Serum Ascorbic Acid Concentration in Patients With Acute *Falciparum malaria* Infection: Possible Significance

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Falciparum **malaria infection is associated with significant destruction of erythrocytes. This leads to the release of toxic metabolic products, including oxidant compounds. We measured the serum concentration of the antioxidant, ascorbic acid, in 129 patients presenting with acute falciparum malaria infection and in 65 healthy individuals. None of the study subjects administered any form of ascorbic acid supplementation within one week prior to participation in this study. The mean serum ascorbate concentration in infected adult males (n = 49, age range 18-50 years) was found to be 2.02 ± 0.20 mg/dL, and it was 2.03 ± 0.24 mg/dL in infected adult females (n = 56, age range 18-50 years). These values were significantly greater than the serum ascorbate levels (1.54 ± 0.10 mg/dL) in healthy adult males (n = 28) and females (n = 28) ($p < 0.05$). In children (age range 3 to 5 years), the serum ascorbate concentration was significantly lower (1.95 ± 0.20 mg/dL) during infection (n = 25) than in their healthy counterparts (2.9 ± 0.24 mg/dL, n = 9) ($p < 0.05$). It is evident therefore that ascorbic acid plays a significant role in the pathogenesis of acute falciparum malaria in adults. Infected children also need to be given supplemental doses of ascorbate in view of the weakness of their immune system.**

Key Words: Malaria, ascorbate, antioxidant.

L-ascorbic acid, and its congener L-dehydroascorbic acid, collectively known as vitamin C (ascorbic acid), has been known to mediate a wide variety of cellular functions [1-3]. This vitamin is required to build and maintain bone matrix, cartilage, dentine, collagen and connective tissue [4]. It is necessary for the conversion of folic acid to folinic acid and for the regulation of the respiratory cycle in mitochondria and microsomes [5]. Ascorbic acid also enhances the absorption of iron via reduction of the ferric form to the more rapidly absorbed ferrous form [6]. It helps in the correction of anemia and in the improvement of iron status in vegetarians [7]. In the formation of hemoglobin and the maturation of red cells, it has a role in the removal of iron from ferritin, particularly in the reticuloendothelial cells of the liver, spleen, and bone marrow [4]. Numerous studies have reported on the effect of ascorbic acid on the immune response. Animal studies have shown that ascorbate is necessary for the differentiation of lymphoid organs during the growth of cockerels and young rats [8], and it enhances the regeneration of lymphoid tissue after X-irradiation [9]. The phagocytic action of leucocytes [10] and the migratory behavior of neutrophils are enhanced by ascorbic acid [11]. Early work by Madison & Manwaring [12], and by Raffel & Madison [13], have shown that addition of ascorbic acid in large amounts to immunizing doses of antigen increases antibody production. However, this effect may be non-specific. Ascorbic acid supplementation has also been reported to raise the levels of circulating interferon [14, 15] and to produce various virucidal effects [16,17]. Later studies have also suggested that ascorbic acid functions in the process that inactivates adipose tissue lipase when energy demands have been met. This

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indicates a role for ascorbic acid in phosphorylation processes. Clinical observations of a number of infections accompanied by fever show a decreased blood level of ascorbic acid, indicating an increased need for this vitamin [18]. Furthermore, Dice & Daniel [19] showed that ascorbate plays a role in carbohydrate metabolism by markedly reducing the insulin requirement in juvenile-onset diabetics and by reducing blood sugar levels in both normal and diabetic subjects. When administered in massive doses, ascorbate has been reported to alleviate a wide variety of pathological conditions, including the common cold, burns, carbon monoxide poisoning, snakebite and infectious hepatitis [20,21]. We examined the effect of falciparum malaria infection on the total serum level of ascorbic acid in patients presenting with the acute, uncomplicated form of this disease.

Material and Methods

Patients

The patients enrolled in this study were male and female adults and children presenting with a history of fever and malaise at the Specialist Hospital Bauchi Outpatient Department. Malaria infection was confirmed by microscopy using Giemsa stain.

Serum

Blood was collected by venipuncture of the antecubital vein in adults and by femoral tapping in children less than five years, using a sterile needle and syringe. The blood was transferred into a clean, sterile container and allowed to clot. After clot retraction and centrifugation, the serum was transferred into a clean sample vial and assayed for ascorbate within 24 hours.

Assay for total serum ascorbate

Total serum ascorbate was assayed with the 2,4-dinitrophenylhydrazine method [22], using 5% trichloroacetic acid as a protein precipitant.

Data analyses

The d-test was used to compare the difference between mean serum ascorbate concentration in patients and their healthy counterparts. One-way analysis of variance (ANOVA) was used to compare the mean serum ascorbate concentration in various categories of infected subjects. P values < 0.05 were considered significant.

Ethical considerations

This work was conducted in accordance with the following ethical declarations: World Medical Association's Declaration of Helsinki [23], APA Ethical Principles in the Conduct of Research With Human Participants [24], World Medical Association's Declaration of Lisbon on the Rights of the Patient [25], and CIOMS/WHO International Guidelines for the Conduct of Research Involving Human Subjects [26].

Results

The mean serum level in healthy males was 1.54 ± 0.10 mg/dL and 2.02 ± 0.20 mg/dL in infected males, ($p < 0.05$). Among the females, the mean serum ascorbate concentration was found to be 1.54 ± 0.10 mg/dL in healthy adults and 2.03 ± 0.24 mg/dL in their infected counterparts, ($p < 0.05$). The mean serum concentration of ascorbate in children was 2.9 ± 0.17 mg/dL in healthy children and 1.95 ± 0.20 mg/dL in infected children. This clearly shows a reversal of trend when compared to the results obtained in adults. The serum ascorbate concentration of 2.9 ± 0.20 mg/dL in healthy children was significantly higher than the normal adult serum concentration of 1.54 ± 0.10 mg/dL ($p < 0.05$), being approximately two times the value in healthy adults. A comparison of the three patient categories however indicates no significant difference in their serum ascorbate levels (Table 1).

Table 1. Mean serum ascorbate concentration in different categories of falciparum malaria patients

Study subjects	Mean serum ascorbate concentration ± SD (mg/dL)
Infected children	1.95 ± 0.20
Infected males	2.02 ± 0.20
Infected females	2.03 ± 0.24

$p > 0.05$.

Discussion

The serum ascorbate concentration was higher in healthy children than in their adult counterparts. This may be attributed to the increased metabolic requirements for this vitamin in children, considering its involvement in the process of growth and differentiation [2,4,5,18]. A potentially significant finding from this work is the indication that ascorbate plays a significant role in the pathogenesis of acute *falciparum* malaria infection. Considering earlier reports by various workers [8,10, 12, 20, 27] on the potentiating role of this vitamin in the immune system, the increased serum ascorbate seen in adult *falciparum* malaria patients may be part of the machinery aimed at boosting host humoral immunity. The reason for this assertion is as follows. Leucocytes are known to participate as components of cell-mediated immunity in the early response of the host to *falciparum* malaria infection [28]. Being rich sources of ascorbic acid, the parasite challenge may stimulate leukocytes to release their ascorbate stores as an additional response to parasite-induced stress. Furthermore, the release of toxic products, including oxidant compounds, as a consequence of erythrocytic merogony, and the associated hemolysis of red blood cells, may further impose a demand on the patient for increased mobilization of antioxidants. Leukocytes can be a readily available source of ascorbic acid, thereby accounting for the increase in serum ascorbate in adult *falciparum* malaria patients. However, considering the complex nature of the pathogenic processes associated with *falciparum* malaria infection [29], other yet to be identified factors may also be at play. The fact that the levels of serum ascorbate in infected patients were significantly above the normal range of 0.5-1.5 mg/dL [22] provides some evidence that adult *falciparum* malaria patients mobilize the tissue stores of this antioxidant as part of the early response to infection. *Falciparum* malaria has also been known to be associated with depressed immune function in children under five years of age. This may account for the decrease in serum ascorbate that we observed in infected children. The implication here is that there is a significant positive correlation between serum ascorbate and immune function, as evidenced by earlier reports [11,15,17]. The picture in children is further complicated by the immature nature of the immune system, which could also be a contributing factor for the ascorbate levels seen in children. Based on earlier studies, which show that ascorbic acid supplementation can lead to significant improvement in serum ascorbate concentration [18], we suggest the need for supplemental doses of ascorbic acid in infected children to aid in boosting their developing immune function and to protect them from the destructive action of oxidant compounds released during the red cell rupture that accompanies infection by the *falciparum* malaria parasite. In addition, we recommend the administration of supplemental ascorbate in both healthy children and adults (healthy and patients inclusive) considering the endemic nature of this disease in the tropics, the stress associated with such endemicity, and the nature of human diets, which are severely restricted in terms of adequate ascorbic acid intake.

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