a higher white blood count than children with HbAA, however *P. falciparum* parasite density was similar in the two groups (Table 1). Among children with SMA, mortality did not differ between children with HbSS (0%) and children with HbAA (0.5%, Table 1). The one child with CM and HbSS survived.

Follow up. Among CM survivors, 2/234 children (0.9%) died during 2-year follow-up. Both had HbAA. Among SMA survivors, 9 children (3.9%) died during follow-up, 1/22 with HbSS (4.6%), 7/207 with HbAA (3.4%, $P=0.77$) and 1/2 with HbAS (50%). Compared to children with SMA and HbAA, children with SMA and HbSS had significantly higher incidence of post-discharge readmissions and a higher incidence of uncomplicated malaria that approached significance ($P=0.06$), but incidence of severe malaria readmissions did not differ significantly between children with HbSS and HbAA (Table 2).

Discussions

We found in contrast to previous studies (7, 8), that children with SCA did not have increased inpatient mortality with severe malaria compared to children with HbAA. Even post-discharge mortality in children with HbSS and severe malaria did not differ significantly from that of children with HbAA (4.5% vs. 3.6%, $P=0.77$), though incidence of all-cause readmissions was higher with HbSS. The findings bring in to question whether children with HbSS have an increased risk of mortality with severe malaria compared to children with HbAA. The findings must be interpreted with caution as the study did not include children with known SCA at the time of admission, and all children were >18 months of age, both factors which could select for a healthier population of children with SCA, as children with SCA in this setting are reported to have significant morbidity and mortality in the first 2 years of life (11, 12).

Previous studies have shown that if blood transfusion service is readily available, as was the case in this study, mortality in SMA is generally low (13, 14). In the present study, this was the case regardless of the presence of concurrent SCA. Prior studies of severe malaria in children with SCA have shown a range of mortality: from 4/86 (4.6%) (15) to 2/21 (9.5%) (8) of children with SCA admitted with parasitemia to 4/5 children (80%) admitted with WHO criteria for severe malaria (7). In the only other study assessing mortality in children with SMA and SCA, SMA was the primary complication of malaria, and none of the 38 children with SMA and SCA died (0% mortality) (15), a finding identical to the present study. Sample sizes of children with both severe malaria and SCA

have been small in all studies, emphasizing the need for large prospective studies of malaria morbidity and mortality in SCA. However, studies such as the present study, in which children with severe malaria are assessed for SCA, are another way of getting at the question, as these studies may “enrich” for children with severe malaria, which appears to be a relatively uncommon complication of SCA.

In conclusion, our study findings suggest that in children >18 months of age with SCA, inpatient mortality from SMA, the most common form of severe malaria, is low and similar to that of children with HbAA if timely blood transfusion is provided, and that post-discharge mortality in children with SMA is also similar in children with HbSS vs. HbAA. However, the exclusion of children with known SCA is an important study limitation, as is the limited study sample size. Therefore, the present study is a call for large prospective studies of malaria morbidity and mortality in children with SCA to definitively address the extent to which severe malaria causes mortality in SCA.

Contributions

ROO, PB, RI, RN and CCJ were involved in the design and conduct of this study. ES carried out the HbS genotyping and critical review of the manuscript. ROO wrote the first draft of the paper. All authors commented on and approved the final version of the manuscript.

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Competing interests: All authors report no conflict of interests.

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Table 1. Demographic and clinical characteristics of children with severe malarial anemia, according hemoglobin AA (HbAA) or hemoglobin SS (HbSS) genotype

	HbAA, n=208	HbSS, n=22	P value ^e
Age, mean (SD)	2.7 (2.0, 4.1)	4.6 (3.7, 5.7)	0.007
Sex, n male (%)	127 (61.1%)	13 (59.1%)	0.86
Hemoglobin, g/dL, mean (SD)	3.7 (0.9)	3.7 (0.6)	0.38
WBC, mean (SD)	12.9 (8.2) ^a	32.7 (16.0)	< 0.001
Thrombocytopenia, n (%)	109 (52.9%) ^a	8 (36.4%)	0.14
<i>P. falciparum</i> parasite density, parasites/ μ L, median (25 th , 75 th percentile)	49,813 ^b (14,678, 229,046)	41,586 ^c (2,261, 140,672)	0.18
Required oxygen, n (%)	19 (9.1%)	1 (4.6%)	0.47
Blood culture, n positive (%)	22 (12.4%) ^d	1 (5.6%) ^d	0.39
Given antibiotics, n (%)	63 (30.3%)	10 (45.5%)	0.15
Death, n (%)	1 (0.5%)	0 (0%)	0.56

WBC, white blood cell count; thrombocytopenia = platelet count < 100,000/ μ L

^a n for HbAA =206, ^b n for HbAA = 205, ^c n for HbSS = 21

^d n for HbAA = 177, n for HbSS = 18

^e Continuous variables compared by Student's t-test except *P. falciparum* parasite density, compared by Wilcoxon rank-sum test. Categorical variables compared by χ^2 analysis.

Table 2. Deaths, readmissions and outpatient clinic visits for children with SMA and HbAA or HbSS during 2 -year follow up

	HbAA N= 207	HbSS N= 22	<i>P value</i>
Died, n (%)	7 (3.4%)	1 (4.5%)	0.77 ^a
Incidence of deaths per 1000 person years	17.29	25.36	0.33 ^b
Incidence of all cause readmissions per 1000 person years	51.89	126.81	0.05 ^b
Incidence of severe malaria readmissions per 1000 person years	42.01	25.36	0.35 ^b
Incidence of uncomplicated malaria visits per 1000 person years	106.26	202.90	0.06 ^b

Children with HbAS not shown because of low numbers (n=2)

^a Compared by χ^2 analysis

^b Incidence rates compared by negative binomial regression.