Vitamin a supplementation and other predictors of anemia among children from Dar Es Salaam, Tanzania.

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VITAMIN A SUPPLEMENTATION AND OTHER PREDICTORS OF ANEMIA AMONG CHILDREN FROM DAR ES SALAAM, TANZANIA

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Abstract. The associations of hemoglobin, hematocrit, and packed cell volume with socioeconomic factors, malaria, human immunodeficiency virus (HIV) infection, and nutritional status were examined among 687 children admitted to hospital with pneumonia participating in a double blind, placebo-controlled trial of vitamin A supplementation. Children were randomized to receive 2 doses of vitamin A (200,000 IU) or placebo at baseline, and additional doses at 4 and 8 months after discharge from hospital. Hemoglobin levels were measured at enrollment and, on a subset of 161 children, during follow-up. At baseline, hemoglobin concentration was positively associated with the number of possessions in the household, maternal level of education and quality of water supply, and inversely related to malaria infection after controlling for potential confounding variables. Children infected with HIV experienced a significant fall in mean hemoglobin levels over time. The risk of developing severe anemia (< 7 g/dL) during follow-up was lower for children who were breastfed for longer than 18 months as compared to those with less than 6 months of breastfeeding (adjusted prevalence ratio = 0.14, 95% confidence interval [CI]= 0.02, 0.93; $P = 0.04$), and higher for children over two years of age as compared to 6 to 11 months-old infants (adjusted prevalence ratio = 8.11, 95% CI = 1.2, 55.8; $P = 0.03$). Children with repeated diagnoses of malaria had 4.1 times the risk of developing severe anemia than did children without the diagnosis (95% CI = 1.3, 13.5; $P = 0.02$). Vitamin A supplements were associated with an overall nonsignificant reduction of 14% in the risk of developing severe anemia (adjusted prevalence ratio = 0.86, 95% CI = 0.37, 1.99; $P = 0.73$). We conclude that malaria, HIV infection, low socioeconomic status, and short duration of breastfeeding are strong and independent determinants of adverse hematologic profiles in this population.

INTRODUCTION

The prevalence of anemia in the developing world is still extremely high. In Africa, 33% of children below age 4, 52% of children from 5–14 years, and 47% of pregnant women have hemoglobin levels below the established adequate cutoff points: 11, 12, and 11 g/dL, respectively.[1] Commonly, anemia is the more severe manifestation of iron deficiency.[2] However, its determinants vary across cultural and geographic settings.[3] In many parts of sub-Saharan Africa, for example, malaria infection accounts for a large proportion of severe anemia among children,[4] but in others, the marginal intake of foods rich in iron is still the most likely cause of anemia.[5] Most of the studies on the determinants of anemia are cross-sectional surveys that fail to differentiate the temporality of the associations between exposure and outcome. Longitudinal designs, such as the one presented here, are more appropriate for identifying specific risk factors that would allow targeting of interventions aimed at reducing the burden of anemia in children.

The coexistence of multiple micronutrient deficiencies may elevate the risk of developing anemia. There is an increasing body of evidence from observational and intervention studies suggesting a positive biological relationship between vitamin A status and iron metabolism,[6] although the specific mechanisms remain unknown. Administration of vitamin A to anemic children[7,8] and pregnant women[9] has resulted in improvements of hematologic parameters and recovery from anemia. Thus, vitamin A supplementation of anemic populations could be an effective, low cost intervention for improving hemoglobin concentrations.[10] There are limited data on trials from sub-Saharan Africa addressing the effect of vitamin A on the hematological status of children. Moreover, the potential interactions between other determinants of anemia and the administration of vitamin A remain unclear. We examined the cross-sectional and longitudinal associations between sociodemographic and health-related predictors of hemoglobin, as well as the effect of vitamin A supplements on the risk of anemia in preschool children from Dar es Salaam, Tanzania.

MATERIALS AND METHODS

The Tanzania Vitamin A Study was a randomized, double-blind, placebo-controlled trial conducted between April 1993 and March 1997 among 687 children 6 to 60 months of age who were admitted with pneumonia to Muhimbili Medical Center in Dar es Salaam. The trial design has been described elsewhere.[11,12] Briefly, children were enrolled to determine the effect of vitamin A on severity of infection during hospital stay. Pneumonia was diagnosed by a pediatrician if children presented with cough and one or more of the following signs: respiratory rate ≥ 40 breaths (50 breaths for infants below one year of age) per minute, chest retractions, inability to eat or drink, decreased air entry, crackling sounds, or dullness to percussion. Exclusion criteria were: presence of eye signs and symptoms of vitamin A deficiency, intake of vitamin A supplements in the preceding 4 months, weight-for-age less than 60% of the reference median, measles, pulmonary tuberculosis, diphteria, and whooping cough. In-hospital treatment of pneumonia was provided according to the standard of care in Tanzania. Children were followed for at least one year after discharge from hospital for survival, health, and growth endpoints. Informed consent was granted by the mother or primary caretaker of the child. The protocol was approved by the Research and Publications Committee of Muhimbili University College of Health Sciences, the Research and Ethics Committee of Tanzania Food

and Nutrition Center, and the Human Subjects Committee of The Harvard School of Public Health.

The participants were randomly assigned to receive four oral doses of either 200,000 IU of vitamin A and 40 IU of vitamin E or placebo (40 IU of vitamin E only). Half of this dose was given to children aged < 1 year. The first dose was administered on the day of admission and the second on the following day. The third and fourth doses were given at four and eight months respectively after discharge from hospital. Compliance with the first two doses was virtually 100%, since they were administered in the hospital by a research supervisor. The two remaining doses were given at a study clinic where mothers and children presented during follow-up. Seventy-five percent of the children were given the third dose and 74.2% received the fourth. Laboratory assays of the vitamin A solution yielded 95% potency after 2 years of field storage.

Information collected at baseline included feeding practices, immunizations, and sociodemographic characteristics such as the parents' level of education, number of siblings, water and sanitary conditions in the household, and possessions available at home (radio, television, bicycle, car, and refrigerator). Height (or length in children < 2 years of age) and weight were measured on the first day of hospital admission by trained personnel using standard techniques and calibrated instruments. A blood specimen was drawn from all children at baseline. At 4 and 8 months, concurrent with the administration of the third and fourth doses, authorization was granted from a subset of the mothers to obtain additional blood samples from their children. Hemoglobin, packed cell volume, and red cell counts were determined by standard techniques.[13] *Plasmodium falciparum* malaria was assessed by thick and thin blood smears. The latest blood specimen available for each child was tested for human immunodeficiency virus (HIV) antibodies using ELISA (Murex Biotech Ltd, Dartford, UK), and confirmed by Western Blot (Biorad Laboratories, Ltd, Hertfordshire, UK). Infection with HIV among children younger than 15 months with positive or undetermined results was also tested using heat-denatured HIV-p24 antigen assays with confirmatory neutralization assays (Dupont, Wilmington, DE).

In a cross-sectional analysis of 617 children in whom a full blood picture was obtained at recruitment, we studied the relationships between baseline hemoglobin concentration and socioeconomic status, malaria and HIV diagnoses, breastfeeding, and nutritional status. Differences among medians of hematological parameters across levels of the covariates were tested using Wilcoxon rank sum and Kruskal-Wallis tests. A linear regression model for hemoglobin was fitted including the independent variables that were significant predictors in the univariate analysis ($P < 0.20$) and those considered relevant from the biological point of view.

The subset of 161 children who had blood pictures at both baseline and follow-up did not differ from the entire study population at baseline in terms of their sociodemographic characteristics, health status, or assignment to treatment. Distribution of potential confounders between vitamin A and placebo groups was compared in the follow-up subsample. We conducted intent-to-treat analyses to examine the effect of supplements on hemoglobin levels over time using mixed effects regression models.[14] Hemoglobin at baseline and at 4 and 8 months was introduced as the dependent variable and vitamin A supplementation, sociodemographic variables, HIV status, and malaria infection as predictors. Robust estimators of variance[15] were used in the regression models.

Severe anemia was defined as a hemoglobin level below 7 g/dL.[16] This corresponds to the 25th percentile of the hemoglobin distribution in our study population. The effect of vitamin A supplements and other variables on severe anemia at either 4 or 8 months was studied using the latest value available as the follow-up endpoint. Prevalence ratios and 95% confidence intervals of anemia at follow-up were estimated by binomial regression with the log link function.[17] The likelihood ratio test was employed to determine whether treatment effects were significantly different across strata of potential modifiers. Data were analyzed with the Statistical Analyses System software (SAS Institute Inc., Cary, NC).

RESULTS

Sociodemographic characteristics did not differ significantly by treatment group at baseline (Table 1). Mean hemoglobin level was 8.5 g/dL (SD = 2.3). About 40% of children were below the age of one year. In the follow-up subsample, children on placebo were more likely to be male, younger, with less educated mothers, fewer belongings, and slightly shorter duration of breastfeeding. Mean hemoglobin at the latest follow-up visit was 9.4 g/dl (SD = 1.7). The prevalence of malaria was 26% at enrollment and 23% at the last visit.

Determinants of hemoglobin, packed cell volume and red cell count at baseline are presented in Table 2. Hemoglobin was positively associated with child's age, and with indicators of the household's socioeconomic status. Mean hemoglobin of children whose mothers had secondary schooling was 1.7 g/dL higher than that of children whose mothers had no primary schooling ($P < 0.001$). Hemoglobin was also significantly higher among children from households with more possessions and better quality of water supply. Duration of breastfeeding at baseline was also positively related, albeit not significantly, to the hemoglobin levels. Mean hemoglobin concentration of children diagnosed with malaria was 1.4 g/dL—significantly lower than among those without malaria. Hemoglobin, packed cell volume, and red cell count were positively associated with the mother's level of education, number of possessions and quality of water, and inversely related to malaria. The child's HIV status was not a significant predictor of hemoglobin levels at enrollment.

In analysis adjusted for sex, age, duration of breastfeeding, and socioeconomic variables, malaria infection was a strong predictor of hemoglobin level at the first visit (Table 3). The difference in mean hemoglobin between children with or without malaria diagnosis was comparable to that found in unadjusted analysis (1.3 g/dL higher in children free of the disease, 95% CI = −1.74, −0.88). Children of mothers with a secondary level of education or from households with two or more possessions had significantly higher hemoglobin concentrations than those of mothers with the lowest level of education or households with no possessions, respectively. Better household water supply was also associated with increased hemoglobin level. Children from dwellings with tap water had, on average, 1.2 g/dL higher

TABLE 1
Characteristics of the study population at baseline in vitamin A and placebo arms

	Placebo no. (%)	Vitamin A no. (%)
No. randomized	307	310
Sex		
Female	135 (44.3)	141 (45.6)
Male	170 (55.7)	168 (54.4)
Child's age (months)		
6–11	137 (44.6)	123 (39.7)
12–23	98 (31.9)	115 (37.1)
≥24	72 (23.4)	72 (23.2)
Mother is literate*	264 (86.0)	273 (88.0)
Mother's education		
Low†	51 (16.6)	46 (14.8)
Elementary	231 (75.2)	249 (80.3)
Secondary	25 (8.1)	15 (4.8)
Mother's occupation		
Housewife	234 (76.2)	219 (70.7)
Petty trader	52 (16.9)	60 (19.4)
Messenger/clerk	7 (2.3)	9 (2.9)
Professional	7 (2.3)	9 (2.9)
Other	7 (2.3)	13 (4.2)
Mother's parity		
No previous births	88 (29.2)	96 (31.4)
1–2	124 (41.2)	133 (43.5)
3–4	63 (20.9)	56 (18.3)
≥5	26 (8.6)	21 (6.9)
Household number of possessions‡		
0	47 (15.3)	34 (11.0)
1	142 (46.3)	161 (51.9)
≥2	118 (38.4)	115 (37.1)
Water supply		
Tap in house	52 (16.9)	52 (16.8)
Tap in compound	86 (28.0)	91 (29.4)
Tap outside compound	151 (49.2)	154 (49.7)
Public well	18 (5.8)	13 (4.2)
Duration of breastfeeding (months)		
0–6	50 (16.3)	41 (13.3)
7–12	110 (35.8)	117 (37.9)
13–18	78 (25.4)	78 (25.2)
≥19	69 (22.5)	73 (23.6)
Child's nutritional status§		
Stunted	62 (22.2)	73 (25.3)
Wasted	42 (15.1)	36 (12.5)
Stunted and wasted	9 (3.2)	12 (4.2)
Malaria prevalence¶	63/258 (24.4)	72/266 (27.1)
HIV positive	23/294 (7.8)	29/301 (9.6)

* Ability to read a sentence.
† Low education includes mothers with no education and those who attended Koranic school or adult education classes.
‡ Radio, television, car, bicycle, and refrigerator.
§ Wasted children were below −2 z-scores in weight-for-age, stunted children were below −2 z-scores (National Center for Health Statistics reference) in height-for-age.
¶ Presence of malaria parasites in thick and thin blood smears.
# HIV = human immunodeficiency virus.

hemoglobin than children whose mothers used public wells (95% CI = 0.19, 1.87 g/dL). Duration of breastfeeding was positively associated with hemoglobin at baseline, although this relationship was not statistically significant.

Vitamin A supplementation resulted in a non-significant, average increase of 0.19 g/dL in hemoglobin levels per visit, after adjusting for sex, age, number of household possessions, HIV status, and malaria, in a longitudinal regression model for repeated measurements of hemoglobin. Children with malaria at baseline had a hemoglobin concentration that was, on average, 1.38 g/dL lower than that of children with a negative blood smear (95% CI = −1.79, −0.97 g/dL; $P < 0.01$). Over time, there was a significant increase in hemoglobin levels, which was higher among children with malaria at the first visit than in those free of the infection (1.13 g/dL per visit versus 0.31 g/dL per visit). Hemoglobin levels at any visit were, on average, 1.30 g/dL lower (95% CI = −1.66, −0.94 g/dL; $P < 0.01$) among children with malaria diagnosis at that same visit than in children without parasites in their blood smears. The short-term change in hemoglobin during any four-month interval between two visits was significantly influenced by the child's HIV status and the hemoglobin level at the beginning of the particular interval. Hemoglobin change was higher among children with lower levels at the beginning of the interval. HIV-positive children had an average 0.8 g/dL decrease in hemoglobin levels during any four-month interval (95% CI = 0.09, 1.49 g/dL; $P = 0.03$).

The overall prevalence of severe anemia (hemoglobin below 7.0 g/dL) at the last follow-up visit was 10.6%. Adjusted prevalence ratio (PR) estimates for anemia by several predictors are presented in Table 4. Children older than two years of age were at a higher risk of anemia than children below one year (Prevalence Ratio = 8.1, 95% CI = 1.2, 55.8; *P* value, test for trend = 0.03). Children with malaria diagnosis at both baseline and follow-up visits had a 4.1 times higher risk of anemia as compared to children who never had malaria. Children who had malaria at baseline but not at follow-up had the lowest risk for anemia in the last visit. Breastfeeding was associated with lower risk of anemia: children who had been breastfed for more than 18 months at the time of enrollment had a large and significant reduction in their risk of developing severe anemia during the follow-up period when compared to children who had been breastfed for six months or less (PR = 0.14, 95% CI = 0.02, 0.93; *P* value, test for trend = 0.04).

Overall, vitamin A supplementation was associated with a non-significant 14% reduction in the risk of developing severe anemia (PR = 0.86, 95% CI = 0.37, 1.99). We examined the effect of the supplements by strata of some other predictors (Table 4). There was a borderline significant interaction between treatment and anemia at baseline ($P = 0.07$). Among children with severe anemia at the first visit, vitamin A had a protective effect against the risk of having anemia by the end of follow-up (PR = 0.27; 95% CI = 0.03, 2.19; $P = 0.22$).

DISCUSSION

Few studies have addressed the determinants of longitudinal changes in hemoglobin levels and anemia risk in a pediatric population with high prevalence of both malaria and HIV infection. We conducted cross-sectional and longitudinal analyses for the predictors of hemoglobin and severe anemia in preschool Tanzanian children who participated in a randomized clinical trial of vitamin A supplementation.

Malaria infection and low socioeconomic status, as measured by the level of maternal education, the number of possessions in the household, and the quality of water supply were strong determinants of low hemoglobin concentration at first visit. In addition, shorter duration of breastfeeding,

TABLE 2
Univariate relationships of child and maternal characteristics and hematologic indicators at baseline

	No. (%)	Mean chemoglobin g/dL (SD)	Mean packed cell volume % (SD)	Mean red cell count × $10^6/\mu l$ (SD)
Sex				
Female	276 (45)	8.7 (2.2)	26.1 (6.7)	3.67 (1.05)
Male	338 (55)	8.5 (2.3)	25.6 (6.3)	3.67 (0.98)
Child's age (months)				
6–11	260 (42)	8.4 (2.1)	25.4 (6.3)	3.63 (1.00)
12–23	213 (35)	8.4 (2.1)	25.6 (6.0)	3.76 (1.00)
≥24	144 (23)	9.1 (2.5)*	26.8 (7.4)	3.59 (1.04)
Mother is literate¶				
No	80 (13)	8.7 (2.2)	26.0 (6.4)	3.60 (0.95)
Yes	537 (87)	8.5 (2.2)	25.7 (6.5)	3.68 (1.02)
Mother's education				
Low¶	97 (16)	8.6 (2.3)	26.1 (6.1)	3.62 (0.93)
Elementary	480 (78)	8.4 (2.2)	25.4 (6.3)	3.63 (1.02)
Secondary	40 (6)	10.3 (2.0)†	30.1 (6.6)†	4.29 (0.87)†
Mother's occupation				
Housewife	453 (73)	8.5 (2.2)	25.7 (6.5)	3.65 (1.02)
Petty trader	112 (18)	8.2 (2.1)	25.2 (6.0)	3.64 (0.94)
Messenger/clerk	16 (3)	9.5 (2.7)	28.7 (8.1)	3.84 (1.27)
Professional	16 (3)	9.3 (1.9)‡	27.8 (5.6)	3.67 (0.93)
Other	20 (3)	9.6 (2.0)	28.5 (6.5)	4.08 (0.88)
Mother's parity				
No previous births	184 (30)	8.5 (2.3)	25.4 (6.7)	3.61 (1.08)
1–2	257 (42)	8.6 (2.2)	25.8 (6.5)	3.70 (1.00)
3–4	119 (20)	8.3 (2.3)	25.8 (6.1)	3.65 (0.94)
≥5	47 (8)	9.0 (2.0)	27.0 (6.3)	3.74 (0.93)
Household possessions				
None	81 (13)	7.7 (2.2)	23.2 (6.7)	3.21 (1.02)
1	303 (49)	8.4 (2.2)	25.4 (6.2)	3.62 (0.99)
≥2	233 (38)	9.0 (2.3)†	27.0 (6.4)†	3.88 (0.98)†
Water supply				
Tap in house	104 (17)	9.2 (2.3)	27.7 (6.7)	3.99 (1.00)
Tap in compound	177 (29)	8.4 (2.1)	25.6 (6.1)	3.78 (0.97)
Tap outside compound	305 (49)	8.5 (2.2)	25.6 (6.3)	3.57 (0.99)
Public well	31 (5)	7.2 (2.3)†	21.7 (7.3)*	2.97 (1.03)†
Breastfeeding (months)				
0–6	91 (15)	8.4 (2.5)	24.9 (6.5)	3.44 (0.97)
7–12	227 (37)	8.4 (2.1)	25.5 (6.2)	3.71 (1.01)
13–18	156 (25)	8.6 (2.3)	26.0 (6.4)	3.75 (1.03)
≥19	142 (23)	8.8 (2.4)	26.4 (6.8)	3.64 (1.00)
Child's nutritional status¶				
Adequate	334 (59)	8.6 (2.3)	25.7 (6.4)	3.72 (1.01)
Stunted	135 (24)	8.6 (2.4)	26.0 (7.0)	3.63 (1.08)
Wasted	78 (14)	8.5 (2.1)	25.9 (5.7)	3.67 (0.90)
Stunted and wasted	21 (4)	8.2 (2.2)	24.0 (7.0)	3.33 (1.19)
Malaria				
No	389 (74)	8.9 (2.1)	26.9 (6.1)	3.87 (0.95)
Yes	135 (26)	7.5 (2.2)†	22.6 (6.2)†	3.13 (0.94)†
HIV§ status				
Negative	543 (91)	8.6 (2.3)	25.7 (6.5)	3.68 (1.02)
Positive	52 (9)	8.2 (2.1)	25.1 (6.1)	3.53 (0.94)
Treatment group				
Placebo	307 (50)	8.5 (2.2)	25.7 (6.1)	3.68 (0.96)
Vitamin A	310 (50)	8.6 (2.3)	25.9 (6.8)	3.68 (1.06)

* $P < 0.01$.
† $P < 0.001$.
‡ $P < 0.05$; for differences among medians of hematological parameters across levels of the covariates (Wilcoxon Rank Sum and Kruskal-Wallis tests).
§ HIV = human immunodeficiency virus.
¶ See definitions, Table 1 footnotes.

malaria, and age greater than 2 years at enrollment were associated with the development of severe anemia after four to eight months of follow-up. Infection with HIV was a strong predictor of low hemoglobin levels during follow-up.

Our results are consistent with those from cross-sectional surveys that have related low socioeconomic status to decreased hemoglobin levels in women of reproductive age and preschool children. In India, among pregnant women attending urban health centers, those with ten or more years of education had significantly higher mean hemoglobin concentration than those with less than 9 years,[18] and among urban school children, there was a monotonic increase in mean hemoglobin by five categories of social class, from 8 g/dL in the lowest to 13 g/dL in the highest one.[19] The prev-

TABLE 3
Determinants of hemoglobin levels at baseline

	No.	Multivariate adjusted hemoglobin difference (g/dL) between groups (95% CI)*	*P* for trend†
Sex			
Male	288	Reference	
Female	234	0.32 (−0.04, 0.67)	
Age (months)			
6–11	221	Reference	
12–23	180	−0.20 (−0.64, 0.61)	
≥24	122	0.38 (−0.39, 1.15)	0.21
Mother's education			
Low‡	89	Reference	
Elementary	404	−0.61 (−1.07, −0.15)	
Secondary	29	0.96 (0.10, 1.82)	0.13
Household possessions			
0	69	Reference	
1	252	0.48 (−0.03, 1.00)	
≥2	201	0.62 (0.06, 1.19)	0.04
Water supply			
Tap in house	92	1.20 (0.26, 2.13)	
Tap in compound	154	0.74 (−0.12, 1.61)	
Tap outside compound	248	1.03 (0.19, 1.87)	
Public well	28	Reference	
Breastfeeding (months)			
0–6	75	Reference	
7–12	196	0.17 (−0.37, 0.71)	
13–18	130	0.32 (−0.48, 1.12)	
≥19	121	0.33 (−0.52, 1.18)	0.38
Malaria			
No	387	Reference	
Yes	135	−1.31 (−1.74, −0.88)	

* Coefficients from a linear regression model in which the effect of each variable is controlled for that of the others presented in the table. 95% CI = 95% confidence interval.
† *P* value, test for trend, when the variable is introduced into the model as continuous.
‡ See definitions, Table 1 footnotes.

alence of mild, moderate, and severe anemia in children under five was also lowest among those from mothers with higher level of education in the Central Asian republics of Kazakstan, Uzbekistan, and Kyrgyz.[20] Low socioeconomic status (SES), defined as the absence of a refrigerator and television in the household, was associated with significantly lower average hematocrit levels among children 6 to 59 months of age, admitted to an emergency ward in Kinshasa, Zaire.[21] Other socioeconomic indicators that have been identified as independent risk factors for anemia include the percentage of outcome income spent in food, the quality of water supply and sewage, house ownership,[22] and maternal height.[23] Low SES is likely to reflect nutritional deficits including marginal intake of foods rich in highly bioavailable iron, poor sanitary practices, and recurrence of infectious diseases.

Previous studies have suggested that malaria is an important risk factor for the development of severe, life-threatening anemia in hyperendemic areas. Most of these studies have reported simultaneous associations between elevated parasitemia or clinical manifestations of malaria and low hemoglobin or hematocrit levels,[21,24–28] but have failed to estimate the effect of time-varying patterns of malaria on hematologic parameters. Additional indirect evidence arises from studies of interventions aimed at reducing the incidence of malaria, that have proven effective in diminishing the risk of anemia.[4,29] In a group of Tanzanian infants, the administration of malaria prophylactic treatment every week resulted in a 57% reduction in the risk of a first episode of severe anemia (packed cell volume $< 25\%$).[4] After controlling for a number of potential confounding variables, including the anemia status at the outset of the observation period, we found a 4-fold increased risk for development of anemia at the last visit among children who had a positive blood smear at both baseline and follow-up visits, as compared with children who were negative in both assessments. Children with positive parasitemia at baseline but not at follow-up had the lowest risk of anemia at the last visit. This could be attributed to the fact that malaria at any visit led to treatment of the infection. We were unable to differentiate whether a positive blood smear at follow-up among children with baseline malaria was due to reinfection or to failure of the treatment. The physiopathologic mechanisms proposed to explain how malaria causes anemia include parasitic damage of erythrocytes and subsequent hemolysis mediated by complement,[30,31] dyserythropoiesis,[32,33] hypersplenism,[34] and secondary folate deficiency.[35] It was not possible for us to distinguish the effects of dietary-related iron deficiency on anemia from those of malaria infection. Both mechanisms are likely to interact,[36] more so in a population with low intake of iron from the diet such as the one under study.[5]

Associations between breastfeeding and anemia have been reported from cross-sectional studies. Among Palestinian refugee children in their second year of life, the prevalence of anemia in those who had never been breastfed was 70% above that of children receiving breast milk at the time of the survey;[37] however, no reference to length of breastfeeding was made. In our prospective study, duration of breastfeeding was strongly protective against the development of anemia during follow-up, after controlling for age and other potential confounding factors. All children had to be older than six months at enrollment and were receiving complementary foods at the time of recruitment. The maximum protective association was observed among children who were breastfed for more than 18 months. This is consistent with the fact that breast milk can be a readily available source of highly bioavailable iron (50% as compared to 10% or less of iron from formula or cow's milk)[38–40] in populations with poor-quality diets, even after six months of age. The length of breastfeeding assessed at the time of enrollment could be positively correlated with the iron stores of the baby, and thus children who had been breastfed for a longer period may have been protected against developing anemia during follow up. Similar conclusions can be drawn from reports of better iron status among children who have been exclusively breastfed for prolonged periods, when compared with non-breastfed children on poor iron diets.[41] Breastfeeding alone, however, does not provide an adequate source of iron beyond the age of 6 months,[42] and there is consensus regarding the need for supplementing the diet of exclusively breastfed children with iron after this age.[43–46] In addition to providing bioavailable iron, breastfeeding may also lead to improved immunity, and hence to a reduced risk of malaria, which constitutes a potential explanation for the protective effect of breastfeeding on the development of anemia. Even though there is some transfer of antimalarial antibodies from mother to child through breast milk,[47] this type of passive immunity is thought to last less than one month. Breast milk may also have nonspecific enhancing functions on cellular immunity.[48]

TABLE 4
Predictors of anemia at follow-up*

	No.	% with anemia	Multivariate adjusted prevalence ratio (95% CI)†	Effect of vitamin A versus placebo on anemia, within each category	
				Prevalence ratio (95% CI)	*P* for interaction‡
Sex					
Male	92	9.8	1.00	1.10 (0.27–4.44)	
Female	69	13.0	1.14 (0.50–2.57)	0.56 (0.16–2.02)	0.22
Age (months)					
6–11	76	10.5	1.00	1.08 (0.33–3.56)	
12–23	54	11.1	2.82 (0.87–9.13)	0.92 (0.18–4.66)	
≥24	31	12.9	8.11 (1.18–55.8)§	0.39 (0.03–4.43)	0.93
Breastfeeding (months)					
0–6	22	22.7	1.00	1.35 (0.11–16.7)	
7–12	72	11.1	0.90 (0.32–2.57)	0.29 (0.06–1.33)	
13–18	37	8.1	0.23 (0.05–1.06)	1.35 (0.10–17.8)	
≥19	30	6.7	0.14 (0.02–0.93)¶	0.74 (0.06–8.92)	0.10
Malaria					
Never	60	11.7	1.00	0.89 (0.27–2.95)	
Baseline, not follow-up	21	4.8	0.30 (0.04–1.95)	1.07 (0.06–18.1)	
Follow-up, not baseline	20	15.0	0.90 (0.26–3.14)	0.68 (0.10–4.81)	
Always	10	50.0	4.14 (1.26–13.5)	1.64 (0.26–10.2)	0.75
Anemia at baseline					
No	121	9.9	1.00	1.34 (0.41–4.32)	
Yes	40	15.0	1.06 (0.37–3.06)	0.27 (0.03–2.19)	0.07
Treatment					
Placebo	80	12.5	1.00		
Vitamin A	81	9.9	0.86 (0.37–1.99)		

* Anemia = hemoglobin <7.0 g/dL.
† From a main-effects binomial regression model: outcome variable = ''anemia at follow-up''. The model includes indicator variables for each factor, and one indicator for missing information on malaria status. 95% CI = confidence interval.
‡ *P* value, test for interaction, from the likelihood ratio test.
§ *P* value, test for trend = 0.03.
¶ *P* value, test for trend = 0.04.

In populations where the limiting factor for anemia is a diminished dietary intake of iron, the prevalence of iron deficiency peaks at ages 6 to 12 months or 1 to 2 years, when more than 50% of the requirements arise from the accelerated growth velocity.[49] In our study, the risk of severe anemia at the last visit was unexpectedly greater for children of 2 years or older. A direct association between malaria and age is not likely an explanation, since the prevalence of malaria actually decreased with age in this group (15% in children of 2 years or older versus 25% before 24 months), similar to that described in other settings.[25,50] In some populations, however, the fall in malaria prevalence occurs only after 36 months of age.[28,51] In low SES areas where short spacing between gestations is prevalent, older children may be introduced to diets low in bioavailable iron or that interfere with iron absorption, such as cow's milk,[52] as priority of care is given to newborns. An alternative explanation is that the increased prevalence for anemia after 2 years of age may be an indicator of persistent anemia after treatment of malaria, due to prolonged immune response mediated by T-cells and lingering macrophage activation.[53]

We found a nonsignificant protective effect of vitamin A supplements on the risk of developing anemia over time, which appeared to be stronger among children who were severely anemic at the time of administration of the first supplement dose. The 14% risk reduction we found may represent an underestimation of the real effect of vitamin A on anemia, due to lack of compliance with the third and fourth doses by a group of children. It is also possible that we lacked statistical power to demonstrate a significant effect. In a population likely to suffer from combined vitamin A and iron deficiencies, vitamin A could help reduce the risk of anemia through several mechanisms.[54,55] Recovery from anemia in the short term (before 2 months) after treatment with vitamin A has been documented previously in supplementation trials.[7–9,56] In an intervention study conducted among anemic, pregnant women from Indonesia,[9] it was found that 35% out of those receiving daily doses of 2.4 mg retinol became non-anemic (hemoglobin > 11 g/dL) after 8 weeks, as compared to only 16% in the placebo group, a 54% risk reduction. Anemic preschool children from Guatemala who received 3 mg of vitamin A per day for a period of two months experienced a higher increase in hemoglobin levels than those given placebo (0.93 versus 0.32 g/dL, respectively).[7] Similar effects were found with the administration of single doses of the vitamin to anemic preschool children from Indonesia (60 mg retinol equivalent)[8] and to non-anemic children from Thailand (110 mg of vitamin A)[56] after 8 and 2 weeks of follow-up, respectively. A plausible explanation for rapid responses of hemoglobin to the administration of vitamin A is that the vitamin prevents the inhibitory effect of phytates and polyphenols on the absorption of nonheme iron from diet.[57,58] Other trials addressing fortification of foods with vitamin A have also resulted in long-term improvements of hematologic parameters in preschool children.[59,60] The pathophysiology of a sus-

tained, longer-term effect of vitamin A on hemoglobin levels is still unknown. Potential mechanisms include a direct effect of vitamin A or its metabolites on the differentiation of red cells.[61] An indirect enhancement of erythropoiesis is also possible, by increasing the re-utilization of serum and reticulo-endothelial iron, which can be trapped during vitamin A deficiency.[62] Finally, the modulating properties of vitamin A on immune responses during infection could play a role, which would result in the resumption of transferrin production, a protein down-regulated during the acute phase response.[63]

We found an association between HIV infection and decreased hemoglobin levels over time. The development of anemia during HIV disease in children is multifactorial. In this population, it may be the result of an impairment in iron absorption and subsequent iron deficiency, compromised hematopoiesis, and the presence of opportunistic infections.[64,65] Anemia prevalence among HIV-infected children varies with underlying conditions, but is usually high, ranging from 12% to 94%.[66–68] Moreover, anemia is a prognostic factor for negative outcomes such as progression to AIDS and early death;[66,69] thus, controlling preventable causes of anemia could also help reduce the burden of HIV-related morbidity and mortality.

In conclusion, the predictors of low hemoglobin levels and severe anemia in this population are largely modifiable conditions, including poor socioeconomic status, malaria, and HIV infection, and short duration of breastfeeding. Public health actions aimed at enhancing the level of maternal education and fostering breastfeeding practices, improving the quality of water supply, and preventing malaria, HIV, and other infectious diseases are likely to significantly reduce the burden of anemia among children from sub-Saharan Africa.

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