Applied nutritional investigation

Effect of Brazil nut supplementation on the blood levels of selenium and glutathione peroxidase in hemodialysis patients

M.B. Stockler-Pinto M.S. a,*, D. Mafra Ph.D. b, N.E. Farage R.D. c, G.T. Boaventura Ph.D. b, S.M.F. Cozzolino Ph.D. a

a University of São Paulo, Faculty of Pharmaceutical Sciences, São Paulo, Brazil
b Clinical Nutrition Department, Faculty of Nutrition, Federal Fluminense University, Niterói, Brazil
c Renal Cor Clinic, NutriRim, Rio de Janeiro, Brazil

Article info

Article history:
Received 27 April 2009
Accepted 13 August 2009

Keywords:
Chronic kidney disease
Oxidative stress
Selenium
Glutathione peroxidase

Abstract

Objective: In patients who have undergone hemodialysis, large amounts of reactive oxygen species (ROS) are produced and, at higher concentrations, ROS are thought to be involved in the pathogenesis of cardiovascular disease. It has been proposed that selenium (Se) may exert an antiatherogenic influence by reducing oxidative stress. The richest known food source of selenium is the Brazil nut (Bertholletia excelsa, family Lecythidaceae), found in the Amazon region. We evaluated the effect of Brazil nut supplementation on blood levels of Se and glutathione peroxidase (GSH-Px) activity in patients on hemodialysis.

Methods: A total of 81 patients on hemodialysis (52.0 ± 15.2 y old, average time on dialysis 82.3 ± 91.4 mo, body mass index 24.9 ± 4.4 kg/m2) from the RenalCor and RenalVida Clinics in Rio de Janeiro, Brazil, were studied. All patients received one nut (around 5 g, averaging 58.1 μg Se/g) a day for 3 mo. The Se concentrations in the nuts and in plasma and erythrocytes were determined by atomic absorption spectrophotometry with hydride generation (Hitachi, Z-500). GSH-Px levels were measured using Randox commercial kits.

Results: Plasma Se (18.8 ± 17.4 μg/L) and erythrocyte (72.4 ± 37.9 μg/L) levels were below the normal range before nut supplementation. After supplementation, the plasma level increased to 104.0 ± 65.0 μg/L and erythrocytes to 244.1 ± 119.5 μg/L (P < 0.0001). The activity of GSH-Px also increased after supplementation, from 46.6 ± 14.9 to 55.9 ± 23.6 U/g of hemoglobin (P < 0.0001).

Before supplementation, 11% of patients had GSH-Px activity below the normal range (27.5–73.6 U/g of hemoglobin). After supplementation, all patients showed GSH-Px activity within the normal range.

Conclusion: The data revealed that the investigated patients presented Se deficiency and that the consumption of only one Brazil nut a day (5 g) during 3 mo was effective to increase the Se concentration and GSH-Px activity in these patients, thus improving their antioxidant status.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Oxidative stress in patients who have undergone hemodialysis (HD) has been reported to be the result of an increased pro-oxidant activity (advanced age, high frequency of diabetes, chronic inflammatory state, uremic syndrome, bioincompatibility of dialysis membranes) and of a reduced antioxidant system (vitamin C levels, intracellular levels of vitamin E, glutathione system, and selenium [Se]) [1–3]. It has been proposed that Se may exert an antiatherogenic influence by lowering oxidative stress in the endothelium [4–7].

The best known biological role of Se is its presence in glutathione peroxidase (GSH-Px), which is one of the enzymes that protect membrane lipids and other cellular and extracellular components from oxidative damage. GSH-Px also catalyses hydrogen peroxide and lipid hydroperoxide reduction [8–12]. Long-term Se deficiency results in markedly decreased GSH-Px activity. Several investigators have reported decreased levels of plasma and erythrocyte Se and decreased GSH-Px activity in...
patients on HD and the majority of the studies recommended Se supplementation for patients on HD [13–15]. Food sources of Se are preferable to alternative supplementation practices to improve the nutritional status of a population. This is because food sources are sustainable, less expensive, and present a lower risk of toxicity [16] compared with supplementation.

In Brazil, there is large consumption of the Brazil nut (Bertholletia excelsa, family Lecythidaceae) from the Amazon region. This nut is known to be the richest food source of Se, with reported mean concentrations of 8–83 μg Se/g [17,18]. Daily consumption of only one Brazil nut could increase dietary Se intakes to the recommended levels, which were estimated in the National Nutrition Survey to be 56 μg for men and 39 μg for women [19].

Studies have suggested that the bioavailability of Se in the Brazil nut is similar to that of sodium selenite for the restoration of tissue Se and selenoprotein activity [20]. However, to our knowledge, no studies have assessed the efficacy of the Brazil nut in increasing Se status in patients on HD. Taking into account that patients on HD are deficient in Se and that Brazil nuts are the best source of this mineral, the aim of the present work was to assess the effects of Brazil nut supplementation on plasma and erythrocytes Se levels and erythrocyte GSH-Px activity in Brazilian patients on HD.

Materials and methods

Eighty-one patients on HD (52.0 ± 15.2 y old, 55 men and 26 women, average time on dialysis 82.3 ± 51.4 mo) were enrolled from the RenalCor and RenalVida Clinics in Rio de Janeiro, Brazil. Inclusion criteria were an age older than 18 y and patients on maintenance dialysis for at least 6 mo. Patients with inflammatory diseases, cancer, positive for human immunodeficiency virus, autoimmune diseases, phosphorus levels above 5.5 mg/dL, use of catheter access for HD, and antioxidant vitamin supplements were not included. The dialysis duration was 3–4.5 h/session, three times a week, the blood flow was greater than 250 mL/min, and the dialysate flow was 500 mL/min. The study protocol was reviewed and approved by the ethics committee of the Faculty of Pharmaceutical Sciences of the University of São Paulo (no. 422) and all patients signed an informed consent.

Nutritional assessment

Anthropometric measurements were made by a trained staff member using standard techniques immediately after the HD session. Body mass index was calculated as weight in kilograms divided by height in meters squared [21]. Waist circumference, triceps skinfold, arm muscle area, and body fat percentage were used in these analyses. The non-dominant arm was used for these measurements, except when there was an arterial venous fistula. Triceps skinfold was measured with a Lange Skinfold Caliper (Cambridge Scientific Products, Cambridge, MA, USA). The arm muscle area was calculated according to the following formula: \[MC (cm) \times \pi \times TSF (mm)/10^2\] – \(n\), where \(n\) = 10 for male and 6.5 for female, MC refers to midarm circumference, and TSF refers to triceps skinfold. Fat-free mass was calculated by subtracting fat mass from body weight. The corresponding percentiles were determined based on the tables developed by Frisancho [22]; values between percentiles 15 and 95 were defined as normality.

Supplementation

Each patient received one nut (around 5 g, with 290.5 μg of Se) a day for 3 mo. According to European Best Practice Guidelines (EBPG) Se supplementation for 3–6 mo should be considered in patients on HD; furthermore, 4–6 wk is considered the minimum period to evaluate Se supplementation, so 3 mo was considered a suitable period for supplementation. The blood samples were collected before and after this study period. The specified supplement of Brazil nut used for the study presented 0.75 g of proteins, 0.45 g of carbohydrates, and 3.53 g of lipids, with each nut supplying a total of 36.7 kcal.

Analytic procedures and sample processing

Blood samples were drawn from each subject in the morning, after an overnight fast. Blood was drawn from the arteriovenous fistula into a syringe containing ethylenediaminetetra-acetic acid (1.0 mg/ml) before the dialysis session. Plasma and erythrocytes were separated (15 min, 3000 × g, 4 °C). Erythrocytes were washed three times with isotonic physiologic solution and reconstituted to the original blood volume to produce a 50% saline suspension and then stored at –80 °C until analysis.

Selenium concentrations in plasma, erythrocytes, and Brazil nuts were determined through atomic absorption spectrophotometry with hydride generation (Hitachi, Z-5000, Tokyo, Japan). The samples were subjected to an acid digestion with nitric acid, followed by a hydrochloric acid reduction before Se analysis. The accuracy of Se analysis was verified against Seronorm (Trace Elements in Serum, Sero AS, Billingstad, Norway) standard reference material.

Erythrocyte GSH-Px activity was determined by using the Random test combination (Ransel, Antrim, UK). According to the method of Paglia and Valentine [23], GSH-Px catalyzes the oxidation of glutathione (at a concentration of 5 μmol) using cumene hydroperoxide in the presence of glutathione reductase (concentration >0.75 × 10–3 U) and 0.35 μmol of reduced nicotinamide adenine dinucleotide phosphate to oxidized nicotinamide adenine dinucleotide. The decrease in absorbance was recorded at 340 nm and 37 °C. The necessary enzyme activity to convert 1 μmol of reduced nicotinamide adenine dinucleotide phospho- to nicotinamide adenine dinucleotide phosphate in 1 min was defined as 1 U of GSH-Px. The results were expressed as units of GSH-Px per liter.

Serum albumin, urea nitrogen, creatinine, albumin, calcium, phosphorus, and potassium were measured using standard laboratory methods.

Diet analysis

Food intake was obtained from three 24-h food records. This was analyzed for mean energy, protein, lipids, and Se intake calculation using NutWin software (developed by the Department of Nutrition, Federal University of São Paulo-UNIFESP, São Paulo, Brazil). The Se concentrations were based on concentrations determined by Ferreira et al. [24] who analyzed Se concentrations in Brazilian foods [24].

Statistical analysis

Results were expressed as mean ± standard deviation or percentage of change, when applicable. Student’s t test was used to examine the difference between means, and the Kruskal-Wallis test was used for non-parametric data. Pearson’s correlation coefficient was calculated to examine the relation between variables. Statistical significance was accepted as \(P < 0.05\). Statistical analyses were performed with SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Nutritional status and routine blood parameters

The anthropometric measurements are listed in Table 1. The etiologies of renal failure were hypertension (59.5%), diabetes (12.7%), and others (27.8%). Mean body mass index was 24.9 ± 4.4 kg/m² and was in the normal range according to criteria from the World Health Organization [25]. Only 3 patients (4%) presented body mass index values below 18.5 kg/m² and 34 (42%) presented values above 25 kg/m². The waist circumference was above normal values in 75% of all patients. According to body fat percentages, 53 patients (70%) presented high values. Regarding arm muscle area, 24 patients (32%) presented normal values, and only 4 (5%) presented reduced muscle mass. Biochemical characteristics of the subjects are listed in Table 2.

Diet analysis

The mean of protein intake was 1.32 ± 0.53 g · kg⁻¹ · d⁻¹. The intake of 31 patients (19%) presented an intake lower than the recommended values (1.2–1.4 g · kg⁻¹ · d⁻¹). The energy intake analysis showed that 88% of patients presented values lower than 35 kcal · kg⁻¹ · d⁻¹. According to Se intake analysis, 93% of patients presented low intake when compared with normal values (45 mg/d). The mean was 27.7 ± 14.3 mg/d.

Selenium analysis

The plasma Se concentration before supplementation was 18.8 ± 17.4 μg/L, and 98.7% of patients presented values below
the normal range (60–120 μg/L). After supplementation the levels increased to 104.0 ± 65.0 μg/L (P < 0.001; Fig. 1). The mean of erythrocyte Se before supplementation was 72.4 ± 37.9 μg/L, and after supplementation the level increased significantly to 244.1 ± 119.5 μg/L (P < 0.0001; Fig. 2). According to GSH-Px activity, 11% of patients presented activity below the normal range (27.5–73.6 U/g of hemoglobin) before supplementation (mean 46.6 ± 14.9 U/g of hemoglobin). After supplementation the levels increased significantly to 55.9 ± 23.6 U/g of hemoglobin (P < 0.0001; Table 3). A negative correlation was found between GSH-Px before supplementation and age (r = −0.28, P = 0.01; Fig. 3).

Discussion and conclusion

The present study is the first to investigate the response of plasma and erythrocyte Se levels and the activity of the selenoprotein GSH-Px to Brazil nut supplementation in patients on HD. We have shown that the daily consumption of just one Brazil nut is effective in raising plasma and erythrocyte Se concentrations and GSH-Px activity.

The literature has reported low levels of Se concentration and GSH-Px activity in patients on HD [26–29]. Our results confirm this Se deficiency in Brazilian patients on HD. The activity of GSH-Px was below the normal range in only 11% of patients. According to some studies the activity of GSH-Px in erythrocytes is within the normal range in these patients [30–32]. In addition, El-Far et al. [12], after assessing the activity of plasma and erythrocyte GSH-Px, concluded that the erythrocyte GSH-Px is not dependent on kidney function, whereas plasma GSH-Px is synthesized in the kidneys.

Several mechanisms have been reported to explain the altered Se status observed in uremic patients, such as low dietary intake, increased urinary and dialytic losses, impaired intestinal absorption, abnormal binding to Se transport proteins, and drug therapy [4,31]. Our results showed low Se intake in patients on HD, but there was no correlation with plasma or erythrocyte Se levels. In the present work, we found a negative correlation between age and GSH-Px, probably because aging may lead to oxidative stress and, being so, the enzyme GSH-Px acts as an antioxidant. It is known that Se concentration declines slightly in the elderly [33].

The interpretation of the biomarkers is complex because Se concentrations depend not only on the total intake but also on the forms of Se, on Se metabolism, and on pathophysiologic responses to conditions associated with increased oxidative stress or inflammation [34]. Although there are few studies reporting Se supplementation in patients on HD, this kind of intervention is already being done. In addition, most studies were performed using formulas of Se, inorganic selenite and selenate, respectively.

Temple et al. [35] observed increased plasma Se concentrations in patients on HD after supplementation with selenate. Others studies also observed [36,37] an increase in the levels of plasma Se concentrations in patients on HD after supplementation with selenite. Others studies also observed [36,37] an increase in the levels of plasma Se concentrations in patients on HD after supplementation with selenite. Others studies also observed [36,37] an increase in the levels of plasma Se concentrations in patients on HD after supplementation with selenite.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Nutritional status of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Patients</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.9 ± 4.4</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>85.5 ± 11.3</td>
</tr>
<tr>
<td>Men</td>
<td>93.1 ± 12.0</td>
</tr>
<tr>
<td>Triceps skinfold (mm)</td>
<td>15.8 ± 10.6</td>
</tr>
<tr>
<td>Arm muscle area (cm²)</td>
<td>31.6 ± 9.4</td>
</tr>
<tr>
<td>Women</td>
<td>36.2 ± 13.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>34.8 ± 7.0</td>
</tr>
<tr>
<td>Men</td>
<td>24.5 ± 9.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Routine blood measurements of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Before supplementation</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dL)</td>
<td>140.0 ± 264.4</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>10.4 ± 3.0</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.1 ± 0.7</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td>Potassium (mg/dL)</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.5 ± 0.3</td>
</tr>
</tbody>
</table>

* P < 0.05.

Fig. 1. Comparison of levels of plasma Se before and after supplementation. Se, selenium.

Fig. 2. Comparison of levels of erythrocyte Se before and after supplementation. Se, selenium.
and in the activity of GSH-Px in erythrocytes after Se supplementation. However, the enzyme activity reaches a plateau when the intake of Se reaches the recommended level [35,37].

The characterization of the Se species in the Brazil nut in other studies indicates that selenomethionine is the main species. According to Thomson et al. [38], studies in rats showed that the bioavailability of the Brazil nut is equal to that of sodium selenite. However, in vivo studies have shown that selenite can cause pro-oxidant effects [35,39]. The greater increase in whole blood Se after Brazil nut consumption suggests that Se from this nut may be more bioavailable than others forms of Se supplementation.

Public health recommendations to include as few as one Brazil nut per day in the diet would avoid the need for fortification of food supplements to improve their Se status [38]. Food sources are preferable to alternative supplementation practices because they are sustainable, less expensive, and present a lower risk of toxicity compared with supplementation. Moreover, because the Brazil nut is naturally found in Brazil and this nut is the richest known food source of Se, its consumption should be encouraged. This study demonstrates that some deficiencies could be avoided with adequate dietary counseling recommending nutrients found locally.

It is important to note that our study presented some limitations. First of all, we could have analyzed C-reactive protein to observe inflammation; however, this was not the main aim of the study. The employment of only GSH-Px activity as a biomarker of oxidative stress also limited our study because this enzyme reaches a plateau when Se intake attains the recommended level.

Furthermore, we should have done the analysis of Se in foods commonly consumed in Brazil; however, this posed some difficulties and high costs to be carried out.

From these results, we concluded that the patients had deficiency in relation to the nutritional Se status, and this deficiency was overcome with the supplementation of one Brazil nut a day for 3 mo. Furthermore, the significant increase in the levels of GSH-Px after supplementation suggests that the Brazil nut can really improve the condition of oxidative stress in patients.

References


