Brief Original Articles

Serum retinol concentration in patients with acute falciparum malaria in Aligarh, India

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Abstract

Background: Vitamin A (retinol)-deficiency and falciparum malaria are two major public health problems in developing countries. Falciparum malaria is associated with significant destruction of erythrocytes and can lead to severe anaemia.

Methodology: The present study was designed to estimate the serum retinol concentration in 150 acute falciparum-malaria patients (aged two to five years) by High Performance Liquid Chromatography (HPLC). Serum retinol concentrations of 20 healthy (age and sex matched, population based) volunteers were included as controls.

Results: The mean serum retinol concentration of healthy controls was 34.31 ± 1.274 µg/dl and that of diseased cohort was 12.562 ± 0.276 µg/dl. The mean parasitemia was 1239.2 ± 33.609 per µL.

Conclusions: The diseased cohort demonstrated significant reduction in concentrations of retinol in comparison to healthy controls ($p < 0.001$) and there was an inverse relationship (coefficient of correlation $r = -0.899$) between parasitemia and serum retinol concentration.

Key words: falciparum- malaria, serum retinol, parasitemia, India

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Introduction

Malaria, which is a disease of antiquity, has proved to be a formidable deterrent to the cultural and socioeconomic progress of mankind throughout the globe especially in the tropical, subtropical and monsoon prone regions. Malaria imposes great socioeconomic burden on humanity and with six other diseases (diarrhoea, HIV/AIDS, tuberculosis, measles, hepatitis-B, and pneumonia) accounts for 85% of the global infectious disease burden [1,2]. Malaria afflicts more than 90 countries and territories in the tropical and subtropical regions and almost one half of them are in Africa, South of Sahara. About 36% of the world population (*i.e.* 2020 million) is exposed to the risk of contacting malaria. The World Health Organization (WHO) estimates 300-500 million malaria cases annually, with 90% of this burden in Africa [3]. In addition, the estimated annual mortality attributed to malaria ranges from 0.7 to 2.7 million globally and more than 75% of the total morbidity occurs in children and expectant mothers [4,5]. According to the WHO, out of approximately 1.4 billion people living in 11 countries in the southeastern Asian region, 1.2 billion are at risk of contracting malaria, the majority of whom are from India [3,6], and Southeast Asia contributed 76% of the total cases.

In India, most cases of malaria occur in Orissa state. Although Orissa has a population of 36.7 million (3.5% of India), it contributed 25% of a total of 1.5-2.0 million reported malaria cases annually, 39.5% of *Plasmodium falciparum* malaria, and 30% of deaths caused by malaria in India. Utter Pradesh (UP), India's largest state, contributes only 5% of total cases [7].

Vitamin A deficiency is associated with common childhood diseases and poor immune function [8], and poses a higher risk for childhood mortality [9]. Moreover the importance of vitamin A to the health and visual acuity in children has been known since the 1920s [10]. Children aged one to three years are affected more due to the fast rate of growth and higher physiological requirement of vitamin A at that age [11].

Table 1. Serum retinol concentrations* versus parasitemia in the study population.

Parasitemia per µL	Serum retinol concentration µg/dl		No. of patients N = 150
	Range	Mean± SE	
600-800	13.33-21.33	16.69±0.441	26
801-1000	12.09-20.13	14.95±0.279	38
1001-1200	12.03-14.35	13.33±0.312	8
1201-1400	8.03-14.08	12.09±0.336	16
1401-1600	8.38-12.87	10.25±0.117	35
1601-1800	7.03-13.23	8.99±0.769	8
1801-2000	7.03-8.98	8.23±0.151	16
2001-2200	5.83-6.98	6.60±0.383	3

*Serum retinol concentration in healthy volunteers = 34.31 ± 1.274 µg/dl.

There are few reports indicating the decrease in serum retinol concentration during malarial infection and also the relation of retinol concentration with *P. falciparum* parasitemia [12,13, 14]. Moreover, such studies are lacking from the Indian subcontinent even though India contributes a major percent of *P. falciparum* infections. Therefore, the present preliminary study was designed to analyze the serum retinol concentration in *P. falciparum* infected patients, and to analyze the relation of serum retinol concentration with *P. falciparum* parasitemia.

Materials and methods

Study population

The study was conducted in confirmed patients of *P. falciparum* infection who attended out-patient clinics or those admitted to the wards of J N Medical College and Hospital, AMU, Aligarh, India, during May 2006 to September 2007. The study population was comprised of 150 children with the age range of two to five years. Twenty age and sex matched, population-based healthy volunteers were also included as controls. There were no ocular signs and symptoms of vitamin A deficiency and night blindness in healthy controls.

Thick and thin Giemsa-stained blood films were screened for the presence of *Plasmodium* species. The parasite count (parasites/µL) was done by counting 200 white blood cells and the number expressed on the basis of 8,000 WBC /µL [15,16].

Calculation of parasitemia

$$= \frac{\text{no.of parasites seen}}{\text{no.of leucocytes seen}} \times 8000$$

Serum samples

Venous blood was collected aseptically from the patients and was kept in a dark environment for less than 6 hours before centrifugation. Serum was obtained by centrifugation at 1,500 × g for 5 minutes at room temperature, and aliquots were prepared and immediately stored at -70^{0}C until processed further.

Vitamin A was measured by high-performance liquid chromatography (HPLC) per the method of Omu *et al.* [17]. Briefly, α-tocopherol acetate and retinol acetate was pipetted into Eppendorf tubes. Respective serum was added to the tubes and vortex. Hexane extract of vitamin A was aspirated in a glass tube, dried under nitrogen stream, and dissolved into methanol. Finally, this preparation was injected into HPLC fitted with a reverse phase C-18 stainless steel column. The vitamin was eluted with methanol at the flow rate of 1.5 ml/min for 15 minutes. The peak heights and the curve areas of vitamin A and its acetate were measured to calculate the amount of vitamin A in plasma in an ultraviolet detector using 292-nm filters.

Statistical analysis

Statistical analysis was done using SPSS, version 14, Statistics software. Unpaired Student's t was applied for the comparison of serum vitamin A levels of cases with those of controls, and a p value of < 0.001was regarded as significant. Descriptive statistics including mean, SDs and SEs were calculated for each continuous variable. Pearson correlation analyses were performed to determine the degree and direction of association between two variables (parasitemia and serum vitamin A concentration).

Results

In this study the mean serum vitamin A level in the patient-cohort was 12.50 ± 0.276 µg/dL against the mean value of 34.311 ± 1.274µg/dL in healthy controls and it was found statistically significant ($p < 0.001$). Moreover, there was a gradual fall in serum vitamin A level as the parasitemia increased (Table 1 and Figure 1). The Pearson coefficient of the correlation between parasitemia with serum vitamin A was -0.899, which demonstrates an inverse relationship. Figure 1 shows the correlation between parasitemia and serum vitamin A concentration.

Discussion

Interestingly, in this study, a gradual fall in serum retinol concentration was observed as the parasitemia increased. In a previous study by Das *et al*. [14], that involved cases of severe and mild malaria, a similar reduction of serum retinol concentration was reported. The serum concentration of retinol, the alcohol form of vitamin A, decreases during malarial infections. The reduction has been characterized as a direct consequence of the inflammatory response to *Plasmodium* infection [12,13]. Increased release of interleukin-6 causes a reduction in the serum level of retinol binding proteins and pre-albumin, impeding the transport of vitamin A from the liver to the target tissues [13].

Malnutrition is one of the leading causes of childhood morbidity and mortality worldwide. In developing countries, 15% or more of children born each year die before they reach the age of 5 years, mainly due to malnutrition, parasitic infections and low birth weight. Malaria has been shown to impair growth of younger but not older children [18].

Beneficial protective effects of vitamin A or zinc on malaria-related morbidity have been demonstrated in Papua New Guinea, Peru and Zanzabari [19-21]. Two randomised, controlled trials conducted in Ghana did not find an overall significant effect of vitamin A on malaria parasitemia rates or parasite densities although the studies showed a reduction of 23% and 32% of probable malaria illness in supplemented children [22]. However, the number of children with probable malaria was so small that this study lacked adequate power to demonstrate an effect of vitamin A on slide-confirmed malaria morbidity.

In a randomised control trial in Burkina Faso done in 148 children (6 months to 6 years), by administering a single dose of vitamin A (200,000 IU) with a daily zinc supplementation, a 30% reduction in slide confirmed malaria fever were reported [23]. A recent population based study [24] evaluated the of impact of vaccine, use of insecticide treated bed nets, intermittent presumptive or preventive therapy in infants (IPTi) and the impact of vitamin A supplementation on the malaria morbidity and reported similar views of reduced malaria morbidity with vitamin A supplementation.

Another study done in Papua New Guinea (children aged 6 to 60 months were supplemented with vitamin A) showed that the highest morbidity rates due to *P. falciparum* infection were in the age group of 12 to 36 months when the immune system is actively developing. Lesser effects were seen in the 37- to 60-age group in which some degree of protection might have already developed. Furthermore, the number of episodes of fever with parasitemia was 30% lower in the vitamin A supplemented group, and while vitamin A supplementation reduced the morbidity in the cases of falciparum malaria, there was no effect on the incidence of falciparum malaria. [25].

It has been demonstrated that free retinol has a pharmacological effect against malarial parasites [26], but the low concentration of free retinol in the serum make its hypothetical effect inconclusive [27]. Therefore, based on the present study and those discussed above, we conclude that reduced retinol concentrations are associated with *P. falciparum* malaria, and that vitamin A supplementation might help reduce malaria morbidity.

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