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A randomised, double-blind, placebo-controlled clinical trial of vitamin A in severe malaria in hospitalised Mozambican children

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Summary This paper reports a randomised, double-blind, placebo-controlled clinical trial of the effect of routine vitamin A supplementation given on admission to children with severe malaria with regard to survival, recovery during hospitalisation and outcome 6 weeks after discharge. Children aged between 6 and 72 months admitted to the paediatric wards of the Central Hospital of Maputo (CHM), Mozambique with a diagnosis of severe malaria were randomly assigned either to a control group (placebo) or an experimental group (vitamin A) and were followed up 6 weeks after discharge. There were 280 children in the experimental and 290 in the placebo group. Seven (2.5%) and 13 (4.5%) children died in the experimental and the placebo groups, respectively, a relative risk of death of 0.56 (95% CI 0.23–1.38, $p = 0.201$). During the 1st 5 hours of admission, the relative risk of death in the vitamin A-supplemented group was 2.54 (0.50–12.96); after 5 hours of admission it was 0.19 (95% CI 0.04–0.85; $p = 0.015$). In the supplemented group, 4/82 (4.9%) of the children developed neurological sequelae *vs* 2/78 (2.6%) in the placebo group (RR = 1.90; 95% CI 0.36–10.09; $p = 0.682$). Although the overall reduction in the risk of death observed for all children receiving vitamin A is not statistically significant, it might be clinically important. This finding cannot, however, be accepted as a firm conclusion and requires validation by future trials.

Introduction

Plasmodium falciparum malaria remains a major cause of mortality and disability in African children, with an estimated minimum of a million deaths and 3,000 cases of neurological sequelae annually.[1] The emergence and spread of chloroquine resistance in some areas of tropical Africa has more than doubled the risk of death from malaria and these numbers could increase.[2,3] Many malaria-endemic areas of Africa are also areas of endemic vitamin A deficiency.[4] Vitamin A is essential

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for normal immune function[5] and resistance to infection. Some community-based studies have shown increased morbidity and mortality in children with mild vitamin A deficiency.[6–8]

The extent of vitamin A deficiency in Mozambique is not known. Data from the 1950s and 1960s suggest that mild vitamin A deficiency has been common but severe forms extremely rare.[9] The only recent data are from a 1990 study in Maputo and two other towns in Mozambique where 0.7% of 10,267 children aged 6–72 months had eye signs of vitamin A deficiency (0.3% at stage XN, 0.3% at stage X1B and 0.1% at stage X3/XS).[10] Serological data are even scarcer. During 1974, 13% of a random sample of 50 children in Maputo without clinically apparent vitamin A deficiency had a serum level of vitamin A below 10 μg/dl and 82% were below 20 μg/l.[9] If these data are still valid, they suggest an important public health problem.

Malaria is a serious problem in Mozambique. In Maputo, it is responsible for about 11% of inpatient deaths at central hospital level.[11]

Several studies suggest an interaction between vitamin A and malaria. Animal studies have shown that vitamin A-deficient animals are more susceptible to malaria and that this susceptibility is reversed by vitamin A supplementation.[12,13] *In vitro*, the replication of *P. falciparum* has been reduced by retinol.[14] Cross-sectional studies in children with malaria have shown low plasma retinol concentrations, especially in severe disease.[15,16] However, it is unclear by what mechanism and to what extent vitamin A status influences immune and clinical responses to malaria. There are a few community intervention studies of the effect of vitamin A supplementation on malaria morbidity and mortality but their results are contradictory. In Ghana,[17] vitamin A supplementation had no impact on malaria morbidity or mortality, but the study had limited statistical power.[18] A recent trial in Papua New Guinea shows a significant 30% decrease of *P. falciparum* febrile episodes in children supplemented with vitamin A.[19] The preliminary results of a randomised, double-blind, placebo-controlled clinical trial using a single oral dose of 200,000 IU of vitamin A given to children admitted with clinical malaria to a hospital in Tanzania suggest 'a tendency to earlier disappearance of symptoms'.[20]

We report a randomised, double-blind, placebo-controlled clinical study of the effect on survival and recovery during hospitalisation and on outcome 6 weeks after discharge of vitamin A given on admission to children with severe malaria.

Patients and methods

After obtaining informed consent from the accompanying guardian, children aged between 6 and 72 months admitted to the paediatric wards of the Central Hospital of Maputo (CHM), Mozambique with a diagnosis of severe malaria were assigned to either an experimental (receiving vitamin A) or a control (receiving placebo) group. They were followed up 6 weeks after discharge. The study was approved by the ethics committees of the Prince Leopold Institute for Tropical Medicine, Antwerp, Belgium and of CHM.

Assignment to groups was done according to a pre-determined simple random list. Capsules of vitamin A or placebo were put into an envelope labelled with a code number and the number was available only to a team member not involved with recruiting and treating patients. The vitamin A capsules contained 100,000 IU of vitamin A plus 20 IU of vitamin E and the identical-looking placebo capsules contained only 20 IU of vitamin E.

The inclusion criteria for cerebral malaria were: coma without a directional response to a painful stimulus (or no motor response in children less than 8 months old) 6 hours after the last convulsion, clear CSF, positive parasitaemia or positive polymerase chain reaction to *P. falciparum*. Other forms of severe malaria were diagnosed by clinicians on the basis of the WHO criteria[21] and confirmed by positive parasitaemia.

The exclusion criteria were: children with a history of measles or measles vaccination in the 4 weeks preceding admission, clinical signs

of vitamin A deficiency, signs of kwashiorkor or marasmus or other severe diseases (apart from severe malaria and requiring hospital admission, e.g. dehydrating diarrhoea and acute lower respiratory infections), and being younger than 6 years or older than 72 months.

On admission, the history, clinical findings and demographic characteristics were recorded on standardised forms. The level of consciousness was assessed by means of the Adelaide Coma Scale (ACS)[22] (a modification of the Glasgow Coma Scale) which takes account of the child's age. Children with a depressed level of consciousness but able to localise a painful stimulus were considered to have impaired consciousness and were deemed prostrated if they could not sit up without support. Respiratory distress was considered present when there were one or more of the following signs: alar flaring, chest recession (intercostal or subcostal), use of the accessory muscles of respiration or abnormally deep (acidotic) breathing. A venous blood sample was obtained for a thick blood film and routine haematological and biochemical measurements. Children with a haemoglobin level < 5.0 g/dl were classified as severely anaemic. All children with evidence of neurological involvement underwent lumbar puncture to exclude meningitis. In the hospital ward, the children were observed every 8 hours while recovering from the coma and every 12–24 hours thereafter. Children were supposed to be discharged as soon as coma had cleared and/or neurological status stabilised, when the child was able to feed by mouth and had completed the treatment schedule.

All children were treated initially with intravenous or intramuscular quinine hydrochloride[23] every 8 hours for 7 days or, alternatively, if coma cleared in the 1st 48 hours, at least five doses of quinine hydrochloride as above, followed by a single dose of pyrimethamine-sulfadoxine. Supportive treatment such as extra liquids and glucose, electrolytes, blood, phenobarbitone, paracetamol, diuretics and antibiotics were given as necessary. All children received a single oral dose of 200,000 IU of vitamin A or placebo immediately after being recruited into the trial (100,000 IU if the child was < 12 months old).

The size of the sample needed was calculated using the formula:[24]

$$n = \frac{p_1 \times (100 - p_1) + p_2 \times (100 - p_2)}{(p_2 - p_1)} \times f(_{\alpha,\beta})$$

Sample size was calculated on the assumption that the percentage of children discharged on day 5 would increase from p_1 (placebo group) $= 60\%$ to p_2 (supplemented group) $= 85\%$. If $\alpha = 0.01$ and $\beta = 0.05$ then $f = 17.8$ and n, the minimum sample size necessary in each treatment group, would be 104 cases and 104 controls.

Data were collected using piloted, standard schedules. Each schedule included up to five components: data extracted from hospital-based clinical documents; data extracted from home-based health records (Road to Health card); data obtained by interviewing the child-minder; results of laboratory tests; and trial identification data.

The following outcome variables were compared between the two groups: (i) inpatient outcome: duration of hospital admission in complete days; mortality prior to discharge; incidence of neurological sequelae; time of resolution of coma (hours); time of clearance of fever (hours); and rate of negative parasitaemia for *P. falciparum* at the time of discharge; (ii) reported health service use for the 6 weeks after discharge; (iii) reported illness in the 2 weeks preceding the 6-week follow-up (diarrhoea, acute lower respiratory infections, fever and other illnesses).

Routine clinical laboratory determinations were done using the methods currently in use in the CHM clinical pathology laboratories. Serum concentration of retinol (by high-pressure liquid chromatography)[25] was determined for all cases at the Department of Human Nutrition, University of Stellenbosch, South Africa. Blood samples were stored in the dark at –80° to –20°C. Each month the collected samples were put on dry ice and shipped to the reference laboratory. *Plasmodium* micro-

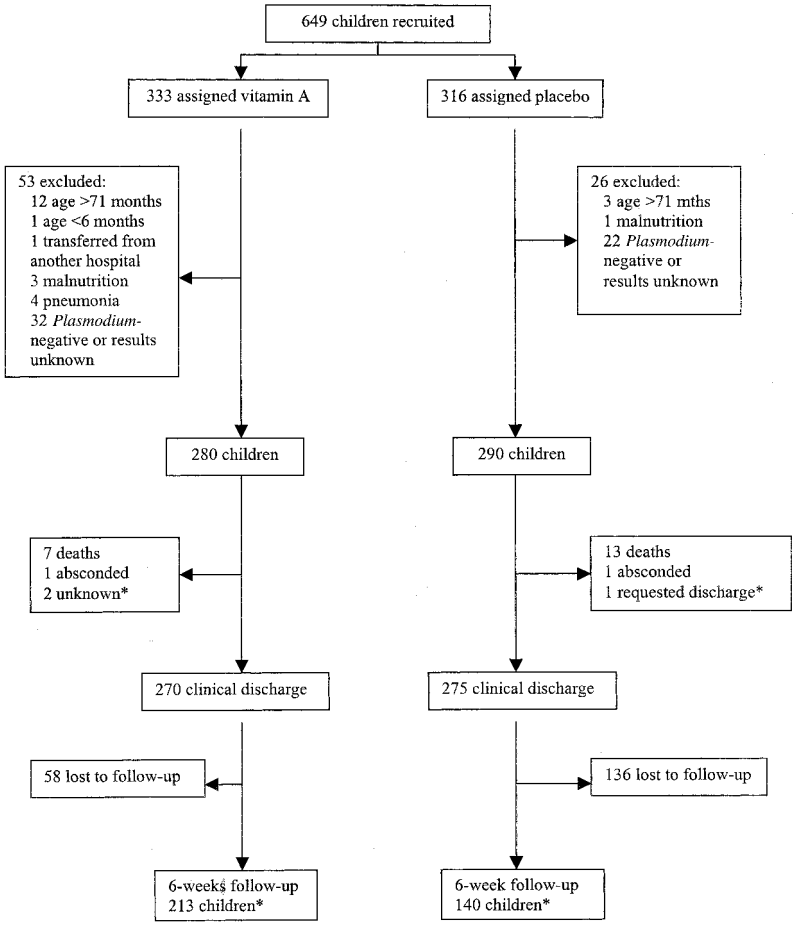

FIG. 1. Trial profile (*one child in each group attended for follow-up).

scopy was done for all children, as is routine in the CHM. A volume corresponding roughly to 100 μl of blood was collected from individual patients on filter paper (Whatmann no. 4), dried and stored at room temperature and then sent to a reference laboratory in Lisbon for DNA extraction and PCR, as described.[26,27]

After a field pilot study in early 1995, the trial was implemented in February 1995 until April 1997 and 649 children were recruited (Fig. 1). Of these, 24 (21 in the vitamin A group and three in the placebo group) were excluded because of the pre-defined exclusion criteria (out of age range, severe malnutrition, pneumonia) and one because he was transferred from another hospital 3 days after intravenous quinine therapy. A thick blood film for *P. falciparum* was negative in 48 and unknown in 24 children and these 72 cases were checked for PCR results. In 39 children, a blood sample for PCR was unavailable and in five the PCR was negative and they were excluded; the remaining 28 children had a positive PCR.

The clinical data from these 28 children were analysed separately and blindly by two paediatricians (one from the research team, LV, and one from the hospital's Department of Paediatrics, NP) to decide whether to include or exclude them from the trial. The discordant results were decided by consensus after discussion and analysis of all available data. The results were concordant in 25 (16

TABLE I. Main manifestations of severe malaria*

Manifestation	No. of children for whom data available	Prevalence No. (%)
Coma on admission	570	189 (33.2)
Severe anaemia on admission	460	190 (41.3)
Respiratory distress on admission	551	145 (26.3)
Hypoglycaemia on admission	132	8 (6.1)
Repeated convulsions**	544	44 (8.1)
Impaired consciousness	562	190 (33.8)
Prostration	562	68 (12.1)

* More than one manifestation observed in the same child; ** more than two in 24 hours.

children with severe malaria) and discordant in three (by consensus, two were classified as having severe malaria). As a result, ten more children were excluded from the trial. A total of 54 children (32 in the vitamin A group and 22 in the placebo group) were excluded because the inclusion criteria were not met. The excluded children reported vomiting before admission more often ($p = 0.031$) and dyspnoea ($p = 0.000$), acidotic breathing ($p = 0.038$) and conjunctival pallor ($p = 0.002$) less often. This suggests a less severe clinical picture.

The usable sample size was 570 children, 280 in the vitamin A group and 290 in the placebo group. At 6 weeks after discharge, 213 and 140 children were still being followed up in each group, respectively.

Analysis was by 'intention to treat'. Normally distributed continuous variables were compared using Student's *t*-test for independent samples and non-normally distributed continuous and discrete data were compared using the non-parametric Mann–Whitney test. Categorical data were compared using the χ^2 or two-tailed Fisher's exact test, as appropriate. Kaplan–Meier survival analysis with a log–rank test was used to assess the effect of vitamin A on survival. The analysis was done with EpiInfo® 6.04 (Centers for Disease Control, Atlanta) and SPSS® 9.0 (SPPP, Chicago).

Results

There were no relevant or significant differences in socio-demographic variables (age, sex, maternal education, household size, number of children in the house, type of housing) or pattern of breastfeeding between the supplemented and placebo groups.

The main clinical manifestations of severe malaria are listed in Table I. Fourteen of the cases included had a diagnosis of severe, not cerebral, malaria but we have no data on the criteria used to categorise them as such. Diagnosis on admission was cerebral malaria for 34.3% (96/280) of the supplemented group and 32.4% (94/290) of the children in the placebo group. The balance was made up by other forms of severe malaria.

Pre-admission clinical data (duration of illness, vomiting, diarrhoea, loss of consciousness, difficulty feeding, fever and self-medication with anti-malarials) were also similar, with the exception of convulsions, 50.9% (141/277) of the supplemented group and 64.5% (185/287) of the placebo group ($p = 0.001$), and consumption of traditional medicines, 5.4% (97/274) of the supplemented group and 51.9% (147/273) of the placebo group ($p = 0.000$) (Table II). Consumption of traditional medicines was associated with a higher frequency of convulsions before admission (OR 2.3, 95% CI 1.6–3.3).

Clinical characteristics observed by health staff on admission (coma, respiratory distress, convulsion) were not significantly different, except for a higher rate of acidotic breathing in the placebo group, 16.6% (48/289), 10.8% (30/277) in the supplemented group ($p = 0.046$) (Table II).

TABLE II. Selected pre-admission and admission clinical characteristics of the children in the vitamin A-supplemented and placebo groups

	Vitamin A		Placebo	
	No.	Prevalence No. (%)	No.	Prevalence No. (%)
Fever	279	277 (99.3)	289	285 (98.6)
Vomiting	279	139 (49.8)	288	137 (47.6)
Convulsions*	277	141 (50.9)	287	185 (64.5)
Loss of consciousness	274	123 (44.9)	286	147 (51.4)
Headache**	145	49 (33.8)	137	49 (35.8)
Traditional medicine†	274	97 (35.4)	283	147 (51.9)
Chloroquine	261	63 (24.1)	272	71 (26.1)
Cerebral malaria	280	96 (34.3)	290	94 (32.4)
Respiratory distress	271	64 (23.6)	280	81 (28.9)
Acidotic breathing‡	277	30 (10.8)	289	48 (16.6)
Convulsions observed by health staff in the emergency room	239	37 (15.5)	207	40 (16.2)

* $p = 0.001$; ** children aged > 24 months; † $p = 0.000$; ‡ $p = 0.046$.

Mean (SD) retinol (μg/dl) levels were not significantly different between the two trial groups, 7.25 (4.75), $n = 202$, in the supplemented group and 7.96 (5.52), $n = 233$, in the placebo group. Levels in survivors and children who died were also similar, 7.67 (5.22), $n = 421$, and 6.74 (4.12), $n = 12$, respectively ($p = 0.491$), reflecting a population with a high prevalence of vitamin A deficiency. In the supplemented group, 77.7% (157/202) of children had retinol levels below 10 μg/dl *vs* 72.1% (168/233) in the placebo group (NS), and 97% (196/202) in the supplemented group *vs* 97.8% (228/233) in the placebo group had retinol levels below 20 μg/dl (NS). Mean (SD) haemoglobin [5.98 g/dl (2.33) in the supplemented and 5.65 g/dl (2.21) in the placebo group] and mean (SD) glucose values [5.18 mmol/l (2.30) in the supplemented and 5.67 mmol/l (2.48) in the placebo group] were also similar. In the supplemented group, 39.6% (89/225) of the children had haemoglobin levels below 5 g/dl compared with 43.0% (101/235) in the placebo group. This difference is not significant.

Seven of 277 (2.5%) and 13 of 288 (4.5%) children died in the supplemented and placebo groups, respectively, a relative risk of death of 0.56 (95% CI 0.23–1.38, $p = 0.201$) (Table III). The survival curve for the two groups is shown in Fig. 2 ($p = 0.187$). In children with cerebral malaria, the relative risk of death in the vitamin A-supplemented group compared with the placebo group is 0.63 (95% CI 0.26–1.55, $p = 0.440$). The only two deaths in children with other forms of severe malaria were in the placebo group. In the cerebral malaria group, the rate of death-in-coma was 6/90 (6.7%) in the supplemented and 10/88 (11.4%) in the placebo group (RR 0.59, 95% CI 0.22–1.55, $p = 0.273$).

In the supplemented group, 4/82 (4.9%) children developed neurological sequelae compared with 2/78 (2.6%) in the placebo group (RR 1.90; 95% CI 0.36–10.09, $p = 0.682$). Children with sequelae had a longer duration of coma [74.20 (32.36)] than children without [29.21 (28.87), $p = 0.003$]. Two of four children observed at the 6-week follow-up were considered better than on discharge.

The median duration of admission of the children who died was 4 hours in the supplemented group and 16 hours in the placebo group ($p = 0.032$). Five of seven children in the supplemented group died in the 1st 5 hours of admission compared with only two of 13 in the placebo group. The remaining two

TABLE III. Mortality, neurological sequelae and frequency of convulsions and blood transfusions in the vitamin A supplemented and placebo groups

	Vitamin A		Placebo			
	No.	Prevalence No. (%)	No.	Prevalence No. (%)	Relative risk (95% CI)	*p*-value
Deaths	277	7 (2.5)	288	13 (4.5)	0.56 (0.23–1.38)	0.201
Neurological sequelae*	82	4 (4.9)	78	2 (2.6)	1.90 (0.36–10.09)	0.682
Frequency of convulsions	266	51 (19.2)	278	58 (20.9)	0.92 (0.66–1.29)	0.623
Blood transfusion	258	206 (79.8)	265	224 (84.5)	0.94 (0.87–1.02)	0.161

* Only children with cerebral malaria

children in the vitamin A group died 19 and 24 hours after admission. In the supplemented group, the relative risk of death during the 1st 5 hours of admission was 2.54 (0.50–12.96) and 0.19 (95% CI 0.04–0.85, $p = 0.015$) after the 1st 5 hours of admission. (This cut-off point was selected considering that peak serum retinol is reached 6 hours after ingestion.[28]) Median duration of hospitalisation in both groups was 3 days ($p = 0.060$) (Table IV). There were no differences in median duration of hospitalisation for children with cerebral malaria (median 4 days in both groups, $p = 0.596$). However, in the other forms of severe malaria, median days of hospitalisation was significantly prolonged in the supplemented group (three in the vitamin A group, two in the placebo group, $p = 0.010$). The rates of discharge on day 5 were similar,

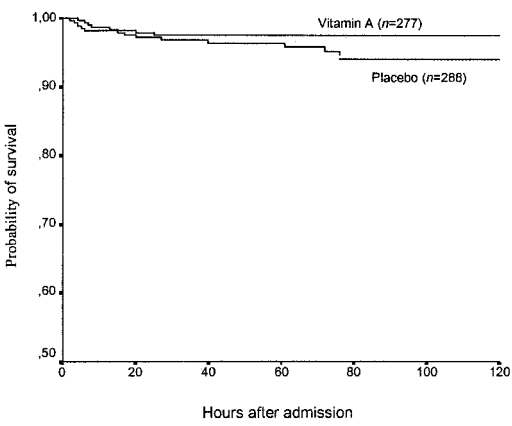

FIG. 2. Kaplan–Meier analysis of survival in 565 children in the vitamin A-supplemented and placebo groups.

85.9% (233/271) in the supplemented group and 84.7% (233/275) in the placebo group ($p = 0.679$).

More children recovered from coma in the supplemented group (94.1%, 96/102) than in the placebo group (88.9%, 88/99), but the difference is not statistically significant. Twenty-two children developed coma while in hospital, 7.1% (12/169) in the supplemented group and 5.6% (10/178) in the placebo group. There were no further differences in the clinical course while in hospital (duration of fever, duration of coma, blood transfusions received and convulsions). At discharge, results of microscopy for *P. falciparum* were negative in only 55.3% (73/123) of the treatment group and 53.7% (88/164) of the placebo group.

Among the children who were followed up 6 weeks after discharge, there were no significant differences in reported frequency of health care use or reported illness (fever, cough or diarrhoea), but the number lost to follow-up is important.

Discussion

As far as we know, this is the first report of the potential effect of vitamin A supplementation in children with severe malaria. The number of deaths in this study is similar to reports from elsewhere in Africa[29–36] and similar also to the rate reported by a previous study in this hospital unit.[23] Most deaths occur within 24 hours of admission, and in this trial five of seven children in the vitamin A-supplemented

TABLE IV. Duration of hospitalisation and length of time to clearance of parasites, resolution of fever and recovery from coma in the vitamin A-supplemented and placebo groups

	Vitamin A			Placebo			
	No.	Mean (SD)	Median (25–75%)	No.	Mean (SD)	Median (25–75%)	*p*-value
Duration of hospitalisation (days)*	270	3.17 (1.58)	3 (2–4)	275	3.18 (2.73)	3 (2–4)	0.060
Duration of hospitalisation in children with cerebral malaria (days)*	82	3.78 (2.11)	4 (3–4)	77	4.42 (4.35)	4 (3–5)	0.596
Duration of hospitalisation in children with other forms of severe malaria (days)*	188	2.91 (1.19)	3 (2–4)	198	2.69 (1.48)	2 (2–3)	0.010
Time to resolution of fever (hours)**	233	35.75 (28.42)	27 (13–56)	226	31.31 (28.04)	21 (13–38)	0.151
Time to recovery from coma (hours)†	95	28.45 (31.87)	17.5 (8–40)	86	26.15 (26.45)	16 (8–32)	0.746
'Clearance' of parasitaemia**	73	39.40 (26.00)	29 (18–60)	88	38.46 (26.53)	28 (18–52)	0.833

* Excluding children who died; ** excluding those discharged with fever and those who died with fever; † excluding those who died in a coma and those who never became comatose

group died within the 1st 5 hours. An acute toxic effect of vitamin A cannot be ruled out but is extremely unlikely, given the dosage used, the absence of signs of toxicity in the other children and the global protective effect on mortality.

The overall reduction in the risk of death among all supplemented children from 4.5% to 2.5%, although clinically important, is not statistically significant. However, vitamin A might be effective in reducing the case fatality rate in those who survive the 1st 5 hours of admission. This result was found on *a posterior* subgroup analysis of the data and should be regarded as a hypothesis for further investigation rather than a firm conclusion. A synergistic interaction between quinine and retinol against *P. falciparum in vitro* was recently described,[37] and we could speculate that such positive interaction is also possible *in vivo* and is linked to the decreased number of deaths after 5 hours. On the other hand, clinical trials comparing quinine with artemether have shown that improving the rate of parasite killing does not affect mortality[38–41] and it is most unlikely that a minor improvement in quinine's antiparasitic action would be beneficial, although only a larger study could take this further.

The rate of gross neurological sequelae is in the range of values described in some other trials[23, 31, 34, 42, 43] but lower than in others.[44–46] However, the numbers are too small and do not allow firm conclusions.

Although duration of hospitalisation was similar in the whole sample and in the children with cerebral malaria, it was prolonged in the supplemented children with other forms of severe malaria. This could be a chance finding or reflect a harmful effect of vitamin A. It contrasts with a recently published clinical trial which shows a significant reduction in duration of admission in vitamin A-supplemented Mozambican children admitted with non-measles acute lower respiratory tract infections.[47]

The number of children lost to follow-up, especially in the placebo group, limits the value of the 6-week post-discharge assessment. Although not significant, there was a slight tendency to more frequent health care use by children in the placebo group. This is in line with results of a large community trial in Ghana which reported a significant reduction in attendance at clinics in vitamin A-supplemented children.[48] Active surveillance after discharge might also be necessary for correct assessment of the effect of vitamin A supplementation, as an increase in mortality soon after discharge was described in Brazzaville,[43] Kenya[49,50] and Mozambique (M. Degdge, National Institute of Health, unpublished data, 1995). The apparent lack of impact at follow-up could also be related to the dose and formula of vitamin A used. If only 30–50% of a large oral dose of vitamin A is retained[51] and in the presence of very low levels of serum retinol, as in this trial, a larger dose should probably be used. In fact, previous trials in acute lower respiratory infections have shown that only doses of 200,000–400,000 IU of vitamin A result in a sustained increase of serum retinol levels in the convalescent period.[52,53] In measles trials, the highest impact of vitamin A supplementation was in those using a dose of 400,000 IU of a water-miscible vitamin A[54,55] rather than the oil-based preparation used in our trial. These considerations should be taken into account in designing future trials.

This trial neither proves nor disproves a beneficial effect of vitamin A supplementation in severe malaria. The data are sufficiently suggestive of therapeutic benefit to warrant further trials of sufficient power, using more effective administration of vitamin A and a detailed assessment of neurological sequelae to evaluate the effect and to ascertain that gains in survival are not offset by increases in neurological sequelae.

Acknowledgments

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