Research Article

Copper, zinc and selenium imbalance in Moroccan haemodialysis patients and its correlation to lipid peroxidation

Rachid Eljaoudi[1]*, Mourad Errasfa[2], Mohammed Benyahia[3], Abdelali Bahadi[3], Yahia Cherrah[1], Azeddine Ibrahimi[4], Driss Elkabbaj[3]

[1]Pharmacology and Toxicology Department, [3]Nephrology Department, [4]Medical Biotechnology lab (Med Biotech), Faculty of Medicine and Pharmacy, University Mohammed V, 10000 Rabat, Morocco
[2]Pharmacology Department, Faculty of Medicine and Pharmacy, University Sidi Mohammed Ben Abdellah, 30000 Fes, Morocco

*Correspondence:
Prof. Rachid Eljaoudi,
E-mail: eljaoudi_rachid@yahoo.fr

ABSTRACT

Background: Haemodialysis patients are at risk of developing trace elements imbalance and lipid peroxidation. The present study was aimed to assess plasma levels of copper (Cu), zinc (Zn), selenium (Se) and malondialdehyde (MDA) of haemodialysis patients and to investigate the possible effect of haemodialysis on these trace elements and MDA imbalance.
Methods: Blood samples of fifty hemodialysis patients and forty healthy controls subjects were analyzed for determination of hemoglobin, albumin, creatinine, urea and high-sensitivity C-reactive protein (hs-CRP). Cu, Zn and Se were determined in plasma (before and after hemodialysis) and erythrocytes and MDA in plasma before and after hemodialysis.
Results: The study showed that, plasma Zn and Se concentrations were lower in haemodialysis patients compared to that of healthy controls, while plasma Cu, MDA and Cu/Zn ratio were higher. Plasma Cu/Zn ratios were positively correlated to MDA and weakly correlated to hs-CRP levels whereas plasma Se concentrations were inversely correlated to MDA. In addition, MDA levels increased after haemodialysis session.
Conclusions: Based on the results of the present study regarding the imbalance of trace elements in haemodialysis patients, it seems reasonable to periodically assess the trace elements status and consider possible correctional therapy in case of deficiency.

Keywords: Trace element, Oxidative stress, Haemodialysis, Lipid peroxidation

INTRODUCTION

Oxidative stress (OS) is a loss of the balance between antioxidant systems and the excess of oxidation, it occurs when the intracellular concentration of reactive oxygen species (ROS) increases over the physiological values.[1] Mammalians cells have integrated antioxidant defense systems to prevent hazardous events such as lipid peroxidation, proteins and oxidative DNA damage.[1,2] This system includes enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (Vitamins A, C, E, copper, zinc and selenium).[1] Malondialdehyde (MDA) is the end product of lipid peroxidation and is considered as a good biomarker of oxidative stress.[2]

Chronic renal failure or end stage renal disease (ESRD) refers to permanent serious damage to the kidney's function resulting in loss of the normal kidney ability to remove the toxic molecules from the body.[3] ESRD is

usually associated with a state of OS, antioxidant depletion and an imbalance of some trace elements such as copper, zinc and selenium concentrations in the body.[3,4] Different factors affect serum concentrations of trace elements, such as diet, failure of renal excretion, degree of renal failure and metabolic alterations associated with renal failure.[4]

Hemodialysis (HD), which is the most common form of treatment for ESRD, removes uremic toxins primarily by allowing equilibration of plasma and dialysate across a semi-permeable membrane. Substances present in dialysate, but not in blood, will tend to accumulate in the patient, while, those, which have lower concentrations in dialysate than in blood tend to be removed by dialysis. HD represents, therefore, another concentration disturbance factor of trace elements and OS is aggravated during every HD session.[5,6]

Disturbances of trace elements have adverse consequences in the general population.[7-8] For example, copper (Cu) is an important trace element and a pro-oxidant factor, it participates in metal-catalyzed formation of free radicals and plays a role in the antioxidant defense system like metalloenzymes.[9] Cu is necessary for the catalytic activity of Cu/Zn superoxide dismutase (Cu/Zn SOD), ceruloplasmin and intra-cellular thioneins.[9,10]

On the other hand, zinc (Zn) is an essential mineral that is a component of more than 200 enzymes such as alkaline phosphatases, superoxide dismutase, protein kinase C and carboxy-peptidase A, B.[11] Zn acts as an antioxidant by protecting the sulfhydryl groups of proteins and enzymes against free radical damage in the body.[12]

Selenium (Se) is an essential component of the antioxidant enzyme such as glutathione peroxidase and is required for other important roles in the human body: synthesis of thyroid hormones, production of prostaglandins and promotion of growth and fertility.[13]

Because of their importance as biomarkers of cell's integrity and healthy body, many researchers have been interested in essential trace elements status and lipid peroxidation in HD patients. Serum Cu was found either to be decreased or more often increased.[14] However, Zn and Se levels tend to be lower than those in normal controls.[5,14-16]

In Morocco, there are no data available on the issue of trace elements status in patients undergoing HD. This study was conducted to determine in HD patients, the levels of MDA in plasma and Cu, Zn and Se levels both in erythrocytes and plasma. We also evaluated the effect HD on these markers of OS.

METHODS

This cross-sectional study was conducted according to the principles of the declaration of Helsinki and was approved by the local Ethical Committee from the Faculty of Medicine and Pharmacy in Rabat, Morocco (Reference 479/2012). Informed consent was sought and obtained from individuals before enrollment into the study.

The study included 50 patients with ESRD and 40 healthy controls. All ESRD patients were on maintenance HD for at least 12 months and none of them received any vitamin complex or antioxidant drugs. Hemodialysis process was performed with online-produced ultrapure dialysis fluid based on Diasafe and heat disinfection with hot feed Fresenius Medical Care, with reverse osmosis, deionization, and carbon filtration. All patients received single-use biocompatible synthetic low-flux membranes (Polyamide, Polyflux Renal Products Gambro). Blood flow rates were chosen between 300 and 350 mL/min, and ultra-filtration rates were set according to individual needs. Dialysate flow rate was fixed at 500 mL/min.

Venous blood was collected from control individuals and patients after 12h of fasting in Ethylenediaminetetraacetic acid (EDTA) and heparinized tubes. The plasma was separated from blood cells by centrifugation and erythrocytes were prepared by removing any residual plasma and the buffy layer and washed five times with physiological saline. Routine blood chemistry parameters: Hemoglobin, albumin, creatinine, urea and high-sensitivity C-reactive protein (hs-CRP) were analyzed in fresh blood samples using Cobas Integra 400 plus (Roche Diagnostics, Germany) autoanalyser.

MDA was determined by high-performance liquid chromatography as previously described with some adjustments.[17] For analysis purposes, an ACQUITY UPLC® system coupled to a fluorescence detector (Waters), controlled by MassLynx Software (version 4.1), was used. The separation was carried out on an ACQUITY UPLC® BEH C18 1.7µm 2.1x50mm column. The fluorescence detection excitation was occurred at 515 nm, while emission was at 553 nm. Calibrators and controls for MDA (Recipe, Munich, Germany) were used during the analysis. MDA in plasma samples was measured after thiobarbituric acid reaction and the generation of a fluorescent adduct. A mixture of acetonitrile: water (7:3, v/v) was used as a mobile phase.

Plasma Cu and Zn were measured by atomic absorption spectrometry (AAS) equipped with an ASC-7000 auto sampler at flame-air/acetylene (AA-7000; Shimadzu) while erythrocytes Cu and Zn were measured by AAS at furnace mode. Plasma and erythrocytes Se levels were measured with hydride generation-AAS (HVG-1, Shimadzu).

Deuterium and self-reversal background correction were used at flame and furnace mode respectively. For plasma Cu and Zn, samples were diluted 10 folds with Milli-Q water before analysis, while Se levels (plasma and erythrocyte) and erythrocytes Cu and Zn, samples were determined following digestion in 65% (w/w) Suprapur® HNO_3 (Merck) in the Microwave Digestion System (Multiwave PRO device, Anton Paar, Austria). In order to reduce Se (VI) to Se (IV), the 2.5 M hydrochloric acid was added and samples were warmed at 100°C for 30min. The mineral contents of serum and erythrocytes were determined at the following wavelengths: for Cu, 324.8 nm, for Zn, 213.9 nm and for Se, 196.0 nm. All measurements were performed in duplicate, adjusted automatically against the blank and carried out as standard addition experiments. Plasma quality controls purchased from Recipe (Munich, Germany) were used during the analysis. No quality control was available for erythrocyte trace elements. The results of erythrocyte trace elements were reported as a ratio to hemoglobin concentration.

All statistical analyses were performed using the SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). Depending on their normal or skewed distribution, data are reported as mean ± standard deviation (SD) or median (full range). Comparison between variables was performed using the t-test, Wilcoxon's or chi square test. Pearson or Sperman rank correlation analysis was used to evaluate the correlations between serum Cu, Zn, Se, Cu/Zn ratio, MDA and laboratory parameters. Value of $p < 0.05$ was considered statistically significant.

RESULTS

The main characteristics of the fifty HD patients and forty healthy controls enrolled in the study are summarized in Table 1. Sex ratio, age and body mass index, were similar between the two groups ($p > 0.05$).

Table 2 shows that in comparison to the control group, HD patient had high urea, creatinine and hs-CRP but low hemoglobin and albumin levels. Plasma MDA concentrations were found to be higher in HD patients compared to healthy subjects.

The levels of Zn and Se in plasma and erythrocyte were significantly lower in HD patients, while plasma Cu levels were higher. No significant difference was found in erythrocyte Cu levels between the two groups. In HD patients, Cu/Zn ratio was higher and the diabetic patients in the group had plasma Cu concentrations higher than the non-diabetic ones (1.56±0.24 vs. 1.39±0.32; p=0.045).

Plasma Cu/Zn ratio was positively correlated to MDA levels (r=0.48, p<0.01) and weakly correlated to hs-CRP (r=0.28, p=0.053), whereas plasma Se concentrations were inversely correlated to MDA (r=0.45, p<0.01 respectively). No other significant correlation was found between the other parameters in this study.

Table 3 indicates that before dialysis MDA levels were significantly lower than those after dialysis (p<0.001) and no change was observed in traces elements concentrations.

Table 1: Characteristics of patients with end stage renal disease undergoing hemodialysis (n=50) and healthy controls (n=40).

Variable	Hemodialysis group	Control group	p
Sex M/W	27/23	21/19	0.42
Age (years)	52.5 ± 17.8	49.8 ± 18.5	0.31
BMI (Kg/m2) m ± sd	23.7 ± 2.6	24.3 ± 3.7	0.21
HD duration (months)	38 (12–133)	-	-
Frequency of dialysis	3 x 4 hours session/week	-	-
Diabetic nephropathy	24 (48%)	-	-
Tubulointerstitial	9 (18%)	-	-
Glomerular nephropathy	7 (14%)	-	-
Amyloidosis	1 (2%)	-	-
Myeloma	4 (8%)	-	-
Undetermined nephropathy	5 (8)	-	-

M/W: Men/women; BMI: Body Mass Index; HD: Hemodialysis.

Table 2: Baseline blood parameters in hemodialysis and control group.

Variable	Hemodialysis group	Control group	p	Normal range**
Albumin	35.0 ± 4.0	42± 3.5	< 0.001*	35-50
Creatinine (mg/L)	95.9± 15.7	8.5± 2.1	< 0.001*	6-13
Urea (g/L)	1.78± 0.31	0.21± 0.15	< 0.001*	0.15-0.5
CRP (ng/mL)	9.5 (5.5–18.2)	1.3 (0.5-3.5)	< 0.001*	< 5
Hemoglobin	9.8± 1.2	15.1± 2.2	< 0.001*	11.5-16.5
MDA (μMol/L)	5.93± 0.81	1.05± 0.11	< 0.001*	0.36-1.24
Plasma Cu (mg/L)	1.45 ± 0.30	1.29 ± 0.22	0.029*	0.7-1.4
Erythrocyte Cu (μg/g Hb)	3.75± 1.11	3.41± 1.81	0.27	-
Plasma Zn (mg/L)	0.62 ± 0.13	1.11 ± 0.16	< 0.001*	0.6-1.2
Erythrocyte Zn (μg/g Hb)	36.6± 6.4	42.7± 9.2	0.013*	-
Plasma Se (μg/L)	50.5 ± 15.6	95.1 ± 18.5	< 0.001*	70-130
Erythrocyte Se (ng/g Hb)	313.7 ± 85.1	413.3 ± 71.5	<0.01*	-
Plasma Cu/Zn ratio	2.48 ± 0.89	1.21 ± 0.24	< 0.001*	1.14-1.29

MDA: Malondialdehyde; Cu: Copper, Zn: Zinc, Se: Selenium; Hb: Haemoglobin.
*: Statistically significant
**: Normal range admitted by our laboratory

Table 3: Effect of hemodialysis session on plasma MDA, copper, zinc and selenium.

Variable	Before Hemodialysis	After Hemodialysis	p
MDA (μMol/l)	5.93± 0.81	8.53± 0.53	< 0.001*
Plasma Cu (mg/l)	1.45 ± 0.30	1.48 ± 0.34	0.24
Plasma Zn (mg/l)	0.62 ± 0.13	0.63 ± 0.14	0.44
Plasma Cu/Zn ratio	2.48 ± 0.89	2.47± 0.88	0.82
Plasma Se (μg/l)	50.1 ± 15.6	47.2 ± 15.3	0.29

MDA: malondialdehyde; Cu: Copper, Zn: Zinc, Se: Selenium
*: Statistically significant

DISCUSSION

The association of Cu, Zn, Se status and lipids peroxidation with ESRD undergoing HD has been extensively studied.[18-25] Previous studies have shown that plasma Zn and Se levels were generally decreased in HD patients, while Cu levels were found to be either decreased or increased.[14,20,26] These trace elements disturbances are usually accompanied by high MDA concentrations.[27] To our knowledge, this is the first study to report the status of Cu, Zn, Se and their relationship with lipid peroxidation among HD patients in Morocco and the effect of HD on the plasma levels of theses biological parameters. The results show that the levels of Zn and Se decrease in HD patients and that plasma Cu and MDA concentrations were increased.

Cu, Zn and Se have antioxidant properties and protect biological systems from oxidative stress associated with ESRD. However, the metabolism and the status of these elements are usually altered in chronic renal failure as reported by several researchers.[14,18-24] In our study, Plasma levels of Cu were found significantly higher in HD patients compared with normal controls. These results were in keeping with previous reports.[20,27] However some contradictions still remain since even if the majority of studies have reported normal or high plasma levels of Cu in HD patients, others have showed decreased levels.[3,19,20,26,27] They may be explained, in part, by the diminution of Cu renal excretion capacity and/or the increase of intestinal Cu absorption due to Zn deficiency.[18,27,28] A second explanation, of our results, could be the high proportion of diabetics in HD group. Indeed, increased levels of plasma Cu were reported in diabetic, this association may be due to a reduction in the link of Cu with ceruloplasmin, which is carbamilated in diabetic patients.[29] Increased plasma Cu concentrations may also arise from the release of Cu during inflammatory tissue damage.[30] On the other hand, it was reported that plasma ceruloplasmin levels as well as ceruloplasmin-bound Cu fraction were decreased, whereas, free Cu fraction was increased in HD patient.[31] Unfortunately, we have not determined the ceruloplasmin for our patients. Even if Cu is an imperative molecule in life, the excess of this trace element is highly toxic by inducing oxidative stress.[32]

In our study, we were able to show low erythrocyte and plasma levels of Zn in HD patients when compared to healthy controls, independently of the presence or not of diabetes. This confirms other reports of low plasma Zn concentration in HD patients.[18-20] Diet with a high Zn content including meat, fish, cheese, chicken, nuts, etc. is restricted in ESRD, this dietary restriction appears as a primary cause of Zn deficiency in HD patients.33 Other causes may contribute to this deficiency, namely, loss of appetite, poor absorption of Zn by gastro-intestinal tract, lower serum albumin levels, decrease of the affinity of Zn to albumin and increased excretion of Zn.[33,34] In another hand, in ESRD, blood pH is generally acidic, this disturbance in acid-base balance can cause the shift of Zn into cells and therefore low levels of Zn.[28] However, both plasma and erythrocyte concentration of Zn were decreased in HD group, this result is in favor of a Zn deficiency and not a redistribution of this element in the different compartments of the body.

The Cu/Zn ratios were higher in HD patient when compared to healthy controls as also shown by previous reports.[35] Cu/Zn ratio is clinically more effective in evaluating Cu and Zn status than considering separately either one of these trace metals and it was shown that elevated Cu/Zn ratios are associated with numerous diseases such as immune dysfunction, neurological and psychiatric disorders, inflammation, and immune dysfunction, human malignancies and cardiovascular diseases.[35,36] This ratio is also a good indicator of enhanced oxidative stress, decreased blood antioxidant capacity and increased inflammatory response.[36] In this study, Cu/Zn ratios were positively correlated to MDA used as an indicator of lipid peroxidation but the correlation to hs-CRP was borderline. ESRD is a chronic state of inflammation and the levels of hs-CRP are usually elevated, this is a marker inflammatory process with endothelial injury and the release of pro-inflammatory cytokine, which can causes a loss of kidney function.

As for Zn, Se erythrocytes and plasma concentrations were lower in HD patients than in control group. Similar results have been reported in previous studies.[3,5,6,14,19,21] The kidney plays an important regulatory role in homeostasis of the Se and contains the highest level of this element, which could explain the Se deficiency in HD group.[21] Other authors suggest a decreased absorption of Se in the small intestine in ESRD patient.[37] Se is particularly important to the antioxidant defense system and the activity of different glutathione peroxidases, which are selenoenzymes, has been shown to be depressed in Se deficiency.[13] A strong correlation between plasma glutathione peroxidases and Se level was found and a decreased activity of this enzyme has been detected in ESRD patients.[38]

In our study, HD has no effect on Cu, Zn and Se concentration. Results with regard to these trace elements levels in HD are conflicting with some studies showing a decrease[30] while others reporting an increase after dialysis.[28] On the other hand, our results showed that there is a significant increase of plasma MDA levels after HD session. These finding are in agreement with the previous studies, which have reported that the HD session may be accompanied by a significant increase of lipid peroxidation and over production of oxidative stress mainly due to cell activation by membrane dialyzer and cellular-cellular interactions during hemodialysis.[25] Others studies report a decrease in MDA levels to normal after dialysis due to its clearance.[40]

The main limitation of this study was the relatively small sample size and there is a need to perform such studies with a larger sample. However, even if it was a small sample, it has allowed us to present some interesting preliminary results for Moroccan nephrologists. The antioxidant status of patients with ESRD was found depressed, as reflected by low plasma concentrations of Zn and Se, elevated Cu/Zn ratio and MDA concentrations. It would be wise to recommend a periodic determination of Cu, Zn and Se plasma levels in HD patient with the possibility of Zn and Se supplementation for patients with documented deficiency.

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