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CLINICAL STUDIES

Relationship between zinc levels and plasma leptin in hemodialysis patients

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ABSTRACT

Recent evidences suggested a possible relationship between zinc deficiency and leptin levels in pathogenesis of anorexia in chronic kidney disease. The present study addressed the relationship between zinc and leptin in hemodialysis (HD) patients.
Methods: Fifty HD patients (54.3 ± 12.7 years old, 62% men) were studied and compared to 21 healthy volunteers (50.7 ± 15.7 years old, 43% men). Biochemical data, serum zinc, plasma leptin, IL-6, TNF-α and C-Reactive Protein levels were determined. Anthropometric parameters, food intake and appetite score were also assessed.
Results: The leptin levels were higher in HD patients (16.1 μg/mL (0.21–118.25) *vs* 6.0 μg/mL (0.50–23.10)) in healthy volunteers ($p = 0.04$), whereas serum zinc levels were lower (54.5 ± 16.3 μg/dL) compared to healthy volunteers (78.4 ± 9.4 μg/dL) ($p = 0.0001$). The plasma leptin was correlated negatively with plasma zinc ($r = -0.33$; $p = 0.007$), energy ($r = -0.38$; $p = 0.002$) and protein intake ($r = -0.34$; $p = 0.006$) and, positively correlated with BMI ($r = 0.54$; $p = 0.0001$), % body fat ($r = 0.70$; $p = 0.0001$) and conicity index ($r = 0.46$; $p = 0.001$). Plasma zinc was associated with hemoglobin ($r = 0.30$; $p = 0.04$) and negatively associated with TNF-α ($r = -0.37$; $p = 0.002$) and C-Reactive Protein ($r = -0.37$; $p = 0.004$). There was no correlation among Zn, leptin and appetite score in these patients.
Conclusion: This study showed that low plasma zinc levels are negatively associated with high leptin levels in HD patients.

Introduction

Anorexia is a common complication in hemodialysis (HD) patients and contributes to protein–energy wasting (PEW) increasing risk of morbidity and mortality [1–3]. Multiple mechanisms may be involved with diminished appetite in chronic kidney disease (CKD), however, the pathogenesis of anorexia in HD patients is unknown [4–6].

Zinc (Zn) is an essential metal involved in many biochemical process, and its deficiency is associated with anorexia, weight loss, and growth retardation [7,8]. The Zn deficiency have been observed in HD patients [9,10] and the disturbances of zinc might occur because of reduced renal function, proteinuria, leading to losses of protein-bound elements, alterations in gastrointestinal absorption, inflammation, hypoalbuminemia and the dialysis procedure *per se* [11,12].

Zinc deficiency induces anorexia by different mechanisms, but some authors suggest that hormone leptin may provide a tool for elucidating the physiology of zinc deficiency-induced anorexia [13]. Leptin is a 16-kDa circulation protein, encoded by the ob gene, produced by adipocytes and metabolized in the kidney [14]. The leptin is released into the blood in proportion to the amount of white adipose tissue and its signals the body's nutritional status to the brain to regulate energy balance [15]. CKD patients have inappropriately high serum leptin levels it has been speculated that hyperleptinemia in these patients may be one of the factors mediating anorexia and wasting [16,17].

Some researchers have tried to investigate the zinc status and its acting on plasma leptin levels, finding a positive correlation between their levels [18,19]. CKD patients have hypozincemia and hyperleptinemia that are associated with anorexia and malnutrition, however, the relationship between Zn deficiency and leptin levels these patients is poorly understood to date. Therefore, the objective of this study was to evaluate the relationship between zinc levels and plasma leptin in hemodialysis patients.

Subjects and methods

Subjects

Fifty maintenance HD patients were enrolled from Renal-Cor Clinic at Rio de Janeiro, Brazil and compared to 21 healthy

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individuals. Inclusion criteria were: age > 18 years and patients on maintenance dialysis for at least 6 months. Patients with autoimmune diseases and infectious diseases, cancer and AIDS or patients using drugs catabolic and antioxidant supplements were excluded. The dialysis duration was 3–4.5 h/session three times/week, blood flow greater than 250 mL/min, dialysate flow 500 mL/min and bicarbonate buffer. Etiology of renal failure was hypertension ($N = 26$), diabetes ($N = 11$), chronic glomerulonephritis ($N = 4$), and others ($N = 9$). The study protocol was approved by the Ethics Committee of the Faculty of Medicine at the Federal University Fluminense (number 040/09).

Methods

Nutritional assessment

Anthropometric measurements were obtained immediately after the HD session by a trained researcher. Body mass index (BMI) was calculated as body mass divided by squared stature and used to assess the nutritional status according to the World Health Organization cut-off points [20]. Biceps and triceps skinfolds (TSF) and upper midarm circumference (MAC) were measured in the non-fistula side using standard techniques [21]. Three sets of measurements at each site were averaged and used in the analyses. Midarm muscle area (MMA) was calculated using the following equation: $\text{MMA} = [[\text{MAC (cm)} - \pi \times \text{triceps skinfold (cm)}]^2/4\pi] - N$, where, $N = 10$ for male and 6.5 for female [22]. The TSF and MMA were classified in percentiles according to age and gender. Muscle and fat-mass wasting were defined as values less than the 15th and 5th percentiles, respectively [23]. Percentage of body fat (%BF) was assessed according to the method of Durnin and Womersley [24]. Abdominal fat deposition was assessed by means of a conicity index (Ci), which estimates fat accumulation in the abdomen as the deviation of body shape from a cylindrical toward a double-cone shape (*i.e.* two cones with a common base at the waist level) [25]. The waist circumference was measured with tape, with the midpoint located between the last rib and the iliac crests, and the end of expiration, with the patient in a standing position. The cut-off points used for elevated waist circumference were ≥102 cm for men and ≥88 cm for women [26].

The average daily intake of calories and protein were estimated using three days (dialysis day, non dialysis day and the weekend) 24-h food records. Average daily ingestion of nutrients was calculated using diet software (NutWin Software developed by the Department of Nutrition, Federal University of São Paulo – UNIFESP, São Paulo, Brazil). Appetite was rated by asking the following question: "How would you rate your appetite?" (*1*) very good, (*2*) good, (*3*) fair, (*4*) poor, or (*5*) very poor [27].

Biochemical variables

Blood samples (10 mL) were obtained from the arterial line of the hemodialysis before the start of session, after the patients had fasted overnight. Blood samples were centrifuged at $3000 \times g$ for 15 min for separation of plasma. Aliquots of plasma samples were stored at −80 °C until analyzed.

The blood was collected in tubes containing 30% sodium citrate as anticoagulant for determination of zinc in plasma. Precautions were taken to avoid trace mineral contamination during sample collection and processing. All materials were immersed for at least 12 h in a solution of nitric acid at 10% followed by thorough rinsing (ten times) with ultrapure water (Milli-Q plus, Milipore, USA). Plasma zinc was assayed by flame atomic absorption spectrophotometry (Varian AA240FS Fast Sequential AAS Inc, Palo Alto, CA, USA) at the standard wavelength of 213.9 nm. A linear standard curve resulted and a correlation coefficient (r) of 0.999 was calculated from it.

Table 1
Demographic and anthropometric characteristics in hemodialysis patients and healthy individuals.

Parameters	HD patients ($N = 50$)	Healthy individuals ($N = 21$)
Age (years)	54.3 ± 12.7	50.7 ± 15.7
Men/Women	31/19	9/12
BMI (kg/m^2)	24.4 ± 4.1	25.6 ± 4.0
Men	24.6 ± 3.8	27.7 ± 2.6*
Women	24.0 ± 4.6	23.8 ± 4.3
WC (cm)	92.9 ± 13.9	85.5 ± 12.3
Men	96.5 ± 13.2	98.7 ± 8.9*
Women	86.5 ± 13.0	80.2 ± 9.2
MMA (cm^2)	35.1 ± 11.8	40.0 ± 7.5
Men	37.2 ± 12.6	47.9 ± 3.6*
Women	31.5 ± 9.7	36.8 ± 6.3
% BF	29.4 ± 7.3	30.0 ± 5.7
Men	27.0 ± 6.6	27.5 ± 5.7
Women	33.5 ± 6.6	31.0 ± 5.7
Conicity index	1.3 ± 0.1	1.2 ± 0.1
Men	1.4 ± 0.9	1.3 ± 0.1*
Women	1.3 ± 0.1	1.2 ± 0.1

HD, hemodialysis; BMI, body mass index; WC, waist circumference; MMA, midarm muscle area; %BF, percentage of body fat.
* $p < 0.05$.

The concentrations of plasma leptin, tumor necrosis factor α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) were measured with a multiplex assay kit manufactured by R&D Systems Minneapolis, MN, in a Luminex® 100/200™ System (Austin, TX, USA).

Serum albumin, urea, creatinine, hemoglobin and hematocrit were measured using standard laboratory methods. Dialysis dose (Kt/V) was calculated from values of blood urea nitrogen, pre- and post-dialysis, weight, and dialysis duration using the Daugirdas formula [28].

Statistical analysis

Results were expressed as mean ± SD (Standard Deviation), median and range or percentage change, as needed. The patients were divided in two groups: BMI < 25 kg/m^2 (normal range) and BMI ≥ 25 kg/m^2. The patients with BMI values below 18.5 kg/m^2 ($N = 2$) were excluded for this analysis. Student's t test was used to test the difference between means and the Mann–Whitney test was used for non-parametric data. Spearman or Pearson correlation coefficients were searched to examine the relationship between variables. Statistical significance was accepted as $p < 0.05$. The statistical analyses were performed through the SPSS 16.0 program (Chicago, USA).

Results

Clinical and biochemical characteristics of the subjects are shown in Table 1. Two (4%) HD patients presented BMI values below 18.5 kg/m^2, twenty-four (48%) presented normal values for BMI, nineteen (38%) were overweight and five (10%) were obese. According to the % of body fat, 93.2% of HD patients had values above normal [21]. According to TSF and MMA, 11.1% and 57.8% patients, respectively, presented values below 15th percentile and, 63.6% of patients presented elevated waist circumference. According to Ci the HD patients presented values higher when compared to healthy individuals (1.3 ± 0.1 *vs* 1.2 ± 0.1, $p = 0.03$).

The mean values of urea, creatinine, hemoglobin and hematocrit in patients were 143.5 ± 30.7 mg/dL, 10.3 ± 2.8 mg/dL, 10.9 ± 1.6 g/dL and 31.5 ± 5.1%, respectively. Serum albumin was 3.88 ± 0.2 g/dL and 18% patients had a serum albumin level lower than 3.8 g/dL. Adequacy of dialysis was indicated by Kt/V_{urea} of 1.5 ± 0.3.