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Effects of supplementation of antioxidant vitamins and lipid peroxidation in critically ill patients

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Abstract

Introduction: **Critical patients present systemic inflammatory process that can be followed by decrease in plasma concentrations of antioxidant vitamins.**

Objetive: **The aim of this study was to evaluate the effect of the supplementation of antioxidant vitamins in critical patients and their relation with lipid peroxidation.**

Methods: **23 patients went on a standard diet (G1) and 11 went on a diet with daily supplementation of 10,000 IU of vitamin A, 400 mg of vitamin E and 600 mg of vitamin C (G2). The APACHE II score was made. Serum concentrations of retinol, β-carotene, vitamins C and E, malondialdehyde (MDA) and C-reactive protein was measured before (T0) and on the 8th day after the beginning of the nutritional therapy (T1). The groups had been monitored on T0, T1 and T2, (at discharges or death) on the following parameters: mechanical ventilation; hospitalization days; mortality; infection incidence.**

Results: **Serum concentrations of MDA and vitamin E were significantly lower in G2 after intervention and strong tendency to increase vitamin C. There were not significant differences between the groups regarding the clinical parameters.**

Conclusions: **The doses of vitamin A, C and E that were indicated were effective for the current lipid peroxidation reduction.**

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Key words: *Vitamin antioxidant. Malondialdehyde. Critical patient. Lipid peroxidation.*

EFECTOS DE LA SUPLEMENTACIÓN CON VITAMINAS ANTIOXIDANTES Y LA PEROXIDACIÓN LIPÍDICA EN PACIENTES CRÍTICOS

Resumen

Introducción: **Los pacientes críticos presentan un proceso inflamatorio sistémico que puede seguirse por un descenso en las concentraciones plasmáticas de vitaminas antioxidantes.**

Objetivo: **El objetivo de este estudio fue evaluar el efecto de la suplementación de vitaminas antioxidantes en los pacientes críticos y su relación con la peroxidación lipídica.**

Métodos: **23 pacientes realizaron una dieta estándar (G1) y 11 una dieta con suplementación diaria de 10.000 UI de vitamina A, 400 mg of vitamina E y 600 mg de vitamina C (G2). Se realizó la puntuación APACHE II. Las concentraciones séricas de retinol, alfa-caroteno, vitaminas C y E, malondialdehído (MDA) y proteína C reactiva se midieron antes (T0) y al octavo día de comenzar con la terapia nutricional (T1). Se monitorizaron los siguientes parámetros en los grupos en T0, T1 y T2, (en el momento del alta o del fallecimiento): ventilación mecánica; días de hospitalización; mortalidad; incidencia de infección.**

Resultados: **Las concentraciones séricas de MDA y vitamina E fueron significativamente menores en el grupo G2 tras la intervención con una fuerte tendencia a aumentar la vitamina C. No hubo diferencias significativas entre los grupos con respecto a los parámetros clínicos.**

Conclusiones: **Las dosis de vitaminas A, C y E que se indicaron fueron eficaces en la reducción actual de la peroxidación lipídica.**

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Palabras clave: *Antioxidante vitamina. Malondialdehído. Paciente crítico. Peroxidación lipídica.*

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Abbreviations

APACHE II: Acute Physiology and Chronic Health Evaluation.
CRP: C-reactive Protein.
G1: Control Group.
G2: Supplemented group.
HPLC: High-pressure liquid chromatography.
ICU: Intensive care units.
MDA: Malondialdehyde.
TBARS: Thiobarbituric acid reactive substances.
T0: Time 0.
T1: Time 1.
T2: Time 2.

Introduction

Critical inpatients in intensive care units (ICU) present systemic inflammatory process that can be followed by decrease in plasma concentrations of antioxidant vitamins.[1-2-3]

As a result of the systemic inflammatory process and the decrease of aerobic metabolism, the oxidative stress occurs,[4] and it is able to induce the lipid oxidation and, in the presence of oxygen, it can cause the lipid peroxidation of cell membranes.[5-6]

One of the most used indicators for the assessment of oxidative stress is the malondialdehyde (MDA), which is a product of lipid peroxidation (the process induced by free radicals) in which is estimated by measurement of thiobarbituric acid reactive substances (TBARS nmol/L), the method most used in studies of lipid peroxidation is simple and sensitive.[4-7]

The defining features of this inflammatory response are the oxidative damage caused by proteases and free oxygen radicals from leukocytes and the endothelium and the excess production of mediators with immunosuppressive effects, such as cytokines and eicosanoids. This inflammatory response can result in organ damage and an increased risk of nosocomial infections with high rates of morbidity and mortality.[7-8]

Critical inpatients are usually exposed to oxidative stress increase, which is proportional to the gravity of their clinical condition. The presence of oxidative stress has been more and more acknowledged as an important component in the subjacent physiopathology in critically ill patients, especially when it comes with the development of organ failure. In this context, the existence of an antioxidant endogenous defense mechanism protects the tissues from cell damages induced by free radicals.[1-2] The profound oxidative stress that occurs during critical illness leads to early depletion of many endogenous antioxidants.[9-10-11]

Among non-enzymatic antioxidant systems, vitamins A, C and E are found. They have a protective effect against lipidic peroxidation.[1-3] 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. Carotenoids exhibit potent antioxidant activity by radical trapping or singlet oxygen quenching activity and are therefore important for the prevention of lipid peroxidation and inactivation of metabolically generated free radical species. β-carotene has an antioxidant activity five times higher than retinol. Vitamins E and C are chain-breaking antioxidants that prevent the peroxidation of lipids in cell membranes and lipoproteins.[4-12-13-14] Vitamins E and C act synergistically, resulting in an α-tocopheroxyl radical that is then reduced back to α-tocopherol by vitamin C.[15]

Some studies have been evaluating the association of the antioxidant nutrient supplementation with the clinical outcome of critical patients, as a shorter time use of mechanical ventilation, reduction in infection incidence, mortality and hospitalization days, yet the results are still conflicting.[2-3-9-10-16-17-18-19]

Thus, the current study aimed at evaluating the effect of the supplementation of antioxidant vitamin in the evolution of clinical parameters in critical patients and their relation with lipid peroxidation, aiming at the subsidy of the establishment and revision of the nutritional protocol addressed to critical patients, with the intention to help clinical practice, and through this, contribute with the decrease of tissue damages frequently observed in these patients.

Materials and methods

Study design

The study was randomized and controlled in an intensive care unit in Rio de Janeiro (Brazil).

Patients

Adult patients who had been indicated for enteral nutrition therapy from February 2007 to February 2010 were inclued. Inpatients under 20 years old were exclued, as well as those with enteral nutrition during the preoperative period and the ones in the bariatric surgery postoperative period.

The study was approved by the Research Ethics Committee of Hospital Universitário Clementino Fraga Filho/Universidade Federal do Rio de Janeiro on February 8th, 2007, registration number 240/06.

Nutritional intervention

Patients were randomized to receive either the supplemented diet or control diet in a 1:2 ratio.

G1-Control group: Patients who received enteral nutrition therapy according to a hospital routine: G2-Supplemented group: Patients who received 10,000 UI supplementation of retinol acetate, 400 mg of vitamin E and 600 mg of vitamin C.

Caloric needs were set at 25 kcal/kg/day (ESPEN)[20] and protein intake at 1.5 g/kg/day. An evaluation on how critical the patients' problems were, was made through APACHE II score (Acute Physiology and Chronic Health Evaluation).[21]

Laboratory determinations

Biochemical dosages were calculated during T0 (T0, before nutritional therapy) and T1 (T1, 8th day after the beginning of nutritional therapy) in the same laboratory:

- Vitamin A (retinol and β-carotene) and Vitamin E levels. The dosages were calculated through the method of high-pressure liquid chromatography (HPLC), according to the technique described by Arnaud et al.[22] Reference values for vitamin E of 15- 40 μmol/L were considered; and cut-off points for serum inadequation of retinol and β-carotene were < 1.05 μmol/L and ≤ 40 μg/dL.
- Vitamin C level. The dosages were calculated through the method of HPLC, according to the technique described by Wayner and Burton.[23] The cut-off point used for inadequation of ascorbic acid concentrations was < = 0,80 mg/dL.
- Lipid peroxidation. The levels of MDA was analyzed through the HPLC-UV method.[24]
- C-reactive Protein (CRP). It was dosed through the nephelometric method, applying the following reference values: < or = 3.5 mg/L, as indicated by the manufacturer's kit (DADEBEHRING). The CRP was dosed simultaneously to the other patterns.
- Lipid. For cholesterol dosage and its fractions (HDL and LDL), the enzymatic colorimetric method was used. The triglycerides were measured with a photometer, after enzymatic reaction.[25]

The groups had been monitored on T0, T1 and time 2 (T2, at discharges or death) on the following parameters: mechanical ventilation; hospitalization days; mortality; infection incidence.

Statistical analysis

The measures of central tendency and dispersion were calculated. Due to the non-parametric behavior of the variables, the Mann-Whitney and the Wilcoxon tests were used for continuous variables.

Spearman's correlation coefficient was employed to verify the correlation among continuous variables in the study, following the control and the supplemented groups.

Software SPSS version 17.0 was used and the significance level with 5% of probability was adopted ($p < 0.05$).

Results

The study included 70 patients, among those, 34 went through it. Half patients who did not get to T1 (n = 36) came to death and the other 50% were discharged from ICU.

After Randomization, 23 patients composed G1 and 11 patients composed G2, 50% (n = 17) female and 50% (n = 17) male.

As for the principal diagnosis of patients by the time they began the study, 20% (n = 7) were in the postoperative period and 80% (n = 27) were in a medical clinic.

Table I shows the mean, the median, the minimum and the maximum values of continuous variables in groups G1 and G2 during T0.

There was no significant difference between the groups during T0 in relation to these variables. The average of energy received by the groups was the same (G1 = 1,502.2 ± 191.9 kcal and G2 = 1,372.6 ± 276.2 kcal - p = 0.20).

There was a positive correlation between the β-carotene and the HDL (r = 0,65/p = 0,008).

In table II values of retinol, β-carotene, vitamin C, vitamin E, CRP and MDA in G1 and G2 during T1 are observed.

The concentrations of CRP were high in both groups during T0 and T1. The serum concentration of MDA was significantly lower in G2 after intervention.

When comparing T0 with T1, the serum values of MDA decreased in such a significant way in G2 after supplementation (W* = -2.31/p = 0.021), unlike G1 (W* = -0.79/p = 0.429) (fig. 1).

A significant increase of serum concentration in vitamin E stands out in G2 after supplementation (W* = -2.81/p = 0.005), unlike G1 (W* = -1.75/p = 0.08). As for the vitamin C, it is observed a high tendency towards increase of serum concentrations in G2 after supplementation (W* = -1.84/p = 0.06), differing from the unsupplemented group (W* = -1.14/p = 0.255). As for the retinol and β-carotene such benefit was not observed in G2 (W* = -1.29/p = 0.197 and W* = -0.11/p = 0.916) (fig. 2). Even though the inadequate percentage of β-carotene is lower (18%) in G2, when compared with G1 (48%) after supplementation.

In the control group, 75% of the patients who had inadequation of retinol during T1 presented inadequate of β-carotene as well. As for the supplemented group, this difference decreased in 50%.

When comparing days of mechanical ventilation, infection outbreak and mortality, there were no differences between the groups on T1 (p = 0.928, p = 0.897 and p = 1,000, respectively) and T2 (p = 0.853, p = 0.157 and p = 0.452, respectively), although on T2 the G1 presented 75% of death and on G2 25% (fig. 3), as well as the need of mechanical ventilation has been higher on G1 (72%) when compared with G2 (28%).

The entire hospitalization days on ICU were similar between the groups (G1 = an average of 27 ± 13 days/ G2 = 30 ± 11 days - p = 0.440).

Table I
Mean, standard deviation (SD), median, minimum and maximum values of continuous variables in group 1 (G1) and group 2 (G2) during T0

Variables	G1 (n = 23)		G2 (n = 11)		p-value
	Mean ± DP	Median (min-max)	Mean ± DP	Median (min-max)	
Age (years)	75.4 ± 14.3	81.0 (43.0-91.0)	80.8 ± 13.5	80.0 (52.0-96.0)	0.299
Retinol (µmol/L)	1.5± 0.8	1.2 (0.6-3.3)	1.5 ± 0.5	1.6 (0.7-2.4)	0.690
β-carotene(µmol/L)	0.5 ± 0.5	0.2 (0.1-1.8)	0.6 ± 0.5	0.3 (0.2-1.3)	0.251
Vitamin C(mg /dL)	1.2 ± 1.8	0.7 (0.08-7.70)	0.5 ± 0.4	0.5 (0.02-1.26)	0.251
Vitamin E(µmol/L)	25.1 ± 13.6	20.5 (9.6-52.8)	17.3 ± 5.3	16.0 (10.8-28.8)	0.243
Cholesterol (mg/dL)	123.4 ± 38.7	105.0 (71.0-209.0)	124.2 ± 22.1	128.0 (89.0-164.0)	0.585
HDL (mg/dL)	40.7 ± 17.5	40.0 (12.0-91.0)	46.4 ± 13.6	54.0 (21.0-61.0)	0.154
LDL(g/dL)	58.0 ± 22.0	52.0 (34.0-119.0)	67.0 ± 16.0	67.0 (40.0-96.0)	0.143
Triglycerides(mg/dL)	107.9 ± 77.3	79.5(30.0-290.0)	64.3 ± 20.3	60.0 (40.0-102.0)	0.104
CRP (mg/L)	141.1 ± 102.5	113.2 (3.2-349.5)	155.5 ± 95.6	146.8 (7.0-263.0)	0.778
MDA (nmol/L)	2.9 ± 1.9	2.8 (0.4-9.0)	2.13 ± 1.1	2.4 (0.2-3.4)	0.291
APACHE	13.8 ± 5.6	13.0 (6.0-33.0)	13.3 ± 6.3	11.0 (5.0-28.0)	0.612

Mann Whitney Test.
HDL: High density lipoprotein; LDL: Low density lipoprotein; CRP: C-Reactive Protein; MDA: Malondialdehyde; APACHE: Acute Physiology and Chronic Health Evaluation.

Table II
Biochemical parameters group 1 (G1) and group 2 (G2) during T1

Variables	G1 (n = 23)		G2 (n = 11)		p-value
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Retinol (µmol/L)	2.1 ± 1.3	152 (0.6-5.1)	2.0 ± 1.1	1.6 (0.8-4.1)	0.800
β-carotene (µmol/L)	0.4 ± 0.4	0.3 (0.0-1.5)	0.6 ± 0.4	0.4 (0.2-1.4)	0.121
Vitamin C (mg /dL)	0.6 ± 0.4	0.6 (0.03-1.27)	1.33 ± 1.8	0.9 (0.1-5.9)	0.152
Vitamin E (µmol/L)	28.5 ± 16.9	21.6 (4.8-67.2)	25.0 ± 10.2	22.8 (13.5-48.0)	0.923
CRP (mg/L)	61.98 ± 79.3	35.1 (3.8-319.3)	117.1 ± 93.7	152.0 (5.2-291.0)	0.143
MDA (nmol/L)	2.7 ± 2.1	1.9 (0.4-9.2)	1.2 ± 0.6	1.0 (0.2-2.2)	0.0019*

Mann Whitney Test.
* $p < 0.05$.
SD: Standard Deviation; CRP: C Reactive Protein; MDA: Malondialdehyde.

Discussion

In the current study, the average age of patients corroborates the data found in the studies of Thomas et al.[26] and Nogueira et al.,[4] which prove that most inpatients in ICU are elders. Older patients are prone to suffer a wider range of nutritional depletions, poor wound healing, changes in body composition and in the functions of organs, among other factors that lead to a higher probability of inflammatory occurrences, eschars and other complications.

Half of the patients who did not complete the study died in the 1st week of hospitalization; similar data were found by Preiser et al.[17] The high level of mortality observed in this study can be attributed to the high number of old inpatients who suffer from co morbidity disorders and frequent complications, which increase death risks. Patients older than 65 years of age have 60% of hospital mortality rate when they present a dysfunction in their organ system, 90% when there is more than one dysfunction and 100% when three or more organ dysfunctions occur.[21-27]

In the current study, high mean values of CRP were observed in both groups during both times, which implies the increased presence of a systemic inflammatory response or the existence of an infectious process; data that corroborate with diverse studies like Castelli et al.[28] and Nogueira et al.[4]

The mean values of APACHE II found in the current study corroborate with Goode et al.,[29] Mishra et al.[30] and Nogueira et al.,[4] which corresponds to 15% of chances of death in both groups of patients. The gravity of the diseases described in APACHE contributes with the level of oxidative stress, as it is described by Crimi et al.[27] The current study did not find any significant association between these two variables, possibly due to the sample homogeneity concerning the values of APACHE, presenting low standard deviation.

The positive correlation between serum β-carotene and HDL values matches recent study.[31] The β-caro-

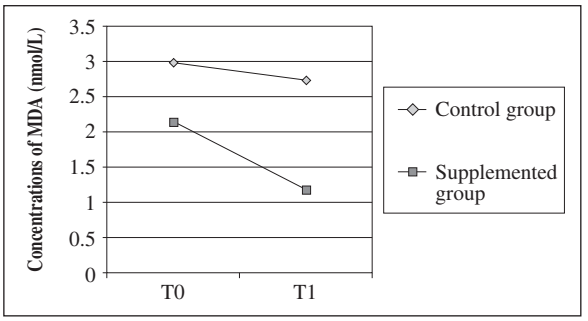

Fig. 1.—Mean values of MDA (malondialdehyde) before (T0) and after (T1) supplementation group and supplement group.

tene acts as an important protector against LDL oxidative damage.

This study shows that critical patients who received antioxidant vitamin supplements ended up with a significant reduction in TBARS, showing evidence to fight or to reduce oxidative stress. These data are corroborated by the findings of Crimi et al.[15] that show an intake of 500 mg of vitamin C and 400 UI of vitamin E per day. Goode et al.[29] and Quasim et al.[32] found significantly increased concentrations of MDA in critical patients, when compared to healthy individuals.

Nogueira et al.[4] evaluated serum concentration of retinol, carotenoid and oxidative stress in ICU inpatients suffering from sepsis and found they had high serum inadequation of these nutrients (65,2% and 73.9%, respectively). The most frequent inadequation was found among those inpatients with higher serum concentration of TBARS.

In the current study, outstanding concentrations of vitamin E and C were found in the supplemented group, showing that the doses prescribed were enough, not only to maintain adequate plasma concentrations of such nutrients but to reduce oxidative stress. Similar data for vitamin E were found by Preiser et al.[17]

Long et al.[14] conducted isolated doses in critical patients, from 300 to 3,000 mg of vitamin C per day, and observed the increase in serum concentrations only when doses of 3,000 mg per day were taken for 48 hours. In the light of the above, it was concluded that to effectively increase the serum concentrations of ascorbic acid as to reduce oxidative stress in patients suffering from trauma and/or infection, it would be recommended to follow the dose prescribed. Such a study neither evaluated any inflammatory and infectious markers nor followed the clinical evolution of patients. The dose of vitamin A proposed by the study acted in an effective way against oxidative stress, which indicated significant decrease in the supplemented group, although no significant difference in serum concentrations of the same vitamin (retinol and β-carotene) had been noticed between the groups. It is relevant to point out that a dose of 5,000 UI was not enough to reduce oxidative stress in critical patients observed by Nogueira et al.[4] Preiser et al.,[17] after supplementary 6,800 UI of vitamin A, did not find higher serum concentrations of retinol in the supplemented group of critical patients either.

The inadequation percentage of β-carotene was lower in the supplemented group, although it had no statistical significance. Besides, it was observed that the increase in β-carotene serum concentrations occurred as there was

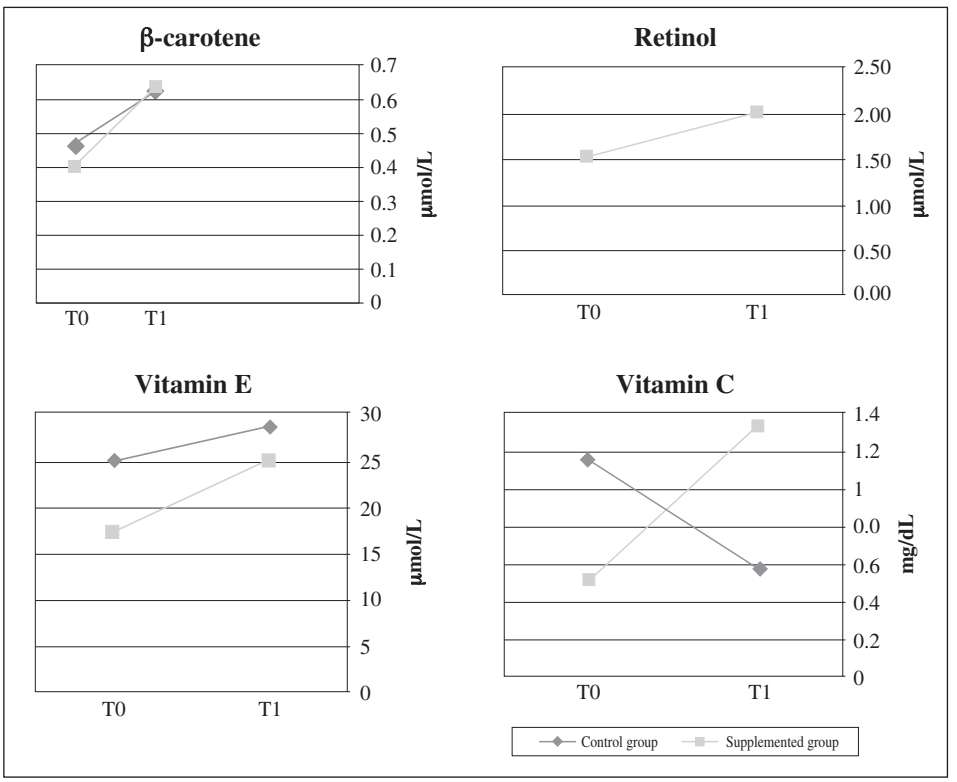

Fig. 2.—Mean values of antioxidant vitamin concentrations before (T0) and after (T1) supplementation in control group and supplement group.

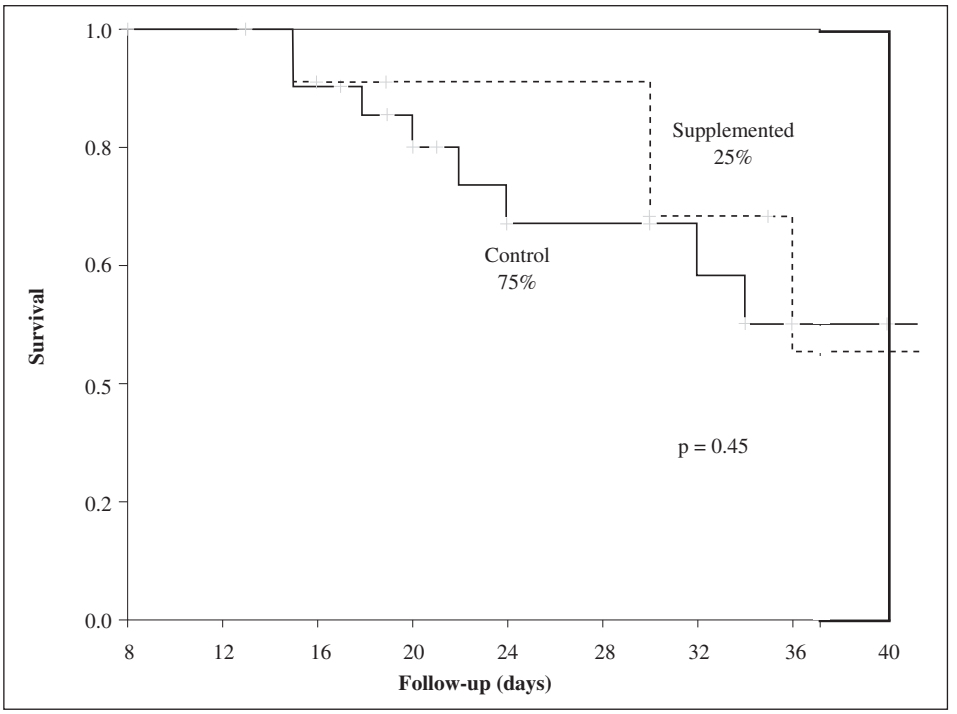

Fig. 3.—Mortality control group and supplemented group.

an increase in circulating retinol values, implying that the higher the retinol offer is, the lower the conversion of β-carotene to retinol becomes, preserving its important antioxidant function to fight free radicals of the critical patients in this current study. According to Mecocci et al.,[33] the adequate nutritional state of vitamin A reduces the conversion of carotenoids to retinol, demonstrating that there is a relation between the nutritional state of retinol and carotenoids. The β-carotene is known as the most powerful precursor of retinol.

When the outbreak of infectious complications, mortality, ICU stay and mechanical ventilation days were compared, no significant difference was observed between the groups, although the death percentage and the mechanical ventilation time had been shorter in the supplemented group. Similar data had been found by Preiser et al.[17] and Berger et al.[2-3-18]

Collier et al.[9] did not find significant difference on the days of mechanical ventilation between the control groups and the supplemented with vitamin E, C and selenium, although the outcome had been positive. Corcoran et al.[19] did not find association between the concentrations of serum in vitamins A and E and the reduction of mortality in critical patients.

Conversely, other studies diverge from such results, observing reduction in mortality, infection and ICU stay in patients receiving antioxidant nutrient supplementation.[9-10-16] It is important to highlight that the study by Nathens 10 used 1,000 mg of vitamin E and 1 g of vitamin C and the results were better in the supplemented group, though it has not presented statistical significance. Similarities in results had been found in the study by Collier[9] which also used equal doses as in the previous study, finding significant reduction in mortality and ICU stay.

Crimi et al.[15] found reduction on mechanical ventilation days and mortality, however, they did not find reduction on hospitalization days in the group of supplemented patients with 500 mg/day of vitamin C and 400 mg/day of vitamin E.

In a systematic review evaluating vitamin supplementation and trace elements (selenium, vitamin C and E, zinc and cooper) on critical patients, a reduction in mortality was observed in the groups that received some of these supplementations through parenteral via ($p < 0.0001$), but there were no effects on the infectious complications. Due to the low number of eligible studies, the enteral and parenteral vias have been combined in analysis. This meta-analysis has observed that the combination of early endogenous antioxidant micronutrients during the illness acute phase is correlated with a better clinical result and it excels in isolated administration of such micronutrients. And it even points out that the isolated administration can produce organic disorder, once it can become pro-oxidant.[1]

Conclusion

The use of vitamins with antioxidant effects is able to reduce lipid peroxidation in critical patients, as shown in this study.

The doses of vitamins C and E proposed were enough to raise the serum concentrations after supplementation significantly.

According to the proposed supplementation protocol, the supplemented group did not present significant differences related to the clinical parameters analysed. However, it is worth highlighting that the death numbers

and mechanical ventilation days were shorter in the supplemented group, which has a great clinical relevance.

The lipid peroxidation can be reduced with antioxidant supplementation, even though this supplementation is not enough to increase serum concentrations, as occurred with vitamin A. Such results can guide clinical practice to act upon this group of patients in a safe and effective way.

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