Selenium plasma levels in hemodialysis patients: Comparison between North and Southeast of Brazil

Níveis de selênio plasmático em pacientes em hemodiálise: Comparação entre Norte e Sudeste do Brasil

Autores
Milena Barcza Stockler-Pinto[1,5]
Olaf Malm[1]
Suellen Regina Geraldo Azevedo[2]
Najla Elias Farage[3]
Paulo Renato Dorneles[1]
Silvia Maria Franciscato Cozzolino[4]
Denise Mafra[5]

[1] Federal University of Rio de Janeiro (UFRJ).
[2] CDR Clinic.
[3] RenalCor Clinic.
[4] University of São Paulo (USP).
[5] Fluminense Federal University (UFF).

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Correspondência para:
Milena Barcza Stockler-Pinto.
Universidade Federal do Rio de Janeiro.
Rua Tiradentes, nº 108, bloco B, 703.
Niterói, RJ, Brasil. CEP: 24210-510.
E-mail: milbarcza@gmail.com

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Abstract

Introduction: Patients with chronic kidney disease present selenium (Se) plasma deficiency which is an essential trace element with important biological functions and, the best known biological role is attributed to its presence in the antioxidant enzyme, glutathione peroxidase (GPx). The Se content of foods depends on soil and some authors have suggested that Amazon soil (North Brazilian region) has high Se concentrations when compared to other regions of Brazil. **Objective:** The objective of this work was to compare the Se status in hemodialysis (HD) patients from North and Southeast of Brazil. **Methods:** Thirty-eight patients from Southeast region (22 men and 16 women, 15% diabetic, 53.5 ± 26.4 yrs) were compared to 40 patients from North region (28 men and 12 women, 22.5% diabetic, 63.5 ± 11.9 yrs). Se in plasma was determined through atomic absorption spectrophotometry with hydride generation. **Results:** The plasma Se levels in patients from Southeast region were significantly lower (17.5 ± 11.9 µg/L) when compared to patients from the North (37.1 ± 15.8 µg/L) ($p < 0.001$). However, both patient groups presented low Se plasma levels when compared to recommended values (60-120 µg/L). There was no correlation between plasma Se levels and analyzed parameters. **Conclusion:** We concluded that patients from North (Amazon) region present higher plasma Se levels when compared to the patients from Southeast of Brazil. However, independently of the region, HD patients presented Se deficiency.

Keywords: Brazil; hemodialysis; selenium.

Resumo

Introdução: Pacientes com Doença Renal Crônica apresentam deficiência de selênio (Se), um elemento essencial, com importantes funções biológicas, como a de ser componente da enzima antioxidante glutationa peroxidase (GPx). A concentração de Se nos alimentos depende de sua concentração no solo e autores relatam que o solo da Amazônia possui elevados níveis de Se. **Objetivo:** O objetivo do trabalho foi comparar o estado nutricional do Se em pacientes em hemodiálise (HD) das regiões Norte e Sudeste do Brasil. **Métodos:** Trinta e oito pacientes da região Sudeste (22 homens e 16 mulheres, 15% diabéticos, 53,5 ± 26,4 anos) foram comparados com 40 pacientes da região Norte (28 homens e 12 mulheres, 22,5% diabéticos, 63,5 ± 11,9 anos). O Se no plasma foi determinado por espectrofotometria de absorção atômica por geração de hidretos acoplados a cela de quartzo. **Resultados:** Os níveis de Se dos pacientes em HD da região Sudeste foram significativamente menores (17,5 ± 11,9 µg/L) comparados aos pacientes da região Norte (37,1 ± 15,8 µg/L) ($p < 0,001$). Entretanto, ambos os grupos apresentaram níveis de Se abaixo da recomendação (60-120 µg/L). Não houve associação entre os níveis de Se e os parâmetros analisados. **Conclusão:** Com base nos resultados, concluímos que os pacientes da região Norte apresentaram elevados níveis de Se quando comparados com os pacientes da região Sudeste do Brasil. Entretanto, independentemente da região, ambos os grupos apresentaram deficiência com relação ao estado nutricional do Se.

Palavras-chave: Brasil, hemodiálise, selênio.

Introduction

Selenium (Se) is believed to exert a number of beneficial health effects on immunocompetence, reproductive capacity, as well as cardio and neuro-protective properties and prevention of cancer.[1] The best known biological role of Se is attributed to its presence in glutathione peroxidase (GPx), which is one of the enzymes that protect membrane lipids and other cellular and extracellular components from oxidative damage.[2-4]

In Brazilian Amazon, Se status of communities ranges from normal to very high, varying between 142 and 2447 µg/L in blood. Important local dietary Se sources, such as Brazil nut (the seed of *Bertholletia excelsa*), chicken, meat and certain fish species, have been identified. The Se content of these foods depends on soil Se concentration and bioavailability as well as the ability of plants to accumulate Se from soils[5] and, some authors have suggested that Amazon soil has high concentrations of Se.[6]

Patients with chronic kidney disease (CKD) commonly present Se deficiency and, low dietary intake, increased urinary and dialytic loss, impaired intestinal absorption, abnormal binding to Se transport proteins, and drug therapy could explain this deficiency.[7,8] It has been reported in the literature the presence of oxidative stress and deficiency in the Se antioxidant mechanisms in the hemodialysis (HD) patients, and is it known that oxidative stress is a classical risk for cardiovascular events. Decreased serum Se levels may have a role in endothelial dysfunction, disruption of coronary flow and accelerated atherosclerosis.[9] Furthermore, serum Se levels are inversely associated with mortality risk in HD patients.[10]

In fact, our previous studies have shown that HD patients from Southeast Brazilian region present Se deficiency.[11,12] This region is characterized by having low concentrations of Se in soil when compared to North (Amazon) region;[6] however, there is no study comparing Se status in CKD patients between these regions. Thus, the present study aimed to compare the Se status in HD patients from North and Southeast Brazilian regions.

Materials and methods

Subjects

Thirty-eight HD patients were enrolled from RenalCor Clinic in Rio de Janeiro, Southeast Brazilian region, and 40 HD patients were enrolled from CDR Clinic in Manaus, North Brazilian (Amazon) region. Inclusion criteria were: age higher than 18 yr, dialysis duration of 3-4.5 h/session thrice weekly, blood flow greater than 250 ml/min, dialysate flow of 500 ml/min and bicarbonate buffer, being on maintenance dialysis for at least 6 months. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine of the Fluminense Federal University (nº 018/09) and all the patients gave the informed consent.

Analytical procedures and sample processing

Blood samples were drawn from each subject in the morning before the dialysis session. EDTA (1 mg/ml) was used as anticoagulant. Plasma was separated (15 min, 3000 x g, 4 °C) and stored in -80 °C until analysis. Serum albumin, creatinine, phosphorus, potassium and calcium were measured by routine procedures with automatic analysers. Dialysis dose and the adequacy were measured through the Kt/V values. The Kt/v was calculated from values of blood urea nitrogen, pre and post-dialysis, weight, and dialysis duration using the Daugirdas formula.[13] Body mass index (BMI) was computed as weight (kg) divided by squared height (m^2) according to World Health Organization.[14]

Determination of selenium concentrations

Plasma Se concentrations were determined through hydride generation atomic-absorption spectrometry (HG-AAS) according Hao *et al.*,[15] using a HITACHI® Z-500 spectrometer. Samples (300 ml) were digested with nitric acid, followed by a hydrochloric acid reduction. After acid digestion, the sample was made up to 25 mL with high purity deionised water (18.2 M Ω cm) from a Milli-Q system. Blanks were carried through the procedure in the same way as the sample. All chemicals used were of analytical reagent grade. Standard solutions were prepared in the working range for adequate calibration curves. Reference material, SERONORM® Trace Elements Serum (Sero AS, Billingstad, Norway), was treated and analysed in the same way. Our analytical results (in µg/L) for the determination of Se in SERONORM® (61.4 µg/L, n = 3) were in good agreement with certified value (53.6-64.8 µg/L).

Statistical analysis

Results were expressed as mean ± SD (Standard Deviation) or percentage change or median, maximum and minimum values, as applicable. Depending

on data normality (Shapiro-Wilk's *W* test), parametric (Student's *t*-test and Pearson's (r) correlation test) or non-parametric tests (Mann-Whitney *U* test and Spearman's correlation test) were used. Statistical significance was accepted as $p < 0.05$. The statistical analyses were performed using SPSS 19.0 software (Chicago, IL, USA).

Results

Clinical characteristics of patients are shown in Table 1. According to BMI, 35% and 27.5% of patients presented values above 25 kg/m^2, in Southeast and North regions, respectively. Plasma Se levels in HD patients from Southeast region were significantly lower (17.5 ± 1.9 µg/L) compared with patients from the North (37.1 ± 15.8 µg/L) ($p < 0.001$) (Figure 1). However, despite this difference, all patients presented Se plasma level deficiency when compared to normal values (60-120 µg/L)[16,17] and when compared with the healthy subjects from the Southeast region (54.1 ± 22.5 µg/L) and North region (83.9 ± 18.0 µg/L).[18,19]

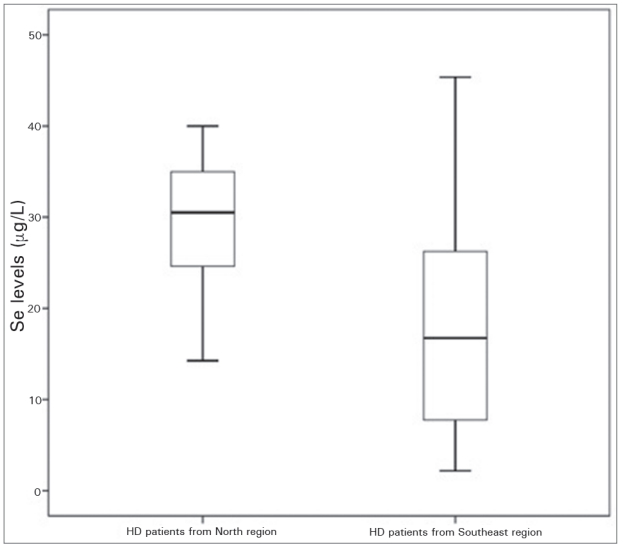
Table 1 Clinical characteristics of HD patients

Parameters	Patients from the Southeast region (n = 38)	Patients from the North region (n = 40)
Men/Women	22/16	28/12
BMI (kg/m^2)	24.3 ± 5.0	24.2 ± 3.8
Age (yrs)	53.5 ± 26.4	63.5 ± 11.9
HD time (mo)	62.4 ± 46.3	47.6 ± 28.0
Diabetics (%)	15.0	22.5
Creatinine (mg/dL)	7.5 ± 2.5	10.6 ± 5.9
Urea (mg/dL)	162.5 ± 31.8	127.5 ± 30.5*
Phosphorus (mg/dL)	5.4 ± 1.3	5.2 ± 1.8
Potassium (mg/dL)	4.7 ± 0.5	6.2 ± 7.1
Calcium (mg/dL)	8.9 ± 0.5	9.4 ± 1.5
Kt/V	1.42 (1.1-1.8)	1.25 (1.0-1.5)*

BMI: Body mass index; HD: Hemodialysis; * $p < 0.0001$.

Significant difference between Kt/v levels was found between patients of Southeast and North region ($p < 0.0001$). However, there was no correlation between plasma Se concentrations and age, HD time, routine parameters (creatinine, urea, phosphorus, potassium and calcium levels) or Kt/v. Furthermore, we did not found significant differences between women and men.

Figure 1. Comparison of plasma Se levels in HD patients from the North and Southeast region of Brazil.



Discussion

Our study has compared Se plasma levels in HD patients from different Brazilian regions (North and Southeast). We showed that patients from North (Amazon) region presented higher Se levels when compared to patients from Southeast; however, all HD patients presented Se deficiency.

Several studies have shown Se deficiency in HD patients.[20-22] A meta-analysis that collected 128 studies of trace elements in HD patients and control healthy showed that, levels of Se were lower in HD compared with controls in 46 studies. Most studies used absorption spectrometry to measure de trace elements levels and were conducted in Europe (47%), Asia (30%) and North America (14%).[23]

The Se deficiency is related to oxidative stress in these patients which have 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 Se).[24]

Low Se intake and low Se soil concentration are also an important cause of this deficiency; however, it has been indicated that Se deficiency might occur in HD patients in nonspecific areas where environmental Se is sufficient.[25,26]

Diet represents, by far, the principal route of Se intake and levels in food reflect soil Se concentrations. Bioavailability of Se in soils varies greatly in different regions. Fish and mammal organs, such as kidney and liver, are known to accumulate significant levels of Se and are, potentially, good dietary sources of this essential element for humans. In general, fruits and vegetables contain low Se levels, however, depending on Se bioavailability; cereals may constitute an important source of Se.[27]

In North (Amazon) Brazilian region, high Se concentrations were found in chicken and meat, and their regular consumption contributed substantially to Se intake. Brazil nuts from the Amazon region are mostly consumed as such and they constitute the richest known food source of Se.[28] Recently, our research group showed that Brazilian HD patients present Se deficiency, as well as that supplementation with one Brazil nut (290 mg of Se) from Amazon region a day during 3 months was effective to improve the Se status.[11,12]

According to Donadio & Cozzolino,[18] healthy subjects from Southeast Brazilian region present low plasma Se levels (54.1 ± 22.5 µg/L) due the low concentrations of Se in the soil from the Southeasth region. In addition, Shaltout *et al*.[29] found significant higher Se concentrations in Amazon soil than in the same matrix from the South Brazilian region.

In the world several places presented richest soil in Se, for example, some plains in Canada and some states in the United States of America, such as, Nebraska, Dakota, Utah and Wyoming. In China there are regions where the selenium content is excessively high enough to cause intoxication and others where it is so low enough to cause deficiency.[27]

Selenium deficiency is a serious problem that commonly occurs in a very restricted area where there is environmental depletion of Se. In 2011, Gibson *et al*.[30] demonstrated that Se concentrations in pregnant women were significantly different in countries with low (Malawi) and high soil Se levels (Philippines).

A public health recommendation to include as few as one Brazil nut/day in the diet would avoid the need for fortification of food supplements to improve the Se status. The European Best Practice Guideline (EBPG) on Nutrition recommend a daily intake of 55 mg of Se for chronic kidney disease (CKD) patients, being the same value for healthy males and females[31,32] and one Brazil nut contains about 240 mg of Se.[11]

Studies have observed an increase in the levels of plasma Se and improve the oxidative stress after Se supplementation.[33-35] In fact, Zachara *et al*.[33] observed that DNA damage decreased significantly in HD patients after Se supplementation (200 µg as Se-rich yeast). The same authors did not show that Se supplementation improved the plasma GPx in HD pantients.[34] Adamowicz *et al*.[35] also showed that GPx activity did not respond to Se supplementation in HD patients (300 µg as Se-rich yeast). Few data describe the impact of Se supplementation on clinical outcomes in HD patients, however, this supplementation may be of benefit to the antioxidant defense mechanisms.

Although the Kt/V values were lower in patients from the North when compared to patients from the Southeast, Se levels were not correlated to Kt/V and, in contrast, Ochi *et al*.[36] observed a significant positive correlation between Se concentrations and Kt/V in HD patients, as higher of Kt/V levels as higher Se levels.

It is important to note that our study presented several limitations. First, the sample sizes were small. Second, we did not have a control with healthy subjects, and we did not have meaning data from the diet of patients, because it is very difficult to compare the consumption in two areas where the foods have different concentrations of Se and there is no accurate software to calculate the diets. Besides, one of the major problems in dietary assessment is the inaccuracy in reporting dietary intake. Underreporting has been shown prevalent in CKD. In 2012, Mafra *et al*.[37] described a high prevalence of underreporting of energy intake in patients in HD with high BMI. Third, the results are not representative of the population with CKD on HD in the North and Southeast regions.

Based on these results we concluded that HD patients presented Se deficiency, regardless of the region. Thus, more attention should be paid to Se deficiency, recommending Se supplementation for HD patients.

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