

Malaria parasitaemia in relation to HIV status and vitamin A supplementation among pre-school children

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Summary

OBJECTIVES To ascertain whether malaria parasitaemia in children is associated with HIV status. To examine the effect of vitamin A supplementation on malaria parasitaemia in children.

METHODS We studied the cross-sectional associations between HIV status and malaria parasitaemia among 546 children 6–60 months of age who participated in a double-blind, randomized clinical trial of vitamin A supplementation. Prevalence ratios and 95% confidence intervals (CI) were estimated for the presence of malaria parasites at baseline by HIV status in uni- and multivariate models that adjusted for sociodemographic and environmental variables. Among children with malaria, correlates of high parasite loads were identified. Next, we examined the effect of vitamin A supplementation on the risk of malaria parasitaemia and high parasite density at 4–8 months of the first dose in a subset of children.

RESULTS The prevalence of malaria parasitaemia was 11.4% among HIV-infected children, compared with 27.6% among uninfected. After adjusting for season, anaemia, use of bednets, maternal education and indicators of socioeconomic status, we found some evidence for lower prevalence of parasitaemia among HIV positive compared with HIV-negative children (prevalence ratio = 0.56; 95% CI = 0.29, 1.09; $P = 0.09$). Other important correlates of malaria parasitaemia at baseline included low level of maternal education, poor quality of water supply, and the presence of animals at home. Vitamin A supplementation did not have a significant effect on malaria parasitaemia at 4–8 months of follow-up, overall or within levels of potential effect modifiers.

CONCLUSION HIV infection appears to be negatively correlated with malaria parasitaemia in this group of children. Investing in women's education is likely to decrease the prevalence of malaria parasitaemia in children. Vitamin A supplementation does not seem to have an effect on malaria parasitaemia in this population; possible benefits against clinical episodes and severe malaria deserve further examination.

keywords malaria, HIV, vitamin A, pre-school children

Introduction

An estimated 0.9–2.3 million annual child deaths are attributed to malaria in Sub-Saharan Africa alone. In addition, the burden of childhood malaria includes disability from malaria-related conditions such as severe anaemia, neurological sequelae from cerebral compromise, respiratory distress, hypoglycaemia, and sub-optimal cognitive and behavioural development (Holding & Snow 2001; Murphy & Breman 2001).

HIV infection, another major public health challenge in Africa, appears to interact with the risk of malaria. HIV infection impairs the immune response to malaria during pregnancy (Moore *et al.* 2000), and children born to

HIV-infected mothers seem to be at higher risk of death from severe malaria (Bloland *et al.* 1995). While HIV infection was postulated to increase the risk of malarial infection among children, a positive association between HIV and malaria parasitaemia among children has not been found in several studies examining this question (Muller & Moser 1990; Greenberg *et al.* 1991; Taha *et al.* 1994; Kalyesubula *et al.* 1997).

Specific micronutrient deficiencies usually co-exist with both malaria and HIV infection in Sub-Saharan Africa, and may contribute to poor outcomes related to these infections. Vitamin A deficiency in animals increases susceptibility to malaria (Roos *et al.* 1946; Krishnan *et al.* 1976; Stoltzfus *et al.* 1989), and observational studies in humans

also suggest such an association (Sturchler *et al.* 1987; Thurnham & Singkamani 1991; Friis *et al.* 1997; Hautvast *et al.* 1998). Yet, the beneficial effect of vitamin A supplementation to children on malaria outcomes has been documented in only one clinical trial (Shankar *et al.* 1999). If a protective effect is demonstrated against malaria, supplementation with vitamin A could constitute an inexpensive tool to reduce the burden of malaria in childhood.

Using data from a vitamin A clinical trial conducted among Tanzanian pre-school children, we examined the cross-sectional associations between HIV infection and the prevalence of malaria parasitaemia and high parasite density at baseline, as well as the relationships between parasitaemia and sociodemographic variables. We also ascertained the effect of vitamin A supplements on malaria parasitaemia in a subset of children who were followed-up for 4–8 months.

Materials and methods

Study design and population

A total of 687 children admitted to hospital with pneumonia in Dar es Salaam were enrolled in a double-blind vitamin A supplementation trial. Details of the trial have been published before (Fawzi *et al.* 1998). Briefly, the study was designed to examine the effect of vitamin A supplements on the severity of pneumonia during hospital stay, and morbidity and mortality endpoints up to 1 year after discharge. Children, aged between 6 and 60 months, diagnosed with pneumonia by a study doctor according to clinical criteria were eligible for enrolment. Severely malnourished children and those with measles, pulmonary tuberculosis, diphtheria, and whooping cough were excluded from the study, as well as children with clinical signs of vitamin A deficiency or who had taken vitamin A supplements during the preceding 4 months. At baseline, trained personnel collected information on sociodemographic characteristics, feeding patterns, and immunizations from the child's caregiver, and a paediatrician performed a complete clinical examination. Height (or length in children <24 months), weight, and mid-upper arm circumference (MUAC) were measured using calibrated instruments and standardized procedures.

Children were randomized to receive 200 000 IU of vitamin A (60 mg of retinol as retinyl palmitate) or placebo on the day of admission, a second dose on the next day, and third and fourth doses at 4 and 8 months after discharge, respectively. Half the dose was administered to children <12 months. Regimens were dispensed from identically looking opaque bottles marked with batch

numbers. Compliance with the first two doses was virtually 100% since they were administered in-hospital by a single research supervisor. Seventy-five percent of the children received the third dose, and 74% the fourth; the majority of those who received the third also received the fourth. Compliance below 100% with these follow-up doses was due to death during the initial hospitalization, address changes that could not be traced, and missed visits. Follow-up for growth, morbidity and mortality after discharge was carried out during biweekly visits, alternatively at hospital and the child's home.

On admission, a blood sample was drawn from all children for haemoglobin, blood cell counts, HIV and malaria assessments. In a random subset of children, additional blood samples were obtained at 4 and/or 8 months after discharge from the initial hospitalization, concurrent with the administration of the supplement. Haemoglobin, packed cell volume, and red cell counts were determined by standard techniques (Dacie & Lewis 1994). The latest blood specimen available for each child was tested for HIV antibodies using enzyme-linked immunosorbent assay (Murex Biotech Ltd, Dartford, UK) and confirmed by Western blot (Bio-Rad Laboratories, Ltd, Hertfordshire, UK). HIV status of children who were younger than 15 months and had positive or indeterminate results was also tested using heat-denatured HIV-p24 antigen assays with confirmatory neutralization assays (DuPont, Wilmington, DE, USA). The presence of malaria parasites in peripheral blood was assessed by thick and thin blood smears stained with Giemsa. One trained laboratory technician carried out independent readings of each blood smear in three different fields of the slide. Parasite density per cubic millimetre was estimated from the number of parasites per 200 leucocytes and the white cell count from each child's blood examination.

Data analysis

The primary outcome of interest was malarial infection, defined as the presence of at least one parasite in the blood film. Among children with malaria, the natural logarithm of parasite density was a secondary endpoint.

HIV infection and correlates of malaria

Children with a blood film were analysed cross-sectionally during the initial hospitalization. We estimated the prevalence of malarial infection, overall and within levels of baseline covariates. These included, in addition to HIV infection, the child's age, sex, and nutritional status, breast feeding history, haemoglobin concentration, erythrocyte sedimentation rate (ESR), and total lymphocytes count; whether the child sleeps under a bednet and the season of

the year at enrolment; the mother's age, parity, education level, occupation and marital status; and indicators of the socioeconomic status such as the number of household possessions, the number of people sleeping in the same room as the child, the level of water supply, the presence of electricity at home, and the possession of animals. Children were classified as stunted if their height or length-for-age was 2 or more standard deviations (SD) below the median of the sex- and age-specific NCHS/WHO reference median (World Health Organization 1983), whereas wasted children were those with weight-for-height ≤ -2 SD from the median reference. Haemoglobin levels were categorized according to conventional cut-off points (anaemia if <110 g/l, severe anaemia if <70 g/l), and ESR and lymphocyte count according to quartiles of the population distribution.

Malaria prevalence ratios and 95% confidence intervals (CI) were estimated from 2×2 tables for each covariate. Covariates associated with malaria at $P < 0.10$ were introduced into a multivariate binomial regression model (Wacholder 1986) from which adjusted prevalence ratios and 95% CI were estimated. Only variables that remained statistically significant at $P \leq 0.05$ and those considered relevant from the biological viewpoint were kept in the final multivariate model.

Among children with malaria, we used characteristics of the population's log parasite density distribution to define low or high parasitaemias. The distribution was bimodal, with peaks at around 7.5 (1800 parasites/mm³) and 10.5 (36 300 parasites/mm³), and an overall median of 8.4 (4400 parasites/mm³). The mid-point between the two data clusters around the peaks was approximately 9 (8000 parasites/mm³). We used this point as a cut-off to define high (≥ 8000 /mm³) *vs.* low (1–8000/mm³) parasite densities. Uni- and multivariate prevalence ratios and 95% CIs for high *vs.* low parasite load were estimated overall and by categories of baseline predictors.

Effect of vitamin A supplements on malaria parasitaemia
In the subset of children with a blood film available after 4 or 8 months from hospital discharge, we conducted an intent-to-treat analysis to ascertain the effect of vitamin A supplements on the incidence of parasitaemia. In children with malaria assessments at both 4 and 8 months, the latest was selected in order to maximize the probability of exposure to vitamin A supplements. We examined the distribution of baseline characteristics by treatment arm in this subset of children. Next, we estimated relative risks for malaria parasitaemia associated with vitamin A supplementation from 2×2 tables, and used the Fisher's exact test to examine the statistical significance. The effect of vitamin A was also examined by levels of potential effect

modifiers at baseline, and a test of heterogeneity for the effect of vitamin A across strata was conducted. Although all children were randomly assigned to vitamin A or placebo, we also examined the effect of vitamin A on malaria after adjustment for potential residual confounding by baseline factors, given that follow-up measurements of the outcome were obtained only from a subset of children. A binomial regression model was used to obtain multivariate-adjusted relative risk and 95% CI.

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.

Results

Of 687 children included in the study, 546 (80%) had a blood film at the time of hospital admission. These constituted the study base for the cross-sectional analyses of malaria correlates. Mean age of the children was 17.2 months (SD = 11.8), 29% were stunted and 18% were wasted. All children had been introduced to complementary foods, and the majority (70%) was being breastfed at the time of the study. Eighty-eight per cent were anaemic (haemoglobin <110 g/l) and 8.4% were infected with HIV-1. Children's mothers were mostly housewives (74%) and the majority (83%) had completed primary school. Only 18% of children came from homes with tap water, and 43% had electricity at home. The majority of children had moderate or mild pneumonia at admission; 11% had high fever (≥ 39 °C), 10% had severe hypoxaemia (oxygen saturation $<90\%$), and 35% had a rapid respiratory rate (frequency >60 /min).

The prevalence of malaria was 25.8% (141/546). In univariate analyses (Table 1), HIV infection was associated with 59% (95% CI = 5%, 82%) significantly lower prevalence of malaria parasitaemia. This association was not statistically significantly modified by child's age; the prevalence ratios (95% CI) for malaria among HIV-infected children compared with uninfected were 0.22 (0.03, 1.50), 0.70 (0.25, 2.01) and 0.29 (0.04, 1.95) for the age groups 6–11, 12–23, and >24 months, respectively (P -value, test for heterogeneity of prevalence ratios across strata of age = 0.50). Children admitted during the long rainy season had decreased malaria rates compared with children admitted during the dry season. Higher level of maternal education and indicators of improved socioeconomic status such as better water supply and electricity at home were associated with decreased malaria prevalence. The number of people who slept in the same room as

E. Villamor *et al.* **Malaria, HIV and vitamin A in children****Table 1** Prevalence of malaria parasitaemia in children 6–60 months of age admitted to hospital with pneumonia, Dar es Salaam, Tanzania 1993–97

	N (%) [*]	Prevalence n (%)	Unadjusted prevalence ratio (95% CI) [†]
Overall	546 (100.0)	141 (25.8)	
<i>Child's sex</i>			
Female	244 (44.7)	65 (26.6)	1.00
Male	302 (55.3)	76 (25.2)	0.94 (0.71, 1.26)
<i>Child's age (months)</i>			
6–11	231 (42.3)	56 (24.2)	1.00
12–23	189 (34.6)	50 (26.5)	1.09 (0.79, 1.52)
24–35	63 (11.5)	19 (30.2)	1.24 (0.80, 1.93)
≥36	63 (11.5)	16 (25.4)	1.05 (0.65, 1.69)
<i>Child is stunted[‡]</i>			
No	359 (71.1)	95 (26.5)	1.00
Yes	146 (28.9)	33 (22.6)	0.85 (0.60, 1.21)
<i>Child is wasted[‡]</i>			
No	415 (82.2)	102 (24.6)	1.00
Yes	90 (17.8)	26 (28.9)	1.18 (0.82, 1.69)
<i>Breastfed at time of study</i>			
No	165 (30.2)	50 (30.3)	1.00
Yes	381 (69.8)	91 (23.9)	0.79 (0.59, 1.06)
<i>Duration of exclusive breast feeding</i>			
≤4 months	428 (84.7)	109 (25.5)	1.00
>4 months	77 (15.3)	22 (28.6)	1.12 (0.76, 1.66)
<i>Child's HIV status</i>			
Negative	482 (91.6)	133 (27.6)	1.00
Positive	44 (8.4)	5 (11.4)	0.41 (0.18, 0.95)
<i>Haemoglobin concentration (g/l)</i>			
>110	64 (12.3)	6 (9.4)	1.00
70–110	339 (64.9)	75 (22.1)	2.36 (1.07, 5.19)
<70	119 (22.8)	53 (44.5)	4.75 (2.16, 10.44)
<i>Erythrocyte sedimentation rate (mm/h)</i>			
0–15 (quartile 1)	124 (26.3)	22 (17.7)	1.00
16–60 (quartiles 2 and 3)	216 (45.8)	45 (20.8)	1.17 (0.74, 1.86)
>60 (quartile 4)	132 (28.0)	52 (39.4)	2.22 (1.44, 3.43)
<i>Lymphocytes count (/mm³)</i>			
≥4200 (quartiles 2–4)	393 (75.3)	104 (26.5)	1.00
<4200 (quartile 1)	129 (24.7)	30 (23.3)	0.88 (0.62, 1.25)
<i>Season</i>			
Jan–Feb (dry)	113 (20.7)	37 (32.7)	1.00
Mar–May (long rains)	163 (29.9)	31 (19.0)	0.58 (0.38, 0.88)
Jun–Oct (dry)	212 (38.8)	59 (27.8)	0.85 (0.60, 1.20)
Nov–Dec (short rains)	58 (10.6)	14 (24.1)	0.74 (0.44, 1.25)
<i>Child sleeps under bednet</i>			
No	59 (10.8)	23 (39.0)	1.00
Yes	487 (89.2)	118 (24.2)	0.62 (0.44, 0.89)

Table 1 (Continued)

	N (%) [*]	Prevalence n (%)	Unadjusted prevalence ratio (95% CI) [†]
<i>Mother's age (years)</i>			
≤20	78 (14.3)	26 (33.3)	1.00
21–25	224 (41.0)	57 (25.5)	0.76 (0.52, 1.12)
26–30	154 (28.2)	34 (22.1)	0.66 (0.43, 1.02)
>30	90 (16.5)	24 (26.7)	0.80 (0.50, 1.27)
<i>Mother's education</i>			
None – illiterate	91 (16.7)	31 (34.1)	1.00
Completed elementary	426 (78.0)	106 (24.9)	0.68 (0.48, 0.96)
Completed secondary	29 (5.3)	4 (13.8)	0.38 (0.15, 0.99)
<i>Mother's occupation</i>			
Housewife	406 (74.4)	109 (26.9)	1.00
Worker	140 (25.6)	32 (22.9)	0.85 (0.60, 1.20)
<i>Number of possessions at home[§]</i>			
None	75 (13.7)	19 (25.3)	1.00
1	343 (62.8)	94 (27.4)	1.08 (0.71, 1.65)
2–5	128 (23.4)	28 (21.9)	0.86 (0.52, 1.44)
<i>Persons sleeping in same room as child</i>			
1–2	89 (16.3)	29 (32.6)	1.00
3	260 (47.6)	64 (24.6)	0.76 (0.52, 1.09)
4	139 (25.5)	39 (28.1)	0.86 (0.58, 1.28)
≥5	58 (10.6)	9 (15.5)	0.48 (0.24, 0.93)
<i>Water supply</i>			
Tap in house	96 (17.6)	14 (14.6)	1.00
Tap in compound	159 (29.1)	43 (27.0)	1.85 (1.07, 3.21)
Tap outside compound	261 (47.8)	70 (26.8)	1.84 (1.09, 3.11)
Public well	30 (5.5)	14 (46.7)	3.20 (1.73, 5.93)
<i>Electricity at home</i>			
No	309 (56.6)	95 (30.7)	1.00
Yes	237 (43.4)	46 (19.4)	0.63 (0.46, 0.86)
<i>Animals in household</i>			
No	481 (88.1)	118 (24.5)	1.00
Yes, in separate space	51 (9.3)	19 (37.3)	1.52 (1.03, 2.24)
Yes, in same space where child sleeps	14 (2.6)	4 (28.6)	1.16 (0.50, 2.71)

* Sums may not add up to 546 due to missing values.

† From univariate binomial regression models in which malaria (presence or absence of parasites in the blood film) is the outcome variable; indicator variables for each correlate are included as predictors.

‡ Stunted children were below –2z-scores (NCHS/WHO reference) in height-for-age. Wasted children were below –2z-scores in weight-for-height.

§ Sum from a list of five items: car, refrigerator, radio, bicycle, and television.

the child was inversely related to the prevalence of malaria. In contrast, the presence of animals in the same room was not associated with malaria; however, having animals in

a separate space of the dwelling was related to a 52% increase in malaria prevalence. Low haemoglobin concentration and high ESR were related to higher malaria prevalence. Indicators of severity of pneumonia during hospital admission were not related to the presence of malaria parasites in peripheral blood.

After adjusting for anaemia, season, maternal education and indicators of socioeconomic status (Table 2), the inverse association between HIV infection and malaria became marginally statistically significant (prevalence ratio = 0.56; 95% CI = 0.29, 1.09; $P = 0.09$). In the same multivariate-adjusted model, children with illiterate mothers had a 43% higher malaria prevalence compared with children with mothers who had completed elementary school ($P = 0.05$). The prevalence of parasitaemia increased monotonically as the level of water supply declined: children from homes with access to water only from public wells had a 1.9 (95% CI = 1.07, 3.47) times higher prevalence than those from dwellings with tap water. The association between the presence of animals at home and the prevalence of malaria remained statistically significant after adjustment for potential confounding variables.

Among children with malaria ($n = 141$), we examined the correlates of high ($\geq 8000/\text{mm}^3$, $n = 63$) *vs.* low ($1\text{--}8000/\text{mm}^3$, $n = 78$) parasite density. In univariate analyses, stunting and poor water supply were related to increased prevalence of high parasite density, whereas low ESR, sleeping under a bednet, the number of household amenities and the presence of electricity were related to decreased parasite loads. Severe anaemia was not related to parasite density; the proportion of children with haemoglobin <70 g/l was the same among children with high (24/61) or low (29/73) parasite densities.

In multivariate analyses, ESR <16 mm/h was related to a 74% (95% CI = 26%, 91%) lower prevalence of high parasitaemia compared with ≥ 16 mm/h. The prevalence of high parasitaemia was 37% (95% CI = 17%, 52%) lower in children from households with at least one amenity compared with no amenities; and living in homes with electricity was related to a significant 45% (95% CI = 10%, 66%) reduced prevalence of high parasite density, compared with homes without electricity.

We next examined the effect of vitamin A supplementation on the incidence of parasitaemia in a subset of 157 children who had a blood film at 4 and/or 8 months after the first dose. The distribution of baseline characteristics did not differ between the study base and this subset of children. We chose the last available blood film as the follow-up measurement for malaria parasitaemia. For 83 (53%) children, the last measurement was available at

Table 2 Correlates of malaria parasitaemia in children – multivariate analysis; Dar es Salaam, Tanzania 1993–97

	Adjusted prevalence ratio (95% CI)*	P-value
HIV status		
Negative	1.00	
Positive	0.56 (0.29, 1.09)	0.09
Severe anaemia (Hb < 70 g/l)		
No	1.00	
Yes	1.98 (1.47, 2.67)	<0.001
Season		
Jan–Feb (dry)	1.00	
Mar–May (long rains)	0.67 (0.46, 0.99)	0.05
Jun–Oct (dry)	0.90 (0.64, 1.27)	0.55
Nov–Dec (short rains)	0.65 (0.39, 1.08)	0.10
Child sleeps under bednet		
No	1.00	
Yes	0.78 (0.55, 1.12)	0.19
Mother's education		
None – illiterate	1.43 (1.00, 2.04)	0.05
Completed elementary	1.00	
Completed secondary	0.97 (0.43, 2.19)	0.94
Persons sleeping in same room as child		
1–2	1.00	
3	0.81 (0.56, 1.17)	0.26
4	1.04 (0.75, 1.45)	0.81
≥ 5	0.59 (0.32, 1.07)	0.08
Water supply		
Tap in house	1.00	
Tap in compound	1.58 (0.97, 2.59)	0.07
Tap outside compound	1.75 (1.09, 2.83)	0.02
Public well	1.93 (1.07, 3.47)	0.03
Animals in household		
No	1.00	
Yes, in separate space	1.66 (1.05, 2.65)	0.03
Yes, in same space where child sleeps	1.00 (0.47, 2.14)	1.00

* Prevalence ratios and 95% CIs estimated from a multivariate binomial regression model. Malaria (the presence or absence of parasites in the blood film) is the outcome variable. Predictors include indicator variables for HIV status (1 indicator plus 1 indicator for missing), severe anaemia (1 indicator plus 1 indicator for missing), season of the year (3 indicators), whether the child sleeps under bed net (1 indicator), maternal education (2 indicators), persons sleeping in the same room as child (3 indicators), water supply (3 indicators), and animals in household (3 indicators). Anaemia and ESR were not introduced simultaneously into the model due to collinearity between these two variables.

4 months after discharge from hospital, whereas 74 (47%) children had the last measurement at 8 months after discharge. Children assigned to vitamin A ($n = 81$) were comparable with those who received placebo ($n = 76$) with respect to baseline characteristics including

age, sex, prevalence of malaria at first visit, HIV infection, anaemia and indicators of the socioeconomic status. The timing of the last follow-up measurement (at 4 months *vs.* 8 months after discharge) was not related to treatment assignment.

Overall, vitamin A supplements were not significantly associated with the risk of malaria parasitaemia during follow-up [relative risk (RR) = 1.27; 95% CI = 0.69, 2.36; $P = 0.56$]. A comparable association was found after adjustment for malaria at baseline, HIV infection, maternal literacy, quality of water supply, mother's occupation, and use of bednet (adjusted RR = 1.37; 95% CI = 0.74, 2.54). We also examined the effect of vitamin A on parasitaemia by levels of potential baseline effect modifiers including malaria at recruitment, HIV status, presence of anaemia and lymphopenia, nutritional status, severity of pneumonia and socioeconomic indicators (Table 3). There was no difference in the effect of vitamin A within strata of these potential effect modifiers. Among children with malaria at follow-up, vitamin A supplementation was not related to parasite density. The relative risk for high ($\geq 8000/\text{mm}^3$, $n = 9$) *vs.* low ($1\text{--}8000/\text{mm}^3$, $n = 34$) parasite density was 1.01 (95%

CI = 0.44, 2.29; P -value from Fisher's exact test = 0.85). Median parasite densities in the vitamin A and placebo arm were 1080 and 1140/ mm^3 , respectively (P -value from Wilcoxon rank-sum test = 0.89).

Discussion

We examined the cross-sectional associations between HIV infection and malaria parasitaemia among pre-school children, and found a marginally significant negative association after adjusting for potential confounders. Sociodemographic and environmental correlates of malaria were also characterized. Finally, we examined the effect of vitamin A supplements on malaria parasitaemia in a subset of children followed-up for 4–8 months. The supplements were without a statistically significant effect.

The cross-sectional nature of the negative association found between HIV infection and malaria parasitaemia prevents us from establishing its temporal directionality. In this context, lower prevalence of malaria among HIV-infected children compared with those uninfected could also be interpreted as reduced prevalence of HIV infection among children with malaria compared with children

Baseline effect modifier	<i>n</i> with malaria/ <i>N</i>		Relative risk (95% CI)	<i>P</i> -value*	<i>P</i> -value† test for interaction
	Placebo	Vitamin A			
Overall	14/76	19/81	1.27 (0.69, 2.36)	0.56	
Malaria at enrolment					0.43
No	8/47	13/51	1.50 (0.68, 3.29)	0.34	
Yes	4/15	4/18	0.83 (0.25, 2.78)	1.00	
HIV status					0.65
Negative	12/67	19/74	1.43 (0.75, 2.73)	0.31	
Positive	2/7	0/7	0	0.46	
Severe anaemia					0.78
No (Hb ≥ 70 g/l)	11/53	14/56	1.21 (0.60, 2.41)	0.65	
Yes (Hb < 70 g/l)	3/16	5/18	1.48 (0.42, 5.24)	0.69	
Child's MUAC					0.84
>25 th percentile	8/46	13/57	1.31 (0.59, 2.89)	0.62	
≤ 25 th percentile	5/25	6/20	1.50 (0.54, 4.21)	0.50	
Mother is literate					0.39
No	4/15	5/9	2.08 (0.75, 5.79)	0.21	
Yes	9/53	14/69	1.20 (0.56, 2.55)	0.82	
Water supply					0.54
Tap in house or compound	9/37	11/42	1.08 (0.50, 2.31)	1.00	
Tap or well outside compound	5/39	8/39	1.60 (0.57, 4.46)	0.55	

* From two-tailed Fisher's exact test.

† From test of heterogeneity of the vitamin A effect across strata of the modifiers.

Table 3 Effect of vitamin A supplementation on the incidence of malaria parasitaemia during 8 months of follow-up; Dar es Salaam, Tanzania 1993–97

without parasitaemia. There could be a number of non-causal explanations for an inverse association between malaria and HIV infection. Some evidence suggests that pre- (Bloland *et al.* 1995) or post-natal (Chintu *et al.* 1995) exposure to both infections increases the risk of child mortality; if in reality HIV infection predisposes children to malaria or vice versa, and children infected with both infections were more likely to die before reaching hospital, we could observe a spuriously negative association due to selection bias, e.g. co-infected children would be less likely to become part of the study base than children infected with only one of the diseases, or uninfected ones. Confounding may also constitute an explanation; after adjustment for maternal education and indicators of the socioeconomic status, the association was attenuated and became statistically marginally significant ($P = 0.09$). However, given the initial strength of the association, the degree of residual confounding is probably not considerable.

The interactions between HIV and malaria are complex and have not been completely elucidated yet. A systematic review of studies published before 1996 that examined the association between HIV serostatus and the prevalence of malaria parasitaemia concluded that there was no evidence for an interaction except between placental malaria and HIV infection (Chandramohan & Greenwood 1998). More recent studies have confirmed that the risk of malaria parasitaemia and parasite loads are higher in HIV-infected pregnant women compared with those uninfected (Parise *et al.* 1998; Verhoeff *et al.* 1999; van Eijk *et al.* 2001; Wabwire-Mangen *et al.* 2001), possibly due to an impairment of placental cellular immunity against malaria (Moore *et al.* 2000). Among HIV-infected pregnant women malaria parasitaemia is a significant correlate of high viral loads (Kapiga *et al.* 2002). Studies in non-pregnant adults also support a positive association (Whitworth *et al.* 2000; Francesconi *et al.* 2001; French *et al.* 2001), and suggest that the degree of immunosuppression is positively related to parasite loads. Several studies in children, however, have shown no association (Muller & Moser 1990; Muller *et al.* 1990; Greenberg *et al.* 1991; Taha *et al.* 1994) or an inverse relationship (Kalyesubula *et al.* 1997; Kawo *et al.* 2000) between the two infections. In a prospective study from Uganda, the rate of malaria episodes was significantly lower among HIV-1 infected children than in HIV-uninfected ones, and children with malaria had delayed mortality and progression to AIDS (Kalyesubula *et al.* 1997). Among hospitalized children from Dar es Salaam, the prevalence of malaria parasitaemia in HIV-uninfected children was four times (significantly) higher than that of HIV-positive children (Kawo *et al.* 2000), consistent with our present findings.

Various explanations have been offered for such an inverse association. Infection with *Plasmodium* could be suppressed by HIV infection through increased oxidative stress in the erythrocytes that would impair the parasite's growth (Kalyesubula *et al.* 1997). Chloroquine may have some anti-HIV properties (Savarino *et al.* 2001; Paton *et al.* 2002) and has been shown to accumulate in breast-milk cells (Boelaert *et al.* 2001). We did not have specific data on chloroquine use by the children or their mothers, but at the time of the study chloroquine was still the standard of antimalarial treatment in Tanzania. Even if chloroquine's antimalarial efficacy was low due to increasing parasite resistance, its frequent use in this setting could have exerted a beneficial effect against HIV infection in children. Similarly, prophylactic use of clotrimoxazol among HIV-infected children could reduce their malaria parasitaemia. Although HIV-infection does not appear to increase malaria parasitaemia in children, it may augment the risk of severe malaria and malaria-related death (Bloland *et al.* 1995; Chintu *et al.* 1995; Kalyesubula *et al.* 1997).

In addition to HIV infection, we found that indicators of the socioeconomic status were important correlates of the prevalence of malaria parasitaemia. After adjusting for potential confounders, the level of maternal education, water supply and presence of animals at home were statistically significantly associated with malaria. Children born to illiterate mothers were 43% more likely to have a positive blood film than children from mothers who had completed elementary school. High levels of maternal education have been previously related to decreased risk of mortality and of other adverse health outcomes in children from developing countries, independent of their socioeconomic status (Terra de Souza *et al.* 1999). In the case of child malaria, higher maternal education could be an indicator of enhanced knowledge of preventive practices and improved access to health services.

A monotonic significant increase in the prevalence of parasitaemia was observed as the level of service of the child's dwelling water supply worsened. Poor water supply may not only represent the child's socioeconomic status, but could also indicate increased exposure to mosquito breeding sites such as public water wells (Miller & Warrell 1990). Similarly, the increased prevalence of parasitaemia found among children from homes with animals could be related to the presence of breeding places in the animals' drinking troughs. Among children with positive blood films, indicators of poor Socio-economic status (SES) including lack of electricity and household amenities were important predictors of high parasite loads, in contrast to previous reports which found no associations between poverty and malaria (Koram *et al.* 1995; Luckner *et al.* 1998).

Vitamin A is indispensable for maintaining adequate humoral and cellular immune responses (Semba 1999). Supplementation of pre-school children with vitamin A results in significant reductions in mortality (Beaton *et al.* 1993; Fawzi *et al.* 1993) and severity of infections including measles and some forms of diarrhoea (Villamor & Fawzi 2000). More limited information is available regarding the relationship between vitamin A status and malaria. Vitamin A deficiency has been associated with increased susceptibility to malaria in animal studies (Roos *et al.* 1946; Krishnan *et al.* 1976; Stoltzfus *et al.* 1989); and several cross-sectional studies in humans have also shown that low serum retinol concentrations are related to higher risk of malaria (Galan *et al.* 1990; Thurnham & Singkamani 1991; Tabone *et al.* 1992; Filteau *et al.* 1993; Friis *et al.* 1997; Hautvast *et al.* 1998) and more severe manifestations of the disease (Das *et al.* 1996; Lewallen *et al.* 1998). Some (Sturchler *et al.* 1987), but not all (Binka *et al.* 1995; Metzger *et al.* 2001; Cooper *et al.* 2002), longitudinal studies in children have also shown significant associations between baseline low serum retinol and malaria parasitaemia at follow-up. The effect of vitamin A supplementation on malaria outcomes has been investigated in four clinical trials conducted among pre-school children, with varying results. In Ghana, supplementation with 200 000 IU vitamin A every 4 months (half the dose if <1 year of age) had no effect on death from malaria, fever episodes, malaria parasitaemia, parasite load, or probable malaria illness (Binka *et al.* 1995). Statistical power in this study was probably not adequate to examine these questions (Shankar 1995). Among pre-school children in Papua New Guinea, the same dose administered at 3-month intervals resulted in a significant 30% reduction in clinical *P. falciparum* episodes, particularly in children aged 12–36 months (Shankar *et al.* 1999). In that age bracket, supplementation also reduced parasite density and the risk of spleen enlargement.

In our Tanzanian study population, vitamin A supplements were associated with a statistically marginally significant 86% reduction in malaria-related deaths (Fawzi *et al.* 1999). Among infants with malaria parasitaemia at baseline, vitamin A supplements resulted in significant body weight gains after a year (Villamor *et al.* 2002). However, as we currently report, no effect was observed on the presence of circulating parasites or total parasite load. The fact that we did not observe an effect could be related to limited statistical power. In a trial conducted in Mozambique, the administration of a single 200 000 IU dose of vitamin A to children admitted to hospital with cerebral and other forms of severe malaria had no significant effects on hospital death, duration of hospitalization, time to resolution of fever, clearance of

parasitaemia, or development of neurological sequelae (Varandas *et al.* 2001). Statistical power in this study was probably too low to detect an effect of vitamin A on these outcomes.

Several specific explanations for the potential role of vitamin A on malaria outcomes have been suggested. In mice, malaria mortality and parasitaemia could be modulated by a genetic locus which includes cellular retinol-binding protein I (Foote *et al.* 1997). The addition of retinol to *P. falciparum* cultures appeared to impair the parasite's reproductive cycle in one *in vitro* study (Davis *et al.* 1998) but not in another (Samba *et al.* 1992). Retinoic acid has been shown to increase macrophage phagocytosis of parasitized erythrocytes from non-immune hosts, augment the number of CD36 lymphocytes involved in parasite clearance, and decrease monocytic secretion of proinflammatory cytokines such as TNF- α (tumour necrosis factor α), which could partially explain the potential protective effect of vitamin A against malarial death (Serghides & Kain 2002).

The epidemiological evidence reviewed suggests that the beneficial effect of vitamin A supplementation on malaria could depend on setting-specific conditions which affect the bioavailability and utilization of the supplement (Fawzi 1997). These may include the underlying prevalence of vitamin A and other nutrient deficiencies, the prevalence of other 'competing' infectious diseases such as diarrhoea, respiratory infection and HIV, and the degree of immunity to malaria. In our study, we did not find an effect of vitamin A supplementation on parasitaemia overall or within levels of some of these potential effect modifiers, probably due to lack of statistical power. We lacked measurements of the vitamin A status of these children at baseline, and could not ascertain whether vitamin A could have had an effect among those suffering from vitamin A deficiency.

We characterized the correlates of malaria parasitaemia in a paediatric population from Tanzania, and examined the effect of vitamin A supplementation on parasitaemia. Improvement of maternal education, and promotion of specific preventive measurements including the use of bednets and control of mosquito breeding sites are likely to reduce the burden of malarial infection in children from Sub-Saharan Africa. Prevention of mother-to-child transmission of HIV could result in decreased incidence of child mortality from other infections including malaria. The effect of such interventions on other malaria outcomes in the child needs to be investigated. Additional longitudinal studies are needed to ascertain the role of HIV infection on the immune response to malaria in children. Independent of its potential role on malaria outcomes, vitamin A supplementation constitutes a valuable public health tool

to improve child health and survival in countries at risk of deficiency (Villamor & Fawzi 2000). Future research on the relationships between the nutritional status and malaria should consider setting-specific conditions that may affect these associations. Given the high prevalence of multiple nutrient deficiencies in countries where malaria is highly endemic, the effect of multiple nutrient supplementation on malaria outcomes deserves examination.

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