Serum concentrations of vitamin A and oxidative stress in critically ill patients with sepsis

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Abstract

Introduction: Sepsis is one of the main causes of mortality in patients in Intensive Care Units. As a result of the systemic inflammatory response and of the decrease of the aerobic metabolism in sepsis, the oxidative stress occurs. Vitamin A is recognized by the favorable effect that it exerts on the immune response to infections and antioxidant action.

Objective: To bring new elements for reviewing of the nutritional support addressed to critically ill patients with sepsis, with emphasis to vitamin A.

Methods: Critically ill patients with sepsis had circulating concentrations of retinol, β-carotene, thiobarbituric acid-reactive substances (TBARS) and C-reactive protein (CRP) measured in Medicosurgical Intensive Care Unit in the city of Rio de Janeiro, Brazil. The patients were divided into two groups: patients who were receiving nutritional support and those without support. At the act of the patient’s admission, APACHE II score was calculated.

Results: 46 patients were studied (with diet n = 24 and without diet n = 22). Reduced levels of retinol and β-carotene were found in 65.2% and 73.9% of the patients, respectively. Among the patients who presented lower concentrations of CRP it was found higher β-carotene inadequacy (64.8%) and 50% of retinol inadequacy. There was no significant difference as regards retinol, TBARS and APACHE II levels among the patients with and without nutritional support. However, higher levels of CRP (p = 0.001) and lower levels of serum β-carotene (p = 0.047) were found in patients without nutritional support.

Conclusions: Sepsic patients presented an important inadequacy of retinol and β-carotene. The present study bring elements to the elaboration/review of the nutritional protocol directed to the group studied, especially as regards vitamin A intake.


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Concentraciones séricas de vitamina A y estrés oxidativo en pacientes críticos con sepsis

Resumen

Introducción: La sepsis es una de las principales causas de mortalidad en pacientes en las Unidades de Cuidados Intensivos. Como consecuencia de la respuesta inflamatoria sistémica y de la disminución del metabolismo aeróbico en la sepsis se produce estrés oxidativo. La vitamina A es reconocida por el efecto favorable que ejerce sobre la respuesta inmunitaria a las infecciones y por su acción antioxidante.

Objetivo: Aportar nuevos elementos a la hora de revisar el soporte nutricional de los pacientes críticos con sepsis, con un énfasis sobre la vitamina A.

Métodos: Se midieron las concentraciones circulantes de retinol, β-caroteno, ácido tiobarbitúrico-sustancias reactivas (ATBSR) y proteína C reactiva (PCR) de pacientes críticos con sepsis en la Unidad de Cuidados Intensivos Medicosurgírúgica de la ciudad de Río de Janeiro, Brasil. Se dividió a los pacientes en dos grupos: pacientes que recibían soporte nutricional y aquellos que no. Se calculó la puntuación APACHE en el momento de su ingreso.

Resultados: Se estudiaron 46 pacientes (con dieta n = 24 y sin dieta n = 22). Se hallaron concentraciones disminuidas de retinol y β-caroteno en el 65.2% y 73.9% de los pacientes, respectivamente. De entre los pacientes que presentaron menores concentraciones de PCR, se halló una mayor inadecuación de β-caroteno (64.8%) y un 50% de inadecuación de retinol. No hubo diferencias significativas con respecto al retinol, ATBSR y las puntuaciones APACHE II entre los pacientes con y sin soporte nutricional. Sin embargo, se hallaron mayores concentraciones de PCR (p = 0.001) y menores concentraciones séricas de β-caroteno (p = 0.047) en los pacientes sin soporte nutricional.

Conclusiones: Los pacientes sépticos presentaron una inadecuación importante de retinol y β-caroteno. El presente estudio aporta elementos a la elaboración/revisión del protocolo nutricional dirigido al grupo estudiado, especialmente con respecto de la toma de vitamina A.

Vitamin A and oxidative stress in sepsis

Introduction

The survival of critical patients depends on a complex and careful immune response through all organic systems. Generally, the immune response dysfunction is present in two different ways: excessive performance of the cellular immune response that is clinically manifested as Systemic Inflammatory Response Syndrome (SIRS) and reduction of the immune response, leading to a significant increase in susceptibility to infections that result in sepsis.1

Sepsis, which may be considered as SIRS face to an infectious stimulus, is one of the main causes of mortality in patients in Intensive Care Units (ICUs), despite improvements in supportive and antimicrobial therapies.2 It is characterized by multiple manifestations which can determine dysfunction or failure of one or more organs, or even death.3 Mortality rate for severe sepsis varies between 30-50% in critical patients,4 and it is the cause of around 2% of hospital admissions.5 Mean sepsis incidence is 50-95 cases for every 100,000 patients and has been rising around 9% every year.6

As a result of systemic inflammatory response and decrease in aerobic metabolism in sepsis, oxidative stress occurs in extracellular and in intracellular spaces.7 Oxidative stress has been implicated in human diseases by a growing body of scientific evidences;8 and it may be defined as the situation where an increase of the physiological levels of the reactive oxygen species (ROS) occurs, resulting either from the decrease of antioxidant defense levels or from high production of ROS.9 Oxidative stress is capable of causing lipid peroxidation of cellular membranes. Lipid peroxidation is a complex process, whereby polyunsaturated fatty acids of cellular membranes undergo reaction with oxygen to yield lipid hydroperoxides. The generation of products of lipid peroxidation after oxidative stress may be measured in the form of TBARS (thiobarbituric acid-reactive substances), where malondialdehyde stands out.10

Acute severe pathological conditions such as sepsis are associated with the increase of ROS production and other species of radicals with a consequent oxidative stress that will be able to exacerbate organic injury.11

Alterations in concentrations of some plasmatic proteins occur in critical patients such as C-reactive protein (CRP) increased production by the liver. CRP is one of the main proteins of the acute phase that has been utilized as a precocious and sensitive biomarker of the response to infectious or inflammatory processes, and it may increase from 19 to 100 times in the first 12 hours after aggression.9

The potential toxicity of ROS may be counteracted by antioxidants.12,13 Antioxidants may be defined as any substance that when present in low concentrations, compared to those of oxidable substrates, significantly postpones or inhibits the oxidation of these substrates.14 Antioxidant systems include glutathione, vitamins (A, C and E) and several enzymes.15 Previous studies have demonstrated that, in sepsis, an increase in the levels of oxidative stress and a decrease in the circulating concentration of antioxidant components of the defense system occur, including vitamins C, E, A and beta-carotene.16 Antioxidant concentrations seem to increase the quantity of the immune system cells when compared to other cells.17 Vitamin A stands out, since the favorable effect it exerts on immune response to infections is recognized.

Vitamin A participates in several primordial functions in human systems playing a role in visual acuity, cellular proliferation and differentiation, antioxidant action and immunological activity. The term vitamin A embodies the terminologies retinol and carotenoids, which are, respectively, the pre-formed vitamin and its precursors. Among these, β-carotene is recognized as the most potent retinol precursor.18 As much its biologically active form, as its provitamin forms, have gained prominence for their role against ROS, protecting the organism against oxidative stress and, consequently, preventing damages and tissue lesions.19 Retinol possesses antioxidant activity as it associates with peroxil radicals before these are able to propagate peroxidation to the cellular lipid component and to generate hydroperoxides. As regards carotenoids, they neutralize peroxil radicals and singlet oxygen.20 β-carotene has an antioxidant activity five times higher than retinol.21

In view of this context, the present study aimed to assess the serum concentrations of retinol, β-carotene and oxidative stress and their relationship to CRP in septic patients hospitalized in ICU as a means of bringing new elements to the review of the nutritional support addressed to this group, with emphasis on the intake of vitamin A.

Materials and methods

The present study was conducted in Medicosurgical Intensive Care Unit in the city of Rio de Janeiro, Brazil. The study was approved by the Research Ethics Committee of Hospital Universitário Clementino Fraga Filho/ Universidade Federal do Rio de Janeiro (under the n.174/05). Informed consent was obtained from the patients’ relatives.

All the adult patients hospitalized with sepsis diagnosis according to the International Sepsis Definitions Conference22 were included in the study from January through December 2006. Patients who had chronic renal insufficiency, liver cirrhosis, and pregnant women, as well patients in immediate postoperative period and with parenteral nutritional support, were excluded.

At the time of enrollment in the study, patients were divided into two groups at random: patients who were receiving enteral nutritional support and those without support. According to hospital routine, the patients with nutritional support received hypercaloric enteral diet (1.5 kcal/ml) and hyperproteic diet (18 ± 2% of protein/day), and medical supplement (containing 5,000 IU/day of vitamin A).
At the act of the patient’s admission, APACHE II score was calculated\(^9\) and the following biochemical assessments were conducted:

- **Vitamin A:** Plasma retinol and \(\beta\)-carotene were determined by high performance liquid chromatography (HPLC).\(^{20}\) Levels of serum retinol were presented by interval classes of 0.35 \(\mu\)mol/L (or 10 \(\mu\)g/dl) to allow its classification according to the recommendations of the World Health Organization.\(^{21}\) This enables to detect the groups with values of severe deficiency (< 0.35 \(\mu\)mol/L or < 10 \(\mu\)g/dl), moderate marginal (0.35 \(\mu\)mol/L ≤ 0.70 \(\mu\)mol/L or 10 \(\mu\)g/dl ≤ 20 \(\mu\)g/dl) and doubtful values (0.70 \(\mu\)mol/L ≤ 1.05 \(\mu\)mol/L or 20 \(\mu\)g/dl ≤ 30 \(\mu\)g/dl). The cutoff utilized to indicating inadequacy of serum values of \(\beta\)-carotene was \(\geq 40 \mu\)g/dl, as suggested by Sauberlich et al.\(^{22}\)

- **Oxidative Stress:** Thiobarbituric acid-reactive substances (TBARS) were measured by the method described by Ohkawa et al.\(^{23}\)

- **C-Reactive Protein (CRP):** It was assessed through the method of nephelometry.

### Statistical Analysis

Measures of central tendency and dispersion were calculated. Pearson and Spearman correlations were performed according to each variable behavior, adopting as strong correlation values higher than 0.6, and regular correlation from 0.3 to 0.6. Student “t” and Mann-Whitney tests were applied for comparison of continuous variables. To assessing association between categorical variables, Chi-square (C\(^2\)) was applied. The significance level of 5% of probability (P ≤ 0.05) was adopted. Statistical analysis was performed through the statistical program SPSS for Windows (version 13; SPSS INC., Chicago, IL, USA).

### Results

Forty-six individuals were studied. In the act of enrollment in this study, it was observed that 52.2% (\(n = 24\)) of the patients received enteral diet, whereas 47.8% (\(n = 22\)) were without nutritional support. The collection of the patients with nutritional support occurred between Day 2 and Day 4 after they received the diet and the supplement (the patients had received the 90 to 100% their nutritional needs and regarding the supplement of vitamin A, the dose was full from the entrance of the patient). Among those who were without nutritional support, the time ranged from 24 to 72 hours. There was no report of diarrhea in any of the patients enrolled.

The general characteristics are shown in table I.

As regards the serum concentrations of retinol, it was observed that 39.1% of the patients (\(n = 18\)) presented severe deficiency, 15.2% (\(n = 7\)) marginal deficiency, 10.9% (\(n = 5\)) doubtful values, and 34.8% (\(n = 16\)) presented normal concentrations (>1.05 \(\mu\)mol/L). As regards \(\beta\)-carotene, it was observed an inadequacy of 73.9% (\(n = 34\)).

It was observed that the inadequacy of retinol and \(\beta\)-carotene was more frequent (77% ; \(n = 23\) and 74%; \(n = 25\), respectively) in patients with higher circulating levels of TBARS (allocated in the 50\(^{th}\) and 75\(^{th}\) quartiles), however no statistical significance was presented (\(p = 0.58\) and \(p = 0.39\), respectively).

It was not found a relationship among the continuous variables studied, except as regards age and APACHE II score which were positive and significantly correlated (\(p = 0.009\) and \(r = 0.381\)).

Patients with adequate retinol presented high frequency of inadequate \(\beta\)-carotene (62.5%), even without statistical significance (\(p = 0.29\)).

Among the patients who presented lower concentrations of CRP (allocated in the 25\(^{th}\) quartile) it was found a higher \(\beta\)-carotene inadequacy (64.8%) and 50% of retinol inadequacy.

Mean offer of vitamin A was 8,622 ± 4,090 IU/day, corresponding to 288% of the value of the recommended daily dietary intake (DRI)\(^{11}\) (fig. 1).

Proportion of serum inadequacy of retinol in the groups with and without diet was 54% (\(n = 13\)) and 77% (\(n = 17\)), respectively, 62.5% (\(n = 15\)) of \(\beta\)-carotene in the group with diet, and 86% (\(n = 19\)) in the group without diet.

There was no significant difference between the groups with and without diet as regards the mean serum retinol concentrations, TBARS and APACHE II score, differently from what occurred as regards serum concentrations of \(\beta\)-carotene and of CRP (table II).

### Discussion

The present study conducted an assessment of the nutritional status of vitamin A and oxidative stress in individuals admitted in ICU with sepsis, aiming to contribute to the review of the nutritional support addressed to this group, with emphasis on the intake of vitamin A.
One of the factors which may lead to vitamin A deficiency (VAD) is the frequency of infectious episodes. Nowadays it is known that even subclinical VAD (when Xerophthalmia signs are absent) intensifies the severity of infirmities and of several infectious processes, and it may provoke immunodeficiency status of an exclusively nutritional origin\textsuperscript{21} added to a higher metabolic utilization of vitamin A against oxidative stress to which individuals with infectious processes are more exposed to.\textsuperscript{1}

In the group studied, besides the high prevalence of deficiency of retinol and $\beta$-carotene found, it was observed evidences of lipid peroxidation associated with high CRP, which jeopardizes the clinical condition of the septic patients assessed in a more intense way.

The hypercatabolic status derived from the septic status increases the demand of antioxidant vitamins as vitamin A. Low organic levels of this vitamin may contribute to sepsis aggravation, mainly when other conditions of risk are added as, for example, advanced age.\textsuperscript{2}

In the present study an association of APACHE II score was found with age, which could possibly be explained by the fact that the referred score attributes a higher score according to increase of age. Aging may be related to an infectious status aggravation by compromising organ functions, and by all consequences that normally accompany this process.\textsuperscript{24}

In the present study, it was observed high mean values of CRP compatible with the inflammatory systemic response and existence of infectious process. This finding corroborates others presented by Andriolo et al\textsuperscript{9} who observed similar levels of this marker (146.1 mg/L) in the serum of septic patients who evolved to death. The same was pointed out by Castelli et al\textsuperscript{25} who assessed the serum concentrations of CRP in patients with SIRS, sepsis and trauma, finding a mean of 150 mg/L of CRP in the group of septic patients.

A statistical difference ($p = 0.001$) was observed when the mean serum concentrations of CRP of the groups with and without diet were compared. Concentrations of CRP significantly higher in the group without diet may be related to the very absence of nutritional support whose impact would contribute to the increase of the catabolic demand.

Among the patients who presented lower concentrations of CRP, higher inadequacy of $\beta$-carotene was

### Table II

<table>
<thead>
<tr>
<th>Retinol (mmol/l)</th>
<th>$\beta$-carotene ($\mu$g/dl)</th>
<th>TBARS (mmol/ml)</th>
<th>CRP (mg/l)</th>
<th>APACHE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional support</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>n</td>
<td>24</td>
<td>22</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Mean</td>
<td>1.03</td>
<td>0.85</td>
<td>44.98</td>
<td>30.27</td>
</tr>
<tr>
<td>SD</td>
<td>1.02</td>
<td>1.15</td>
<td>47.04</td>
<td>28.89</td>
</tr>
<tr>
<td>p</td>
<td>0.33</td>
<td>0.047*</td>
<td>0.24</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

SD: standard deviation; CRP: C-reactive protein; TBARS: reactive substances to thiobarbituric acid; APACHE II: Acute Physiology and Chronic Health Evaluation.

* $p < 0.05$ considered statistically significant.
found, and half of them presented retinol inadequacy. Data of the National Health and Nutrition Examination Survey in the USA (NHANES III) showed that individuals with values of CRP higher than 10 mg/L presented risk of VAD up to 8.6 times more than individuals with normal inflammatory activity. VAD has already being described as a risk factor for mortality in a large number of infectious diseases. The most consensual justification is that the presence of infections would increase the organic utilization of vitamin A, thus causing firstly a decrease in the serum levels of retinol, which would promote depletion of the liver stores of this micronutrient. On the other hand, VAD associates with damage in the immunological response creating more susceptibility to infections, therefore generating the cycle “infection, deficiency, infection”. Such statements may in part explain the increase of severity and mortality in the presence of the status of deficiency of vitamin A.

It is important to point out the findings related to urinary retinol loss associated with infectious processes. Stephensen et al conducted a study with 29 patients in ICU who presented pneumonia or sepsis, and they found an increase in the urinary excretion of retinol in the group studied when compared to healthy individuals. The patients presented an APACHE II score of 18 ± 7 in the first 24 hours of admission to the ICU (a finding similar to the values found in the present study: 16.13 ± 4.68). It was observed that the most severe patients (score > 20) excreted 10 times more retinol than those who were the less severe ones (p < 0.0022). Neves et al observed a strong correlation between low serum levels of retinol and higher urinary loss of this nutrient in individuals with infection. Such findings must be taken into account in the understanding of the physiopathological mechanisms related to the development of VAD in patients with infection, mainly during severe infectious processes.

Goode et al studied 16 patients interned in an ICU with a diagnosis of septic shock and secondary organ dysfunction and they found evidences of oxidative stress, as well reduced values of vitamins A and E, and carotenoids (lycopene and β-carotene). Mean serum retinol found was 26.5 μg/dL, mean APACHE II score was 16.6, and the concentrations of TBARS showed a strong negative correlation with those of serum retinol (p < 0.01). In the present study, mean retinol was 0.95 μmol/L (27.14 μg/dl) and mean APACHE II was 16.13, results similar to the study previously cited; however it was not found an association between serum levels of TBARS and blood retinol. It was observed that retinol and β-carotene inadequacy was more frequent in patients with higher circulating levels of TBARS (allocated in the 50th and 75th quartiles), nonetheless, no statistical significance was found. All patients studied by Goode et al presented β-carotene inadequacy. Such findings are in agreement with the high serum β-carotene inadequacy observed in the present study.

In the present study, it was not found a statistical difference as regards serum levels of retinol, TBARS and APACHE II score in the groups with and without diet. Up to the present moment, literature does not present results similar to those of the present study, which would enable comparisons with these findings.

As regards β-carotene, it was observed serum concentrations significantly lower in the group without diet. β-carotene is considered provitaminic forms on account of their capacity of bioconversion to retinol, which is the active form of vitamin A. They are formed by an extensive conjugated system of double binds, and are five times more efficient than retinoids as regards protection against oxidative stress. According to Mecocci et al, the adequate nutritional status of vitamin A reduces the conversion of carotenoids into retinol, hence demonstrating that there is a relationship between the nutritional status of retinol and carotenoids. In the present study, besides a reduction of β-carotene in the group without diet, it was verified that individuals with adequate serum concentrations of retinol presented a higher frequency of inadequacy of β-carotene. Such factors could be explained by the mobilization of β-carotene as antioxidants in the fight against oxidative stress (which was higher in the group without diet), and by bioconversion in order to maintain serum retinol concentrations that, despite the inadequate concentrations for the assemblage of patients, did not present statistically significant difference between patients with and without nutritional support. These findings demonstrated that critical patients with sepsis, included in the present study and who did not receive the diet, would be prevented from the antioxidant power of β-carotene taking into account the lower mean serum concentrations found in this subgroup.

Dietary Reference Intake (DRI) of vitamin A for adults is 900 μg/day (males) and 700 μg/day (females) and the UL (Tolerable Upper Intake Level) is 3,000 μg/day.

In the present study, the mean vitamin A administered to patients was 8,622 IU, corresponding approximately to three times the recommended intake for adults; therefore suggesting that the dose of vitamin A routinely offered to these patients was not able to meet the demand of this group, due to the high frequency of serum inadequacy of retinol and β-carotene found in the study.

Conclusion

Septic patients presented an important inadequacy of retinol and β-carotene. The results found in the present study brought elements to the elaboration/review of the nutritional protocol addressed to the group studied, especially as regards the intake of vitamin A as a means of improving the prognosis and evolution of these patients.
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