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Evaluation of serum ascorbic acid levels in acute falciparum malaria

Adil Raza[1], Haris M. Khan[1], Fatima Shujatullah[1], Trivendra Tripathi[2], Mohd. Ashraf Malik[3], Mohd. Shahid[1], Abbas A. Mahdi[4]

[1]Department of Microbiology, J.N. Medical College & Hospital, AMU, Aligarh-202002, UP, India
[2]Department of Biochemistry, J.N. Medical College & Hospital, AMU, Aligarh-202002, UP, India
[3]Department of Paediatrics, J.N. Medical College & Hospital, AMU, Aligarh-202002, UP, India
[4]Department of Biochemistry, Chhatrapati Shahuji Maharaj Medical University (Formerly King George's Medical University), Lucknow, UP, India.

Abstract

Vitamin C (Ascorbic acid) is a water soluble vitamin which is an antioxidant and has a wide variety of biological functions for growth and development of the human body. It is very essential for immune enhancement in human beings. Vitamin C-deficiency and falciparum malaria are two major public health problems in developing countries. Falciparum malaria is associated with significant destruction of erythrocytes and leading to severe anaemia. The present study was delineated to estimate the serum ascorbic acid concentration in 150 acute falciparum-malaria patients (aged two to ten years). Serum ascorbate level concentrations of 20 healthy volunteers (aged two to ten years) were included as controls. The mean serum ascorbic acid concentration of healthy controls was 1.163 ± 0.059 mg/dL and that of diseased cohort was 0.685±0.0145 mg/dL. The mean parasitemia was 1239.2±33.609 per µL. The diseased cohort demonstrated significant reduction in concentrations of ascorbic acid in comparison to healthy controls ($p<0.001$) and there was inverse relationship (coefficient of correlation $r = -0.98$) between parasitemia and serum ascorbic acid concentration.

Key words: Falciparum malaria, serum ascorbic acid, parasitemia, Vitamin C, Indian children.

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Introduction

Malaria 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 [1]. Malaria inflicts socioeconomic burden on humanity with six other diseases such as diarrhoea, HIV/AIDS, tuberculosis, measles, hepatitis-B and pneumonia accounts for 85% of global infectious disease burden [1,2,3]. Malaria afflicts more than ninety countries and territories in the tropical and subtropical regions and almost one half of them are in Africa and South of Sahara. About 36% of world population is exposed to the risk of contacting malaria. The World Health Organization (WHO) calculated approximately 300-500 million malaria cases annually with 90% of this burden in Africa [1,4]. Additionally, the approximated annual mortality attributed to malaria ranges from 0.7 - 2.7 million globally and > 75% of the total morbidity accounts in children and expectant mothers [1,5,6] In the South-Eastern Asian Region of WHO, ~ 1.4 billion people living in 11 countries, 1.2 billion are exposed to the risk of malaria, and majority of them are from India [4,7]. However, Southeast Asia contributed 76% of the total cases [1].

Malaria is mostly contributed in India by 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 [1,8]. Uttar Pradesh (UP), one of the biggest states of India, contributes only 5% of total cases [8]. However, documented evidences have highlighted that malaria cases and their investigations have been increased in UP zone of India. Falciparum malaria, which has been an important cause of acute renal failure in certain highly endemic zones of India, is showing an increasing prevalence in other parts such as Eastern UP due to an imbalance between the increasing population and inadequate sanitary facilities, which further worsen during floods [9]. In recent community based study conducted in Solana Village, Meerut district, Uttar Pradesh, India the prevalence of falciparum malaria was found to be 79%, vivax

malaria was of 18.7% and that of mixed infection was 2.3% [10], which reflects the prevalence of malaria in western Uttar Pradesh [10].

Moreover, several studies have showed the decrease of vitamin-C levels in malarial patients [6,7,8]. However, oxidative stress has been demonstrated an important role in the development of malarial anemia [11,12]. Malarial infection activates the immune system of the body and thereby leads to release of reactive oxygen species (ROS). The malarial parasite itself generates large quantities of ROS and also through its interaction with phagocytes [13-15]. Vitamin-C is known as an antioxidant because by donating its electrons and prevents other compounds from getting oxidized [16]. Ascorbic acid is also an strong immune modulator [17,18].

Thus, keeping in mind the existing studies showing increasing prevalence of malaria in the UP zone of India, we have designed our study to delineate the therapeutic importance of antioxidant (Vitamin C) by analyzing the serum ascorbate level in acute falciparum malaria in children and their relation with parasitemia that is fragmentary in reported literature.

Materials and Methods

Study population

The study was conducted on in- and out-patients of J. N. Medical College and Hospital, Aligarh Muslim University, Aligarh, India during a period from August 2006 to July 2007. The study population was comprised of 150 children with the age range of two to ten years. Twenty age and sex matched, population-based healthy volunteers were also included as controls. The cases were presented with fever with chills and rigors, prostration, headache, nausea and vomiting, abdominal pain, splenomegaly etc, and the study was approved by Institutional Ethical Committee, J. N. Medical College and Hospital, A.M.U., Aligarh, India.

Diagnostic methods

Diagnosis was made by demonstration of parasite in Giemsa-stained thin and thick blood smear and Quantitative Buffy Coat (QBC) or by rapid malaria antigen detection test, DIAGNOS MALARIA STIX (Biomed industries, India).

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 (WBCs) and the number expressed on the basis of 8000 WBCs/µL [19,20].

Following formula was used to calculate Parasitemia as shown by Raza et al. [1].

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

Estimation of ascorbic acid levels

Ascorbic acid levels were determined by the method of Natelson [21]. Briefly, 0.5 mL of serum was treated with 10% trichloro acetic acid (TCA), centrifuged at 10000 g, and 0.2 mL of protein free supernatant was collected for the detection of ascorbic acid. The collected supernatant was mixed with 0.2 mL of 3-[4,5 dimethyl thizolyl-2]-2,5-diphenyl tetrazolium bromide (MTT), and 0.2 mL of citrate phosphate buffer and incubated at 37°C for 30 minutes after incubation 0.5 mL of acetic acid was added to stop the reaction. The amount of formazon formed was measured at 578 nm (double beam UV Spectrophotometer 2203, Sistronics) against the reference vitamin-C tube run simultaneously.

Data analysis

Student's *t*-test was used to compare the difference between mean plasma ascorbate level in patients and their healthy controls. Statistical significance was concluded at probability $p < 0.05$. However, $p < 0.001$ were considered highly significant. Analyses were performed on SPSS for windows (version 12.0, Inc., Chicago, IL).

Results

The malarial patients were diagnosed as acute falciparum malaria on laboratory investigations and clinical features, and age ranging of this group was 2 to 10 years. In our study, the male female ratio in malarial patients (n = 150) and healthy control (n = 20) was randomized 5:3. The mean parasitemia in patients belonging to acute falciparum malaria was 1239.2±33.61 parasites/µL. The mean serum ascorbate level was 0.685±0.0145 mg/dL, refer to Table 1.

A distinct downward trend of serum ascorbate level was noticed as the parasitaemia increased (Table 1) which shows inverse relation of parasitaemia with serum ascorbate level and this was found to be statistically significant ($p < 0.001$). The mean ascorbate level of healthy control group was 1.163 ± 0.059 mg/dL. Also Pearson coefficient of correlation between parasitemia and serum ascorbate level was -0.98, which shows an inverse relationship.

Table 1: Serum ascorbic acid concentration* versus parasitemia in the study population

Parasitemia per µL	Serum ascorbate concentration mg/dl		Patients No. n= 150
	Range	Mean± SE	
600-800	0.882-0.988	0.923±0.035	26
801-1000	0.74-0.862	0.818±0.041	38
1001-1200	0.698-0.73	0.717±0.004	8
1201-1400	0.62-0.68	0.645±0.021	16
1401-1600	0.548-0.612	0.577±0.003	35
1601-1800	0.49-0.532	0.517±0.005	8
1801-2000	0.36-0.432	0.398±0.005	16
2001-2200	0.296-0.308	0.302±0.003	3

**Serum ascorbic acid concentration in healthy volunteers 1.163 ± 0.059 mg/dL*

Discussion

Ascorbate plays a pivotal role in protecting plasma lipids from reactive oxygen attack. However it is rapidly oxidized when challenged by oxidant released from activated polymorphs [22]. Moreover, reduced ascorbic acid levels have been reported in *Plasmodium falciparum* infection [23-25].

Plasmodia cells accumulate protective enzymes (catalase, glutathione peroxidase and superoxiade dimutase) that are depleted in the red blood cells of the host. Enhanced production of hydrogen peroxide (H_2O_2) and free oxygen radicals and a decrease in antioxidant enzymes have been observed in parasitised erythrocytes [13,25]. Moreover, reduced antioxidant enzymes defense in the RBCs of *P. falciparum* infected patients may be responsible for higher levels of lipid peroxidation and oxidative stress in children with moderate and high parasitaemia. Thus, the enhanced oxidative stress to erythrocytes in children with moderate and high parasiteamia was endogenously stimulated by malaria parasites during their consumption of haemoglobin and further leading to anemia [25]. Ascorbic acid plays an important role in conserving plasma lipids from ROS. However, it is rapidly oxidised when challenged by oxidants released from activated polymorphonuclear neutrophils [22,25]. Ascorbic acid level was significantly reduced by *P. falciparum* infection and this coincided with enhanced level of MDA. Once ascorbic acid has been used up, there is initiation of lipid peroxidation [25].

Furthermore, ascorbic acid is also known for the conversion of folic acid to folinic acid and for the regulation of respiratory cycle in mitochondria and microsomes [26], absorption of iron through reduction of ferric to ferrous form [27], correction of anemia and maturation of RBCs [28], and in removal of iron from ferritin, particularly in the reticuloendothelial cells of the liver, spleen and bone marrow [29]. Clinical observation of a number of infections accompanied by fever shows decreased blood levels of ascorbic acid, indicating an increased need for this vitamin [30].

In nutshell plasma ascorbate levels in, acute falciparum malaria was lower in comparison to healthy controls and there was inverse relation between parasitaemia and serum ascorbate levels. This is among the premier reports evaluating the serum ascorbate levels and their relation to falciparum malaria. We therefore feel that Vitamin C supplementation could be of therapeutic help in clinical outcome of the patients and thus suggest Vitamin C supplementation in malaria therapy. However, further researches with large study population are required to establish the firmness of this fact.

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Correspondence to:

Adil Raza
Department of Microbiology
J.N. Medical College & Hospital
Aligarh Muslim University
Aligarh 202002, India.
E.mail: dradil786_786@yahoo.co.in