Determination of trace elements in the diet of non-dialyzed renal patients

Determinação de elementos traço na dieta de pacientes renais não dialisados

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ABSTRACT

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Patients with chronic kidney disease (CKD) often have abnormal levels of plasma minerals. Among some factors that may account for an abnormal mineral metabolism in this group is the low or high dietary intake. However, the mechanism of trace-element disturbances has not been thoroughly established. The purpose of this study was to evaluate the concentrations of eleven trace elements (As, Br, Co, Cr, Cs, Fe, Rb, Se, Th, U and Zn) in the diet of non-dialyzed CKD patients. While Fe, Co, Cr, Br, Rb, Se and Zn are elements playing a role in nutrition, As, Cs, Th and U are toxicologically important trace elements. These elements were determined using Instrumental Neutron Activation Analysis (INAA) on samples of total diets consumed by typical non-dialyzed CKD patients in Brazil. Each subject's food consumption was calculated by using an individual 24h-consumption record and the diets were prepared according to a modified duplicate portion method based on the analysis of 39 diet samples. In this group, Br, Co, Fe, Se and Zn intakes were lower and there was a positive correlation with protein intake. The intakes of toxic elements were within recommended levels. The patient's diets presented low concentrations of important trace elements, which can cause some alterations in the metabolism. The supplementation with a determined trace element may be indicated when its depletion is documented in body compartments, and when there is evidence of the positive effects of this element on the life quality of CKD patients.

**Keywords: Nutrition. Diet.
Chronic kidney disease.
Trace elements. Instrumental Neutron
Activation Analysis.**

RESUMEN

Pacientes con enfermedad renal crónica (ERC) pueden presentar niveles anormales de minerales. Uno de los factores que puede contribuir para este metabolismo anómalo es la dieta. Entretanto, los mecanismos de esas anormalidades no están completamente comprendidos. El objetivo de este estudio fue evaluar las concentraciones de once elementos trazos (As, Br, Co, Cr, Cs, Fe, Rb, Se, Th, U y Zn) en la dieta de pacientes con ERC no dializados. Los minerales como Fe, Co, Cr, Br, Rb, Se y Zn desempeñan funciones nutricionales importantes, al contrario de As, Cs, Th y U que son tóxicos. Esos elementos fueron determinados en 39 muestras de dietas obtenidas del consumo alimentar de 24 horas relatado por los pacientes con ERC y el método analítico utilizado fue Análisis Instrumental por Activación de Neutrones (INAA). Las concentraciones de Br, Co, Fe, Se y Zn eran bajos y hubo correlación positiva con el consumo de proteínas. La ingestión de elementos tóxicos estaba dentro de lo permitido. La dieta de los pacientes presentó bajas concentraciones de elementos trazos importantes lo cual podría causar alteraciones metabólicas importantes que exigirían suplemento en caso que los niveles sanguíneos alterados fuesen confirmados. Los elementos tóxicos no estaban en concentraciones elevadas.

Palabras clave: Nutrición. Dieta. Enfermedad renal crónica. Elementos trazos. Análisis Instrumental de Activación de Neutrones.

RESUMO

Pacientes com doença renal crônica (DRC) podem apresentar níveis anormais de minerais. Um dos fatores que pode contribuir para esse metabolismo anormal é a dieta. Entretanto, os mecanismos para essas anormalidades não são completamente entendidos. O objetivo desse estudo foi avaliar as concentrações de onze elementos traço (As, Br, Co, Cr, Cs, Fe, Rb, Se, Th, U e Zn) na dieta de pacientes com DRC não dialisados. Os minerais como Fe, Co, Cr, Br, Rb, Se e Zn são elementos com importantes papéis na nutrição. Em contrapartida As, Cs, Th e U são tóxicos. Esses elementos foram determinados em 39 amostras de dietas obtidas do consumo alimentar de 24h relatado pelos pacientes renais crônicos, e o método utilizado para verificação dos minerais foi pela Análise Instrumental de Ativação de Nêutrons (INAA). Os níveis de Br, Co, Fe, Se e Zn foram baixos e houve correlação positiva com o consumo protéico. A ingestão de elementos tóxicos estava dentro das recomendações. A dieta dos pacientes apresentou baixas concentrações de importantes elementos traço, o que poderia causar algumas alterações no metabolismo desses nos pacientes renais, sendo a suplementação necessária se os níveis sangüíneos alterados fossem confirmados. Os elementos tóxicos não estavam em elevadas concentrações.

Palavras-chave: Nutrição. Dieta. Doença renal crônica. Elementos traço. Análise Instrumental de Ativação de Nêutrons.

INTRODUCTION

Many trace elements have been reported to be either essential or beneficial to humans. All essential minerals have a regulatory pathway that maintains optimal tissue concentration in spite of variations in dietary supply. Some elements, such as Cu, Mn and Zn, are essential micronutrients with a human requirement of no more than a few milligrams per day. However, micronutrients may become harmful when their ingestion rates are too high, such as Cd and Pb which are well known as toxic if their intake through ingestion or inhalation is excessive (WORLD HEALTH ORGANIZATION, 1996).

Also, deficiencies, excesses, or imbalances in the supply of inorganic elements from dietary sources can have an important deleterious influence on human health (SANTOS et al., 2004).

It has been reported that patients with chronic kidney disease have an abnormal metabolism of zinc, selenium and many other minerals (KALANTAR-ZADEH; KOPPLE, 2003; ZIMA et al., 1998). The reasons for these abnormalities are not clear, and the blood and tissue levels of these elements may be affected in renal patients by several factors, including renal excretory function, environmental and occupational exposure, the concentrations in dialysate, and possibly the mode of dialytic therapy (KALANTAR-ZADEH; KOPPLE, 2003; MAFRA; ABDALLA; COZZOLINO, 1999; ZIMA et al., 1998). However, the decrease in mineral dietary intake may contribute to alter the metabolism of these trace elements.

Patients on special low-protein diets take an additional risk of developing mineral deficiencies because foodstuffs rich in trace elements (e.g. meat, milk, sea fish and eggs) are often restricted in such diets (MAFRA et al., 2004). On the other hand, renal patients can be exposed to a variety of contaminants from the environment and some of these are considered harmful, such as arsenic, thorium and uranium (VANHOLDER et al., 2002).

There are many problems involved in measuring trace-element intakes, like lack of sensitive and specific analytical techniques and the difficulty in obtaining reliable information on food consumption. The duplicate portion method has been used to study the trace-element intakes for institutional surveys due to the cost and time involved (MAIHARA et al., 1998).

The recommended dietary allowances (RDA) (FOOD AND NUTRITION BOARD, 2001) and adequate intakes of micronutrients are well studied in the general population. However, dietary allowances are less accurately examined in uremic patients (KALANTAR-ZADEH; KOPPLE, 2003).

Deficiency of trace elements is commonly observed in hemodialyzed patients, but there are few studies with pre-dialysis patients. The present study provides the dietary intakes of trace elements (Fe, Co, Cr, Br, Rb, Se and Zn) and some toxic trace elements (As, Cs, Th and U) from a group of non-dialyzed CKD patients in Brazil.

PATIENTS AND METHODS

GROUP SELECTION

This study was performed with 39 stable non-dialyzed CKD patients at the average age of 54.7 ± 16.1 years and with an average creatinine clearance of 34.6 ± 13.4mL/min, treated at the outpatient clinic of the Division of Nephrology at the Federal University of São Paulo. Subjects younger than 18 years old or suffering from diabetes mellitus or autoimmune, malignant or infectious diseases were not admitted into the research. During the study protocol, all patients were instructed to eat approximately 35Kcal/Kg/day and 0.6g to 0.8g of protein/Kg/day.

The study protocol was reviewed and approved by the Ethics Committee of the Federal University of São Paulo (UNIFESP). All patients signed an informed consent.

BIOCHEMICAL PARAMETERS AND BMI

Biochemical variables were measured by standard techniques. Body mass index (BMI) was calculated according to Keys, Fidanza and Kcarvonen (1972). The overall compliance with dietary protein prescriptions has been assessed by calculation of protein equivalent of nitrogen appearance (PNA) using Maroni's formula (MARONI; STEINMAN; MITCH, 1985).

MODIFIED DUPLICATE PORTION TECHNIQUE (DIET COLLECTION)

Each patient handed in daily 24-hour food-consumption records. Before the collection of food records, each patient attended a standard session on how to keep the records, including instructions on how to estimate the portion sizes. After this, all patients' meals were prepared according to their records in the Laboratory of Experimental Nutrition of the University of São Paulo, mixed and homogenized in a domestic blender demineralized in nitric acid. The meals included solid food and beverages, but did not include drinking water. After this step, the meals were freeze-dried in a large equipment (FTS SYSTEMS™) and again mixed and homogenized in a blender. The dried samples were kept in demineralized polyethylene bottles for analysis. For analytical purposes, each sample was divided into 2 portions. One of the portions was kept at the laboratory for macronutrient analysis and the other one was sent to the Neutron Activation Laboratory of IPEN/CNEN-SP for determination of trace elements.

DETERMINATION OF THE PROXIMATE COMPOSITION

The diets were analyzed for their moisture content at 105°C, fixed mineral residue (550°C), protein (micro Kjeldahl method) and ether extract (Soxhlet method), according to the methodology of the Association of Official Analytical Chemists (1990). The carbohydrate amount was obtained as the difference between total dry weight and the sum of protein, fat and ash content. All samples were sent to the Neutron Activation Laboratory of IPEN/CNEN-SP for the determination of trace elements.

INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS (INAA)

Aliquots (about 200mg) of diet samples and reference material (weighed in previously cleaned polyethylene bags) with the synthetic standards were placed into aluminum containers and irradiated in the IEA-R1 nuclear research reactor. Samples and standards were irradiated for 8 hours under a thermal neutron flow of 10^{12} – 10^{13} $n.cm^{-2}.s^{-1}$. After different cooling times, the following radioisotopes were determined: ^{76}As, ^{82}Br, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{59}Fe, ^{86}Rb, ^{75}Se, ^{239}Np (U), ^{232}Pa (Th) and ^{65}Zn.

The gamma-ray spectra were obtained using an ORTEC EG&G counting system (high resolution solid state Ge detector, type POP TOP, Model 20190) with a resolution of 1.9keV and a 1,332keV peak of γ-ray from ^{60}Co. This detector was coupled to an EG&G ORTEC ACE8K card and associated electronics. Spectrum analysis was performed using the VISPECT2 software in TURBOBASIC language.

Validation of the methodology - The following reference materials were used for checking the precision and accuracy of the method: Typical Diet (SRM NIST 1548^a), Peach Leaves (SRM NIST 1547) and Orchard Leaves (SRM NIST 1541).

STATISTICAL ANALYSIS

Data are given as mean ± SD, unless otherwise stated. Correlations were sought using linear regression analysis or Spearman's rank correlation coefficient for nonparametrically distributed data. All statistical analyses were carried out using a SPSS statistical analysis computer package (Version 8.0). A *p value* <0.05 was considered significant.

RESULTS

The main demographic, clinical and biochemical characteristics of the patients are shown in table 1. The patients' age ranged from 18 to 79 years and creatinine clearance from 14.0 to 62.0mL/min/1.73 m^2. BMI ranged from 20.5 to 41.6Kg/m^2. The analysis of the diets showed a protein intake of 0.89 ± 0.2g/Kg/day. The intakes of protein and other nutrients are shown in table 2.

The estimated intakes of essential elements were compared to the Recommended Daily Allowances (FOOD AND NUTRITION BOARD, 2001), while the estimated intakes of potentially toxic elements were compared to reference according to World Health Organization (1996) (Tables 3 and 4).

Table 1 – Demographic, clinical and biochemical characteristics of the patients

N	**39**
Gender (m/f)	25/14
Age (y)	54.7 ± 16.1
Creatinine clearance (mL/min/1.73m^2)	34.6 ± 13.4
Plasma creatinine (mg/dL)	2.7 ± 1.1
BMI (kg/m^2)	25.3 ± 4.5
Etiology of CKD	HA=26, PKD=3, CG=4, Other=7

HA = Hypertension, PKD = Polycystic kidney disease, CG = Chronic glomerulonephritis.

Table 2 – Daily intakes of energy and macronutrients in the CKD patients' diets and recommended levels

Energy / Nutrients	Patients	Recommended level
Energy (kcal/d)	1444 ± 409 (799 – 2427)	–
Energy (kcal/kg/d)	26.2 ± 7.7 (13.7 – 39.7)	30 - 35
Protein (g/kg/d)*	0.89 ± 0.2 (0.4 – 1.4)	0,6 – 0,8
PNA (g/kg/d)	0.96 ± 0.26 (0.55 – 1.66)	0,6 – 0,8
Carbohydrate (%)	64.4 ± 7.9 (46.3 – 81.2)	Rest of calories
Lipids (%)	20.4 ± 7.0 (7.1 – 36.7)	30

*There was no statistical difference from the PNA results.

Table 3 – Daily intakes of trace elements in the CKD patients' diets and recommended levels (FOOD AND NUTRITION BOARD, 2001)

Element	Mean ± SD	Range	Recommended level
Br (mg/day)	1.75 ± 0.73	0.61 – 3.7	2 – 8
Co (µg/day)	11.5 ± 6.0	1.12 – 26.9	15
Cr (µg/day)	35.6 ± 14.6	8.56 – 63.8	20 to 30
Fe (mg/day)	6.28 ± 2.5	2.7 – 12.4	8 –18
Rb (mg/day)	4.26 ± 7.23	1.21 – 47.8	1 – 5
Se (µg/day)	22.8 ± 11.8	10.2 – 48.1	55
Zn (mg/day)	6.09 ± 2.3	2.9 – 12.4	8 - 11

Table 4 – Daily intakes of toxic trace elements in the CKD patients' diets and recommended levels (WORLD HEALTH ORGANIZATION, 1996)

Element	Mean ± SD	Range	Recommended level
As (μg/day)	54.0 ± 32.0	14.0 – 106.9	140.0
Cs (mg/day)	23.3 ± 21.7	4.9 – 119.4	ND
Th (μg/day)	1.7 ± 1.45	0.35 – 4.51	< 3.0
U (μg/day)*	2.56 ± 0.98	1.27 – 4.87	36.0

The recommended level is 0.6μg/Kg (people weighing an average 60Kg).
ND – Not Determined.

The data obtained in the present work showed a positive correlation between the intakes of protein and zinc (r= 0.66, *p* = 0.0001), iron (r= 0.44, *p* =0.005), selenium (r= 0.5, *p* =0.01), rubidium (r= 0.45, *p* =0.003) and cobalt (r= 0.42, *p* =0.01).

DISCUSSION

In patients with uremia, trace-element disturbances might occur because of (a) reduced renal function; (b) proteinuria, leading to losses of protein-bound elements; (c) alterations in the gastrointestinal absorption; (d) the dialysis procedure itself and/or reduced intake (D'HAESE et al., 1999). Animal sources of protein are primarily reduced when low-protein diets are prescribed. This can cause not only a quantitative fall in mineral intake, but also a fall in the percentage of the ingested minerals that is absorbed, as the percentage of minerals absorbed from animal protein is much higher than the percentage absorbed from vegetable protein. In the present work, it could be concluded that protein restriction caused reduction of some minerals in the diet.

Some works show that Br plasma levels tend to decrease with renal disease (THOMSON et al., 1983). But there are no studies about Br intake in CKD patients and the present research showed a reduction in Br intake by uremic patients (1.75 ± 0.73mg/day). However, bromide has not been conclusively shown to have any essential function in plants or animals (PAVELKA, 2004).

Pehrsson and Lins (1983) determined the concentration of 23 trace elements in heart tissue in a postmortem study on 8 uremic patients and observed that cobalt may be an important etiological agent in uremic heart failure. In the present work, some patients showed a Co intake lower than the recommended level.

The ultratraces like Rb are a disparate group in terms of their possible nutritional importance for human health and welfare, and probably they have some bits of creditable evidence to suggest, at least positive effects under some situations (NIELSEN, 1996). Some works reported similar findings and noted a decreased concentration of Rb in plasma and erythrocytes in CKD patients (ALLAIN et al., 1984; CANAVESE et al., 2001). However, Rb intake is not emphasized in these researches and a normal intake was observed in the present work.

Canavese et al. (2001) observed Rb deficiency in uremic patients and concluded that further studies are needed to clarify the roles of many factors in Rb deficiency, including the effects of uremia itself, pre-dialysis factors (diet, impaired renal function and drugs), dialytical procedures (frequency, length, diffusive/convective components) or other biochemical/clinical parameters (hemoglobin, body mass index, age). The finding of Rb deficiency in uremia is important as it plays a role in neurobehavioural functions, mainly as an antidepressant.

Selenium, as a component of glutathione peroxidase active site, plays an important role in protecting cell membranes from oxidative damage. Decreased blood Se plasma levels and glutathione peroxidase (GSH-Px) activity are common in CKD patients (ZACHARA et al., 2004). In this study, Se intake was quite low (22.8 ± 11.8μg/day). Other studies about daily Se intake carried out with different population groups in the city of São Paulo (Brazil) have shown very low levels. One of the reasons can be attributed to the low Se content in soils of this region (MAIHARA et al., 1998; ZACHARA et al., 2004).

Selenium deficiency is suspected in dialyzed patients and selenium supplementation may be beneficial for CKD patients due to the increasing in glutathione peroxidase activity, cardioprotective effect and immunostimulatory properties (FÁVARO et al., 1994; KALANTAR-ZADEH; KOPPLE, 2003; ZIMA et al., 1999). Further studies are necessary to confirm this deficiency in non-dialyzed patients before prescription of Se supplementation.

Zinc is an element which plays an important role as a component of proteins, enzymes and antioxidants in biological systems. Alterations in blood and tissue concentrations of trace elements in patients with chronic kidney disease have been extensively investigated (PIETRZAK; BLADEK; BULIKOWSKI, 2002).

Some studies observed low Zn intake. Blendis et al. (1981) observed a Zn intake of 8.2mg/day, while Komindr et al. (1997) related an ingestion of 3.2mg/day in hemodialyzed patients. In the present work, the patients' intake was about 6.09 ± 2.3mg/day.

A low-protein diet reduces zinc intake and is correlated with meat consumption. Zinc supplementation may be recommended for patients with proven zinc deficiency. However, it is doubtful whether it applies to all chronic kidney disease patients, since serum zinc concentrations are often low and erythrocyte zinc is often elevated in these patients (MAFRA et al., 2004).

Anemia is an almost invariable feature of progressive renal disease and its most important cause is decreased production of erythropoietin, but reduced iron intake may also contribute to anemia. In the present work, a reduced iron intake (6.28 ± 2.51mg/day) was observed.

The prevalence of anemia increases as kidney function decreases and certain subgroups are at increased risk of anemia. Several studies have recently begun to investigate the effects of preventing progressive renal anemia (McCLELLAN et al., 2004).

Patients in the pre-dialytical period have reduced intestinal iron absorption. At least part of this reduced absorption could be explained by the changes in the intestinal mucosa observed in uremia. Moderate to severe histological changes, including reduction of villous height, increased crypt depth, and infiltration of inflammatory cells, as well as functional changes such as decreased activity of dipeptidases and increased activity of disaccharidases, are commonly seen in the small intestines of patients with CKD (SILVERBERG et al., 1999).

On the other hand, several trace elements have been implicated in the decline of renal function. These elements include arsenic, cadmium, copper, germanium, lead and mercury (VANHOLDER et al., 2002). Gawlik et al. (1989) observed high Cs concentration in renal patients, although it has not yet been possible to distinguish whether these changes in blood cesium levels in the patients are due to a decrease in renal excretion or only a secondary effect of unnoticed changes in dietary habits.

There are not available data about As intake in renal patients, but the results obtained in the present work were very similar to other studies with Brazilian regional diets, where the As intake was below 200μg/day[21], and lower than the WHO recommended value (WORLD HEALTH ORGANIZATION, 2004).

Chromium intake was above the recommended levels (20 a 30mg/day). Some works have shown serum chromium concentration in CKD patients significantly higher than in healthy subjects (MALECKA et al., 1995).

The daily exposure to thorium was estimated by the International Commission on Radiological Protection (ICRP) at 3μg/day (LIDE, 2004). The average values obtained in this study (1.7 ± 1.45μg/day) are in agreement with the value set by the ICRP.

Uranium is one of the primordial radioactive elements widely distributed in the earth's crust, and the hazards due to high intake of this element are double – chemical toxicity and radiological damage (BERLIN; RUDELL, 1986). Uranium intake (2.56 ± 0.98μg/day) was low when compared with the "Tolerable Daily Intake" of 0.6μg/Kg (average 36μg/day) (WORLD HEALTH ORGANIZATION, 2004).

There are several limitations in the present study. First, not having measured BMI for nutritional status and not having analyzed plasma levels of trace elements are to acknowledge, and since plasma concentrations were not known, it was not possible to conclude whether this intake is enough, too much or not enough. Another important limitation is the number of patients.

CONCLUSIONS

Besides a low-protein diet and careful analysis of macronutrients and energy, the nutritional strategies for treating CKD patients should include measures such as the control of mineral intake. In spite of little information about the matter, minerals are important in the diet therapy for these patients.

This paper reports on a sensitive and reliable method for analysis of trace elements in diet samples. From the results presented it can be concluded that the daily dietary intakes of Br, Co, Fe, Zn and Se were below the recommended levels and the intake of the analyzed toxic elements were within the recommended levels. So, it is important to analyze the relationship between mineral intake and metabolism in these patients. Supplementation with a trace element may be indicated when its depletion has been documented and there is evidence of the positive effects of this element on the CKD patients' life quality.

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