

Original

Nutritional status of zinc and activity superoxide dismutase in chronic renal patients undergoing hemodialysis

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Abstract

Introduction: Chronic kidney disease promotes changes in the zinc nutritional status and in the antioxidant defense system. This study assessed the relationship between the parameters of the zinc nutritional status and the activity of superoxide dismutase in patients with chronic renal failure who are receiving hemodialysis.

Methods: 134 individuals, aged between 18 and 85 years, were divided into two groups: case group (hemodialyzed patients, n = 63) and control group (n = 71). Zinc concentrations in plasma and erythrocytes were determined using the flame atomic absorption spectrophotometry technique. The activity of superoxide dismutase enzyme was determined according to Ransod kit.

Results: The mean values of plasma zinc were $62.02 \pm 13.59 \mu\text{g/dL}$ and $65.58 \pm 8.88 \mu\text{g/dL}$, and for erythrocytary zinc the values were $54.52 \pm 22.82 \mu\text{gZn/gHb}$ and $48.01 \pm 15.08 \mu\text{gZn/gHb}$ for the chronic renal patients and the control group, respectively. The activity of superoxide dismutase was significantly lower in patients when compared with the control group ($p < 0.05$).

Conclusion: The activity of superoxide dismutase in patients with chronic renal failure undergoing hemodialysis, which is influenced by zinc concentrations, was significantly lower. There was an inadequate response of this enzyme to oxidative stress in patients undergoing hemodialysis.

(Nutr Hosp. 2011;26:1456-1461)

DOI:10.3305/nh.2011.26.6.5400

Key words: *Chronic kidney disease. Hemodialysis. Zinc. Superoxide dismutase.*

ESTADO NUTRICIONAL DEL ZINC Y ACTIVIDAD DE LA ENZIMA SUPERÓXIDO DISMUTASA EN PACIENTES CON ENFERMEDAD RENAL CRÓNICA EN HEMODIÁLISIS

Resumen

Introducción: La enfermedad renal crónica produce cambios en el estado nutricional del zinc y en el sistema de defensa antioxidante. Por lo tanto, este estudio investigó la relación entre parámetros del estado nutricional del zinc y la actividad de la enzima superóxido dismutase en pacientes con enfermedad renal crónica en hemodiálisis.

Métodos: Se incluyeron 134 personas, de 20 a 59 años de edad que fueron divididos en dos grupos: grupo caso (pacientes en hemodiálisis, n = 63) y grupo control (n = 71). El zinc plasmático y eritrocitario fueron analizados según el método de espectrofotometría de absorción atómica. La actividad de la enzima superóxido dismutasa fue analizada de acuerdo con Kit Ransod.

Resultados: Los valores medios de zinc plasmático fueron $62,02 \pm 13,59 \mu\text{g/dL}$ y $65,58 \pm 8,88 \mu\text{g/dL}$ y los valores de zinc eritrocitario fueron $54,52 \pm 22,82 \mu\text{gZn/gHb}$ y $48,01 \pm 15,08 \mu\text{gZn/gHb}$, en los pacientes en hemodiálisis y en el grupo control, respectivamente. La actividad de la superóxido dismutasa fue significativamente inferior en los pacientes que en controles ($p < 0,05$).

Conclusiones: La actividad de la superóxido dismutasa en pacientes con enfermedad renal crónica en hemodiálisis, que es influenciada por la concentración del zinc, fue significativamente inferior. Hubo una respuesta inadecuada por la enzima al estrés oxidativo en pacientes en hemodiálisis.

(Nutr Hosp. 2011;26:1456-1461)

DOI:10.3305/nh.2011.26.6.5400

Palabras clave: *Enfermedad renal crónica. Hemodiálisis. Zinc. Superóxido dismutasa.*

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Recibido: 26-VI-2011.
1.ª Revisión: 15-VII-2011.
Aceptado: 18-VII-2011.

Abbreviations

EAR: Estimated Average Requirement.

SOD: Superoxide Dismutase.

Kt/V: Measure of Dialysis Adequacy.

Introduction

Chronic kidney disease is a clinical condition that is characterized by progressive and irreversible loss of kidney function, usually accompanied by an imbalance of metals, nutrients and electrolytes, which progresses to functional renal failure and manifests as uremia.¹ The uremic syndrome results from the accumulation of products that are created by the loss of glomerular filtration mechanisms, which are not purified by the dialytic procedures.²

Uremia is an important factor in the creation of reactive oxygen species in hemodialysis patients, and excessive production of these compounds appears to be due to the chronic inflammatory process which in turn is the result of uremic toxins and bioincompatibility of dialysis membranes.^{3,4} In addition, the presence of reactive oxygen species in these patients also seems to be associated with the reduction in plasmatic and intracellular concentrations of substances of the antioxidant defense system.⁵

The literature on this topic has reported alterations in the metabolism of minerals in patients undergoing hemodialysis, and researchers have shown most interest in the element zinc.⁶ Zinc is essential for human nutrition, as it acts as a structural and functional component of several metalloproteins, and participates in cellular metabolism reactions, including physiological processes, such as immune function, growth and development. Zinc is also an antioxidant that reduces free radicals.⁷

Studies have shown that there are alterations in the metabolic behavior of zinc in hemodialysis patients, and that these are usually associated with disturbances in the antioxidant defense system.^{8,9} Lower serum concentrations of zinc have been attributed to reduced food intake and intestinal absorption, uremic toxicity, interaction with calcium and iron, vitamin D deficiency and increased mineral loss during dialysis.¹⁰ Another important factor is the ability of zinc to bind to proteins modified by uremia, thus increasing its deficiency.¹¹

The activity and expression of superoxide dismutase appears to be modulated by the concentration of reactive oxygen species that result from uremia, and changes in the activity of this enzyme have been identified in hemodialysis patients. This enzyme participates in the antioxidant defense system, and plays a role in the dismutation of the superoxide anion radical to hydrogen peroxide.^{12,13}

Some clinical manifestations of chronic renal disease may be associated with an excessive production of reactive oxygen species that are created during ischemia/reperfusion. The reactions of free radicals in

the cell membrane result in changes in its structure, fluidity, permeability, transport, and antigenicity. These changes may affect the morbidity of chronic renal disease patients, and commonly result in symptoms such as cataracts, anemia, amyloidosis, platelet dysfunction and atherosclerosis.¹⁴

The objective of this study was to assess the nutritional status of zinc and the activity of superoxide dismutase in chronic kidney disease patients on hemodialysis. This theme was chosen because the morbidity and mortality rate of these patients is severe and due to the increased production of reactive oxygen species in these individuals, and the fact that zinc and superoxide dismutase participate in the antioxidant defense system.

Patients and methods

This was a cross-sectional, analytical and experimental study involving 134 individuals, aged between 18 and 85 years, of both genders, who were divided into two groups: experimental (hemodialyzed patients, n = 63) and control (healthy, n = 71). Patients were recruited from a dialysis clinic.

The patients were undergoing hemodialysis three times per week, with proportioning machines, bicarbonate bath and biocompatible capillaries, and water was treated using reverse osmosis. Patients were taking human recombinant erythropoietin and intravenous iron, with the goal of achieving a hematocrit of 33-36%, ferritin of 200-300 ng/mL and transferrin saturation of 20-30%.

Patients were selected according to the following study eligibility criteria: minimum of six months of hemodialysis; the presence of an arteriovenous fistula for permanent vascular access; Kt/V equal to or greater than 1.2, as a parameter of treatment adequacy;¹⁵ absence of the use of nutritional supplements and/or other drugs that could interfere in the assessment of the nutritional status of zinc; absence of diagnosis of diabetes, neurological diseases, thyroid diseases and/or liver disease; absence of clinical or surgical complications.

Evaluation of zinc intake

The intake of zinc was obtained by recording alimentation over a 3-day period, and the nutritional analysis was made using Nutwin software version 1.5.¹⁶ The Estimated Average Requirement (EAR) reference values of zinc used were 6.8 mg/day for female, and 9.4 mg/day for male.¹⁷

Collection of biological material and biochemical parameters

Blood samples (15 ml) were taken in the morning, from 7:30 to 8:30 o'clock, after fasting for at least 12

hours. The plasma was separated from the total blood by centrifugation at 1,831xg for 15 minutes at 4°C (Sorvall® 4K15 centrifuge). Three aliquots of each plasma sample were diluted at a ratio of 1:4 with Milli-Q® water and aspirated directly into the flame of the atomic absorption spectrophotometry.¹⁸ Tryptizol® (Merck), prepared by dilution with Milli-Q® water with 3% glycerol at 0.1, 0.2, 0.3, 0.5, and 1.0 µg/ml dilutions was used as a standard.

For the separation of the erythrocytes, the erythrocyte mass obtained from total blood was washed three times with 5 ml of 0.9% saline solution, homogenized by inversion and centrifuged at 2,493xg for 10 minutes (Sorvall® 4K15 centrifuge) at 4°C. After the last centrifugation, the saline solution was aspirated and the erythrocyte mass was carefully extracted using a micropipette, placed in demineralized eppendorfs tubes, and stored at -20°C, for zinc and hemoglobin analysis.¹⁹ To express the results in terms of mass zinc/mass of hemoglobin (µg/gHb), the erythrocyte lysed was measured according to the cyanmethaemoglobin method.²⁰

The analysis of erythrocyte zinc was carried out using atomic absorption spectrophotometry.¹⁹ Tryptizol® was used as a reference, prepared by dilution in Milli-Q® water at concentrations of 0.1, 0.2, 0.3, 0.5, and 1.0 µg/ml. The reference interval for plasma and erythrocyte zinc is 70-110 µg/dl and 40-44 µg/gHb, respectively.^{21,22}

The activity of superoxide dismutase enzyme in erythrocytes (Ransod Kit; Randox Laboratories Ltd., Crumlin, Antrin, UK) was determined, in triplicate, by the method *in vitro*, in a biochemical analyzer Lyasis, according to methodology recommended by the manufacturer. The reference interval for superoxide dismutase is 1,102-1,601 U/gHb, according to the Ransod/Randox Kit.

Statistical analysis

The data were analyzed using the SPSS software for Windows, version 10.0. The student's *t*, Mann-Whitney and Wilcoxon W tests were used to determine whether differences on the main study variables existed between groups. Pearson's correlation analysis was applied to find out the correlation of plasmatic and erythrocytary zinc and superoxide dismutase activity. Significance was established at $p < 0.05$ a priori for all statistical tests.

Results

The table I shows the clinical characteristics of dialysis patients and control group. Table II presents the results of the analysis of the diets consumed by the experimental group and control group. There was no statistically significant difference in food intake

Table I
Clinical characteristics of dialysis patients and control subjects

Parameters	Dialysis patients (n = 63) Mean ± SD	Control subjects (n = 71) Mean ± SD
Age (years)	50.0 ± 18.18	41.07 ± 12.90
Creatinine (mg/dL)	10.89 ± 4.28	–
Dialysis duration (months)	50.83 ± 40.01	–
*Kt/V	1.73 ± 0.43	–

*Handbook of Dialysis¹⁵.

Table II
Mean values and standard deviations for energy, macronutrients and zinc in the diet of patients undergoing hemodialysis and the control group

Energy/ Nutrients	Dialysis patients (n = 63) Mean ± SD	Control subjects (n = 71) Mean ± SD	Significance <i>p</i>
Energy (kcal)	1,376.37 ± 545.51	1,397.74 ± 430.02	0.801
Carbohydrates (%)	54.75 ± 9.20	55.44 ± 6.46	0.619
Proteins (%)	22.60 ± 6.36	22.18 ± 4.24	0.652
Fats (%)	22.66 ± 5.17	22.37 ± 4.78	0.734

Reference values: 10 to 30% of proteins, 45 to 65% of carbohydrates and up to 35% of fats¹⁷. There was no significant statistical difference between groups. Student's *t* test ($p > 0.05$).

between groups in terms of macronutrients and energy ($p > 0.05$). The mean of zinc intake of experimental group was 9.62 ± 3.93mg/d and 7.20 ± 3.30 mg/d, and of the control group was 12.94 ± 5.70 mg/d and 8.66 ± 2.57 mg/d for male and female, respectively ($p > 0.05$). In contrast, Raimundo et al²³ demonstrated a severe depletion in energy, protein and zinc in diets of hemodialysis patients.

Table III presents the mean values and standard deviations of plasma and erythrocyte zinc concentrations and the activity of superoxide dismutase in ery-

Table III
Mean values and standard deviations for the plasmatic and erythrocytary concentrations of zinc and the activity of superoxide dismutase for the experimental group and control

Parameters	Dialysis patients (n = 63) Mean ± SD	Control subjects (n = 71) Mean ± SD	Significance <i>p</i>
Plasmatic zinc (µg/dL)	62.02 ± 13.59*	65.58 ± 8.88*	0.036*
Erythrocytary zinc (µgZn/gHb)	54.52 ± 22.82	48.01 ± 15.08	0.077
SOD (U/gHb)	3,533.35 ± 1643.86**	4,120 ± 1375.18**	0.003**

*Significantly different values in the hemodialysis patients. Wilcoxon W and Mann-Whitney's test ($p < 0.05$). **Significantly different values between the experimental group and the control group. Student's *t* test ($p < 0.05$). SOD= enzyme superoxide dismutase.

Table IV
Correlation between plasmatic zinc and erythrocytary zinc and erythrocytary superoxide dismutase activity

Parameters	Correlation coefficient (r)	
	Dialysis patients	Control subjects
Plasmatic zinc X SOD	0.003	0.209
Erythrocytary zinc X SOD	0.716*	0.805*

SOD = enzyme superoxide dismutase. *Significant positive correlation ($p < 0.05$).

throcytes for the experimental and control groups. There is a significant difference regarding plasma zinc concentrations and superoxide dismutase activity between the groups ($p < 0.05$), but no statistical difference regarding the concentrations of erythrocyte zinc between the groups ($p > 0.05$).

Table IV presents the correlation coefficients for the biochemical parameters of zinc and activity of superoxide dismutase in experimental and control groups. There is a significant positive correlation between the erythrocyte zinc and the enzyme activity in both groups ($p < 0.05$).

Discussion

This study analyzed the parameters of the nutritional status of zinc and superoxide dismutase activity, and investigated the correlation between these variables.

For the results of plasmatic zinc concentrations, there was a statistically significant difference between the groups. Similar data have also been identified in other studies that also found reduced plasma zinc concentrations in patients undergoing hemodialysis.^{10,24,25}

The literature has identified several aspects that contribute to the alteration of plasma zinc of hemodialysis patients. The hemodialysis treatment and the disease's pathophysiology may contribute to the alteration in the mineral profile of these patients.

One of the main reasons for the alteration in plasma zinc is the concentration of zinc in the diets of patients who undergo hemodialysis. This concentration is usually lower due to anorexia and dietary restrictions.⁹ However, the patients in this study consumed the recommended levels of zinc, so this factor does not seem to have influenced the hypozincemia that was found.

The hemodialysis process is another factor that might have led to hypozincemia, since it impairs the excretion of trace elements, which are either retained by or spoiled from the body.¹¹

The concentration of trace elements in the dialysate is unknown. When their concentration in the dialysate is lower than in the blood, they may be removed during dialysis through semipermeable membranes. This can deplete biologically active minerals, such as zinc.²⁶

The mineral content of the water used in the dialysis sessions may also have interfered with the concentra-

tion of zinc in plasma. The levels of trace elements are not periodically controlled, and this could lead to changes in the concentration gradient between blood and dialysate for some trace elements, including zinc.^{9,27}

Another hypothesis that could explain the reduction of zinc in the plasma of patients in this study is the redistribution of body zinc. In this case, zinc seems to be transported from plasma to other tissues. According to some researchers, there is a higher expression of zinc transport proteins due to the presence of chronic inflammation.¹¹

IL-6 is produced during the chronic inflammatory response and induces the production of metallothionein, an intracellular chelator of minerals, which promotes the compartmentalization of zinc and makes hypozincemia more likely.²⁸

The reduction of zinc absorption has also been thought to be a factor in the hypozincemia of hemodialysis patients. This situation occurs due to the reduction in zinc efflux from the enterocytes into the plasma, as well as hypoproteinemia, problems with tubular reabsorption and calcitriol deficiency.²⁹

The reduction of zinc concentrations in the plasma of hemodialysis patients may also be explained by the antagonism between zinc and copper. These elements compete for the same binding sites with the protein metallothionein, which has a higher affinity for copper. This competition reduces the absorption of zinc and causes hypozincemia.³⁰

Iron deficiency may also be an important factor in the redistribution of zinc among the tissues in patients with chronic renal disease. A low level of iron results in a higher concentration of erythrocytary zinc and a lower concentration of plasmatic zinc. This might have influenced the results of this study. Therapy with intravenous iron helps to correct this compartmentalization.³¹

Plasmatic zinc is a useful parameter for diagnosing zinc's nutritional status, since it significantly changes according to any intervention that is carried out.³² Plasmatic zinc accounts for only 0.1% of the body's zinc content, but is the primary source of this mineral for all cells. Zinc's dynamics keep it under constant homeostatic control.³³

Regarding the results of erythrocytary zinc, there was no significant statistical difference between the groups studied. The mean concentrations of zinc found in the erythrocytes of hemodialysis patients were normal. Erythrocytes have a mean life of 120 days, and so erythrocytary zinc is used to evaluate the previous nutritional status of the mineral. Erythrocytary zinc concentration decreases by only 21% after the ingestion of diets with a low level of this mineral for a period of 90 days.²⁰

However, there are differences between the results of studies that use this marker to diagnose zinc's nutritional status in hemodialysis patients. These differences may be due to the interference of the matrix, and the difficulty of separating biological material and measuring erythrocytary hemoglobin, which may be altered due to associ-

ated chronic or acute diseases.²⁰ Some studies have found high levels of erythrocytary zinc in patients with chronic kidney disease^{11,30} while others have found lower concentrations of this parameter.³⁴

The definition of the nutritional status of zinc is still considered challenging for many researchers because of the lack of a sensitive, practical and specific method to determine it.³⁵ Experts recommend the use of different methods in order to obtain a more accurate diagnosis of the nutritional status of zinc in chronic kidney disease patients.

Regarding the activity of superoxide dismutase, there was a significant statistical difference between the groups. Patients with chronic renal disease undergoing hemodialysis had a reduced activity of this enzyme. These results are consistent with other studies.³⁶⁻³⁸

Some factors are thought to play an important role in the alterations of superoxide dismutase activity in these patients, including the deficiency of essential trace elements for its activation, such as zinc. The increase in the production of superoxide and hydrogen peroxide ions that is found in uremia and the exposure to elements such as aluminum, silicon and iron during dialysis, also contribute to the reduction.^{39,40}

There was a significant positive correlation between the erythrocytary zinc and the activity of superoxide dismutase, both in patients with chronic renal disease and in the controls. This result may be explained by the fact that zinc is a cofactor of the enzyme superoxide dismutase, and is an important antioxidant.

The alterations in mineral concentrations and in the activity of enzymes involved in the antioxidant defense system contribute to the manifestation of oxidative stress.⁸ Uremic syndrome is considered to be a pro-oxidant condition, since it increases the levels of lipid peroxidation and decreases antioxidant activity,^{41,42} while hemodialysis can lead to a loss of antioxidant substances of low molecular weight through high permeability membranes.⁴³

The interaction between dialysis membranes and blood neutrophils may activate the production of free radicals, such as superoxide anion, hydrogen peroxide and myeloperoxidase. These molecules contribute to the oxidation of lipids, proteins and nucleic acids.⁴⁴

Another important aspect concerns the role of leukocyte activation induced by hemodialysis in the creation of reactive oxygen species. The hemoreactivity of membranes and the hemoincompatibility of components of the dialysis system are considered to be the main factors involved in the formation of excessive free radicals.⁴⁵

Oxidative stress can also be expressed by blood contamination by endotoxins in the dialysis fluid, by repeated contact of blood with artificial materials in the extra corporeal circuit, with activation of the complement system, and also by the infusion of large amounts of intravenous iron during the hemodialysis sessions.⁴⁶

The oxidative stress of the patients in this study could have increased the activity of erythrocytary

superoxide dismutase. However, this probably did not occur because of the reduced plasmatic zinc concentrations in these patients, but was due to the chronic kidney disease and the hemodialysis treatment.

Further studies should be carried out to clarify the mechanisms involved in the compartmentalization of zinc and in the manifestation of oxidative stress in chronic kidney disease patients undergoing hemodialysis. These studies could also evaluate the possibility of supplementation with zinc, in order to develop approaches that improve the treatment of chronic kidney disease.

Conclusion

Patients with chronic renal disease undergoing hemodialysis evaluated in this study had reduced concentrations of plasma zinc, which may have influenced the activity of superoxide dismutase, since there was no adaptive and compensatory response of this enzyme to oxidative stress.

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