

# Analysis of serum and supplemented vitamin C and oxidative stress in HIV-infected children and adolescents

Karoline Faria de Oliveira,<sup>1</sup> Daniel Ferreira da Cunha,<sup>2</sup> Virginia Resende Silva Weffort<sup>3</sup>

## Abstract

**Objectives:** To assess adequacy of vitamin C intake in HIV-infected children and adolescents; to evaluate serum levels of vitamin C and indicators of oxidative stress; to compare with the uninfected group; to correlate serum vitamin C with oxidative stress and associate them according to the reference values.

**Methods:** Comparative cross-sectional study. Two groups of 27 children and adolescents each, aged between 3 to 19 years. Group 1 (G1) comprised individuals vertically infected with HIV seen at a regional outpatient clinic. Group 2 (G2) comprised invited individuals without history of HIV infection. The groups were matched for age, sex, and socioeconomic status. The following variables were analyzed: body mass index for age; micronutrient intake and consumption; and serum vitamin C, C-reactive protein (CRP), and albumin.

**Results:** The mean age was 12 years old. Most subjects were female (17, 63%), and there was prevalence of the economic class C (27, 50%). The most prevalent nutritional status was normal weight in 20 individuals (74.1%) in G1 and 21 (77.8%) in G2. The intake of vitamin C was significantly higher in G1 ( $p = 0.006$ ;  $t = 2.987$ ) according to the 24-hour dietary recall method. There were significant differences in serum vitamin C concentration between the groups, with a lower level in G1 ( $p = 0.000$ ;  $t = -7.309$ ). In relation to oxidative stress, values of CRP in G1 were significantly higher ( $p = 0.007$ ;  $t = 2.958$ ). There was no association between deficiency of vitamin, CRP, and albumin.

**Conclusion:** Our findings show that HIV-infected individuals have low levels of vitamin C; however, this deficiency is not related to eating habits, since the intake of this nutrient was higher in this group than in the control group. HIV-infected individuals have specific characteristics that increase their oxidative stress, which is evidenced by increased CRP.

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## Introduction

Infection with viruses, such as human immunodeficiency virus (HIV), causes persistent chronic inflammation. Its protein produces a progressive intracellular increase of the oxygen-reactive species, which influences the rate of apoptosis, decreases CD4+ lymphocyte cells and, consequently, increases HIV replication secondary to overproduction of free radicals. Free radicals are produced in normal physiological processes, but their synthesis becomes significantly higher in activated phagocytic cells, as part of their microbicidal response.<sup>1</sup> After cell

invasion, the parasitic phase begins with the use of the synthetic function of the host cell for virus replication. This actually interferes with normal physiological chemistry of the endoplasmic reticulum and cell mitochondria. Such interference is responsible for the generation of oxygen-reactive species and depletion of antioxidants.<sup>2</sup>

Thus, the activation of the immune system can produce significant local or systemic oxidative stress. The oxidative damage resulting from this stress depends on the protection provided by antioxidant systems at the site of inflammation

1. NP. Mestre, Atenção à Saúde, Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, MG, Brazil. Professora assistente, Departamento de Enfermagem na Assistência Hospitalar, UFTM, Uberaba, MG, Brazil.
2. MD, PhD. Professor titular, Departamento de Clínica Médica, UFTM, Uberaba, MG, Brazil.
3. MD, PhD. Pediatra nutróloga. Professora adjunta, Departamento Materno Infantil, UFTM, Uberaba, MG, Brazil.

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or in the organism as a whole.<sup>1</sup> The excessive progress of oxidation and subsequent increase in lipid peroxidation play a critical role in stimulating HIV replication and immunodeficiency. Such damage can be avoided or reduced if the antioxidant defense system is normal.<sup>3</sup>

Vitamin C (ascorbic acid) promotes immunity and, at high levels, it is considered to be a pro-oxidant agent.<sup>4</sup> Vitamin C is a synergistic agent of tocopherol, acting together in the antioxidant process,<sup>5</sup> and it is also considered to be a micronutrient necessary to maintain the physiological functions and integrity of the organism.<sup>6</sup> Therefore, as the body is unable to synthesize Vitamin C, it needs continuous and regular exogenous supply established by the Dietary Reference Intakes and the regulatory agencies.<sup>7</sup>

The absence or deficiency of this nutrient in the diet and their high metabolic need due to the peculiarities of these individuals may affect their treatment and therefore expose them to situations that impair their health, worsening these individuals' prognosis and quality of life.

The objectives of the present study was to assess the adequacy of consumption of vitamin C in HIV-infected children and adolescents; to evaluate serum levels of vitamin C and indicators of oxidative stress; to compare these individuals with the uninfected group, correlating serum vitamin levels with oxidative stress and associating them based on reference values.

## Methods

It is analytical field study, sectional, cross-sectional comparative, quantitative approach, developed at the Universidade Federal de Minas Gerais (UFTM), Uberaba, State of Minas Gerais, Brazil, and data collection performed at the Pediatric Clinic of UFTM.

This institution was chosen because it is a reference macro-region, located in South Triangle of the state of Minas Gerais. It serves 27 municipalities (with an estimated population of 698,785 inhabitants in the year 2009).<sup>8</sup>

Our sample comprised children and adolescents with vertically transmitted HIV infection who were followed up at the Outpatient Clinic of Pediatric Infectious Diseases (DIPP) between January 1993 and December 2008. Participants did not have any other chronic disease, and their guardians agreed with their participation in the study. Participants were receiving highly active antiretroviral therapy (HAART) for more than 1 year, with the association between drug classes, nucleoside analog reverse-transcriptase inhibitors, non-nucleoside analog reverse-transcriptase inhibitors, and/or protease inhibitor.

The control group was matched for sex, age, and socioeconomic status with students of the municipal school Adolfo Bezerra de Menezes. They were invited to participate in the study after being randomly chosen using the Statistical

Package for the Social Sciences (SPSS). Their guardians signed an informed consent form. The inclusion criteria were: no history of chronic or acute infection at the time of the approach and being HIV-negative during antenatal care reported by the guardians. The guardians also participated in the study by providing additional information related to the questions of the socioeconomic questionnaire and the assessment of dietary intakes.

Our initial survey identified a population of 31 children and adolescents aged between 3 and 19 years. These individuals were HIV-positive who had been infected by vertical transmission, and who had previous symptoms of the acquired immunodeficiency syndrome (AIDS). Upon investigation, they did not present with acute infection, were being followed up in the outpatient clinic of the DIPP at the UFTM and were on antiretroviral therapy.

We conducted an active search of four patients who missed the last follow-up visits. Of these, two moved to another city and hence also changed the reference outpatient clinic; and two refused to participate. Our results represent 87.1% (27) of the population being investigated.

We calculated the power targeted for a sample of 27 subjects, considering a significance level of 0.05 and the mean differences for vitamin C, and we reached a power of 100% within the limits of machine precision. Conversely, considering the difference between the groups, we would reach a power of 85%, with a sample size of 10 individuals. In clinical and practical terms, the groups were really different. The effect size was 1.41.

The present investigation was initiated after project approval by the Research Ethics Committee of UFTM under protocol no. 1660/2010.

The Brazilian Economic Classification Criterion was used to assess the subjects' socioeconomic status. This guideline can evaluate the purchasing power of families and the educational level of the household head, classifying them into economic classes based on the points earned: A1 (42 to 46); A2 (35 to 41); B1 (29 to 34); B2 (23 to 28); C1 (18 to 22); C2 (14 to 17); D (8 to 13); and E (0 to 7).<sup>9</sup>

In order to perform the anthropometric assessment, we used the techniques and cutoff points recommended by the Manual of Anthropometry of the Scientific Department of Nutrition, Brazilian Society of Pediatrics,<sup>10</sup> according to the recommendations of the World Health Organization (WHO). The individuals were classified according to the indexes used for diagnosis, such as body mass index for age (BMI/A), according to the WHO reference value adopted by the Ministry of Health (2006/2007).<sup>10</sup>

Research on food intake was performed using a specific questionnaire administered to the guardians and the child or adolescent. They could provide additional information because they recalled their own eating habits in a more

detailed manner. The 24-hour dietary recall method (R24) and the semiquantitative food frequency questionnaire (SFFQ) were applied to evaluate the standard of usual food intake and were analyzed using the computer program Avanutri V. 3.1.1.

Laboratory tests were performed to measure albumin, C-reactive protein (CRP), and vitamin C collected at the laboratory of Hospital de Clínicas of the UFTM.

Blood samples were collected by qualified professionals from the laboratory of the hospital according to the routine of the outpatient clinic. The vitamin C concentration was calculated by the researcher after receiving specific training. The analyses were performed according to the methodology proposed by Arnauld et al. (1991).<sup>11</sup>

The reference values for the concentrations were according to the recommendation of the Brazilian Society of Pediatrics (2009).<sup>10</sup>

Data were stored in Excel® spreadsheets and later entered into the SPSS. The Kolmogorov-Smirnov normality test, Pearson's correlation test, and Student's paired *t* test were performed considering a significance level of 95%.

## Results

The patient group (G1) and the control group (G2) were matched for sex and age, with the minimum difference of 0 years and maximum of 2 years among the paired individuals. There was not significant difference between groups using Student's *t* test ( $p = 0.31$ ;  $t = 1.03$ ). Children's mean age

was 12 years as provided in Table 1. The female population (17-63%) was larger than the male population (10-37%) in the present study.

When comparing the socioeconomic score of G1 and G2 with the paired Student *t* test, we found no significant difference between the groups ( $t = -0.424$ ;  $p = 0.675$ ) (Table 1). The frequency distribution of economic classes in our study indicates a larger number of individuals included in the economic class C in both groups, 44.4% in G1 and 55.6% in G2.

When comparing the groups in terms of BMI using the paired Student's *t* test, we found no significant difference ( $t = -0.671$ ,  $p = 0.508$ ) (Table 1). The classification of groups was performed with the aim of identifying risk factors for obesity and thinness. The BMI parameters were evaluated in combination with the clinical assessment, and thus the classification was closer to reality.

Based on their BMI and age, individuals were classified according to the WHO recommendations (2006/2007). Most individuals (20 and 21) were classified as having healthy weight in both groups (74.1 and 77.8%). We found more cases of overweight and risk of overweight in G2. However, G1 had one case of obesity.

Thinness was higher in G1 (3 to 11.1%) and is in agreement with the classification of low height for age found in two cases (7.4%).

Among the treatment drugs used, we found associations between nucleoside analog reverse-transcriptase inhibitor (NARTI) and non-nucleoside analog reverse-transcriptase

**Table 1 -** Characterization of HIV-infected individuals and control group according to age, economic status, BMI, mean serum CRP, albumin, ascorbic acid, CD4, CD8 and CD4/CD8 in the infected group

	G1 (n = 27)	G2 (n = 27)	t test
	Mean ± standard deviation	Mean ± standard deviation	
Age (years)	12.59±3.43	12.37±3.91	t = 1.03; p = 0.312
Socioeconomic classification (score)	13.52±4.17	13.96±4.05	t = 0.424; p = 0.675
BMI (kg/m <sup>2</sup> )	18.16±2.77	18.63±2.74	t = -0.671; p = 0.508
CRP (mg/L)	3.96±5.36	0.9±0.85	t = 2.958; p = 0.007*
Albumin (g/dL)	4.62±0.36	4.72±0.21	t = -1.205; p = 0.239
Ascorbic acid (mg/dL)	0.24±0.15	0.55±0.19	t = -7.309; p = 0.000*
CD4	782.85±650.12	-	-
CD8	1556.59±2022.76	-	-
CD4/CD8	2.57±9.94	-	-

BMI = body mass index; CRP = C-reactive protein; HIV = human immunodeficiency virus.

\*  $p \leq 0.05$ .

inhibitor (NNRTI), or simple or combined association of these drugs with the protease inhibitor (PI). The frequency of therapies associated with PI in children and adolescents was suggested to be 44.44% in G1.

According to the R24 method, consumption of vitamin C was higher in G1, being significant ( $p = 0.0061$ ,  $t = 2.987$ ) when using the paired Student's  $t$  test. Based on the SFFQ method, consumption of vitamin C did not reach a significant difference using the same statistical test ( $p = 0.21$ ;  $t = 1.286$ ) (Table 2).

Regarding serum micronutrient deficiency, values below 0.4 mg/dL were considered deficient. We also found deficiency in 23 (85.19%) individuals of G1 and five (18.52%) participants of G2. When we compared the means of the groups, we found an extremely significant difference between the groups ( $p = 0.000$ ;  $t = -7.309$ ). The relative risk related to vitamin C deficiency was 4.6, demonstrating that the patient group has a strong risk factor for depletion of this micronutrient (Table 3).

The amount of albumin found in both groups was within the reference values, thus there was no deficiency of this protein. However, CRP was altered as shown in Table 3.

When we analyzed the serum values of these proteins, there was a significant difference between the serum levels of CRP using the paired Student's  $t$  test. Serum CRP was higher in G1 compared with G2 ( $p = 0.007$ ;  $t = 2.958$ ). We found a strong risk factor (7.5) related to the increase in CRP in G1. In relation to albumin, the difference between the means of the groups was not significant ( $p = 0.239$ ,  $t = -1.205$ ), although G2 had a higher mean value (Table 1).

No correlation was found between serum levels of vitamin C and albumin and CRP values by Pearson's test. We could not find a significant difference between the sexes in relation to CRP, albumin, and ascorbic acid, as shown in Table 4.

## Discussion

The mean age found in our study is in agreement with that found in similar studies conducted in São Paulo, Brazil: 11.9 years.<sup>12</sup> All participants of G1 were receiving HAART.

**Table 2** - Mean values regarding the proportion of daily intake of vitamin C (mg) for children and adolescents infected with HIV or not and according to the R24 and SFFQ

	G1 (n = 27)	G2 (n = 27)	t test
R24	1.16±1.46	0.52±0.75	$p = 0.006$ ; $t = 2.987$
SFFQ	8.14±14.58	3.98±6.28	$p = 0.21$ ; $t = 1.286$

R24 = 24-hour dietary recall;

SFFQ = semiquantitative food frequency questionnaire.

**Table 3** - Percentage of children and adolescents infected (G1) or not (G2) with HIV showing normal or altered ascorbic acid and CRP

	G1 (n = 27)		G2 (n = 27)	
	n	%	n	%
Ascorbic acid (C)				
RR = 4.6				
Low	23	85.2	5	18.5
Normal	4	14.8	22	81.5
CRP				
RR = 7.5				
Increased	15	55.6	2	7.4
Normal	12	44.4	25	92.6

CRP = C-reactive protein; HIV = human immunodeficiency virus;  
RR = relative risk.

The emergence of the antiretroviral therapy provides a higher survival rate to schoolchildren; this explains the larger the number of patients seen in health care facilities.<sup>13</sup> However, the use of this therapy in the population can be considered a limitation of our study, since the use of HAART may be associated with increased markers of oxidation.<sup>1</sup>

According to the Brazilian Association of Survey Companies (Associação Nacional de Empresas de Pesquisa), the average wage of the population being studied is R\$ 927.00.<sup>14</sup> Another study involving children and adolescents from São Paulo demonstrated an average monthly income of parents or caregivers of R\$ 436.70.<sup>12</sup>

A higher frequency of economic class C in 40% of the families of HIV-infected children was found by Balbus et al., followed by classes D and E.<sup>15</sup> Caregivers providing care to HIV-infected patients need to have deeper knowledge and better preparation. The low socioeconomic status, which means lower educational level of the head of household, can lead to problems of therapy adherence or lack of precautionary measures related to new health problems.

The fact that we found a better economic condition in our study may this population to better results regarding eating habits, fewer episodes of acute infections, better adherence to the prescribed therapies, and adoption of health promotion and disease prevention strategies, improving the prognosis of these individuals.

The prevalence of normal weight has been found in studies with HIV-positive children and adolescents conducted in São Paulo and Rio de Janeiro, Brazil. In the study of São Paulo, the authors found 103 (86.6%) individuals with normal weight, seven (5.9%) at risk of overweight, and three (2.5%) obese subjects.<sup>12</sup> It is worth mentioning that the classification criterion used in this study was the National Center of Health Statistics (NCHS) 2000, and this may be a factor that underestimates the cases of overweight and obesity.

**Table 4 -** Mean serum values and standard deviation of CRP, albumin, and ascorbic acid in HIV-infected children and adolescents and control group according to sex

	G1 (n = 27)			G2 (n = 27)		
	Mean ± standard deviation		t test	Mean ± standard deviation		t test
	Male	Female		Male	Female	
CRP (mg/L)	4.27±4.52	3.78±5.93	t = 0.22; p = 0.83	0.71±0.7	1.01±0.94	t = -0.88; p = 0.38
Albumin (g/dL)	4.57±0.33	4.66±0.37	t = -0.59; p = 0.56	4.82±0.24	4.66±0.18	t = 1.98; p = 0.06
Ascorbic acid (mg/dL)	0.22±0.14	0.25±0.15	t = -0.58; p = 0.57	0.48±0.14	0.6±0.21	t = -1.59; p = 0.13

CRP = C-reactive protein; HIV = human immunodeficiency virus.

\* p ≤ 0.05.

A study conducted with 43 HIV-infected children and adolescents at the Outpatient Clinic of Infectious Diseases in Pediatrics of the Instituto Fernandes Figueira, Fundação Oswaldo Cruz, found similar results for normal weight, with 35 (81.3%) individuals having healthy weight.<sup>16</sup>

The choice of two methods for the analysis of food intake was intended to enable the investigation of sporadic and frequent eating habits.

Investigating the frequency with which food groups are consumed helps to better identify eating habits. The use of a questionnaire that quantifies and qualifies the eating frequency of individuals has been validated in several epidemiological studies and is considered the most appropriate method for evaluating eating habits.<sup>17</sup>

A study involving HIV-exposed Indian children with low socioeconomic status showed means of vitamin A, C and E significantly lower than those of unexposed children (p < 0.01; p < 0.001; p < 0.02; respectively), and vitamin C deficiency was extremely significant,<sup>18</sup> similarly to what occurred in our study.

Our results corroborate what was found in a study of 241 HIV-infected young adults, which demonstrated an extremely significant deficiency of vitamin C in a comparison with 115 people without the disease. The authors considered that this deficiency was related to oxidative stress, causing high consumption of ascorbic acid due to compensatory mechanisms.<sup>19</sup>

Supplementation of vitamin C strengthens the antioxidant defense system,<sup>20</sup> regardless of whether this supplementation is provided by means of food or drugs. This micronutrient deficiency affects children's physical and mental growth in a negative manner and it may compromise the immune defense<sup>21</sup>; therefore, its replacement is essential.

A study conducted with 68 children (with 34 of them being HIV-infected) assessed the antioxidant capacity of the saliva of these individuals, suggesting decreased total antioxidant capacity in HIV-infected children.<sup>22</sup>

Oxidative stress has specific markers, such as CRP and albumin, which increase or decrease, respectively, as the individual is exposed to stress.<sup>10</sup>

The normal serum albumin values found in the study may be related to an adequate intake of macronutrients considering the nutritional requirements of these individuals. The optimal plasma albumin concentrations are in agreement with a study of 1,240 children exposed to the virus (with 124 infected individuals), who had normal serum albumin levels<sup>23</sup>; as well as a study of 38 children aged 2 to 11 years (10 HIV-infected individuals with delayed growth, 18 HIV-infected with normal growth, and 10 non-infected individuals with normal growth).<sup>24</sup>

Regarding CRP, a study conducted at the Hospital das Clínicas de Ribeirão Preto, state of São Paulo, Brazil, with 41 hospitalized adults with HIV showed that 68.3% had increased CRP levels, with a median of 2.61 mg/dL.<sup>25</sup>

HIV infection maintains serum CRP levels higher compared to the recommended values.<sup>26</sup> CRP levels higher than 0.23 mg/dL in HIV-patients increase in 37% these individuals' risk of having AIDS symptoms.<sup>27</sup> In addition, values greater than 1 mg/dL increase the risk of death by 2.26 times in women.<sup>28</sup>

The results demonstrate ascorbic acid deficiency significantly higher in the deficient group using the R24 method regardless of consumption. In addition, this group has indicator of oxidative stress, CRP, at significantly increased levels, suggesting metabolic changes.

Therefore, financial resources should be invested in the training of the multidisciplinary team, as well as specific

nutritional questionnaires, body composition and specific laboratory tests, and implementation of educational strategies, both for patients and families and for health professionals should be included in the follow-up routine so that these changes attributed to HIV infection, opportunistic infections or toxicity of therapeutic agents<sup>29</sup> do not impair the quality of life of these individuals.

## Conclusion

Our findings show that HIV-infected individuals have low levels of vitamin C, regardless of their eating habits, since the consumption of this micronutrient was higher than that of the control group, which did not present with this depletion. There are peculiarities in the infected individuals, which increase their index of oxidative stress, evidenced by the increase in CRP.

The present study enabled us to identify the serum levels of vitamin C in children and adolescents with HIV, and a significant deficiency of this vitamin was detected. Children and adolescents have high consumption of nutrients related to growth and development. Infection with a virus such as the HIV and its immunosuppressive effect require greater nutrient demand as a result of frequent infectious episodes. Vitamin C is essential for these individuals because it acts as pro-oxidant agent. There is a need for specific eating planning or supplementation according to each case, keeping the quantities of vitamin C at optimal values for improved immune function and antioxidant activity of these children and adolescents.

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## Correspondence:

Virginia Resende Silva Weffort  
 Disciplina de Pediatria da Universidade Federal do Triângulo Mineiro  
 Av. Getúlio Guaritá, 230  
 CEP 38025-440 - Uberaba, MG - Brazil  
 Tel.: +55 (34) 3318.5244  
 E-mail: weffort@mednet.com.br