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Effect of End-Stage Renal Disease and Diabetes on Zinc and Copper Status

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ABSTRACT

The aim of this study was to compare the nutritional status of zinc and copper in patients with and without diabetes submitted to chronic hemodialysis. Thirty-three patients with type 2 diabetes (DM group), 30 nondiabetic patients (NDM group), and 20 healthy individuals (control group) were studied. Plasma, erythrocyte, and urinary zinc and plasma copper were obtained from atomic absorption spectrophotometry and ceruloplasmin by immunonephelometry. The anthropometric parameters were similar among the groups. Plasma zinc was lower and erythrocyte zinc was higher in the DM and NDM groups in relation to the control group. No difference in urinary zinc was observed comparing the groups. Plasma copper was higher in the DM group when compared to the NDM and control groups. Ceruloplasmin was similar in the three groups. Serum urea was a positive independent determinant of plasma zinc concentrations. The determinants of erythrocyte zinc were MAMC midarm muscle circumference and Kt/V dialysis adequacy. The determinants of plasma copper concentration were serum creatinine and serum glucose. The results of this study demonstrate an alteration in the distribution of zinc in

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patients with chronic kidney disease (CKD) independently of the presence of DM. Also, the status of copper seems not to be influenced by CKD, but only by the metabolic derangements associated with diabetes.

Index Entries: Zinc; copper; chronic kidney disease; hemodialysis; diabetes.

INTRODUCTION

Alterations in the metabolism of zinc and copper have been frequently observed in patients with chronic kidney disease (CKD) and in those with diabetes *(1–5)*. These elements have important roles in the biological systems, as components of proteins, enzymes, and antioxidants *(6,7)*. Anorexia, low taste sensibility, hipogeusia glucose intolerance, healing difficulties, and anemia are common features of both CKD and diabetes that can be associated with copper and zinc abnormalities *(4,7–9)*.

The majority of the studies that evaluated the status of these two elements in hemodialysis patients excluded individuals with diabetes, so that scarce information exists on the effects of the association between CKD and diabetes in the metabolism of zinc and copper. The aim of the present study was to compare the nutritional status related to zinc and copper in patients with and without diabetes submitted to chronic hemodialysis.

PATIENTS AND METHODS

This study enrolled 63 outclinic patients with CKD submitted to chronic hemodialysis, 33 of them with diabetes (DM group) and 30 without diabetes (NDM group). There was also a control group with 20 healthy individuals from the clinic staff. Patients under 18 yr of age, with lupus, neoplasias, positive human immunodeficiency virus, and infection were excluded.

The patients were submitted to a 4-h dialysis session, three times per week, using dialyzers of acetate cellulose membranes (Baxter, USA). All patients were dialyzed through an arteriovenous fistula using a blood flow rate of 250 mL/min and a dialysate flow rate of 500 mL/min.

The research project was performed in collaboration with Federal Universities of Rio Grande do Norte and São Paulo. The study was approved by the Ethic Committee of the Federal University of São Paulo and an informed consent was obtained from each subject.

All selected patients were submitted to the evaluations discussed in the following subsections.

Anthropometric Data

The measurements were performed after the dialysis session by the same observer on the opposite side of the vascular access. The following anthropometric parameters were evaluated: weight, height, mid-arm muscle circumference (MAMC), and triceps skinfold thickness (TSF). Body mass index (BMI) was calculated as body weight divided by the height squared *(10).* Ideal body weight was obtained from the Metropolitan Life Insurance tables modified by Grant *(11).* The MAMC was calculated through measures of mid-arm circumference and TSF. The adequacy of TSF and MAMC were obtained using the National Health and Nutrition Examination Survey (NHANES) percentiles distribution tables adapted by Frisancho *(12).*

Subjective Global Assessment

Subjective global assessment (SGA) was performed after the hemodialysis session by the same observer. This method is based on medical history and physical examination as described by Detsky et al. *(13).* Based on these evaluations, patients were classified as well nourished, mild/moderate malnourished, or severely malnourished.

Dietary Evaluation

Food consumption was assessed by a 4-d food diary including 1 d of hemodialysis and one weekend day. Nutrient intake was calculated using the software Virtual Nutri, version 1.0 *(14).* Complementary tables of food composition were used *(15,16).* Protein intake was also estimated from the protein equivalent of total nitrogen appearance (PNA). PNA was obtained using the formula recommended by the National Kidney Foundation Dialysis Outcomes Quality Initiative (K/DOQI) Practice Guideline on Nutrition *(17).*

Laboratorial Parameters

Blood samples were drawn after fasting and before dialysis for the following: hemoglobin, serum glucose, glycated hemoglobin (HbA1c), urea, creatinine, serum iron, transferrin, ferritin and albumin. Glucose, urea, and creatinine were measured in a 24-h urine collection. Dialysis adequacy was assessed by the calculation of Kt/V, calculated by the natural logarithm formula *(18).*

The determinations of ceruloplasmin were obtained by immunonephelometry (Beckman, Ireland). The normal concentrations were considered to be 20–60 mg/dL.

Zinc and Copper Determinations

A blood sample was obtained in a deionized tube containing sodium citrate at 30% as a coagulant. All determinations were performed by atomic absorption spectrophotometry in a Spectra Varian AA-200 model, calibrated with a 324.8-nm wave length, an opening of 0.5 nm, expansion factors of 1.0, and a sample flux of 10 mL/min.

The determination of zinc in the plasma was performed according to the Rodriguez method *(19);* the normal range is 70–110 μg/dL. Erythrocyte zinc was determined according to Cordeiro *(20)*; reference values are 40–44 μg/g Hb. Urinary zinc was obtained by the Kiilerich method modified by Pedrosa *(21,22)*, and normal values are 300–600 μg/24 h.

Plasma copper determinations were performed using the method proposed by Walter *(4)*, normal values are 70–110 μg/dL.

Plasma and urine zinc and copper determinations were performed in duplicate and erythrocyte zinc in triplicate, and were repeated when the coefficient of variation was above 10%. The final result was established from the average of all of the obtained values.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation. The comparisons between the two groups of patients were analyzed by the Student's *t*-test for independent samples and for comparisons among the three groups analysis of variance (ANOVA) and post hoc Tukey test for multiple comparisons were used according to indication. Categorical variables were described using proportions and were analyzed by the χ^2 test or Fisher's test when indicated. Correlation coefficients were calculated using Pearson's test, and multiple regression analysis was applied to assess which variable is independently associated to zinc and cooper parameters. The significance level was fixed at $p < 0.05$. All tests were performed using the True Epistat 5.0 program (Tracy L. Gustaffson, Epistat Services, Richardson, TX).

RESULTS

The main characteristics of the three studied groups are shown in Table 1. Patients in the DM group were older compared to the other two groups. The DM patients were also on dialysis treatment for a longer period of time; however, the difference did not achieve statistical significance ($p = 0.07$). The main causes of CKD in the DM group were diabetic nephropathy ($n = 29$; 88%), hypertensive nephropathy ($n = 2$, 6%), and undetermined ($n = 2$; 6%). In the NDM group, the main causes of CKD were chronic glomerulonephritis ($n = 11$; 37%), hypertensive nephropathy ($n = 9$; 30%), undetermined ($n = 6$; 20%), and other ($n = 4$; 13%). The hematocrit was significantly lower in the DM group; however transferrin saturation was significantly higher. As expected, serum glucose and HbA1c were significantly higher in the DM group. No difference between the DM and NDM groups was found regarding gender, urea, creatinine, hemoglobin, iron, ferritin, and Kt/V.

According to the subjective global assessment, no difference between the DM and NDM groups was found. Mild/moderate malnutrition was present in 16 (49%) of the patients in the DM group and in 13 (43%) in the

Table 1
Demographic, Clinical, and Biochemical Characteristics of Patient and Control Groups

GROUPS	DM	NDM	CONTROL
	N= 33	N= 30	N=20
Sex (M/F)	16/17	15/15	8/12
Age (years)	61.1 ± 8.1^{a,b}	53.3 ± 14.4	52.9 ± 9.7
Time of dialysis (months)	29.0 ± 27.4	43.3 ± 33.5	
Time of diabetes (months)	196 ± 105.2		
Urea (mg/dL)	155.8 ± 57.7	158.0 ± 48.8	
Creatinine (mg/dL)	10.0 ± 3.6^{b}	12.0 ± 3.9^{c}	0.9 ± 0.2
Hemoglobin (g/dL)	9.1 ± 2.2	10.2 ± 2.1	
Hematocrit (%)	26.3 ± 4.9^{a}	29.4 ± 3.0	
Iron (μg/mL)	131.6 ± 134.0	97.7 ± 57.1	
Ferritin (μg/mL)	474.3 ± 467.9	436.1 ± 416.4	
Transferrin saturation (%)	41.9 ± 14.0^{a}	30.7 ± 13.2	
Serum glucose (mg/dL)	137.5 ± 80.5^{a,b}	91.0 ± 12.1	86.3 ± 8.76
HbA1c (%)	9.1 ± 1.7^{a,b}	7.7 ± 1.1	7.0 ± 0.7
Kt/V	1.2 ± 0.3	1.1 ± 0.2	

Note: Values are given as mean ± SD For comparisons among the three groups, the ANOVA test with the post hoc Tukey, test was used. For comparisons between patients groups analysis, the *t*-test was used. $p < 0.05$: a = DM vs. NDM; b = DM vs. control; c = NDM vs. control.

NDM group and severe malnutrition in 4 (12%) and 2 (7%) patients, respectively.

As shown in Table 2, anthropometric parameters were similar among the groups, except for the BMI, which was higher in the control group. Dietary intake results of the three groups are shown in Table 3. A lower energy intake was observed in the DM group. Zinc intake in the DM group, for both males and females, was significantly lower than that in the control group. When the three groups were compared without considering gender, the zinc intake in the DM and NDM groups was significantly lower than in the control group (6.5 ± 4.2 vs. 6.6 ± 2.9 vs. 9.4 ± 5.0 mg/d, respectively; $p < 0.05$). Copper intake did not differ among the groups. Considering only the patients ($n = 63$), a positive correlation between PNA and zinc intake was observed ($r = 0.25$; $p = 0.049$). There was no relation between PNA and protein, energy, or copper intake. Furthermore, protein intake obtained by the dietary diaries did not correlate with zinc and copper intakes.

No differences between DM and NDM groups were observed regarding total serum protein (6.9 ± 0.9 vs. 6.8 ± 0.7 g/dL), albumin (3.8 ± 0.5 vs.

Table 2
Anthropometric Parameters of Patient and Control Groups

	DM	NDM	CONTROL
	N= 33	N= 30	N= 20
Current weight (kg)	64.4 ± 11.9	62.8 ± 14.1	68.7 ± 12.8
Ideal weight (kg)	55.9. ± 6.9	54.3 ± 6.4	56.0.±.8.1
Adequacy to weight (%)	114.9 ± 1.0	114.6 ± 17.6	122.6.±.13.4
BMI	24.3 ± 3.6[b]	24.6 ± 4.2[c]	27.5 ± 3.4
Adequacy to MAMC (%)	92.2 ± 15.5	87.5 ± 10.7	86.1 ± 8.0
Adequacy to TSF (%)	103.2 ± 73.5	125.3 ± 70.0	127.7 ± 45.7

Note: Values are given as mean ± SD ANOVA and post hoc Tukey, test were used. $p < 0{,}005$; a = DM vs. NDM; b = DM vs. control; c = NDM vs. control.

Table 3
Food Consumption and PNA of Patient and Control Groups

	DM	NDM	CONTROL
	N= 33	N= 33	N= 20
Energy kcal/kg/day	30.3 ± 8.4[b]	34.1 ± 8.8	38.5 ± 11.4
Protein (%)	19.2 ± 4.4[a]	16.9 ± 3.1	17.3 ± 2.2
Carbohydrtes (%)	52.6 ± 8.1	53.2 ± 7.1	54.1 ± 6.0
Lipids (%)	27.0 ± 6.6	30.2 ± 6.2	28.7 ± 4.7
Protein (g/kg/day)	1.4 ± 0.5	1.4 ± 0.4	1.6 ± 0.5
PNA (g/kg/day)	1.1 ± 0.3	1.0 ± 0.4	
Zinc (mg/day) M	7.5 ± 3.8 [b]	8.1 ± 3.1	11.6 ± 4.5
F	4.6 ± 2.7 [b]	5.6 ± 2.3	8.1 ± 5.2
Copper (μg/day) M	410 ±120	450 ± 200	520 ± 130
F	440 ± 290	380 ± 170	390 ± 140

Note: Values are given as mean ± SD For comparisons among the three groups, the ANOVA test with the post hoc Tukey tests was used. For PNA analysis, the *t*-test was used. $p < 0{,}05$; a = DM vs. NDM; b = DM vs. control; c = NDM vs. control. M = male; F = female.

3.7 ± 0.5 g/dL), and transferrin (307.2 ± 229.1 vs. 381.5 ± 381.5 mg/dL). However, 14.3% of the patients in the DM group and 12.7% in the NDM group showed albumin values below 3.5 g/dL. The frequency of patients with serum transferrin lower than 200 mg/dL was higher in the DM group (54% vs. 23%; $p = 0.02$).

Zinc, copper, and ceruloplasmin determinations are shown in Table 4. Although plasma zinc, on average, was within the normal range in all groups, DM and NDM groups showed lower levels compared to the con-

Table 4
Biochemical Parameters of the Evaluation of Zinc, Copper, and Ceruloplasmin in Patient and Control Groups

	DM	NDM	CONTROL
	N = 33	NDM = 30	N = 20
Plasma zinc (μg/dL)	84.1 ± 17.7 [b]	81.2 ± 19.8 [c]	93.3 ±12.1
Erythrocyte zinc (μg/g/Hb)	53.2 ± 9.5 [b]	51.3 ± 10.3 [c]	41.8 ± 63.0
Urine zinc (μg/24h)	266.1 ± 152.1*	256.1 ± 177.5**	280.1±158.9***
Plasma copper (μg/dL)	130.02 ± 36.7[a,b]	109.7 ± 26.2	108.0 ± 8.5
Ceruloplasmin (mg/dL)	31.7 ± 8.1	30.9 ± 9.3	29.4 ± 4.3

Note: Values are given as mean ± SD. ANOVA and post hoc Tukey tests were used. **n* = 21; ***n* = 12; ****n* = 20. $p < 0{,}05$; a = DM vs. NDM; b = DM vs. control; c = NDM vs. control.

trol group. Plasma zinc concentrations below 70 μg/dL was observed in 21% of the patients in DM group and 27% in NDM group ($p = 0.94$). The concentrations of erythrocyte zinc were high in both groups in relation to the control group. Erythrocyte zinc above 44 μg/g Hb was observed in 88% of the patients in DM group and in 76% of the patients in NDM group ($p = 0.61$). No difference not was observed for urinary zinc, comparing the three groups. Only 60% and 40% of the patients in DM and NDM groups, respectively, had urinary volume higher than 400 mL/24 h and were considered for analysis.

Plasma zinc correlated positively with urea ($r = 0.53$; $p < 0.01$) and had a correlation of borderline significance with albumin ($r = 0.25$, $p = 0.051$). In the multivariate analysis, urea was a determinant of plasma zinc (β coefficient = 0.15, confidence Interval [CI] = 0.03–0.27; $p = 0.01$). The determinants of erythrocyte zinc were MAMC (β coefficient = 0.17, CI = 0.002–0.35; $p = 0.047$) and Kt/V (β coefficient = –13.9, CI = 24.03–3.86; $p = 0.007$).

Plasma copper was higher in the DM group compared to NDM and control groups and was correlated positively with serum glucose ($r = 0.44$; $p = 0.0003$), HbA1c ($r = 0.25$; $p = 0.04$), Kt/V ($r = 0.30$; $p = 0.01$), and negatively with iron ($r = -0.47$; $p = 0.0001$), the duration of dialysis treatment ($r = -0.35$; $p = 0.005$), hematocrit ($r = -0.33$; $p = 0.009$) and adequacy of TSF ($r = -0.28$; $p = 0.02$). However, in the multivariate analysis only serum creatinine (β coefficient = –3.19, CI = –5.12–1.25; $p = 0.002$) and serum glucose (β coefficient = –0.17, CI = –0.04–0.58; $p = 0.009$) were the independent determinants of copper. In the univariate analysis, ceruloplasmin correlated positively with copper ($r = 0.52$; $p = 0.00002$) and glucose ($r = 0.27$; $p = 0.04$) and negatively with adequacy of TSF ($r = -0.29$; $p = 0.03$). Plasma copper was the only independent determinant of the ceruloplasmin concentration (β coefficient = 0.12, CI = 0.05–0.18; $p = 0.0007$).

DISCUSSION

Various studies have demonstrated that patients with diabetes or CKD, even in the initial phase, have disturbances in the metabolism of zinc and copper *(1–4,8,23–27)*. However, the impact of the association of both diseases on the metabolism of these elements needs to be investigated.

In the present study, a reduction in the plasma zinc and an increase in erythrocyte zinc concentration were found in patients on hemodialysis when compared to healthy individuals, independently of the presence of diabetes. This result raises the question of whether these alterations denote a deficiency of zinc or only a redistribution of this element in the different compartments of the body. Similar findings were described by other authors in dialysis *(3,8,24,25)* and in predialysis patients *(28)*. The causes of this abnormality are unknown. It has been suggested that acidosis could be implicated, as it facilitates the entrance of zinc in the erythrocyte. The zinc redistribution could also be related to the deficient erythropoiesis in uremic patients, similar to what has been observed in other states of chronic anemia *(29)*. Additionally, it has been suggested that the zinc abnormalities observed in this population would be an adaptive mechanism of kidney failure *(30)*. On the other hand, decreased plasma zinc could be, at least in part, the result of a low zinc intake or decreased intestinal absorption *(31)* Accordingly, in the present study, zinc intake of the patients, especially of males, was significantly lower than that of the control group.

It is well known that zinc intake correlates directly with protein intake, as these two nutrients share the same food sources *(32)*. In our study, zinc intake has not been correlated to protein intake estimated by the food diaries, however, using a more reliable method to estimate protein consumption (PNA), a direct correlation between zinc and protein intake was then observed. Moreover, the plasma zinc concentration correlated directly with the urea and albumin, which reinforces the role of the protein intake in zinc metabolism.

In the present study, no relationship between zinc status and the presence of diabetes was observed. In fact, no difference in zinc concentrations was found comparing DM and NDM groups; also HbA1c did not correlate with zinc. Data concerning this issue are conflicting *(4,5,33–36)*. In patients with diabetes, the glycosylation of plasma proteins causes a reduction of zinc affinity with these proteins. This condition leads to an increase of circulating free zinc and hyperzincuria, which is frequently observed in patients with diabetes without CKD *(7,22,27,38–40)*. Thus, a decrease in plasma zinc would be expected. Nevertheless, compensatory increases in zinc absorption maintain plasma zinc in the normal range *(41)*. In the present study, the relation of plasma zinc, metabolic control of diabetes, and zincuria could not be properly evaluated because most of the patients were anuric.

Although elevated, the concentrations of zinc in the erythrocyte in our study did not differ between DM and NDM groups, which is in accordance with reports on DM patients without CKD compared with healthy subject *(22,42)*. For this reason, it is seems reasonable to conclude that CKD is actually the determinant of increased concentration of zinc in erythrocyte, as has already been discussed *(1,8,28)*.

The role of dialysis treatment on zinc metabolism needs to be considered, as an inverse relation between erythrocyte zinc and Kt/V was found. Because Kt/V is a measure of dialysis adequacy, this negative correlation could suggest a redistribution of zinc as a result of the loss of this element during the dialysis procedure. However, studies have not demonstrated a zinc loss during hemodialysis, and an increase in plasma zinc was found during the dialysis procedure, which could be a consequence of hemoconcentration *(2,43)*. It has to be mentioned that these results are related to plasma zinc. No data are available regarding the association between erythrocyte zinc and the dialysis procedure.

In contrast to what has been observed with zinc, CKD does not seem to influence the concentrations of copper. In fact, copper and ceruloplasmin concentrations of the nondiabetic uremic patients were similar to the control group. These findings are in accordance with the majority of studies that evaluated patients with CKD *(1,2,24,31)*. However, there are some studies showing increased *(44)* and decreased *(3,45)* plasma copper concentration in this population. These discrepancies can be partially explained by the differences in the methodologies used for the measurement of copper as well as by the dialysis and/or population characteristics. In fact, the majority of the studies exclude or do not describe whether diabetic patients were included.

Our findings of increased plasma cooper in the DM group are in accordance with studies in DM patients without CKD *(46–48)*. Two possible factors could be speculated in explaining this association: First, an increase of intestinal cooper absorption and, second, a reduction in the link of cooper with ceruloplasmin as a consequence of the elevated degree of carbamilation of this protein in diabetic patients *(49)*. In the present study, the poor glycemic control was associated to a higher concentration of copper in the plasma, which could indirectly suggest a higher level of ceruloplasmin carbamilation. Another factor to be considered is the poor efficiency of hemodialysis in removing the final products of glycosylation *(50)*. In this way, these toxic products would be favoring ceruloplasmin glycosylation *(51)*.

Analyzing all patients, we observed that, in addition to the glucose, serum creatinine was an inverse and independent determinant of plasma copper. The reasons for this finding are not clear. As elevated creatinine is a marker of renal failure, we could suppose that this relationship could be attributed to the loss of renal function *per se*. However, it is known that the serum creatinine in patients undergoing hemodialysis constitute a marker of muscular mass. In fact, low serum creatinine concentration characterizes a reduction in lean body mass *(16).* Although we did not find a significant difference in MAMC comparing DM and NDM groups, the serum creatinine of the diabetic was significantly lower. Moreover, as poorly controlled diabetic patients have a reduction in muscle mass, the inverse correlation between creatinine and copper could be indirectly related to the effect of poor glycemic control on lean body mass, particularly in the group with diabetes. This hypothesis, however, has to be tested in other studies.

It is well demonstrated that an excess of cooper contributes to an increased oxidative stress in CKD patients *(52).* In the general population, the elevated serum copper was independently associated with a higher risk of death from coronary heart disease *(53).* The impact of copper excess on morbidity and mortality in CKD patients remains to be established.

The results of this study demonstrate an alteration in the distribution of zinc in patients with CKD, independently of the presence of diabetes mellitus. On the other hand, the nutritional status of copper seems not to be influenced by CKD, but only by the metabolic dearrangements associated with diabetes.

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