Original Article

Low serum vitamin A is prevalent in underfive children with severe malaria and is associated with increased risk of death

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Abstract

Introduction: Micronutrient deficiencies are prevalent in developing countries and may influence vulnerability to diseases particularly malaria and its severity. This study investigated serum vitamin A profile of under-five children with severe malaria (SM) in South-western, Nigeria and to determine its association with degree of malaria parasitaemia, types of SM and eventual outcome.

Methodology: Using HPLC, serum vitamin A concentrations of 170 under-five children with SM and 170 age- and gender-matched controls were determined. Parasite species identification and density were also determined. Association between serum vitamin A levels and the degree of parasitaemia, type of SM and patients' outcome were examined by both bivariate and logistic regression analyses.

Results: Thirty-five (20.6%) of the children with SM compared with 3 (1.8%) of the controls had hypovitaminosis A, $p < 0.001$, OR = 14.4, 95% Confidence Interval = 4.4 – 47.8. The mean serum vitamin A concentration was also lower in the patients (45.23µg/dL vs. 87.28µg/dL; $p < 0.001$). There was inverse correlation between serum vitamin A levels and malaria parasite density ($r = -0.103$, $p = 0.027$). Higher proportions of children with SM and hypovitaminosis A presented with metabolic acidosis and cerebral malaria ($p < 0.001$ and 0.032 respectively). Children with SM and hypovitaminosis A were 9.1 times more likely to die compared to those without low serum vitamin A levels, OR = 9.1, 95% Confidence Interval = 2.2–38.1, $p = 0.002$.

Conclusion: Children with SM had reduced serum vitamin A and significantly contributed to increased morbidity and mortality.

Key words: Nigeria; severe malaria; under-five children; vitamin A.

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Introduction

Malaria is a leading cause of morbidity and mortality among under five children in sub-Saharan Africa [1,2]. Between 300 and 500 million new cases of malaria, primarily due to *Plasmodium falciparum,* are annually observed in the world with 90% of them in sub-Saharan Africa and accounting for an estimated over a million child deaths [1-3]. In Nigeria, malaria accounts for over 60% of outpatient visits and is responsible for 30% and 11% mortality in under five years old and pregnant women respectively [4]. The disease also has a crippling effect on the continent's economic growth and perpetuates a vicious cycle of poverty and ill health.

Vitamin A, a generic term for all beta ionone derivatives is an essential fat soluble vitamin needed for the normal functioning of the visual system, growth and development, maintenance of epithelial integrity, immune function, reproduction and child survival [5,6]. Retinol, retinaldehyde and retinoic acid which are preformed vitamin A are found only in food of animal origin such as colostrum, liver, fish, fish liver oils, whole milk, egg yolk and fortified margarine [6,7]. However, carotenoids (pro-vitamin A) are found in plant such as green leafy vegetables, yellow fruits, carrots, and red palm oil [6]. The normal serum values of retinol ranges between 20 and 50µg/dl in infants and from 30 to 225µg/dL in older children and adults. The safe daily requirement vary from 180 to 500µg/day of retinol or its equivalent.

Supplementation with vitamin A potentiates host resistance to malaria and subsequently reduces malaria morbidity [7]. The exact underlying mechanism is still largely unknown [1,2]. A study following vitamin A supplementation reported a reduced secretion of Tumor Necrosis Factor (TNF), increased phagocytosis of *Plasmodium falciparum* parasitized erythrocytes and upgraded gene expression on monocytes and macrophages [8], thus, partly explaining the beneficial effects of normal vitamin A status or supplementation with vitamin A in malaria. A case-control study in Ile-Ife, Nigeria showed lower levels of retinol, β-carotene

and α-tocopherol in malaria infected children compared with controls [9]. Also a study in Cameroon to characterize the relationship between malaria and micronutrients reported that the proportions of malaria patients deficient in vitamin A and calcium were significantly higher than the corresponding control group [10].

Inspite of the beneficial effects of vitamin A on malaria pathogenesis, little information exists on the vitamin A profile among under-five children with severe malaria in southwestern Nigeria. This comparative study was therefore conducted to investigate this profile and study the correlation between serum vitamin A level and malaria parasite density.

Methodology

Study location

This prospective comparative study was conducted at the Children's Emergency Ward (CEW) and the Underfive Welfare Clinic of the Wesley Guild Hospital (WGH) Ilesa unit of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria. Ilesa lies on latitude 7° 37' N and longitude 4° 40' E and is located in the rainforest belt that is holoendemic for *Plasmodium falciparum* malaria.

Study participants

Patients were all consecutive children aged 6 months to 5 years with clinical features of severe malaria [11] admitted into the CEW of the hospital during the 7-month study period. The controls were children matched for age, gender and socio-economic status without clinical features of malaria seen at the Under-five Welfare Clinic attending for routine immunization, school entry medical tests or those accompanying their siblings to see a doctor. None of the controls had demonstrable malaria parasite in their blood. Children with other clinical conditions such as sickle cell anaemia, HIV/AIDS, pneumonia, sepsis and hepatitis as well as those whose parents or the accompanying relatives refused consent were excluded from the study.

All children with severe malaria, as defined by WHO [11] were recruited within one hour of admission. Severe malaria anaemia was defined as haemoglobin levels <5g/dL [11]. Information on the sociodemographic variables (age, gender and socioeconomic class as recommended by Olusanya *et al.* [12], signs and symptoms of malaria, its complications and duration of illness were documented.

Growth and nutritional status

Anthropometry data were obtained for all study participants. Weight was measured to the nearest 0.1 kg on a scaletronix digital electronic scale (Scaletronix, White Plains, NY) and height to the nearest 0.1 cm with a stadiometer (Holtain, Crymych, UK). Weight and height were transformed into z scores for height/age (HAZ), weight/age (WAZ) and weight/height (WHZ) and then compared to the reference values using WHO/National Center for Health Statistics (NCHS) reference standards [13]. Weight-for-height z-score (WHZ) ≤ 2 SD from the mean was defined as wasting, HAZ ≤ 2 SD as stunting, Weight-for-age z-score (WAZ) ≤ 2 SD from the mean as underweight. Overweight was defined as WAZ >2 SD but ≤ +3 SD from the mean; and obese if WAZ was >3 SD from the mean. Normal weight was WAZ score within the mean ±2 SD.

Coma scoring

The level of consciousness for those with cerebral malaria was assessed using the Blantyre Coma Scale [14].

Laboratory Investigations

Five milliliters of peripheral venous blood were obtained from each patient before the initiation of treatment into sterile plain bottles to obtain the serum using a clinical macro centrifuge (Hettich Universal 11, Aylesbury, Buckinghamshire, England). The serum was then frozen at -20ºC and transported ice packed to the Central Science Laboratory at the Obafemi Awolowo University, Ile-Ife, Nigeria (a distance of about 20 kilometres) for vitamin A analysis. The analysiswas done using High Performance Liquid Chromatography (HPLC)Agilent Technologies, 1200 series following a standard procedure [15].

Calculation of the vitamin A in serum was done taking into consideration peak area (PA) of sample, peak area (PA) of standard, concentration of standard and dilution factor as follow;

$$Vitamin\ A\ concentration\ = \frac{\mathrm{PAs}\ \times SC\ \times DF}{\mathrm{PAst}}$$

Where: PA_s = PA of the sample; SC = Standard Concentration; DF = Dilution Factor; PA_{st}=PA of the standard.

Serum level of vitamin A < 20µg/dL was defined as hypovitaminosis A, values between 20 and 50 µg/dL as normal and values >50 µg/dL as hypervitaminosis A [16].

Diagnosis of Malaria

Malaria was diagnosed using thin and thick blood smears stained with Giemsa stain to determine the parasite species and density respectively at the Microbiology Laboratory of the Hospital. Quantification of the parasite density on the thick film was determined according to the number of parasite counted against 200 white blood cells (WBC) assuming a total WBC count of 8000/microlitre (μL) of blood (average WBC used where the patient's total WBC counts are not available) [17].

$$Parasite\ (blood\ \mu L) = \frac{NP\ \times EWBC}{NWBC}$$

Where: NP = Number of parasites counted; EWBC = Estimated white blood cell count; NWBC = Number of white blood cells counted.

Other Investigations

Other investigation that were done included haematocrit, random blood sugar, cerebrospinal fluid microscopy, culture and sensitivity, serum bicarbonate estimation, urinalysis and other routine laboratory investigations considered necessary for individual patients were carried out to enhance patient management.

The outcome of the hospitalization was documented as discharged or dead.

Data analysis

Data analysis was done using the SPSS for Windows software version 17.0. Means, standard deviations (SD), proportions and percentages were determined as applicable. Means (SD) were compared using independent sample't' test or Mann Whitney U test as applicable. Proportions and ratios were compared using the Pearson Chi squared (χ^2) or with Yates continuity correction as necessary. The relationship between the degree of malaria parasitaemia and serum vitamin A levels was assessed with Spearmann correlation analysis. Risk estimates (i.e. Odd Ratio (OR) and 95% Confidence Intervals (CI), were calculated for comparison of cases and controls. Logistic regression analysis was further undertaken to determine the influence of severe malaria infection on serum vitamin A levels. In the regression model, serum vitamin A status (dichotomised as hypovitaminosis A or no hypovitaminosis) was used as the outcome/dependent variable, and, age, gender, socio-economic characteristics, anthropometry, malaria diagnoses and parasite density were taken as predictive or independent factors. Values of $P < 0.05$ were accepted as statistically significant.

Ethical consideration

The study was approved by the Institutional Ethics and Research Committee of the hospital (IRB/IEC Number: 00005422). Also, written consents were obtained from the caregivers of all the 340 participants before commencing the study.

Results

One hundred and seventy (28.6%) of the 595 children admitted into the CEW of the hospital during the 7-month study period had severe malaria. One hundred and seventy age- and gender-matched children with no demonstrable malaria parasitaemia who attended the Under-five Welfare Clinic of the hospital during the period were recruited as controls. Hence, a total of 340 children, (170 with severe malaria and 170 age- and gender-matched controls without demonstrable malaria parasitaemia) were recruited and studied.

Table 1. The baseline characteristics of the cases and controls.

Characteristics	Cases, n (%)	Controls, n (%)	P values	OR (95% CI)
Male, n(%)	99 (58.2)	92 (54.1)		
Female, n(%)	71 (41.8)	78 (45.9)	0.444	1.2 (0.8–1.8)
Age, mean±SD	24.8 ± 13.6	23.0 ± 13.3	0.218	(1.1 – 4.7)
Age group				
6 -12 months	40 (23.5)	46 (27.1)		
13-24 months	63 (37.1)	66 (38.8)		
25-36 months	37 (21.8)	27 (15.9)	0.527	(0.5 - 0.6)
37-48 months	23 (13.5)	27 (15.9)		
49-59 months	7 (4.1)	4 (2.4)		
Anthropometry				
Underweight	31 (18.2)	37 (21.8)	0.416	0.8 (0.5 – 1.4)
Overweight	1 (0.6)	2 (1.2)	1.000	0.5 (0.1 – 5.5)
Wasting	14 (8.2)	17 (10.0)	0.572	0.8 (0.4 – 1.7)
Stunting	11 (6.5)	9 (5.3)	0.645	1.2 (0.5 – 3.1)

The age of the cases ranged from 7 to 60 months with mean ±SD of 24.77 ±13.64 months, while that of the control ranged from 6 to 60 months with mean ±SD of 23.02 ±13.34 months. There was no statistical difference in the ages of the two groups, t = 1.20, p = 0.233. Ninety-nine (58.2%) of the cases and 92 (54.1%) of the control were males. Also, the gender distribution was similar, χ^2 = 0.585, p = 0.444. The overall male: female ratio was 1.3:1. As shown in table 1, most of the study participants were children in the age group 13-24 months, while the least represented age group was the 49-59 months. Majority of the cases and the control were from social class II (56.5% vs. 68.2% respectively). The sociodemographic characteristics and anthropometric measurements of the cases and controls were similar (Table 1).

Serum levels of vitamin A

Among the children with malaria, the minimum serum vitamin A was 2.49µg/dL while the maximum was 500.00µg/dL. For the controls, serum vitamin A levels ranged from 18.59 to 853.23µg/dL. The mean ± SD serum vitamin A levels for the cases and the control were 45.23 ± 73.90µg/dL and 87.28 ± 111.09µg/dL respectively, with cases having significantly lower median serum vitamin A levels (p < 0.001 with Mann Whitney U test) as shown in Table 2. In addition, significantly higher proportion of the cases, 35 (20.6%), than the control, three (1.8%) had hypovitaminosis A (i.e. serum level <20µg/dL); p <0.001. Cases were 14.4 times more likely to present with hypovitaminosis A than the controls, OR = 14.4, 95% CI = 4.4 – 47.8.

Parasitaemia

The mean (SD) parasitaemia was 38,200 ± 132,448.36/µL (Range = 360 - 1,360,000/µL). About a third, 60 (35.3%) had severe parasitaemia of ≥10,000 parasite/µL, 97 (57.1%) had moderate parasitaemia i.e. 1000-9999/µL and 13 (7.6%) had mild parasitaemia (<1000/µL). Only 6 (3.5%) had hyperparasitaemia (malaria parasite count >250,000 parasite/µL).

Diagnoses

Severe anaemia accounted for about one-half, 80 (47.1%) of the cases. The least made diagnosis was jaundice and was seen in one (0.6%) patient. The frequencies of other manifestations of severe malaria were shown in table 3.

Association between serum vitamin A and sociodemographic characteristics

There were significant associations between the age groups and socioeconomic class distributions of the patients and serum vitamin A levels as shown in Table 3 (p = 0.033 and 0.032 respectively). Gender was however not significantly associated with serum vitamin A (p = 0.086).

Relationship between serum vitamin A and severe malaria diagnoses

Significantly higher proportion of children with metabolic acidosis, 25 (33.8%) of the 74 compared with 10 (10.4%) of the 96 without metabolic acidosis had low serum vitamin A, p <0.001, OR = 4.4, 95% CI = 2.0 – 9.9. Similarly, children with cerebral malaria were 2.3 times more likely to present with low serum vitamin A. Fifteen (31.3%) of 48 with cerebral malaria vs. 20 (16.4%) of 122 without cerebral malaria had low seum vitamin A, p = 0.031, OR = 2.3, 95% CI = 1.1 – 5.0. The levels of serum vitamin A were not significantly related with the frequency of other malaria diagnoses, as shown in table 3.

Association of serum vitamin A levels with malaria parasite density

With Spearmann correlation analysis, serum vitamin A had significant mild inverse correlation with malaria parasitaemia (r = - 0.103, p = 0.027).

Multivariate analysis

Binary logistic regression analysis was done to show the influence of sociodemographic, nutritional status, malaria diagnosis and parasite density on the

Table 2. Comparison of the serum Vitamin A levels between cases and controls.

Variables	Cases N = 170	Control N = 170	P	OR (95% CI)
Range µg/dL	2.49 - 500	18.59 – 853.23		
Overall mean ± SD µg/dL	45.23 ±73.90	87.28 ± 111.09	<0.001#	18.6 – 65.5
Median µg/dL	28.03	58.60		
Hypovitaminosis n (%)	35 (20.6)	3 (1.8)	<0.001*	14.4 (4.4–47.8)
Normal serum level n (%)	109 (64.1)	71 (41.7)	<0.001*	2.5 (1.6 – 3.9)
Hypervitaminosis n (%)	26 (15.3)	96 (56.5)	<0.001*	0.14 (0.1 – 0.3)

n: number of children; SD: Standard Deviation; # analysed with Mann Whitney U test; *analysed with Chi-squared test; hypovitaminosis A: serum vitamin A <20 µg/dL), normal level (20 – 50 µg/dL), hypervitaminosis (>50 µg/dL).

Table 3. Relationship between serum vitamin A levels and the socio-demographic characteristics and diagnosis among the 170 children with severe malaria

Socio demographic characteristics	Total n = 170	Low serum vit A n (%)	Normal serum vit A n (%)	High serum vit A n (%)	P
Age group					
6 – 12	40	12 (34.3)	23 (21.1)	5 (19.2)	
13 – 24	63	11 (31.4)	45 (41.3)	7 (26.9)	**0.033**
25 -36	37	10 (28.6)	21 (19.3)	6 (23.1)	
37 – 48	23	2 (5.7)	17 (15.6)	4 (15.4)	
49 – 59	7	0 (0.0)	3 (2.8)	4 (15.4)	
Gender					
Male	99	21 (60.0)	23 (21.1)	5 (19.2)	0.086
Female	71	14 (40.0)	51 (46.8)	6 (23.1)	
Social class					
SEC I	8	1 (2.9)	7 (6.4)	0 (0.0)	**0.032**
SEC II	96	16 (45.7)	69 (63.3)	11 (42.3)	
SEC III	66	18 (51.4)	33 (30.3)	15 (57.7)	
Diagnosis					
Severe Anaemia	80	18 (51.4)	57 (52.3)	5 (19.2)	0.561
Metabolic Acidosis	74	25 (71.4)	43 (39.4)	6 (23.1)	**<0.001**
Multiple convulsions	52	12 (34.3)	36 (33.0)	4 (15.4)	0.594
Cerebral malaria	48	15 (42.9)	32 (29.4)	1 (3.8)	**0.031**
Hyperpyrexia	15	8 (22.9)	7 (6.4)	0 (0.0)	0.694
Prostration	15	6 (17.1)	8 (7.3)	1 (3.8)	0.288
Haemoglobinuria	8	3 (8.6)	5 (4.6)	0 (0.0)	1.000
Hyperparasitaemia	6	2 (5.7)	4 (3.7)	0 (0.0)	0.363
Hypoglycaemia	2	1 (2.9)	1 (0.9)	0 (0.0)	1.000

Numbers in parenthesis are percentages of total in each column: 35, 109 and 26 for low, normal and high serum levels of Vitamin A respectively. SEC: Socioeconomic class.

presence or otherwise of hypovitaminosis A in these children with severe malaria. Only metabolic acidosis (OR = 2.8, 95% CI = 1.2 – 7.7, p = 0.046) predicted presence of hypovitaminosis A (Table 4).

Association of serum vitamin A with outcome

Nine (33.3%) of the 27 deaths in the CEW during the study period were as a result of severe malaria. The severe malaria case fatality rate was therefore 5.3%, i.e. nine of the 170 children with severe malaria. Children with severe malaria who in addition had low serum vitamin A were 9.1 times more likely to die compared to those without low serum vitamin A levels. Six (17.1%) of the 35 with low serum vitamin A as against 3 (2.2%) of the 135 without low serum vitamin A died, OR = 9.1, 95% CI = 2.2 – 38.1, $\chi^2 = 9.545$, p = 0.002.

Discussion

During the present study, severe malaria was responsible for 28.6 per cent of childhood hospital admission which is within the quoted 20 to 50 percent contribution of malaria to hospital admission in Africa

Table 4. Binary logistic regression analysis showing the influence of sociodemographic, malaria diagnosis and parasitaemia on presence or absence of hypovitaminosis A in under-five children with severe malaria.

Independent variables	OR	95% CI		P values
		Lower	Upper	
Gender	0.9	0.9	1.1	0.833
Age	1.1	0.4	2.8	0.991
Socioeconomic class	0.8	0.3	1.9	0.572
Underweight	1.0	0.9	1.1	0.997
Wasting	1.1	0.9	1.2	0.459
Stunting	4.1	0.1	0.8	0.977
Severe malaria anaemia	2.1	0.7	2.3	0.063
Metabolic acidosis	2.8	1.2	7.7	**0.046**
Cerebral malaria	1.4	0.8	2.4	0.178
Parasite density	1.2	0.5	2.3	0.883

OR: Odd Ratio, CI: Confidence Interval.

[18]. This shows that malaria is still a major contributor to childhood morbidity and mortality. Though all the patients were under-five and are yet to acquire adequate clinical immunity to malaria, a higher prevalence of severe malaria was recorded in children less than 24 months. This supports the view that children in the early years are particularly more vulnerable to severe attacks of malaria [1,2].

The proportions of children with severe malaria who were deficient in vitamin A were significantly higher than their corresponding control groups ($p < 0.001$). Consistent with some previous findings, severe malaria is associated with micronutrient deficiencies [7,9,10,19]. The reduction of serum micronutrients in the malaria-infected children could be adduced to increased utilization of body nutrients, reduced intake from anorexia and enhanced free radical activities [7]. In this study, a significantly lower mean serum vitamin A concentration in malaria infected children than in the control was observed, ($p < 0.001$). This observation had been reported earlier [3,7,10,20]. Low level of vitamin A observed is probably due to the fact that vitamin A is an anti-infective vitamin, which plays an important role in immunity against infectious diseases [10]. It may also be that vitamin A is utilized in the face of increased parasitaemia [4]. A previous study of Thai children [21], suggested that retinol, being bound to a negative acute phase protein, Retinol Binding Protein (RBP), could move rapidly into extra vascular fluids. This may result in increased availability of retinol to the tissue where it can more efficiently protect tissues exposed to reactive oxygen radicals that are emanating from infections.

Research has shown that *P.falciparum* may use vitamin A from its human host as an anti-oxidant to protect itself from oxidative stress while intra-erythrocytic [22]. The authors also reported that the amount of vitamin A taken up by the parasite in-vitro is small compared with the deficit that sometimes leads to hypovitaminosis A in malaria cases [22]. Chronic inadequate intake of vitamin A rich food plus consumption of vitamin A by parasite may then be the cause of the characteristic low serum vitamin A seen in malaria cases [6,19,22]. It has been documented that free retinol has a pharmacological effect against malaria parasite [23], but the very low concentrations of free retinol in serum of malaria patient makes this hypothetical effect inconclusive, [24] making these children unable to mount necessary host resistant to malaria.

In the present study, 20.6% of the children with severe malaria had low serum vitamin A. This proportion was lower than the proportion of malaria patient deficient in vitamin A (51.85%) reported among under-five children in Cameroun [10]. The lower proportion of children with low serum vitamin A reported in the present study may probably be due to vitamin A supplementation coverage during National Immunization Days (NIDs) in Nigeria [9]. Nigeria, listed by the World Health Organisation as one of the category-1 countries with the highest risk of vitamin A deficiency, has intensified efforts through the periodic distribution of vitamin A capsules to children younger than five years of age [9,25]. Previous data showed that there has been an improvement in immunization coverage to above 70% in children 6-59months in Nigeria, although still less than the expected 90% coverage [26].

Majority, 64.1%, of the children with SM from this study had normal serum vitamin A. It is possible that the serum vitamin A levels of these children were high before malaria infection and these seemingly normal values represented the characteristic fall of serum vitamin A in malaria infection. Despite the risk of hypervitaminosis A, UNICEF proposed that in countries where mortality among young children is high, children aged 6-59 months should receive enough vitamin A. They reported that provision of two high-dose vitamin A capsules a year to young children is safe and may be the single most cost-effective child survival intervention [26].

The prevalence of vitamin A deficiency (1.8%) among the control subjects in this study was remarkably lower than 23.2% reported for under-five children by Nigeria Federal Ministry of Health in 2005 [9] and 29.5% in similar children by Maziya-Dixon *et al.* in 2004 [27]. The mandatory fortification of three Nigerian staple foods: vegetable oil, wheat flour and sugar with vitamin A on a large scale in Nigeria [9,26], may be contributory to the generally low prevalence of vitamin A deficiency even among the controls in the present study. It is plausible that vitamin A deficiency might no longer be a public health issue in Nigeria in the near future, if this trend continues.

Malaria parasite density inversely correlated with serum vitamin A level in this study, ($r = -0.103$, $p = 0.027$). This was consistent with an earlier documentation that the magnitude of depression of serum retinol was inversely proportional to the degree of parasitaemia and disease severity [26]. In addition, types of severe malaria, specifically metabolic acidosis and cerebral malaria, were also associated with vitamin A reduction. Interestingly, we did not find any association between serum vitamin A level and severe

malaria anaemia, or with other SM forms such as multiple convulsions, haemoglobinuria, prostration, hyperpyrexia and jaundice.

The proportion of childhood deaths occuring in association with vitamin A deficiency in the present study is notably high, in tandem with local report that vitamin A deficiency contributes to about 25 percent of infant and child mortality in Nigeria [26].

Our study is limited by the cross-sectional design in which inference about causality or reverse could not be conclusively made. The low vitamin A could simply reflect the metabolic consequences of the severe disease, and have nothing to do with causation. Also, the children with deficiency were not given vitamin A supplementation. A preferred study design would have assessed the retinol concentrations longitudinally following treatment.

Conclusions

This study demonstrates that under-five children with severe malaria have lower serum vitamin A levels compared to children without malaria. It also suggests a possible association between depressed serum vitamin A level, cerebral malaria and metabolic acidosis and increased risk of mortality. It is therefore recommended that serum vitamin A should be determined and supplemented if found to be low, in under-five children with severe malaria, especially those with cerebral malaria and metabolic acidosis.

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