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Cu/Zn ratios are associated with nutritional status, oxidative stress, inflammation, and immune abnormalities in patients on peritoneal dialysis

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ABSTRACT

Objectives: We evaluated the relationship of the plasma copper/zinc (Cu/Zn) ratio with nutritional status, inflammation, oxidative stress, and immune function in peritoneal dialysis patients.

Design and methods: Clinical and laboratory parameters were measured in patients ($n=45$) and age- and sex-matched healthy individuals ($n=30$).

Results: There were significant negative correlations of the Cu/Zn ratio with nutrition-related parameters (body mass index [BMI], creatinine, hemoglobin, and albumin) and antioxidant (vitamin C and E) levels and positive correlations of the Cu/Zn ratio with the levels of high sensitivity C-reactive protein (hs-CRP) and oxidation products (malondialdehyde [MDA] and protein carbonyl). The Cu/Zn ratio was negatively correlated with the percentages of B- and T-lymphocyte subsets and the ratio of CD4/CD8 antigens.

Conclusions: In peritoneal dialysis patients, elevated Cu/Zn ratios are associated with malnutrition, increased oxidative stress, inflammation, and disrupted immune status.

Introduction

Patients with end stage renal disease (ESRD) require transplantation or long-term dialysis, costly procedures that are an increasingly significant public health problem. Taiwan has the highest incidence and prevalence of ESRD worldwide [1]. Recent studies have shown that patients on long-term dialysis have high rates of morbidity and mortality [2]. Risk factors associated with the outcome of these patients include long duration of dialysis, poor nutritional status, oxidative stress, infection, and inflammation [3]. However, little is known about the association of trace element levels and clinical response of ESRD patients.

Previous investigations showed that ESRD patients undergoing long-term dialysis have increased oxidative stress [4,5]. Oxidative stress is closely associated with inflammation status, and the maintenance of redox balance is known to modulate immune system homeostasis [6]. Reduced serum albumin and increased oxidative stress have been observed in ESRD patients; the former may be due to poor nutritional status [7], and latter may be related to alterations in their levels of essential trace elements [8].

Long-term dialysis patients have altered levels of essential trace elements. In particular, ESRD patients on hemodialysis or peritoneal dialysis have significantly lower levels of serum zinc (Zn) and higher oxidative stress [9]. Zn has antioxidant and anti-inflammatory properties, and oral Zn supplementation can increase in serum zinc levels, leading to reduced inflammation in hemodialysis patients [10]. A recent investigation suggested an association between serum zinc levels and nutritional status in dialysis patients [11]. However, some other studies [12,13] and our unpublished results have reported conflicting results.

Copper (Cu), another essential trace element, has a role in hemoglobin synthesis and immune function and is a cofactor for Cu/Zn superoxide dismutase (Cu/Zn-SOD) and ceruloplasmin [14]. Although the actual cause of the changes in Cu concentration and distribution remains to be elucidated, one study reported that long-term dialysis patients have elevated levels of serum Cu and more oxidative stress than healthy controls [15]. In contrast, other studies have reported that dialysis patients had normal levels of Cu [16] or reduced levels of Cu [12]. Chronic inflammation, as indicated by increased level of serum ceruloplasmin, is related to elevated level of C-reactive protein (CRP) and Cu [17]. However, another investigation reported no significant association of CRP with Cu in dialysis patients [18].

Changes in plasma levels of Cu and Zn has also been demonstrated for certain diseases, but an imbalance of the Cu/Zn ratio seems to be a better indicator of infection, vascular complications, and prognosis of diseases than Zn or Cu status alone [19,20]. In addition, a previous study indicated that the Cu/Zn ratio may be a useful

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inflammatory–nutritional biomarker and predictor of mortality in elderly people [21]. In our previous research, we noted a significantly positive association between the serum Cu/Zn ratio and oxidative stress in uremic patients [8].

Taken together, abundant evidence indicates that disruption of Cu and Zn homeostasis can increase the risk of adverse clinical outcomes. However, few studies have evaluated the use of the Cu/Zn ratio as a marker for the prediction of nutritional state, oxidative stress, immune, and inflammation status, which are related to outcome of long-term dialysis patients.

In the present investigation of ESRD patients on peritoneal dialysis, we measured the associations of the Cu/Zn ratios with nutritional status, oxidative stress, inflammation, and immune system dysfunction.

Methods

Patients

Between October 2007 and February 2008, 45 patients undergoing routine continuous ambulatory peritoneal dialysis (CAPD) at the dialysis unit of Kuang Tien General Hospital (Taichung, Taiwan) were enrolled. The mean duration of dialysis treatment was 2.4 ± 1.1 years. All patients received 3 or 4 nocturnal exchanges per day. Thirty-eight patients used a 1.5% glucose bags for one exchange and 2.5% glucose bags for three exchanges; seven patients used 2.5% glucose bags for three exchanges and a 7.5% glucose bags for one exchange or performed four exchanges with 2.5% glucose bags.

None of the patients had liver disease, mental retardation, dementia, psychiatric illness, or cancer, and none received immune suppressant drugs or supplementation with antioxidants vitamins/minerals (e.g. selenium, Cu, and Zn). All patients were clinically stable and free of edema.

The following clinical characteristics were recorded as follows: body mass index (BMI), diabetes mellitus (45%), hypertension (29%), ischemic heart disease (30%), and dyslipidemia (44%). Diabetes mellitus was diagnosed if the patient used insulin or an oral hypoglycemic agent. Hypertension was diagnosed if there was a history of hypertension. Dyslipidemia was diagnosed if fasting triglycerides were 200 mg/dL or greater or if the patient received medical treatment for hyperlipidemia. Ischemic heart disease was diagnosed if there was angina pectoris, history of myocardial infarction, coronary artery bypass surgery, or percutaneous coronary intervention. Some patients used insulin, sulfonylurea, Ca2+ channel antagonists, NSAIDs, and/or beta-blockers. None of the patients used statins, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers.

As controls, 30 healthy subjects of similar age and gender were studied. Exclusion criteria for the healthy controls were presence of a systemic disease, diabetes mellitus, hypertension, heart disease, renal/hepatic disease, dyslipidemia, and consumption of vitamin supplements or medications. All subjects signed informed consent statements. The study protocol was approved by the ethics in human research committee of our hospital.

Biochemical analysis

Blood samples were drawn in the morning after an overnight fast of 12 h. Plasma concentrations of albumin, hemoglobin, blood urea nitrogen (BUN), and creatinine were measured with a Hitachi 7050 automatic analyzer (Hitachi Corp., Tokyo, Japan) and a Sysmex KX-21N hematology analyzer (Sysmex, Kobe, Japan).

Measurement of oxidative stress, antioxidants, and CRP

The extent of lipid peroxidation was determined by assaying the formation of malondialdehyde (MDA). Plasma samples were mixed with 3% sodium dodecyl sulfate, 0.1 N HCl, 10% phosphotungstic acid, and 0.7% thiobarbituric acid and then incubated at 95 °C. The MDA was extracted into n-butanol and the fluorescence of the n-butanol layer was measured at 530 nm with 485 nm excitation [8].

Protein carbonyls reacted with 2,4-dinitrophenyl-hydrazine (DNPH) forming a Schiff base to produce the corresponding hydrazone. Plasma proteins were precipitated with an equal volume of 20% trichloroacetic acid and centrifuged at 10,000*g*. The pellets were re-suspended in the ethanol/ethyl acetate mixture. The results were calculated using the extinction coefficient of 0.022 $\mu M^{-1}\,cm^{-1}$ at 370 nm [8].

For plasma vitamin C determination, samples were immediately treated with 4% metaphosphoric acid/dithiothreitol as a stabilizer. The product was then coupled to *o*-phenylenediamine to produce a chromophore, and absorbance was measured at 340 nm [22]. Vitamin E was measured as described by Borel et al. [23]. Briefly, α-tocopheryl acetate was added as an internal standard and vitamin E was then extracted with *n*-hexane/BHT. The hexane phase was isolated and evaporated to dryness under nitrogen, re-dissolved in the mobile phase, injected into a Gemini C18 column (150 × 4.6 mm, 5 μm)(Phenomenex), and assayed by reverse-phase HPLC (LabAlliance, HPLC Pumps, Systems & Accessories).

Plasma high sensitivity-CRP concentrations were measured using the hs-CRP ELISA kit (DRG Instrument GmbH, Marburg, Germany).

Measurement of enzyme activity

Erythrocyte SOD activity was determined with a RANSOD kit (Randox, San Diego); one unit was defined as the amount of enzyme necessary to produce 50% inhibition in the rate of p-iodonitrotetrazolium reduction. Catalase activity was measured with a commercially available kit (IBL Immunobiological Laboratories, Hamburg, Germany); one unit was defined as the amount of enzyme that caused the formation of 1.0 nmol of formaldehyde per minute at 25 °C.

Glutathione peroxidase (GPx) activity was measured with a kit from Cayman Chemical (cat #703102); the rate of decrease in absorbance at 340 nm is directly proportional to the GPx activity. Glutathione reductase (GR) activity was assessed by monitoring the oxidation of NADPH to $NADP^+$ via addition of oxidized GSH (GSSG); one unit was defined as the amount of enzyme that catalyzed the reduction of 1 mmol of GSSG.

Determination of immunologic parameters

Peripheral blood B- or T-lymphocytes of all subjects were stained with the following monoclonal antibodies, which conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE): CD3–FITC, CD4–PE, CD8–PE, CD19–PE (eBioscience). Briefly, 100 μL of whole blood was incubated with 20 μL of monoclonal antibody reagent for 15 min in the dark at room temperature. Following leukocyte fixation and erythrocyte lysis with the CyLyse lysing reagent kit (Partec, GMBH, Münster, Germany), the percentages of lymphocyte subsets were determined using a Partec CyFlow ML flow cytometer (Partec, GmbH).

Determination of trace elements

The concentrations of Zn and Cu were measured with a flame atomic absorption spectrophotometer (932 plus, GBC, Australia) using an air-acetylene flame without background correction at 213.9 and 324.71 nm, respectively. Samples were digested in a H_2O_2/HNO_3 mixture in a START D microwave-assisted digestion system (Milestone Microwave Labstation ETHOSD), and the volume was increased with double deionized water.