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Determinants of Anemia among Preschool Children in Rural, Western Kenya

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Abstract. Although anemia in preschool children is most often attributed to iron deficiency, other nutritional, infectious, and genetic contributors are rarely concurrently measured. In a population-based, cross-sectional survey of 858 children 6–35 months of age in western Kenya, we measured hemoglobin, malaria, inflammation, sickle cell, α-thalassemia, iron deficiency, vitamin A deficiency, anthropometry, and socio-demographic characteristics. Anemia (Hb < 11 g/dL) and severe anemia (Hb < 7 g/dL) prevalence ratios (PRs) for each exposure were determined using multivariable modeling. Anemia (71.8%) and severe anemia (8.4%) were common. Characteristics most strongly associated with anemia were malaria (PR: 1.7; 95% confidence interval [CI] = 1.5–1.9), iron deficiency (1.3; 1.2–1.4), and homozygous α-thalassemia (1.3; 1.1–1.4). Characteristics associated with severe anemia were malaria (10.2; 3.5–29.3), inflammation (6.7; 2.3–19.4), and stunting (1.6; 1.0–2.4). Overall 16.8% of anemia cases were associated with malaria, 8.3% with iron deficiency, and 6.1% with inflammation. Interventions should address malaria, iron deficiency, and non-malarial infections to decrease the burden of anemia in this population.

INTRODUCTION

Anemia is a widespread public health problem, and severe anemia is a significant cause of childhood mortality.[1] The World Health Organization (WHO) estimates 1.6 billion people are anemic worldwide, and approximately two-thirds of preschool children in Africa and southeast Asia are anemic.[2] The causes of anemia are multifactorial, interlinked, and context-specific. Important known risk factors for anemia in developing countries include micronutrient deficiencies (e.g., iron, vitamin A, folate, vitamin B-12), infections (e.g., intestinal parasites, schistosomiasis, malaria, human immunodeficiency virus [HIV]), and inherited red blood cell disorders (e.g., sickle cell, α-thalassemia).[3]

Iron deficiency is frequently reported to be the major cause of anemia with an estimate that 50% of anemia worldwide is attributable to iron deficiency.[4] However, the frequency of wide-ranging risk factors for anemia in developing countries, including iron deficiency, vitamin A deficiency, infection, and genetic risk factors, are not routinely measured in a single population.[5] In sub-Saharan Africa, where undernutrition, HIV, malaria, helminthiasis, and hemoglobinopathies are prevalent, an iron supplementation intervention alone may not adequately address anemia.[6–11]

Difficulty demonstrating the success of iron supplementation programs in reducing anemia has led some to question their effectiveness.[9] It is important to understand the scope and strength of individual risk factors for anemia in populations where anemia is common to design more effective interventions.[3,9] In this study, we determined the prevalence of iron deficiency, vitamin A deficiency, malaria infection, non-malarial inflammation, wasting, stunting, α-thalassemia, and sickle cell hemoglobin, and investigated the degree to which these nutritional, infectious, and genetic factors contribute to childhood anemia in western Kenya.

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METHODS

Study population and sample. The study was carried out in Nyando Division, Nyanza Province, Kenya (population 80,000). Residents were primarily of Luo ethnicity, engaged in subsistence farming, and lived in compounds consisting of a single main house surrounded by one to three additional households. In 2007, malaria parasitemia was found in 19.1% of preschool children, and stool parasites (schistosomes, *Trichuris*, ascaris, and hookworm) were found in 14.8% of primary school-aged children.[12]

We carried out a cross-sectional, household-based cluster survey of children 6–35 months of age in August 2010 in 60 villages enrolled in the Nyando Integrated Child Health and Education (NICHE) project. NICHE evaluated the effectiveness of the promotion and sale of health products, including a micronutrient powder, Sprinkles, from 2007 to 2010. Details of NICHE are described elsewhere.[12–14]

Using an updated 2010 household census that was conducted in the study area, 19 compounds were randomly selected per village. Lists of selected compounds were provided to the field team, and all children 6–35 months of age living in these compounds were eligible to participate. Written informed consent was obtained from all participating households. Children severely anemic (hemoglobin < 7.0 g/dL) or with clinical malaria (fever with positive malaria smear) were referred for treatment to the nearest hospital or clinic. Institutional review boards of the Kenya Medical Research Institute and the U.S. Centers for Disease Control and Prevention (CDC) approved the study.

There were 1,348 children assessed for eligibility and 1,079 were eligible. Among the eligible children, 882 children were enrolled, 33 refused, 124 were unavailable for enrollment, and 40 children were excluded for other reasons. An additional 24 children were excluded because of missing hemoglobin results, which led to a total of 858 children included in the final analysis. Some children did not have complete laboratory data for all measures because of inadequate blood volume; therefore, the smallest sample size for one of the multivariable models was 792.

Assessment of nutrition and health status. Trained field workers used a questionnaire to obtain demographic and

socioeconomic data, child feeding practices, and child morbidity in the previous 24 hours. Anthropometric measurements of height and length were made using a wooden measuring board accurate to 0.1 cm (Irwin Shorr Productions, Olney, MD). Weight was measured to the nearest 0.1 kg using a digital scale (Seca Corp, Hanover, MD). Trained fieldworkers completed the measurements using standard techniques. Capillary blood samples were collected for hemoglobin (Hb) measurements and the preparation of malaria smears. Aliquots were stored for the later measurement of iron and vitamin A status, C-reactive protein (CRP), α-1-acid glycoprotein (AGP), and genotyping for blood disorders.

Details of the laboratory analyses are described in detail elsewhere.[14,15] Briefly, Hb was determined from the second drop of blood from the finger using a HemoCue B-Hemoglobin machine (Ängelholm, Sweden). Anemia was defined as hemoglobin < 11.0 g/dL, and severe anemia was defined as hemoglobin < 7.0 g/dL.[16] Malaria blood slides were read at the CDC laboratory in Kisian, Kenya. Presence of any parasites on the blood smear was defined as a positive malaria sample. Frozen plasma samples were transported to the VitA-Iron Lab (Willstaett, Germany), where levels of ferritin, retinol binding protein (RBP), CRP, and AGP were measured by sandwich enzyme-linked immunosorbent assay.[17] The following thresholds were used to define abnormal values for these biochemical indicators: ferritin < 12 μg/L; RBP < 0.7 μmol/L; CRP > 5 mg/L; and AGP > 1 g/L.[18] To account for the effects of inflammation across the entire acute phase response, inflammation was defined as elevated CRP or AGP in the entire study population. Using CRP and AGP enables one to detect inflammation in both early (CRP) and convalescent stages (AGP) of inflammation.[15] Non-malarial inflammation was defined as elevated CRP or AGP in children without malaria.

Ferritin was used to measure iron deficiency because it has the highest sensitivity and specificity to detect iron deficiency in those without inflammation in comparison to bone marrow biopsy.[18] Because ferritin and RBP are acute phase proteins, iron deficiency and vitamin A deficiency were defined using a correction factor approach to adjust ferritin and RBP values for the presence of inflammation, as described previously.[15,19]

Genotyping for HbS and the common African form of α-thalassemia caused by a 3.7-kilobase pair deletion in the α-globin gene were conducted by polymerase chain reaction (PCR) at the KEMRI-Wellcome Trust Laboratories in Kilifi, Kenya. Details of genotyping analyses are described elsewhere.[15,20,21] Children who had one β^S mutation of the Hb gene were defined as HbS, whereas homozygotes were defined as sickle cell disease (HbSS). For α-thalassemia, children with a single α-globin deletion (–α/αα) were defined as heterozygotes for α-thalassemia, whereas those with two α-globin deletions (–α/–α) were defined as homozygotes for α-thalassemia.[20]

Statistical analysis. Statistical analyses were done using SAS 9.2 (SAS Institute Inc., Cary, NC) and Stata 10 (StataCorpLP, College Station, TX). To determine the prevalence and 95% confidence intervals (CIs) for characteristics thought to be related to anemia in the study population, SAS PROC SURVEYFREQ was used to account for the cluster survey design. A multivariable PR regression model was developed to determine variables that were associated with anemia and severe anemia using STATA (survey methods for generalized linear models), taking into account the cluster sample design.

We used the WHO Child Growth Standards (WHO Anthro, Geneva, Switzerland) to calculate *z*-scores, and we categorized underweight as a weight-for-age *z*-score of < –2, stunting as a height/length-for-age *z*-score of < –2, and wasting as a weight-for-height/length *z*-score of < –2. To classify respondents by socioeconomic status, we used a principal component analysis to categorize households into quintiles within the study population on the basis of household assets.[22] Low socioeconomic status was defined as the poorest quintile.

To assess for the characteristics that were most highly associated with anemia and have the highest prevalence in the population, anemia prevalence fractions were calculated[23]

$$\text{Prevalence Fraction} = p_{E|D}\frac{(PR-1)}{(PR)}.$$

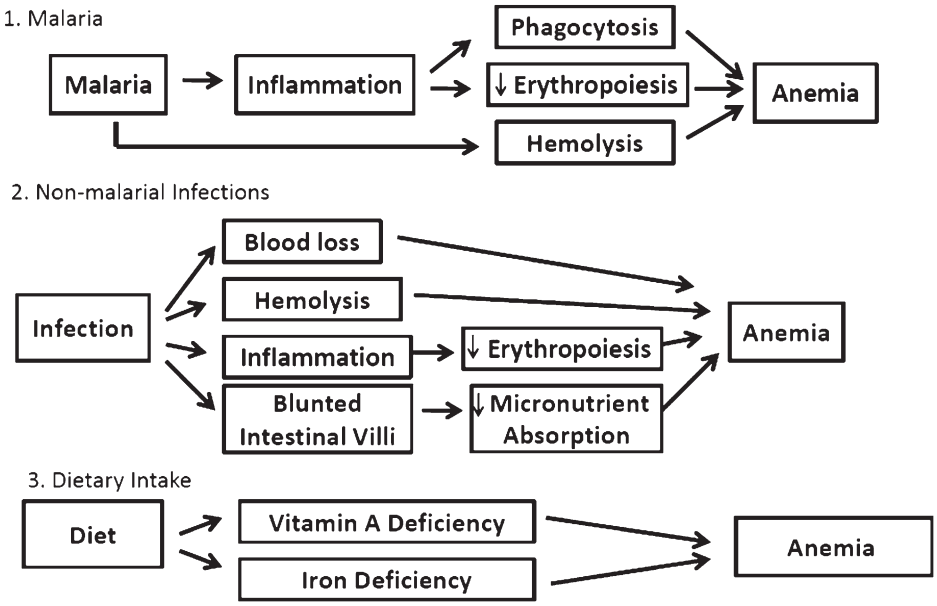

FIGURE 1. Important causal pathways for anemia among children 6–35 months of age, Nyando District, Kenya.

The adjusted PR for anemia was used in the formula for each factor that was significantly associated ($P < 0.05$) with anemia and severe anemia. Prevalence fractions cannot be interpreted as attributable fractions, because this was a cross-sectional study and risk ratios could not be measured. Prevalence fractions have been used to understand both the strength of the association between an exposure and anemia, as well as how common the exposure is in the population.[24,25]

Multivariable modeling approach. The following covariates were considered for the multivariable model to evaluate their association with anemia: low socioeconomic status, less than complete primary school maternal education, male sex, age < 24 months, maternal report of child tea consumption in the last 24 hours, maternal report of child Sprinkles consumption in the last 24 hours, heterozygous α-thalassemia, homozygous α-thalassemia, HbS, HbSS, iron deficiency, vitamin A deficiency, malaria, non-malarial inflammation, child stunting, and child wasting. Our multivariable modeling approach was informed by the hypothesized causal diagrams represented in Figure 1. We did not include "any inflammation" in the multivariable model because inflammation is an intermediate in the malarial causal pathway for anemia and this would change the interpretation of the PR of anemia caused by malaria (Figure 1).[26] Instead, we included inflammation in children without malaria (non-malarial inflammation) as a covariate. We used backward regression to eliminate all terms that had a $P < 0.05$. We then added terms individually back into the model to see if any of the individual terms confounded the relationship between the independent variables and anemia. We kept any term that changed the PR by more than 15%. The same process was repeated using severe anemia as the outcome.

As an additional analysis, we used the exclusion approach to determine the association between iron deficiency and anemia and severe anemia in children that did not have inflammation. This approach has been used to avoid having inflammation bias the association between measures of iron deficiency and anemia.[15] The same additional analysis was also used to assess the association between vitamin A deficiency and anemia and severe anemia. A multivariable model was developed using the same modeling approach described previously to determine the anemia PR for iron deficiency and vitamin A deficiency.

RESULTS

Demographic and health characteristics. Of the 858 children participating in the survey, 50.3% were male, and the mean age was 21.5 months (Table 1). Most children were currently breastfeeding (54.7%). The mean age of mothers was 26.9 years, and 52.5% had completed primary school education. Nearly all households were observed using insecticide-treated bed nets (92.3%). Households were primarily made of mud or dung (95.1%) and were without electricity (98.2%).

Anemia (71.8%) and severe anemia (8.4%) were common; mean hemoglobin concentration was 9.6 g/dL (Table 1). Factors commonly thought to be associated with anemia were found to be widespread, including malaria parasitemia (32.5%), iron deficiency (34.6%), vitamin A deficiency (16.3%), stunting (29.6%), wasting (3.3%), HbS (17.1%), HbSS (1.6%), heterozygous α-thalassemia (38.5%), and homozygous α-thalassemia (9.6%) (Table 1). Non-malarial inflammation was found in 33.0% of children (Table 1). The prevalence of iron deficiency was similar using the correction factor approach or by excluding

TABLE 1

Demographic, anthropometric, and nutritional characteristics of children in Nyando District, Kenya, August 2010*†

	N	% or median (95% CI or interquartile range)
Household		
No electricity (%)	830	98.2 (97.0, 99.4)
Dung or mud walls (%)	830	95.1 (92.6, 97.5)
SES quintiles‡	830	
1 (poorest) (%)		16.6 (12.7, 20.5)
2		22.9 (19.7, 26.1)
3		26.0 (22.2, 29.8)
4		16.5 (13.5, 19.5)
5 (wealthiest)		18.0 (14.3, 21.6)
Mothers		
Age in years (interquartile range)	838	25.0 (21.0, 30.0)
Less than complete primary	833	47.5 (43.0, 52.1)
School education (%)		
Children		
Male (%)	858	50.3 (46.9, 53.8)
Age in months (interquartile range)	858	23.0 (14.0, 28.0)
Ever breastfed (%)	824	91.0 (87.9, 94.1)
Currently breastfeeding (%)	762	54.7 (50.8, 58.6)
Consumed tea in last 24 hours (%)	829	83.1 (80.0, 86.3)
Used Sprinkles in last 24 hours (%)	823	11.1 (8.2, 13.9)
Observed insecticide-treated net in use (%)	829	92.3 (90.0, 94.5)
Hemoglobin (g/dL)	858	9.8 (9.3, 11.1)
Anemia (Hb < 11.0 g/dL) (%)	858	71.8 (68.2, 75.3)
Severe anemia (Hb < 7.0 g/dL) (%)	858	8.4 (6.4, 10.4)
α-globlin genotype	823	
Normal (αα/αα) (%)		51.9 (48.3, 55.5)
Heterozygous α-thalassemia (–α/αα) (%)		38.5 (35.3, 41.7)
Homozygous α-thalassemia (–α/–α) (%)		9.6 (7.6, 11.6)
HbS genotype	854	
Normal (%)		81.3 (78.3, 84.3)
HbS (%)		17.1 (14.3, 19.9)
HbSS (%)		1.6 (0.8, 2.5)
Low ferritin (< 12 μg/L) (%)	847	19.1 (15.8, 22.5)
Iron deficiency (correction factor method)§ (%)	847	34.6 (31.3, 37.9)
Iron deficiency (exclusion method) ¶(%)	322	35.1 (29.5, 40.6)
Low RBP (RBP < 0.7 μg/L)(%)	847	30.9 (27.0, 34.8)
Vitamin A deficiency (correction factor method)‖ (%)	847	16.3 (13.6, 19.0)
Vitamin A deficiency (exclusion method)** (%)	322	12.7 (8.6, 16.9)
Malaria parasitemia (%)	850	32.5 (28.4, 36.6)
Fever in the last 24 hours (%)	855	27.1 (23.3, 31.0)
Elevated CRP (CRP > 5 mg/L) (%)	847	34.1 (29.6, 38.6)
Elevated AGP (AGP > 1 g/L) (%)	847	60.8 (56.1, 65.5)
Any Inflammation†† (%)	847	62.0 (57.3, 66.7)
Non-malarial inflammation‡‡ (%)	845	33.0 (28.5, 37.5)
Stunted (HAZ < –2) (%)	850	29.6 (26.5, 32.8)
Wasted (WHZ < –2) (%)	850	3.3 (1.8, 4.8)

*Values are percent or median with 95% confidence intervals (CI) or interquartile range in parenthesis.

†Abbreviations: SES = socioeconomic status; HbS = sickle cell trait; HbSS = sickle cell disease; RBP = retinol binding protein; CRP = C-reactive protein; AGP= alpha-1-acid-glycoprotein; HAZ = height-for-age z-score; WHZ = weight-for-height or length *z*-score.

‡Households were categorized into quintiles of relative SES based on household assets using a principal component analysis

§Iron deficiency defined as ferritin < 12 μg/L. Ferritin values were adjusted for the presence of inflammation using the following correction factors: correction factor for early inflammation, 0.71; correction factor for early convalescent inflammation, 0.21; correction factor for late convalescent inflammation, 0.50.

¶Iron deficiency defined as ferritin < 12 μg/L excluding children with inflammation.

‖Vitamin A deficiency defined as RBP < 0.7 μg/L. RBP values were adjusted for the presence of inflammation using the following correction factors: correction factor for early inflammation, 1.14; correction factor for early convalescent inflammation, 1.49; correction factor for late convalescent inflammation, 1.09.

**Vitamin A deficiency defined as RBP < 0.7 μg/L excluding children with inflammation.

††Any inflammation was defined as any child with CRP > 5 mg/L or AGP > 1 g/L.

‡‡Non-malarial inflammation was defined as CRP > 5 mg/L or AGP > 1 g/L in children without malaria.

children with inflammation and measuring the prevalence of iron deficiency in the remaining children (34.6% versus 35.1%, respectively). The prevalence of vitamin A deficiency was 16.3% using the correction factor approach and 12.7% if children with inflammation were excluded.

TABLE 2

Characteristics associated with anemia in children 6–35 months of age in Nyando District, Kenya, August 2010

Characteristic	Anemia (%)	Unadjusted PR (95% CI)	Adjusted PR* (95% CI)	*P* value†
SES				
Quintile 1 (poor)	80.4	1.2 (1.0, 1.3)	–	–
Quintiles 2–5	69.9			
Maternal education				
< Complete primary school	75.0	1.1 (1.0, 1.2)	–	–
≥ Primary school	68.2			
Sex				
Male	76.2	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	0.020
Female	67.4			
Age				
< 24 months	75.8	1.1 (1.0, 1.2)	1.2 (1.1, 1.3)	< 0.001
≥ 24 months	67.0			
Consumed tea in last 24 hr				
Yes	71.4	1.0 (0.9, 1.1)	–	–
No	73.6			
Consumed Sprinkles in last 24 hr				
Yes	68.1	0.9 (0.8, 1.1)	–	–
No	72.3			
α-globlin genotype				
Normal (αα/αα)	66.7	Reference		
Heterozygous α-thalassemia (–α/αα)	75.7	1.1 (1.0, 1.3)	1.1 (1.0, 1.3)	0.006
Homozygous α-thalassemia (–α/–α)	83.5	1.3 (1.1, 1.4)	1.3 (1.1, 1.4)	< 0.001
Hemoglobin type				
Normal	72.5	Reference		
HbS	69.2	1.0 (0.8, 1.1)	–	–
HbSS	71.4	1.0 (0.7, 1.4)	–	–
Iron deficiency‡				–
Yes	80.5	1.2 (1.1, 1.3)	1.3 (1.2, 1.4)	< 0.001
No	67.0			
Vitamin A deficiency§				
Yes	75.4	1.1 (0.9, 1.2)	–	–
No	70.9			
Malaria parasitemia				
Yes	90.6	1.4 (1.3, 1.6)	1.7 (1.5, 1.9)	< 0.001
No	62.9			
Non-malarial inflammation¶				
Yes	71.0	1.0 (0.9, 1.1)	1.2 (1.1, 1.4)	0.003
No	72.1		–	–
Stunted				
Yes	79.8	1.2 (1.1, 1.3)	1.1 (1.0, 1.2)	0.017
No	68.5			
Wasted				
Yes	92.9	1.3 (1.1, 1.5)	1.2 (1.1, 1.4)	0.008
No	71.2			

*Adjusted anemia prevalence ratio (PR) is presented for a multivariable generalized linear model that accounted for cluster study design. Only significant factors ($P < 0.05$) or confounders were kept in the multivariable model. Factors included in the multivariable model ($N = 792$) were male sex, age < 24 months, heterozygous and homozygous α-thalassemia, iron deficiency, malaria, non-malarial inflammation, stunting, and wasting.

†*P* value is for association between the given factor and anemia in the multivariable model.

‡Iron deficiency was defined as ferritin < 12 μg/L. Ferritin values were adjusted lower in the presence of inflammation using correction factors.

§Vitamin A deficiency was defined as RBP < 0.7 μg/L. RB*P* values were adjusted higher in the presence of inflammation using correction factors.

¶Non-malarial inflammation was defined as CRP > 5 mg/L or AGP > 1 g/L in children without malaria.

CI = confidence interval; SES = socioeconomic status; HbS = sickle cell trait; HbSS = sickle cell disease.

Characteristics associated with anemia and severe anemia. In bivariate analysis, the following childhood characteristics were associated with anemia ($P < 0.05$): low socioeconomic status, less than complete primary school maternal education, male sex, age < 24 months, heterozygous α-thalassemia, homozygous α-thalassemia, iron deficiency, malaria, stunting, and wasting (Table 2). In multivariable analysis, childhood characteristics associated with having anemia included male sex, age < 24 months, heterozygous α-thalassemia, homozygous α-thalassemia, iron deficiency, malaria, non-malarial inflammation, stunting, and wasting (Table 2). The characteristics most strongly associated with having anemia included malaria (PR: 1.7; 95% CI: 1.5, 1.9), iron deficiency (PR: 1.3; 95% CI: 1.2, 1.4), and homozygous α-thalassemia (PR: 1.3; 95% CI: 1.1, 1.4). Homozygous α-thalassemia (PR: 1.3; 95% CI: 1.1, 1.4) was more strongly associated with anemia than heterozygous α-thalassemia (PR: 1.1; 95% CI: 1.0, 1.3), however this was not statistically significant (PR: 1.1; 95% CI: 0.98, 1.2). If we had used any inflammation in the model instead of non-malarial inflammation, the anemia PR for inflammation increased compared with non-malarial inflammation (1.3 versus, 1.2) and the anemia PR for malaria decreased (1.4 versus 1.7).

We also constructed a multivariable model to evaluate the association between iron deficiency and anemia and vitamin A deficiency and anemia by excluding children with inflammation. Similar to the correction factor approach, vitamin A deficiency was not associated with anemia and iron deficiency was associated with anemia. The anemia PR for iron deficiency using the correction factor method (PR: 1.3; 95% CI: 1.2, 1.4) was not as large as using the exclusion method (PR: 1.6; 95% CI: 1.3, 1.9).

Children with malaria were more likely (PR: 1.8; 95% CI: 1.6, 2.0) to have inflammation than those without malaria, and 88.3% of children with malaria had inflammation. In bivariate analysis, 80.5% of children with iron deficiency had anemia. In children with homozygous α-thalassemia, 83.5% had anemia, and in children with wasting 92.9% had anemia.

In bivariate analysis, the following characteristics were associated with having severe anemia ($P < 0.05$): malaria parasitemia and stunting (Table 3). Childhood characteristics associated with severe anemia in multivariable analysis include malaria (PR: 10.2; 95% CI: 3.5, 29.3), non-malarial inflammation (PR: 6.7; 95% CI: 2.3, 19.4), and stunting (PR 1.6; 95% CI: 1.0, 2.4). If we had used any inflammation in the model instead of non-malarial inflammation, the severe anemia PR for inflammation increased compared with non-malarial inflammation (8.2 versus 6.7) and the severe anemia PR for malaria decreased (1.7 versus 10.2).

In bivariate analysis, 14.9% of children with malaria had severe anemia. All children with severe anemia and malaria had inflammation. Among children with non-malarial inflammation, 9.3% had severe anemia. In children that did not have malaria but had severe anemia, 87% had inflammation. We also attempted to evaluate the association between vitamin A deficiency and severe anemia and iron deficiency and severe anemia by excluding children with inflammation. However, because of small sample sizes this association could not be evaluated.

Prevalence fractions. To identify the characteristics that had the highest prevalence and strongest association with anemia, we calculated prevalence fractions for both anemia

TABLE 3

Characteristics associated with severe anemia in children 6–35 months of age in Nyando District, Kenya, August 2010

Characteristic	Severe anemia (%)	Unadjusted PR (95% CI)	Adjusted PR* (95% CI)	*P* value†
SES				
Quintile 1 (poor)	10.1	1.3 (0.7, 2.3)	–	–
Quintiles 2–5	7.8			
Maternal education				
< Complete primary school	9.1	1.2 (0.7, 2.1)	–	–
≥ Primary school	7.3			
Sex				
Male	9.3	1.2 (0.8, 2.0)	–	–
Female	7.5			
Age				
< 24 months	7.6	0.8 (0.5, 1.4)	–	–
≥ 24 months	9.4			
Consumed tea in last 24 hr				
Yes	8.7	1.7 (0.8, 3.7)	–	–
No	5.0			
Consumed Sprinkles in last 24 hr				
Yes	6.6	0.8 (0.4, 1.7)	–	–
No	8.3			
α-globlin genotype				
Normal (αα/αα)	7.3	Reference	–	–
Heterozygous α-thalassemia (–α/αα)	9.5	1.3 (0.7, 2.3)	–	–
Homozygous α-thalassemia (–α/-α)	11.4	1.6 (0.7, 3.4)	–	–
Hemoglobin type				
Normal	8.6	Reference		
HbS	6.8	0.8 (0.4, 1.5)	–	–
HbSS	14.3	1.7 (0.5, 5.7)	–	–
Iron deficiency‡				
Yes	6.8	0.7 (0.4, 1.3)	–	–
No	9.4			
Vitamin A deficiency§				
Yes	11.6	1.5 (0.9, 2.4)	–	–
No	7.9			
Malaria parasitemia				
Yes	14.9	2.8 (1.8, 4.6)	10.2 (3.5, 29.3)	< 0.001
No	5.2			
Non-malarial inflammation¶				
Yes	9.3	1.2 (0.7, 1.9)	6.7 (2.3, 19.4)	0.001
No	8.0			
Stunted				
Yes	12.3	1.8 (1.2, 2.7)	1.6 (1.0, 2.4)	0.032
No	6.9			
Wasted				
Yes	17.9	2.2 (0.9, 5.1)	–	–
No	8.2			

*Adjusted severe anemia prevalence ratio (PR) is presented from a multivariable generalized linear model that accounted for cluster study design. Only significant factors ($P < 0.05$) or confounders were kept in the multivariable model. Factors included in the multivariable model ($N = 836$) were malaria parasitemia, non-malarial inflammation, and stunting.

†*P* value is for association between the given factor and anemia in the multivariable model.

‡Iron deficiency was defined as ferritin < 12 μg/L. Ferritin values were adjusted lower in the presence of inflammation using correction factors.

§Vitamin A deficiency was defined as RBP < 0.7 μg/L. RB*P* values were adjusted higher in the presence of inflammation using correction factors.

¶Non-malarial inflammation was defined as CRP > 5 mg/L or AGP > 1 g/L in children without malaria.

CI = confidence interval; SES = socioeconomic status; HbS = sickle cell trait; HbSS = sickle cell disease.

and severe anemia (Figure 2). The characteristics that were associated with the largest percentage of cases of anemia were malaria (16.8%), age < 24 months (8.4%), iron deficiency (8.3%), and non-malarial inflammation (6.1%). The characteristics with the largest prevalence fractions for severe anemia were malaria (52.1%), non-malarial inflammation (31.2%), and stunting (16.1%).

DISCUSSION

On the basis of the WHO classification for persistent anemia in a population (40%), our findings indicate that anemia among preschool aged children in Nyando District, Kenya was a severe public health problem that was associated with many known risk factors.[2] This is one of the first studies to measure many factors thought to be associated with anemia including hemoglobinopathies among a single population in a developing country setting where malaria was highly prevalent. We found that malaria, iron deficiency, and homozygous α-thalassemia were the factors that were most strongly associated with having anemia, and that malaria, non-malarial inflammation, and stunting were most strongly associated with severe anemia. Non-modifiable characteristics including α-thalassemia, sex, and age < 24 months were also associated with anemia and commonly found in the population.

The fact that malaria and iron deficiency were the characteristics that were most strongly associated with anemia was plausible for rural, western Kenya. Recurrent malaria infections and a diet deficient in iron are common in this community. However, neither condition was associated with a majority of cases of anemia, which may challenge the estimation that 50% of cases of anemia in malaria-endemic areas is caused by iron deficiency.[4] George and others[27] recently examined the association of genetic disorders and other factors with anemia in Cambodia; however, the association of these disorders with severe anemia, a risk factor for mortality, was not examined. Furthermore, no estimate was made of the relative contribution of each of these factors to anemia in the population. In our Kenyan study, malaria was common, and as a result we were able to determine the strength of association between anemia and malaria, as well as the strength of association between anemia and iron deficiency and hemoglobinopathies, factors thought to be affected by the presence of malaria.

Severe anemia was most strongly associated with malaria, non-malarial inflammation, and stunting. Malaria is a known cause of severe anemia and as expected, was associated with a large percentage of cases.[26] Non-malarial inflammation was also strongly associated with severe anemia, which may be caused by several characteristics commonly found in this population that are known causes of inflammation and anemia including tropical enteropathy, HIV, and resolving inflammation in those that have recently cleared malaria infection. After the resolution of malaria, levels of alpha-1-acid-glycoprotein (AGP) stay elevated for up to 3 weeks after clearance of parasitemia.[28] Therefore, children who clear parasitemia can have continued removal of unparasitized red blood cells by activated macrophages in the spleen. This mechanism is thought to cause severe anemia in children with malaria and may explain, in part, the strong association we observed between non-malarial inflammation and severe anemia.[26] Our results suggest that inflammation may be an intermediate in the causal pathway for malaria and anemia. When the variable "any inflammation" instead of "non-malarial inflammation" was used in the multivariable model, the association between both anemia and malaria and severe anemia and malaria decreased.

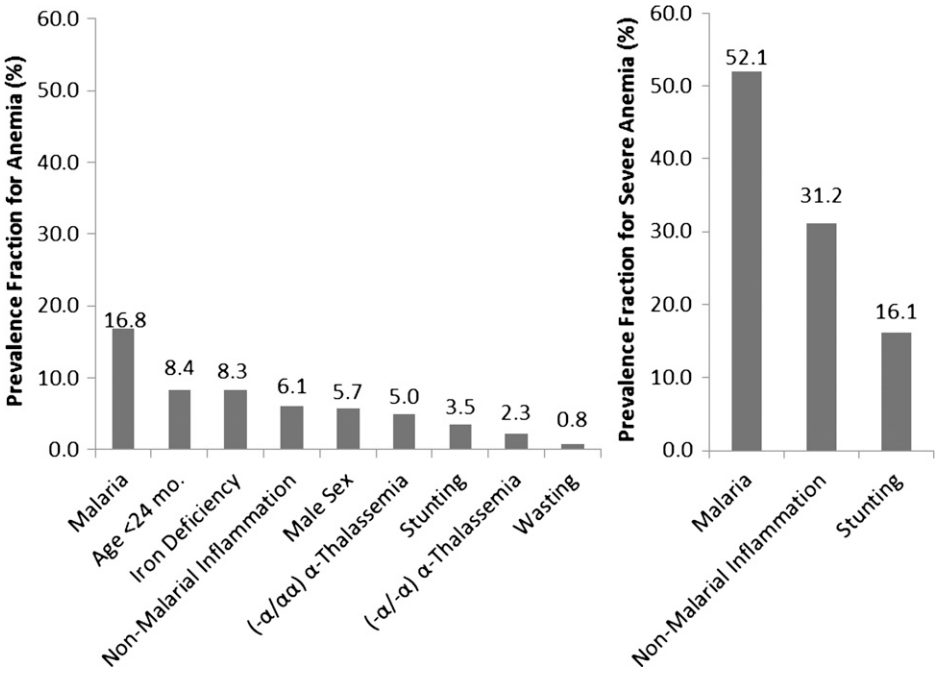

FIGURE 2. Anemia and severe anemia prevalence fractions for associated factors, Nyando District, Kenya.

Furthermore, the association between anemia and severe anemia and "any inflammation" was stronger in comparison to the association between anemia and severe anemia and "non-malarial inflammation."

Another possible source of inflammation that could lead to anemia is tropical enteropathy, whereby elevated inflammatory markers and blunted intestinal villi impair absorption of nutrients and cause anemia of chronic inflammation.[29] Nearly everyone living in developing countries may have signs of tropical enteropathy or intestinal dysfunction, thought to be as a result of poor hygiene and sanitation conditions.[30] Furthermore, HIV prevalence among infants in western Kenya is estimated to be 10%,[31] which could cause inflammation as well as anemia and severe anemia.[32,33] Schistosomiasis, present in school-aged children in the region, can also cause anemia and inflammation.[34,35] It is possible that children had multiple parasitic infections, resulting in inflammation and anemia that has been seen in other developing country settings.[36]

Stunting was also associated with a large fraction of the cases of anemia and severe anemia. Intestinal parasites can cause stunting, anemia, and severe anemia.[32,37,38] Because we did not measure intestinal parasites, and intestinal parasites were present among school-aged children in the study community in 2007, stunting may be an indicator for intestinal parasite infection that could in part explain its association with anemia and severe anemia.[12] Other studies have shown that helminthiasis may not be associated with inflammation, and its association with anemia was not likely captured within the non-malarial inflammation group.[37,39] Stunting and wasting can be associated with HIV infection, and HIV is a known risk factor for anemia present in this region.[31,40] This could be a possible explanation for the association between wasting and anemia and stunting and anemia. Wasting has also been shown to be associated with helminthiasis, and treating helminthiasis has also been shown to reduce both wasting and anemia.[10] Even though we adjusted for some of the possible infectious and nutrition-related pathways, there may be other unmeasured factors that could explain the link between anthropometric measures and anemia.

Sickle cell hemoglobin and sickle cell disease were not associated with anemia. Because this was a cross-sectional study that did not follow a cohort from birth, it was unknown what happened to all children in this population that were born with sickle cell disease. There is a high mortality rate among children with sickle cell disease between 1 and 3 years of age, and low hemoglobin is a risk factor for death among these children.[41] It was possible that in this population, children with sickle cell disease that were more likely to have anemia or severe anemia already died.[42]

There are limited studies with few subjects that have evaluated the prevalence of α-thalassemia in eastern Africa and its association with anemia.[20,43] Our study found that α-thalassemia is an important genetic characteristic that is associated with anemia. Although it is thought that heterozygous α-thalassemia is not associated with anemia, and those with homozygous α-thalassemia will have a mild anemia, we found that both homozygous and heterozygous α-thalassemia were associated with anemia.[44]

Vitamin A deficiency was not associated with anemia or severe anemia in this survey, which is inconsistent with other published findings.[32] It is possible that the other studies did not adjust their retinol or RBP values for inflammation, which could have biased the association between vitamin A deficiency and anemia and severe anemia.[32,45]

The study had several limitations. First, the study was carried out in Nyando District, Kenya, and the findings are likely not representative of Kenya or sub-Saharan Africa. Second, this was a cross-sectional study, therefore causality cannot be determined. In addition, PRs, not risk ratios were measured. As a result, we were not able to calculate population attributable

fractions. However, prevalence fractions are still helpful for measuring population-level associations by helping one understand both the strength of the association and how common the factor is in the population.[25] Third, the correction factor method has never been validated against bone marrow biopsy or liver biopsy, the gold standard tests of iron deficiency and vitamin A deficiency in the presence of inflammation.[46,47] Fourth, the cause of the high prevalence of inflammation was unknown. We did not collect data on additional characteristics that can cause inflammation such as HIV, tropical enteropathy, or schistosomiasis that also were potentially associated with anemia. Finally, we could not examine the effect of polyparasitic infections on anemia, which was shown to be important in other populations where malaria, schistosomiasis, and intestinal parasites were likely present.[36]

In conclusion, anemia is a severe public health problem in Nyando District, Kenya. Malaria and iron deficiency were most strongly associated with anemia, and non-malarial inflammation, malaria, and stunting were most strongly associated with severe anemia. Alpha-thalassemia was an important non-modifiable genetic factor that was associated with anemia and common in this population. There was no single characteristic that was associated with the majority of the cases of anemia. To implement effective public health interventions to prevent anemia in this population, one must take an integrated approach that addresses iron deficiency, as well as infections including malaria, schistosomiasis, HIV, and intestinal parasites.

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