High Prevalence of Anemia but Low Level of Iron Deficiency in Preschool Children during a Low Transmission Period of Malaria in Rural Kivu, Democratic Republic of the Congo

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Abstract. Anemia is a worldwide public health concern especially in preschool children in developing countries and iron deficiency (ID) is generally assumed to cause at least 50% of the cases. However, data on this contribution are scarce. To close this gap, we determined in 2013 the contribution of ID in the etiology of anemia and measured others factors associated to noniron deficiency anemia (NIDA) in 900 preschool children randomly selected during a two-stage cluster nutritional survey in the Miti-Murhesa health zone, in eastern Democratic Republic of the Congo. In these children, we collected sociodemographic, clinical, and biological parameters and determined the nutritional status according to the World Health Organization 2006 standards. Anemia was defined as altitude-adjusted hemoglobin < 110 g/L and ID was defined as serum ferritin < 12 μg/L or < 30 μg/L in the absence or presence of inflammation, respectively. Median (interquartile range) age was 29.4 (12–45) months. The prevalence of anemia was 46.6% (391/838) among whom only 16.5% (62/377) had ID. Among children without signs of inflammation, only 4.4% (11/251) met the ferritin-based (unadjusted) definition of ID. Logistic regression analysis identified ID, history of fever during the last 2 weeks and mid-upper arm circumference < 125 mm as the only independent factors associated to anemia. In conclusion, anemia is a severe public health problem in the Miti-Murhesa health zone, but NIDA is mostly predominant and needs to be further studied. Control of infections and prevention of acute undernutrition (wasting) are some of appropriate interventions to reduce the burden anemia in this region.

INTRODUCTION

Anemia is a clinical condition characterized by a decrease of hemoglobin (Hb) concentration, with as consequence a loss of the oxygen-carrying capacity of the blood. The supply of oxygen to tissues becomes insufficient to meet physiologic needs, especially in conditions of high demand such as exercise, pregnancy, and so on.[1] In children, anemia is associated with increased morbidity and mortality,[2,3] and can, on the long term, affect physical and intellectual developments if not corrected quickly.[4–6]

Anemia is a worldwide public health concern. According to an analysis of the World Health Organization (WHO) Global Database on anemia carried from 1993 to 2005 around one quarter of the world's population is affected.[7] Preschool children are the most affected group with global prevalence estimated at 47.4%, representing 293 million (95% confidence interval [CI] = 282–303 million) children.[7] The condition is more prevalent in Africa and South Asia.[8] In Africa, a prevalence of 64.6% has been reported in children.[7,9] Data from 11 western and central African countries indicated an even higher prevalence of 72% in preschool children.[9] A demographic and health survey (DHS) done in the Democratic Republic of the Congo (DRC) in 2007 reported that in South Kivu 59.8% of children were anemic, with a higher rate in rural areas.[10]

The etiology of anemia is complex and can be uni- or multifactorial.[11,12] Common factors include iron deficiency (ID), malaria, and helminthic infections. According to the WHO, around half of the global cases of anemia may be due to ID.[12] In South Kivu, little is known about etiologies of anemia in children. The results of an intrahospital study carried out in the late seventies at the Lwiro hospital located in the Miti-Murhesa health zone in a selected group of children with edematous severe acute malnutrition (SAM), suggested that anemia during protein-energy malnutrition in South Kivu region cannot be explained by isolated ID.[13]

Thus, in 2013 at the time of designing this study, community level data on the magnitude of anemia and its relation with ID were lacking. The primary objective of this study was therefore to close this gap by determining the contribution of ID in the etiology of anemia and the secondary objective was to identify others factors associated with non-ID anemia (NIDA) in preschool children in the eastern part of DRC.

METHODS

Study area. Miti-Murhesa is a rural health zone located at 35 km north of Bukavu, the capital city of the South Kivu Province in the eastern part of the DRC. Situated between 1,500 and 2,000 m of altitude, the Miti-Murhesa health zone covered about 250,000 people at the time of this study. Subsistence agriculture is the main economic activity. Undernutrition of children under 5 years of age is still endemic and the prevalence of stunting in preschool children was estimated at 66% in 2009,[14] whereas prevalence of global acute malnutrition (GAM) was almost 6% in 2011.[15]

Sample size and study design. A two-stage sampling process was used to determine the study participants in this

cross-sectional study. A representative sample of villages from the Miti-Murhesa health zone was selected, and households were randomly selected using systematic sampling technique. As there was no data available on ID, the expected proportion in this study was based on the prevalence of anemia in children aged 6–59 months in South Kivu of 60%, according to the 2007 DHS.[10] The sample size was determined using the estimates for proportion in a single cross-sectional survey.[16] Considering 95% CI, a precision of 5%, a design effect of 2, a nonresponse and/or concern with blood drown rate of 10%, the sample size required for this study was found to be 812 children. Based on Micronutrient Initiative and the Centers for Disease Control and Prevention guidelines for nutritional surveys,[16] we opted for 30 clusters of 30 children each. Therefore, 900 children were selected from 30 villages.

Village and household selection. In April 2013, the 30 villages were randomly selected using a complete list of all villages of the Miti-Murhesa health zone. The village households' list was not available so households were selected following a "random walk" method. From the geographically central location identified by the local health worker and the chief of the village, a pen was spun to randomly indicate the first direction to follow for household selection. One household was selected for every successive five households. The same process was used to select another direction and household until the number of required children was reached.[17]

Inclusion and exclusion criteria. We included children aged 6–59 months (only one child per household) who were permanent resident of the Miti-Murhesa health zone and whose mothers or guardians granted consent for study inclusion and for blood samples collection.

We excluded severely sick children (including those with psychomotor retardation) and those who were between 6 and 59 months if another child had already been selected in the same household.

Data collection and procedures. *Study questionnaire.* Data were collected by trained enumerators using a specially designed and pretested standardized data collection form. Data collected included demographic characteristics and information on immunization and morbidity, access to nutrition sensitive preventive interventions (vitamin A supplementation, deworming).

Anthropometric measurements. Weight, recumbent length, or standing height (for children aged more than 2 years) and mid-upper arm circumference (MUAC) were measured by trained nurses following the Food and Nutrition Technical Assistance guidelines and using regularly calibrated equipment.[18] Measurements were taken in duplicate, and repeated if the difference between the two first measurements was outside the allowable value for that anthropometric parameter.

Blood samples collection and processing. Hb was measured using a portable HemoCue Hb201+ point-of-care analyzer (HemoCue AB, Ängelholm, Sweden). OnSite Malaria Pf/Pan Ag Rapid Test (San Diego, CA) was used to diagnose malaria. The test has the ability to detect the presence of either *Plasmodium falciparum* antigen or indistinctively detect one of the other *Plasmodium* species including *P. malariae*, *P. ovale*, and *P. vivax*. A venous blood sample (4 mL) was drawn by venipuncture. The collected blood sample was immediately stored in the collection tube in a cooler box until

transfer to the laboratory where the samples were processed and centrifuged to obtain serum. Part of the serum was used for the determination of serum albumin using a spectrophotometer GENESYS 20 (Thermo Fisher Scientific, Waltham, MA). Sera for determination of iron parameters were transferred into 0.2-mL storage tubes and stored at −40°C until shipment to the "VitMin Laboratory" (Willstaett, Germany). During shipment, the frozen serum samples were transported in a styrofoam box with dry ice. The serum was used for determination of ferritin (SF), retinol-binding protein (RBP), C-reactive protein (CRP), and α1-acid glycoprotein (AGP) using the sandwich enzyme-linked immunosorbent assay technique.[19]

Variables transformation. Nutrition status was defined using WHO 2006 growth standards, MUAC, and presence of nutritional edema.[20,21] A child was considered as suffering from wasting when the calculated weight-for-height Z-score (WHZ) was < −2 standard deviations (SD). He was classified as stunted if the height-for-age Z-score was < −2 SD, and as underweight if the weight-for-age Z-score was < −2 SD.[20] A child with a MUAC < 115 mm and/or WHZ < −3 SD and/or with pitting edema was considered as suffering from SAM.[21] A child, without edema, with a MUAC < 125 mm but > 115 mm or a WHZ between −3 and < −2 was considered as suffering from moderate acute malnutrition (MAM). Prevalence of both, SAM and MAM was combined to obtain the prevalence of GAM.[22] Because of the small number of children who had a MUAC < 115 mm, in logistic regression two groups were used with a cutoff of 125 mm.

SF is an acute phase protein and its levels are raised during infection/inflammation. Therefore, the SF was adjusted according to an individual's inflammation status, based on CRP and AGP values.[23] Inflammation was defined as CRP > 5 mg/L and/or AGP > 1 g/L. For this study, ID was defined as SF < 12 μg/L in the absence of inflammation, or SF < 30 μg/L in case of inflammation.[23] RBP was adjusted using corrections proposed by Thurman and others.[24] Since all villages were more than 1,500 m above sea level, Hb concentrations were adjusted and evaluated according to WHO guidelines.[1] Anemia was defined as Hb concentrations < 110 g/L.[1] Hypoalbuminemia was defined as serum albumin < 3.5 g/dL.[25,26]

Data management and statistical analysis. Data were double entered using Microsoft Access 2007 (Microsoft Corporation, Redmond, WA). Nutritional indices were computed making use of Emergency Nutrition Assessment software. All other statistical analyses were performed using STATA for Mac, version 12.1 (StataCorp, College Station, TX).

Participant descriptive characteristics were summarized as mean and SD for continuous variables, or as median and interquartile range for nonnormal continuous variables, and as number or percentages for categorical variables. Analyses were done taking into consideration the multi-step sampling process. Pearson's χ^2, χ^2 of trend were used to examine bivariate relationships between anemia and others variables, and also odds ratio and 95% CI were used. Logistic regression model after stepwise backward removal was used to examine the independent association between different factors and anemia, the dependent variable. For all analysis, the statistical significance level was fixed at < 0.05.

Ethical considerations. The study was approved by the local Institutional Ethics Committee of the Catholic University of Bukavu. It was also authorized by the provincial

representative of the Ministry of Health. Participation was voluntary and children were enrolled only if legal guardians granted informed consent. All data were managed in a manner to guarantee confidentiality for all participants. All children's anthropometric data were plotted on their health vaccination card and when growth faltering was detected

they were referred for counseling at the health facility. Children with SAM identified either by a MUAC < 115 mm, by WHZ < –3 SD, or edema were referred to the local health facility for treatment. Children with positive malaria test were referred to the local health center and managed according to the national malaria program guidelines.

TABLE 1
Demographic clinical and biological characteristics of enrolled children

Variable	n	%	Mean (SD)	Median (P25–P75)
Sociodemographic				
Age (months)	887			29.4 (17.1–44.6)
6 to < 12		13.3		
12 to < 23		26.7		
24 to 59		60.0		
Sex (% girl)	893	51.0		
Morbidity				
Fever within the last 2 weeks	898	25.7		
Diarrhea within the last 2 weeks	898	14.8		
Positive malaria rapid diagnostic test	875	1.7		
Coverage of public health preventive interventions				
Deworming within the last 6 months	789	84.2		
Vitamin A supplementation within the last 6 months	869	86.7		
Sleeping under treated bed net the night before the survey	885	53.8		
Reported measles immunization (children ≥ 9 months)	802	81.6		
Nutrition status				
ZWH	855			
< –3		1.8		
–3 to < –2		5.4		
≥ –2		92.8		
ZWA	857			
< –3		16.1		
–3 to < –2		21.8		
≥ –2		62.1		
ZHA	838			
< –3		39.8		
–3 to < –2		26.2		
≥ –2		34.0		
MUAC (mm)	881		140.0 (13.0)	
< 115		3.0		
115 to 124		8.7		
≥ 125		88.3		
Bilateral pitting edema				
Presence	886	1.6		
Acute malnutrition classification				
Severe acute malnutrition*	835	6.0		
Global acute malnutrition†	835	18.4		
Albumin (g/dL)	859		3.4 (1.3)	
< 3.5		45.7		
Retinol-binding protein adjusted for APP (μmol/L)	843		1.2 (0.3)	
Vitamin A deficiency (< 0.7)		4.6		
Inflammation parameters				
CRP (mg/L)	852			0.9 (0.4–3.4)
> 5		19.7		
AGP (g/L)	852			1.2 (0.9–1.6)
> 1		69.8		
Presence of inflammation‡	852	70.5		
Anemia and iron parameters				
Hemoglobin adjusted for altitude (g/L)	838		110.0 (12.8)	
< 70		0.2		
70 to 109		46.4		
≥ 110		53.4		
Ferritin (μg/L)	852			68.7 (42.3–95.7)
≤ 12		2.2		
> 12 and ≤ 30		13.0		
> 30		84.8		
ID = ferritin adjusted for CRP and AGP	843	10.4		

AGP = α1-acid glycoprotein; APP = acute phase proteins; CRP = C-reactive-protein; ID = iron deficiency; MUAC = mid-upper arm circumference; SD = standard deviation; ZHA = height-for-age Z-score; ZWA = weight-for-age Z-score; ZWH = weight-for-height Z-score.
* Edema or ZWH < –3 or MUAC < 115 mm.
† Edema or ZWH < –2 or MUAC < 125 mm.
‡ CRP > 5 mg/L and/or AGP > 1 g/L.

RESULTS

Table 1 gives demographic data, information about morbidity and coverage of preventive interventions, nutrition status, and biological parameters. The sex ratio was close to 1, the majority of enrolled children was above 24 months and had good coverage of preventive interventions, but fever and diarrhea were common. Proportions of GAM (18.4%), stunting (66%), and hypoalbuminemia (45.7%) were high. Biological signs of inflammation were present in seven children out of 10. Almost half of our population (46.6%, 95% CI = 40.4–52.8) was anemic, with 0.2% (95% CI = 0.1–0.5) being severely anemic. The global group prevalence of ID was 10.4% (95% CI = 7.7–13.1). Among anemic children (N = 377), prevalence of ID was 16.5%. In iron-deficient children (N = 82), anemia was present in 75.6%. In the subsample of 251 children without inflammation, only 4.4% had SF < 12 µg/L (unadjusted).

Bivariate analysis (Tables 2 and 3) shows that children of male sex, those who experienced fever during the past 2 weeks, those who did not receive vitamin A supplementation, those who were not dewormed within the past 6 months, and those with low MUAC were significantly more likely to have anemia. Low SF, either unadjusted or adjusted for inflammation, was significantly associated with anemia.

In a logistic regression (Table 4), ID, low MUAC (< 125 mm), and history of fever during the last 2 weeks were the independent factors associated with anemia. In a second analysis model where children with ID were not included, anemia was associated with low MUAC (< 125 mm) and history of fever during the last 2 weeks.

DISCUSSION

Our study shows that in the Miti-Murhesa health zone, nearly half of children (46.6%) from 6 to 59 months are anemic. This figure is higher than 36% reported in South Kivu by the 2014 DHS.[27] A similar higher rate was also found in another recent survey, carried out in similar geographical rural area of South Kivu, that used adjusted Hb levels.[28] However, although higher than the anemia prevalence of about 34% reported in preschool children in Rwanda, a region in geographical proximity of South Kivu,[29] our prevalence is less than the estimate of 64.6% for African children suggested by McLean and others[7] and even lower than the 72% estimate for western and central African counties.[9]

There appears to be a change in anemia prevalence in South Kivu over time. Indeed, DHS done in DRC in 2007 and in 2014 reported anemia prevalences of about 60% and 36% of children from South Kivu, respectively, while using the same definition and methodology.[10,27] This difference between the two DHSs may be partially explained by the decrease of the incidence of malaria in the region after large-scale public health interventions, including the distribution of impregnated bed net and the use of artemisinin-based combination therapy to treat malarial cases.[30,31] Similar findings have been reported in highland Kenya where the prevalence of anemia decreased after an interruption of malaria transmission.[32]

The results confirm that anemia is highly prevalent but that ID contributes less than a fifth of the observed anemia

TABLE 2
Demographic and clinical risk factors of anemia in children

Variables	n	% Anemia	OR (95% CI)	P
Age (months)				0.186
6 to < 12	104	53.9	1.49 (0.88–2.53)	
12 to < 23	223	49.8	1.26 (0.87–1.84)	
24 to 59	501	43.9	1	
Category of age				0.110
6 to < 23	327	51.1	1.33 (0.93–1.91)	
24 to 59	501	43.9	1	
Sex				0.047
Male	414	50.2	1.32 (1.01–1.72)	
Female	419	43.4	1	
Edema				0.122
Yes	13	69.2	2.61 (0.72–9.43)	
No	815	46.2	1	
History of fever within the last 2 weeks				0.041
Yes	216	55.1	1.58 (1.02–2.45)	
No	622	43.7	1	
Diarrhea within the last 2 weeks				0.112
Yes	123	53.7	1.39 (0.92–2.10)	
No	715	45.5	1	
Treated bed net the night before				0.630
Yes	453	47.5	1.08 (0.77–1.52)	
No	374	45.5	1	
Deworming within the last 6 months				0.010
Yes	620	43.7	1	
No	116	57.8	1.75 (1.15–2.70)	
Vitamin A supplementation within the last 6 months				0.034
Yes	704	44.7	1	
No	105	55.2	1.52 (1.03–2.27)	
ZWH (WHO 2006)				0.958
< −3	130	46.7	1.11 (0.38–3.22)	
−3 to < −2	177	44.2	1	
≥ −2	497	46.2	1.09 (0.58–2.04)	
ZWA (WHO 2006)				0.808
< −3	322	49.2	1.15 (0.69–1.92)	
−3 to < −2	209	45.8	1.00 (0.69–1.45)	
≥ −2	266	45.7	1	
ZHA (WHO 2006)				0.726
< −3	15	48.1	1.13 (0.80–1.61)	
−3 to < −2	43	45.0	1	
≥ −2	744	45.5	1.02 (0.68–1.52)	
Mid-upper arm circumference (mm)				0.016‡
< 115	24	62.5	2.03 (0.81–5.09)	
115 to 124	72	59.7	1.80 (1.07–3.05)	
≥ 125	732	45.1	1	
Acute malnutrition				
SAM*				0.304
Yes	48	53.8	1.38 (0.73–2.59)	
No	739	45.8	1	
GAM†				0.629
Yes	148	48.5	1.11 (0.71–1.75)	
No	639	45.8	1	

CI = confidence interval; GAM = global acute malnutrition; OR = odds ratio; SAM = severe acute malnutrition; WHO = World Health Organization; ZHA = height-for-age Z-score; ZWA = weight-for-age Z-score; ZWH = weight-for-height Z-score.
* Edema or ZWH < −3 or MUAC < 115 mm.
† Edema or ZWH < −2 or MUAC < 125 mm.
‡ χ^2 of trend.

burden. WHO estimates that ID is the "single" biggest contributor to the anemia burden and evokes a proportion around 50% of anemia due to ID. However, there has not been any methodologically sound study to confirm this in our region. This study is among the first few conducted in the region to evaluate the contribution of ID to the high burden of anemia in children under 5 years of age, using reference methods that include measurements of SF and of inflammation to adjust biochemical markers of iron load. Our results suggest that the contribution of ID to anemia

TABLE 3
Biological risk factors of anemia in children

Variables	n	% Anemia	OR (95% CI)	P
RDT malaria				0.616
Positive	15	53.3	1.30 (0.45–3.81)	
Negative	811	46.7	1	
Ferritin unadjusted (μg/L)				< 0.001†
≤ 12	17	88.2	9.82 (1.96–49.10)	
> 12 and ≤ 30	101	64.4	2.36 (1.54–3.64)	
> 30	686	43.4	1	
ID = ferritin adjusted for CRP and AGP				< 0.001
Yes	82	75.6	4.01 (2.34–6.85)	
No	722	43.6	1	
CRP (mg/L)				0.093
< 5	643	45.3	1	
≥ 5	161	53.4	1.39 (0.94–2.04)	
AGP (g/L)				0.157
≤ 1	240	42.5	1	
> 1	564	48.8	1.28 (0.90–1.84)	
Retinol-binding protein unadjusted (μmol/L)				0.084
Deficiency (< 0.7)	75	58.7	1.69 (0.92–3.09)	
Nondeficiency (≥ 0.7)	729	45.7	1	
Retinol-binding protein adjusted for APP (μmol/L)				0.441
Deficiency (< 0.7)	37	54.1	1.35 (0.61–2.98)	
Nondeficiency (≥ 0.7)	767	46.5	1	
Albumin (g/dL)				0.975
< 3.5	432	46.3	1	
≥ 3.5	379	46.4	1.01 (0.69–1.47)	
Inflammation*				0.122
Yes	568	48.9	1.33 (0.92–1.91)	
No	236	42.0	1	

AGP = α1-acid glycoprotein; APP = acute phase proteins; CI = confidence interval; CRP = C-reactive-protein; ID = iron deficiency; OR = odds ratio; RDT = rapid diagnostic test.
* CRP > 5 mg/L and/or AGP > 1 g/L.
† χ^2 of trend.

burden may be context specific. Indeed, the prevalence of iron deficiency anemia (IDA) is remarkably low in our sample but not as low as in another study in the same region that found a prevalence of IDA of 9.4% in children below 24 months and an even lower prevalence of 0.4% in those between 24 and 59 months.[28] Danquah and others reported similar results among Rwandese children. They found ID in only 25% of children with anemia.[33] These findings suggest that ID may not be the principal cause of anemia in children under 5 years of age in the African Great Lake Region. The results are in contrast with findings in other developing countries including Kenya where 84% of anemic children (preschool and early school children) from coastal region had evidence of IDA,[34] and Côte d'Ivoire where 80% of anemic preschool children had IDA.[35] This low ID in our sample is surprising given the high proportion of undernourished children and the absence of a public health intervention that primarily targets ID. Possible explanation of low contribution of ID to anemia burden is consumption of iron-rich food and widespread self-prescription of iron tablets as reported in the 2014 DHS.[27] Indeed, the survey found that in South Kivu 46.5% of 6–23 months old children consumed foods rich in iron the day prior the survey and 14% of 6–59 months old children received iron supplement the week preceding the survey. Caution should still be exercised in attributing the low level of IDA to diet because of the known low consumption of foods of animal origin in this region.

TABLE 4
Logistic regression model for anemia in children

Variables	All children (model 1, N = 794; 374 cases of anemia)*		Children without iron deficiency (model 2, N = 712; 312 cases of anemia)†	
	aOR (95% CI)	P	aOR (95% CI)	P
Iron deficiency		< 0.001	–	–
Yes	4.10 (2.41–6.96)		–	
No	1		–	
Mid-upper arm circumference (mm)		0.006		0.005
< 125	1.87 (1.18–2.94)		1.96 (1.24–3.13)	
≥ 125	1		1	
Fever within the last 2 weeks		0.049		0.05
Yes	1.58 (1.00–2.50)		1.62 (1.00–2.62)	
No	1		1	

aOR = adjusted odds ratio; CI = confidence interval. The following variables have been removed by both two models: age, weight for age Z-score, weight for height Z-score, height for age Z-score, serum albumin, inflammation, retinol-binding protein, vitamin A supplementation the last 6 months, sex, diarrhea in the last 2 weeks, and deworming within the last 6 months.
* F = 11.11; P > F = 0.0001; McKelvey and Zavoina's R^2 = 0.356.
† F = 5.23; P > F = 0.0118; McKelvey and Zavoina's R^2 = 0.169.

The etiology underlying most cases of anemia remains unclear. The fact that likelihood of anemia was increased in presence of a MUAC < 125 mm (not modified by age) suggests that undernutrition might contribute to the development of NIDA. In South Kivu, where our study was done, Fondu and others also found that children with protein-energy malnutrition, defined based on serum albumin level and presence of edema, were more anemic than controls even after 60 days of refeeding.[13] Besides ID, other known nutritional deficiencies associated with anemia include deficiency in vitamin A, vitamin B12, folic acid, zinc, or copper.[36] These deficiencies are common among undernourished children and, in many countries, association between anemia and undernutrition has been reported. A study done in Rwanda revealed that anemia was associated to underweight in preschool children.[37] Foote and others in Kenya reported that anemia was associated with stunting and wasting, whereas severe anemia was associated with stunting.[38] However, the relationship between anemia and nutritional status (stunting and wasting) remains controversial.[39]

A study done in 2014 in the same area as ours among preschool children showed that zinc deficiency is highly prevalent, varying between 23% (6–23 months) and 25% (24–59 months) but vitamin A, folic acid, and vitamin B12 deficiencies are not prevalent.[28] Thus, even though we did not analyze zinc levels in our population, it is possible that zinc deficiency could account for part of the anemia cases in our population as found in Cambodian children for whom anemia was associated with zinc deficiency regardless of ID.[40] Zinc plays a role of catalyst in iron metabolism and is involved in erythropoiesis; hence it is plausible for its deficiency to cause anemia.[41] Also, zinc deficiency enhances oxidative stress,[42] which may induce anemia by decreasing the lifespan of red blood cells.

We found that recent episodes of fever increased the likelihood of anemia. Given that fever is a common sign of infection, this suggests that infection might contribute to NIDA. The relationship between anemia and infection has been reported previously but mechanisms by which infection and/or inflammation cause anemia are not clearly understood.[8,43–45] It is commonly thought that anemia during infection is caused by a complex immunological mechanisms involving cytokines[46] and mediated by many pathways, either by inducing a sequestration of iron in macrophages and a decrease of duodenal enterocyte absorption,[43,45] or by inhibiting erythropoiesis,[43] or by directly inducing hemolysis.[47]

Malaria is by far the infection contributing the most to the anemia burden globally.[8,33,39] This does not seem to be the case in the Miti-Murhesa health zone. However, this possibility cannot confidently be excluded as malaria might have been underestimated in children carrying low parasitemia not detectable by the rapid diagnostic test (RDT) used in the present study.[48] Also false-negative RDT associated with the *P. falciparum* HRP-2 gene deletion has been reported in asymptomatic children from South Kivu.[49] Even when asymptomatic, low parasitemia of *Plasmodium* has been reported to be associated with the occurrence of anemia in preschool children in Rwanda.[29]

In this context of low prevalence of malaria, the fact that a history of fever was associated with anemia suggests that other common infectious diseases of childhood might play a role. With 70% of children presenting biological sign of inflammation, it is clear that infection is a highly common condition in this community. Unfortunately, to our knowledge there is no specific local study of the etiology of mild or moderate febrile illness in children, making it difficult to evaluate which type of infection is contributing more to the burden of anemia.

Our results, after adjustment for MUAC and fever, confirm the findings of a recent systematic review showing that mass deworming has no effect on Hb,[50] but contrast with previous results that suggested a protective effect of deworming.[8,11,51] This discrepancy suggests that the effect of deworming on Hb may be context specific and that further research is needed to explore the link and mechanism. Specifically for our study, we are unable to confirm the exact contribution of helminth infections as there was no systematic screening for soil-transmitted helminths, including *Ancylostoma duodenale*, *Necator americanus*, *Trichuris trichiura*, and *Ascaris lumbricoides*, known to cause anemia.

High prevalence of hemoglobinopathies is another possible explanation of high NIDA. Gahutu and others have shown that in Rwanda, a country sharing border with South Kivu, the prevalence of hemoglobinopathies has been underestimated.[52] In their study, sickle cell trait, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and α(+)-thalassemia were observed in 2.8%, 9.6%, and 15.1%, respectively. Tshilolo and others reported after a neonatal screening for sickle cell anemia in Kinshasa, capital city of DRC, that the prevalence of sickle cell trait was 14.4% in newborns of Kivu origin.[53] However, the above-mentioned rate of sickle cell trait needs to be assessed locally as sickle cell disease has traditionally been reported to be very low in the region. The prevalence of thalassemia has not been assessed in the Kivu and could play a more important role. In Kenya, preschool children with either heterozygous or homozygous thalassemia,[38,54] and in Rwanda[52] and in Tanzania[54] children with homozygous thalassemia were more at risk of anemia compared with those with normal genotype. In Malawian children, the G6PD$^{-220/-376}$ genetic disorder was found to be associated with severe anemia.[55] Further research needs to assess the contribution on NIDA of these inherited traits and of a reported nonnegligible rate of ferroportin mutations in the region.[56]

CONCLUSION

This study has documented high prevalence of anemia. However, the level of ID is low. A large-scale survey is needed to assess the most common etiologies of anemia to better fight this debilitating condition. Screening for hemoglobinopathies and other inherited or acquired conditions, which interact with the synthesis of Hb, the erythropoiesis and/or the survival of red blood cells is urged to design the most appropriate preventive interventions. In the meantime, based on the current evidence, these findings suggest that public health strategies should prioritize interventions for preventing infectious diseases and improving the nutritional status to reduce the burden of anemia among children in the Miti-Murhesa health zone and probably in the whole DRC or African Great Lake Region.

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