Increased severe anemia in HIV-1-exposed and HIV-1-positive infants and children during acute malaria

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Objective: Since the primary hematological complication in both pediatric HIV-1 and malaria is anemia, co-infection with these pathogens may promote life-threatening severe malarial anemia (SMA). The primary objective of the study was to determine if HIV-1 exposure [HIV-1(exp)] and/or HIV-1 infection [HIV-1(+)] increased the prevalence of SMA in children with acute malaria.

Design: The effect of HIV-1 exposure and HIV-1 infection on the prevalence of SMA (hemoglobin < 6.0 g/dl), parasitemia (parasites/μl), and high-density parasitemia (HDP, $\geq 10\,000$ parasites/μl) was investigated in children ≤ 2 years of age presenting at hospital with acute *Plasmodium falciparum* malaria in a rural holoendemic malaria transmission area of western Kenya.

Methods: Upon enrollment, a complete hematological and clinical evaluation was performed on all children. Malaria parasitemia was determined and children with acute *P. falciparum* malaria were evaluated for HIV-1 exposure and infection by two rapid serological antibody tests and HIV-1 DNA PCR, respectively.

Results: Relative to HIV-1(−) group (n = 194), the HIV-1(exp) (n = 100) and HIV-1(+) (n = 23) groups had lower hemoglobin concentrations ($P < 0.001$ and $P < 0.001$, respectively), while parasitemia and HDP were equivalent between the three groups. Multivariate analyses demonstrated that the risk of SMA was elevated in HIV-1(exp) children (odds ratio, 2.17; 95% confidence interval, 1.25–3.78; $P < 0.01$) and HIV-1(+) children (odds ratio, 8.71; 95% confidence interval, 3.37–22.51; $P < 0.0001$). The multivariate model further revealed that HIV-1 exposure or infection were not significantly associated with HDP.

Conclusions: Results presented here demonstrate that both HIV-1 exposure and HIV-1 infection are associated with increased prevalence of SMA during acute *P. falciparum* infection, independent of parasite density.

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Introduction

Plasmodium falciparum and HIV-1 infections are leading causes of childhood morbidity and mortality in many subtropical and tropical countries. Based on the geographic overlap between *P. falciparum* and HIV-1 there has been substantial public health concern. Although earlier reports failed to demonstrate significant interactions between malaria and HIV-1 in co-infected adults [1–7], recent investigations showed that co-infection enhances the pathogenesis of both diseases [7–19].

One of the most common clinical problems in pediatric populations in developing nations is anemia, occurring in approximately 63% of preschool children in Africa [20]. Causes of pediatric anemia are multifactorial and include: nutritional and micronutrient deficiencies, hemoglobinopathies, low birth weight (LBW) infants, bacteremia, hookworm, malaria, and HIV [21]. Several studies failed to demonstrate significant interactions between childhood malaria and HIV-1 [22,23], while others have shown increased severe malaria in HIV-antibody(+) versus HIV(−) children [24]. Although the most prevalent hematological abnormality in childhood malaria and HIV-1 is anemia [25–27], the impact of co-infection on anemia is largely unreported. One study, however, demonstrated that HIV-1(+) infants have lower hemoglobin (Hb) levels during acute malaria than HIV-1(+) children without malaria [28].

To investigate the impact of co-infection on pediatric anemia, a hospital-based study was performed in a rural, holoendemic *P. falciparum* transmission area of western Kenya [29]. In this equatorial community, severe malarial anemia (SMA) is the primary clinical manifestation of life-threatening childhood malaria, with cerebral malaria occurring only in rare cases [30]. Results presented here describe the association between HIV-1 status and SMA and parasitemia.

Methods

Study participants

Children ≤ 2 years ($n = 317$) were recruited in Siaya District, western Kenya during their first hospital contact for acute malaria. Study participants were matched upon enrollment for age, sex, and area of residence. Children with *P. falciparum* parasitemia (any density) were divided into three groups: HIV-1 negative [HIV-1(−), negative results for two serological antibody tests (Determine and Unigold)]; HIV-1 exposed [HIV-1(exp), positive result(s) for one or both serological tests and negative HIV-1 DNA PCR results on two consecutive blood samples approximately 3 months apart]; and HIV-1 positive [HIV-1(+), positive results for one or both serological tests and positive HIV-1 DNA PCR results on two consecutive blood samples approximately 3 months apart]. None of the study participants had cerebral malaria. Children with malaria were promptly treated according to Ministry of Health, Kenya guidelines and provided with the appropriate supportive therapy. Children positive for one or both serological tests were started on trimethoprim–sulfamethoxazole. None of the children were receiving antiretroviral therapy at the time of enrollment. Written informed consent was obtained from the parents/guardians of participating children and pre- and post-test HIV counseling was provided. The study was approved by the University of Pittsburgh and Kenya Medical Research Institute (KEMRI) Institutional Review Boards.

Laboratory measures

Malaria diagnosis was performed using capillary finger-prick blood on thin and thick blood smears stained with 3% Giemsa. The number of asexual *Plasmodium* parasites was determined per 300 leukocytes and parasite density was calculated by multiplying by the white blood cell count. Heel/finger-prick blood (< 100 μl) was collected into EDTA-containing tubes and spotted on FTA Classic cards (Whatman Inc., Florham Park, New Jersey, USA) for HIV-1 DNA PCR. Complete blood counts were performed with a Beckman Coulter AcT diff2 (Beckman-Coulter Corporation, Miami, Florida, USA) on peripheral blood (1–3 ml) collected into EDTA-containing vials.

HIV-1 diagnosis

HIV-1 exposure was determined in venipuncture blood by two rapid serological antibody tests: Unigold (Trinity Biotech, Bray, County Wicklow, Ireland) and Determine (Abbott Laboratories, Chicago, Illinois, USA). Single- or double-positive results were evaluated for HIV-1 infection by nested PCR of proviral DNA extracted from filter papers using the Chelex method [31]. HIV-1 *gp41* primers were selected for highly conserved HIV-1 group M, N, and O sequences for use in western Kenya [32,33]. Nested PCR products were visualized on an ethidium bromide-stained 2% agarose gel. HIV-1 positivity was based on the presence of a 460-bp fragment.

Statistical analyses

Data were analyzed using SPSS (version 11.0, Chicago, Illinois, USA). Pearson's rank χ^2 test was used for comparing proportions. Parameter medians were compared with Kruskal–Wallis tests, followed by pairwise Mann–Whitney U tests. Multivariate logistic regression, controlling for age, sex, and sickle-cell trait was used to determine the association between HIV-1 status and SMA and high-density parasitemia (HDP, $\geq 10\,000$ parasites/μl). Although the World Health Organization (WHO) defines SMA as Hb < 5.0 g/dl [25], SMA in western Kenya is more appropriately defined as Hb < 6.0 g/dl. The modified SMA definition is based on approximately 14 000 repeated Hb measurements in

children 0–4 years of age in western Kenya [34]. However, both definitions were used in the analyses. Differences were considered statistically significant at $P < 0.05$.

Results

Clinical and hematological characteristics

The demographic, clinical, and hematological parameters of the study participants (n = 317) upon enrollment are presented in Table 1. Study participants were divided into three groups based on the presence of malaria parasites and HIV-1 status: HIV-1 negative [HIV-1(−), n = 194, median age 10.5 months; range, 3–31 months]; HIV-1 exposed [HIV-1(exp), n = 100; median age, 8.5 months; range, 2–32 months]; and HIV-1 positive [HIV-1(+), n = 23; median age, 13.0 months; range, 3–26 months]. There was a significant difference in age across the groups ($P < 0.05$) with the HIV-1(−) group being older than the HIV-1(exp) group ($P < 0.05$, Table 1). Temperature, glucose, parasitemia, and HDP were not statistically different between the groups. Red blood cell (RBC) counts differed between the groups ($P < 0.001$), with the HIV-1(exp) and HIV-1(+) groups having lower RBC counts than the HIV-1(−) group ($P < 0.001$ and $P < 0.001$, respectively, Table 1). Relative to the HIV-1(−) group, Hb and hematocrit (Hct) were reduced in the HIV-1(exp) ($P < 0.001$ and $P < 0.001$, respectively) and HIV-1(+) children ($P < 0.001$ and $P < 0.001$, respectively, Table 1). The HIV-1(+) group also had significantly lower levels of Hb and Hct than the HIV-1(exp) group ($P < 0.01$ and $P < 0.05$, respectively, Table 1).

Effect of HIV-1 status on SMA

The effect of HIV-1 status on SMA was also determined. The proportion of children with SMA (Hb < 6.0 g/dl) in the HIV-1(−) group was 19.1% versus 35% in the HIV-1(exp) group ($P < 0.01$), and 65.2% in the HIV-1(+) group [$P < 0.001$ versus HIV-1(−); Table 1]. SMA was more prevalent in the HIV-1(+) group vs. the HIV-1(exp) group ($P < 0.01$, Table 1). Based on the WHO categorization (Hb < 5.0 g/dl), 8.8% of the HIV-1(−) group had SMA, while SMA was present in 13.0% of the HIV-1(exp) cases [$P = 0.256$ versus HIV-1(−)], and 34.8% of the HIV-1(+) group [$P < 0.001$ versus HIV-1(−), and $P < 0.01$ versus HIV-1(exp); Table 1].

Multivariate analyses examining the impact of HIV-1 status on SMA and HDP

The effect of HIV-1 status on SMA and HDP was further investigated by multivariate analyses controlling for age, sex, and sickle-cell trait. The multivariate model revealed that SMA (Hb < 6.0 g/dl) was higher in the HIV-1(exp) group [odds ratio (OR), 2.17; 95% confidence interval (CI), 1.25–3.78; $P < 0.01$] and the HIV-1(+) group (OR, 8.71; 95%CI, 3.37–22.51; $P < 0.0001$) compared with HIV-1(−) children (Table 2). SMA was also more prevalent in the HIV-1(+) group than the HIV-1(exp) group (OR, 4.00; 95%CI, 1.51–10.62; $P < 0.01$, Table 2). Based on WHO criteria (Hb < 5.0 g/dl), HIV-1 exposed and positive children had an increased risk of SMA (OR, 4.33; 95%CI, 1.47–12.72; $P < 0.01$ and OR, 5.93; 95%CI, 2.14–16.45; $P < 0.001$, respectively) relative to the HIV-1(−) group (Table 2).

Compared to the HIV-1(−) group, multivariate analyses demonstrated that HDP did not differ in the HIV-1(exp)

Table 1. Demographic, clinical, and hematological parameters of the study participants.

Characteristic	HIV-1(−)	HIV-1(exp)	HIV-1(+)	P[a]
Subjects (n)	194	100	23	
Age (months)	11.66 (0.45)[b]	10.09 (0.63)	12.04 (1.34)	< 0.05
Sex (male/female)	109/85	51/49	12/11	0.684
Axillary temperature (°C)	37.55 (0.10)	37.72 (0.11)	37.80 (0.17)	0.079
Glucose (mmol/l)	4.94 (0.13)	5.15 (0.12)	5.28 (0.31)	0.378
Parasitemia (per μl)	47 245.40 (4262.21)	47 985.35 (5877.51)	48 356.14 (15 850.16)	0.488
Geomean parasitemia	19 816.28	19 761.46	12 526.26	
High-density parasitemia ≥ 10 000 parasites per μl [n (%)]	138 (71.1)	73 (73.0)	15 (65.2)	0.756
RBC ($\times 10^6$/μl)	3.59 (0.07)[c,d]	3.18 (0.10)	2.75 (0.19)	< 0.001
Hb (g/dl)	7.76 (0.14)[c,d]	6.97 (0.20)[e]	5.90 (0.38)	< 0.001
Hct (%)	24.18 (0.42)[c,d]	21.52 (0.59)[f]	18.64 (1.15)	< 0.001
Hb < 6.0 g/dl [n (%)]	37 (19.1)[g,d]	35 (35.0)[e]	15 (65.2)	< 0.001
Hb < 5.0 g/dl [n (%)]	17 (8.8)[d]	13 (13.0)[e]	8 (34.8)	< 0.001

Data are presented as the mean (SEM) unless otherwise noted. Differences between the groups were compared using Kruskal–Wallis test for medians, while proportions were compared using Pearson's χ^2 test. Pairwise comparisons between the groups were performed using Mann–Whitney U tests and Pearson's χ^2 test for medians and proportions, respectively.
[a]P value for the comparison between all three groups.
[b]HIV-1(−) vs. HIV-1(exp) at $P < 0.05$.
[c]HIV-1(−) versus HIV-1(exp) at $P < 0.001$;
[d]HIV-1(−) versus HIV-1(+) at $P < 0.001$.
[e]HIV-1(exp) versus HIV-1(+) at $P < 0.01$.
[f]HIV-1(exp) versus HIV-1(+) at $P < 0.05$.
[g]HIV-1(−) versus HIV-1(exp) at $P < 0.01$.

Table 2. Association of HIV-1 status with severe malarial anemia and high-density parasitemia.

	SMA (Hb < 5.0 g/dl)[a]			SMA (Hb < 6.0 g/dl)[b]			HDP (≥ 10 000 parasites/μl)		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
HIV-1(−)	1.00	–	–	1.00	–	–	1.00	–	–
HIV-1(exp)[c]	1.37	0.63–2.99	0.430	2.17	1.25–3.78	< 0.01	1.18	0.70–1.96	0.535
HIV-1(+)[c]	5.93	2.14–16.45	< 0.001	8.71	3.37–22.51	< 0.0001	0.64	0.27–1.53	0.320
HIV-1(+)[d]	4.33	1.47–12.72	< 0.01	4.00	1.51–10.62	< 0.01	0.55	0.22–1.37	0.199

Multivariate logistic regression, controlling for age, sex, and sickle-cell trait was used to determine the association of HIV-1 status [i.e., HIV-1(−), n = 194; HIV-1(exp), n = 100, or HIV-1(+), n = 23] with SMA and HDP.
[a]Definition of SMA according to WHO criteria.
[b]Modified definition of SMA based on Hb distributions within an age- and geographically referenced population [34].
[c]Odds ratio (OR) relative to HIV-1(−) group.
[d]OR relative to HIV-1(exp) group. SMA, severe malarial anemia; HDP, high-density parasitemia; CI, confidence interval.

group (OR, 1.18; 95%CI, 0.70–1.96; $P = 0.535$) or the HIV-1(+) group (OR, 0.64; 95%CI, 0.27–1.53; $P = 0.320$; Table 2). Moreover, HDP was not significantly different between the HIV-1(+) group and the HIV-1(exp) group (OR, 0.55; 95%CI, 0.22–1.37; $P = 0.199$; Table 2).

Discussion

Anemia is the most common finding associated with pediatric HIV and malaria in sub-Saharan Africa [30,34–37]. While it is clear that both pathogens promote anemia, only one study to date has directly addressed the role of co-infection as a risk factor for pediatric anemia, showing that HIV-1(+) infants had increased prevalence of anemia, while HIV-1/malaria co-infected infants had lower Hb levels than infants with either infection alone [28]. Although the previous study showed that anemia in HIV-1(−) infants born to HIV(+) mothers [a group comparable to the HIV-1(exp) group investigated here] approached significance ($P < 0.07$), the prevalence of SMA did not differ among the groups [28]. Results presented here in an adjacent, rural area with presumably higher malaria exposures rates demonstrate that both HIV-1 exposure and infection significantly increase severe anemia during acute malaria. In the current study, > 99% of the study participants were from the Luo ethnic tribe, which in this rural setting, have similar malaria exposure patterns and socio-demographic indices [29]. Thus, homogeneity among our study population may explain differences in the present results as compared to previous findings in an urban and peri-urban area with greater diversity in malaria exposure rates and socio-demographic factors [28].

The finding that both HIV-1 exposure and infection is associated with increased SMA suggests that children born to HIV-1(+) mothers may be predisposed to hematological complications during malaria. Although the underlying mechanism(s) is presently unclear, *in utero* HIV-1 exposure may impair hematological and/or immunological development. This hypothesis is supported by investigations showing that fetal liver hematopoiesis in second trimester abortuses from HIV(+) women have hematological abnormalities including reductions in multipotent progenitors (e.g., CD34) [38]. Moreover, examination of 100 symptomatic HIV(+) versus HIV(−) infants with transplacental HIV-1 antibodies demonstrated that 94% of the HIV(+) group had anemia; a major predictor of disease progression in which those with Hct levels < 25% presented with a rapidly fatal course [39]. Additional studies illustrated that HIV-1/malaria co-infection in pregnant women increases the risk of LBW, preterm birth (PTB), post-neonatal mortality, and intrauterine growth retardation [40,41]. Since maternal malaria status and rates of LBW and PTB were unknown in our cohort, the influence of these variables cannot be determined. Alternatively, increased prevalence of SMA could be due to factors associated with breastfeeding. Since all of the children in the current study were breastfed, it is possible that HIV-1(+) mothers had poor nutritional and/or immunological status that increased the development of SMA. Additional investigations are required to confirm this speculation.

Findings presented here also showed that parasitemia is unrelated to Hb concentrations, as evidenced by similar parasitemia in the presence of marked differences in anemia status between the groups. Although previous studies in adults illustrated that co-infection is associated with increased parasitemia [9,42,43], present results support previous findings in African pediatric populations [1,22–24,44] showing no association between HIV-1 and parasitemia.

In summary, data presented here demonstrate that HIV-1/malaria co-infection increases SMA in infants and young children, illustrating that HIV-1 testing is essential in pediatric populations in malaria endemic areas. Extending these cross-sectional results and prospectively following children throughout their first 5 years of life may offer important insight into the number of HIV-1(exp) children that seroconvert and become HIV-1(+),

and additionally, if HIV-1(exp) and/or HIV-1(+) children acquire immunity to *P. falciparum* following repeated exposures.

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