Serum L-Ascorbic Acid Concentration And Its Potential For Scavenging Of Reactive Oxygen Species In Acute, Uncomplicated, Falciparum Malaria Infection

I Garba, U Abraham, U Shuaibu

Citation

I Garba, U Abraham, U Shuaibu. *Serum L-Ascorbic Acid Concentration And Its Potential For Scavenging Of Reactive Oxygen Species In Acute, Uncomplicated, Falciparum Malaria Infection*. The Internet Journal of Infectious Diseases. 2005 Volume 5 Number 2.

Abstract

Introduction: Reactive oxygen species (ROS) are produced during falciparum malaria infection leading to serum lipid peroxidation which is known to overwhelm some of the body's major antioxidant defenses including vitamin A, vitamin E, iron, catalase, glutathione peroxidase and superoxide dismutase.

Methods: The serum concentration of L-ascorbic acid was measured in 252 patients comprising of 90 adult males and 90 adult females (age range = 18-35 years), 34 male children and 34 female children (age range = 3-5years), presenting with acute, uncomplicated falciparum malaria infection and a control group of 76 healthy age-matched adults and 19 children.

Results: Serum L-ascorbic acid concentration was found to be significantly elevated in all the patient groups relative to the control L-ascorbic acid concentration. The male and female adult patients had a serum L-ascorbic acid concentration of 1.07 ± 0.03 mg/dl and 1.24 ± 0.03 mg/dl, while the value was 0.53 ± 0.03 mg/dl in healthy adult controls, $p < 0.05$. Serum L-ascorbic acid concentrations in male and female children were 1.18 ± 0.03 mg/dl and 1.23 ± 0.02 mg/dl. These values are all higher than the serum L-ascorbic acid concentration of 0.55 ± 0.03 mg/dl in healthy children, $p< 0.05$.

Conclusion:The increased serum L-ascorbic acid may arise as result of the mobilization of leukocyte L-ascorbic acid since leukocytes are known to increase in response to acute falciparum malaria infection. It could also be a compensatory homeostatic mechanism by the patients to offset the failure of the other antioxidant defenses during the disease.

INTRODUCTION

The substance widely referred to as vitamin C is an equilibrium mixture containing L-ascorbic acid, semidehydroascorbic acid and L-dehydroascorbic acid with over 80 percent of the vitamin C activity accounted for by L-ascorbic acid at equilibrium [1]. These three forms comprise a reversible redox system making the vitamin an effective quencher of free radicals such as the singlet O_2^- species [2]. Evidence abounds on the role of this vitamin in disease and maintenance of health [3,4,5]. Vitamin C has been reported to be markedly decreased in patients at risk of developing multiple organ failure [6]. In a study on the effect of vitamin C on plasma lipids, Howard and Meyers [7] were able to show some evidence of an inverse relationship between vitamin C intake and the development of atherosclerosis. The mediatory role of vitamin C in this instance may not only be due to its antioxidant activity, but also through a plasma lipid-modifying effect. The oxidative modification of low density lipoproteins (LDL) has been postulated to be one of the early steps in atherogenesis. In this respect ascorbate has been shown to reduce LDL oxidative susceptibility, even though it is not lipophilic [8]. Vitamin C has also been shown to have a positive therapeutic effect in the treatment and control of autoimmune disorders, including diabetes mellitus and acquired Immune Deficiency Syndrome (AIDS), whose immunological background data are in favour of the participation of an autoimmune mechanism in the genesis of the disease [9].

Endothelium-dependent vasodilatation is an also known to be impaired in humans with diabetes mellitus via inactivation of endothelium-derived nitric oxide by oxygen-derived free radicals. Vitamin C has been reported to improve this condition, thus further supporting the hypothesis that nitric oxide inactivation by oxygen-derived free radicals contributes to abnormal vascular reactivity in diabetes [10]. Paolisso et al. [11] have also been able to show that chronic vitamin C administration improves whole body glucose disposal and non-oxidative glucose metabolism in aged non-insulin dependent (type II) diabetic patients. Similarly, a high vitamin C intake has been documented to reduce the risk of cartilage loss and progression in people with osteoarthritis [12]. Thus the specific objective of this work was to assess the serum concentration of L-ascorbic acid in patients presenting with acute, uncomplicated falciparum malaria infection with the aim of establishing its adequacy and availability for reactive oxygen species scavenging or need for supplementation during the disease.

PATIENTS AND METHODS

STUDY LOCALE

The southern and northern limits of Bauchi State, Nigeria, where the study was conducted are demarcated by latitudes 9°30' North and 10°30' North respectively. Its Western and Eastern limits are bounded by longitudes 8°45' East and 11°0' East respectively. Two thirds of the land area is in the south of latitude 11°15'.

PATIENTS AND STUDY DESIGN

Patient selection and pre-qualification was done by simple random sampling of individuals presenting at the Bauchi Specialist Hospital Outpatient Department with a history of fever and malaise within a period of 1-7 days, and who were confirmed to be infected with the falciparum malaria parasite by microscopic examination of Giemsa stained thin blood slides. None of the patients and controls had taken any form of vitamin C supplementation within a period of one week prior to participation in the study. Based on the above criteria, 252 patients were found to be qualified for participation in the study. The qualified patients consisted of a group each of 90 adult males and females in the age range of 18-35 years. A control group of 76 age-matched healthy adults and 19 children were also enrolled for comparative purposes. Selection with this age group was to avoid age-dependent fluctuations in serum L-ascorbate concentration. The children consisted of two groups each comprising of 34 males and females and a control group of 19 children all in the age range of 3-5 years.

SERUM SAMPLE COLLECTION AND PREPARATION

Blood samples from each of the participants were collected between the hours of 9.00 a.m. and 11.00 a.m. by venepuncture of the antecubital vein into clean, sterile, plastic centrifuge tubes. The samples were centrifuged at 3000g for ten minutes after clotting. Sera was collected by aspiration using a Pasteur pipette and assayed within 24 hours.

ASSAY FOR SERUM L-ASCORBIC ACID

Serum L-ascorbic acid concentration was measured using the 2,6-dichlorophenolindophenol method [13].

STATISTICAL ANALYSIS

Data was analyzed using the MINITAB-10 Statistical Software. Results are expressed as mean ± SEM. Comparison of mean serum L-ascorbic acid concentration between the control group and patients were done using one-way analysis of variance (ANOVA). Where P values are < 0.05, the Duncan's Multiple Range Test was used to test the difference between pair of means. p values < 0.05 were considered significant.

ETHICS

This work was conducted in accordance with the CIOMS / WHO International Guidelines for the Conduct of Research Involving Human Subjects [14].

RESULTS

Results obtained showed a significantly higher serum L-ascorbic acid concentration in all categories of falciparum malaria patients as shown in tables 1 and 2.

Figure 1

Table 1: Serum L-ascorbic acid concentration in adult malaria patients and control.

Subjects	Mean ± SEM
Control (adults)	0.53 ± 0.03*[a]
Adult male patients	1.07 ± 0.03*[b]
Adult female patients	1.24 ± 0.03*[c]

* $p < 0.05$ (ANOVA)
[b, c] significantly different from [a] ($P < 0.05$, LSD)

Figure 2

Table 2: Serum L-ascorbic acid concentration in infected children and control.

Subjects	Mean ± SEM
Control (children)	0.55 ± 0.03[#a]
Male children, patients	1.18 ± 0.04[#b]
Female children, patients	1.23 ± 0.02[#c]

[#] $p < 0.05$ (ANOVA)
[b, c] significantly different from [a] ($P < 0.05$, LSD)

Mean serum L-ascorbic acid in both adult male and female patients was over twice the control serum L-ascorbic acid concentration of 0.53 ± 0.03 mg/dl, $p < 0.05$, table 1. A similar pattern was obtained among children presenting with acute, uncomplicated falciparum malaria infection, with values being twice above the control serum L-ascorbic acid concentration of 0.55 ± 0.03 mg/dl, $p < 0.05$, table 2. Among the patients, no difference was found to exist in the mean serum L-ascorbic acid concentration although the value was higher among females (both adults and children) relative to their male counterparts.

DISCUSSION

Potentially damaging free radicals or reactive oxygen species are produced in cells under normal conditions through either homolytic cleavage of a covalent bond, univalent oxidation or reduction [15]. Such free radicals, particularly reactive oxygen species have been implicated in the pathogenesis of various diseases, including atherosclerosis, diabetes mellitus, cancer and Parkinson's disease [16,17,18,19]. In particular, lipid peroxidation induced by free radicals is believed to be one of the major causes of cell membrane damage, leading to cell lysis and dysfunction [20]. Free radical defenses in the body comprise of a complex antioxidant system including vitamins A, E and C, glutathione and enzymatic antioxidants such as glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase [15, 21,22]. Biologically, free radicals are known to exert some physiological functions in the body such as biosynthesis, detoxification and microorganism clearance [23,24]. Therefore it is the duty of the antioxidant defenses to maintain normal homeostatic balance between free radical production and clearance. During acute falciparum malaria infection the host immune system is activated, leading to the release of reactive oxygen species. Specifically, macrophage-generated oxygen species are known to function as non-specific effector molecules in their defense arsenal [25]. In performing their protective role of destroying parasitized red blood cells, macrophage-generated reactive oxygen species also affect non-parasitized cells leading to the accumulation of organic peroxides and oxidation of membrane lipids [26] and cellular destruction. This state of oxidant-induced stress has been known to occur in malaria patients as evidenced by the increased serum levels of malondialdehyde (MDH) in this disease [27]. Equally striking is the failure of some key antioxidant defenses such as vitamin E, serum iron glutathione peroxidase and superoxide dismutase in maintaining reactive oxygen species homeostasis during malaria infection [25]. The observed increase in serum L-ascorbic acid can arise as a result of the mobilization of the available stores of this vitamin from the patient's leukocytes whose population and activity is known to increase in response to acute falciparum malaria infection. This leukocyte L-ascorbate mobilization can serve as a compensatory mechanism by the host to mitigate the effect of the potentially hazardous failure of the antioxidant defenses earlier mentioned. Because L-ascorbate is now primarily channeled to providing first protection against antioxidant damage, it is not available to carryout its other role of regenerating the metabolically active form of vitamin E [15]as evidenced by the reported decrease in serum vitamin E concentration in malaria infection [27]. Although some studies exist which report decreased serum L-ascorbate in falciparum malaria infection [28]. The low serum ascorbate is a reflection of the severity of the infection which has progressed to severe or complicated malaria with multiple organ involvement and a complex scenario of pathogenic determinants each uniquely capable of significantly contributing to the body reactive oxygen species pool [29,30,31,32]. The increase in L-ascorbate concentration observed in this study should not be seen as adequate considering the failure of the other antioxidant defenses. L-ascorbate supplementation is suggested to augment existing serum levels taking into cognizance the potentially vital role of this vitamin in the scavenging of reactive oxygen species produced in acute, uncomplicated falciparum malaria infection.

References

1. England S, Seifter S. The biochemical functions of ascorbic acid. Ann Rev Nutr 6 (1986) 365-406.
2. Frei B. Reactive oxygen species and antioxidant vitamins: mechanisms of action. Am J Med 97(1994) 34-55.
3. Negri E, La Vecchia C, Franceschi S, et al. Intake of selected micronutrients and risk of endometrial carcinoma. Cancer 77 (1996) 917-923.
4. Sardesai VM. Role of antixidants in health maintenance.

Nutr Clin Pract 10 (1995) 19-25.
5. Klenner RF. Significance of high daily intake of ascorbic acid in preventive medicine. J Int Acad Prev Med 1(1974) 45-69.
6. Borrelli E, Roux-Lombard P, Grau GE, Girardin E, Ricou B, Dayer J, Suter PM. Plasma concentrations of cytokine, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. Crit Care Med 24 (1996) 392-397.
7. Howard PA, Meyers DG. Effect of vitamin C on plasma lipids. Ann Pharmacother 29 (1995) 1129-1136.
8. Jialal I, Fuller CJ. Effect of vitamin E, vitamin C and beta-carotene on LDL oxidation and atherosclerosis. Can J Cardiol 11Suppl G (1995) 97G-103G.
9. Kodama M, Kodama T. Vitamin C and the genesis of autoimmune disease and allergy (review). In Vivo 9(1995) 231-238.
10. Ting HH, Timimi FK, Boles KS, et al. Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. J Clin Invest 97(1996) 22-28.
11. Paolisso G, Balbi V, Volpe C, et al. Metabolic benefits deriving from chronic vitamin C supplementation in aged non-insulin dependent diabetics. J Am Coll Nutr 14 (1995) 387-392.
12. McAlindon TE, Jacques P, Zhang Y, et al. Do antioxidant micronutrients protect against the development and progression of knee osteoarthritis? Arthritis Rhuem 39(1996) 684-656.
13. Plummer D. Introduction to practical Biochemistry. Oxford: Oxford University Press: 1971: 293.
14. Council of International Organization of Medical Sciences / World Health Organization (CIOMS/WHO) (1993). International Ethical Guidelines for Biomedical Research Involving Human Subjects. Geneva, Switzerland.
15. Combs GF. The vitamins: Fundamental aspects in nutrition and disease. Academic Press Inc. San Diego, California, 1992.
16. Plachta H, Bartkowska E, Obara A. Lipid peroxides in blood from patients with atherosclerosis of coronary and peripheral arteries. Clin Chim Acta 211(1992) 101-102.
17. Mahboob M, Rahman MF, Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore Med J 46 (2005) 322-324.
18. Sudha K, Rao AV, Rao A. Oxidative stress and antioxidants in epilepsy. Clin Chim Acta 303 (2001) 19-24.
19. Sudha K, Rao AV, Rao S, Rao A. Free radical toxicity and antioxidants in Parkinson's disease. Neurol India 51(2003) 60-61.
20. Barber AA, Berheim F. Lipid peroxidation its measurement occurrence and significance in animal tissues. Adv Gastroenterol Res 2 (1967) 355-403.
21. Halliwell B, Gutteridge JMC. The antioxidants of human extracellular fluids. Arch Biochim Biophys 280 (1990) 1-8.
22. Du WD, Yuan ZR, Sun J, Tang JX, Cheng AQ, Shen DM, Song XH, Yu XF, Zheng SB. Therapeutic efficacy of high dose vitamin C on acute pancreatitis and its potential mechanisms. World J Gastroenterol 11 (2003) 2565-2569.
23. Braganza JM, Scott P, Bilton G, Schofield D, Chaloner C, Shiel N, Hunt LP, Bottiglieri T. Evidence for early oxidative stress in acute pancreatitis. Clues for correction. Int J Pancreatol 17 (1995) 69-81.
24. Wereszczynska S, Dabrowski A, Jedynak M, Gabryelewicz A. Oxidative stress as an early prognostic factor in acute pancreatitis (AP): its correlation with serum phospholipase A2 (pla2) and plasma polymorphonuclear elastase (PMN-E) in different severity forms of human AP. Pancreas 17(1998) 163-168.
25. Kulkarni AG, Suryakar AN, Sardeshmukh AS, Rathi DB. Studies on biochemical changes with special reference to oxidant and antioxidants in malaria patients. Indian J Clin Biochem 18(2003) 136-149.
26. Rath RN, Panigrahi N, Das BK, Das BK. Lipid peroxidation in acute falciparum malaria. Ind J Med Res 93 (1991) 303-305.
27. Nanda R, Mishra PK, Das UK, Rout SB, Mohapatra PC, Panda A. Evaluating role of oxidative stress in determining the pathogenesis of falciparum malaria induced acute renal failure. Indian J Clin Biochem 19(2004) 93-96.
28. Egwunyenga AO, Isamah G, Nmorsi PO. Lipid peroxidation and ascorbic acid levels in Nigeria children with acute falciparum malaria. African J Biotech 3 (2004) 560-563.
29. Sitprija V. Nephrology forum: Nephropathy in falciparum malaria. Kidney Int 34 (1988) 867-877.
30. Sharma AK, Arora M, Gupta R, Makkad RK, Gupta HP. Free radicals in acute renal failure due to falciparum malaria. Indian J Nephrol 8(1998) 101-103.
31. Barsoum RS. Malarial acute renal failure. J Am Soc Nephrol 11(2000) 2147- 2151.
32. Mohan K, Dubey ML, Ganguly NK, Mahajan RC. Plasmodium falciparum- induced perturbations of erythrocyte antioxidant system. Clin Chim Acta 209 (1992) 19-26.

Author Information

Ibrahim Hassan Garba, Ph.D.
Chemistry Programme, School of Science, Abubakar Tafawa Balewa University

Ubom Gregory Abraham, Ph.D.
Department of Biochemistry, Faculty of Medical Sciences, University of Jos

Umar Shuaibu, B.Tech.
Chemistry Programme, School of Science, Abubakar Tafawa Balewa University