

Research Article

# Effects of Zinc Supplementation on Oxidative Stress in Patients Undergoing Maintenance Hemodialysis

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Keywords: Hemodialysis; Zn supplement; Copper to zinc ratio; Catalase activity; Homocysteine; Glutathione; Total bilirubin



## Abstract

**Introduction:** The aim of this study was to examine the effects of Zn supplementation on oxidative stress by evaluating changes in serum Copper (Cu) to Zinc (Zn) ratio, homocysteine (hCys), Glutathione (GSH), Total Bilirubin (TB) and Catalase (CAT) activity in hemodialysis patients.

**Methods:** Seventy-seven HD patients were enrolled in a multicenter simple-blind randomized clinical trial. Only 37 HD patients completed the study. They were randomly divided into two groups and supplemented with zinc sulfate ( $n = 17$ ) or placebo ( $n = 20$ ) for two months. Serum Zn and Cu were measured by atomic absorption spectrophotometry. Serum hCys was measured by immunology method, serum GSH and CAT activity were assessed by spectrophotometry method and TB was measured by colorimetric method. Determinations were performed before and after supplementation.

**Findings:** After zinc supplementation, serum Zn, serum GSH, and Serum Total Bilirubin (STB) significantly increased. Serum Cu to Zn ratio, serum hCys, and CAT activity significantly decreased in the Zn Zn-supplemented group.

**Conclusion:** Zinc supplementation increased serum antioxidant factors such as Zn, GSH, and bilirubin and decreased serum oxidative factors such as copper to zinc ratio, hCys, and decreased CAT activity. The study results suggest that zinc supplementation may be a useful tool for the improvement of oxidative stress in HD patients.

## Introduction

Patients undergoing maintenance hemodialysis exhibit a higher morbimortality due to cardiovascular disease [1] which can be attributed to many factors including oxidative stress [2,3]. Several studies have demonstrated zinc deficiency as a common problem in patients with HD [4]. It has been also shown that zinc deficiency plays considerable roles in the development and progression of CVD in these patients [5-7]. Zn is a trace element that acts as an antioxidant and anti-inflammatory agent [5,6]. Only a few studies have investigated the effects of zinc supplementation in HD patients on oxidative stress [8] also only a few studies

have analyzed several SO parameters at the same time in the same group of patients. The impact on CAT activity and STB has never been analyzed.

The present study aimed to investigate the effects of Zn supplementation in HD patients on oxidative stress by evaluating changes in the levels of zinc, copper, homocysteine, reduced glutathione, catalase activity, and total bilirubin.

## Materials and methods

A multicenter randomized single-blind clinical trial was conducted among 77 HD patients (45 men and 32 women). The patients were recruited from five centers: Rabta Hospital

Nephrology Department, Matri Hospital HD unit, Manouba HD Radial Center, Manouba HD Public Center as well and HD Udial Center. Causal nephropathies are distributed as follows: glomerulonephritis (27 patients), hypertensive nephropathy (16 patients), interstitial polynephritis (11 patients), polycystic disease (9 patients) and undetermined cause (14 patients).

The selection of patients included the following criteria: age over 18 years old, HD treatment for at least six months, HD performed three times per week (each for 4 hours) through a polysulfone membrane against a dialysis liquid containing the following ions: Na<sup>+</sup>: 138 mmol/L; K<sup>+</sup>: 2 mmol/L; Ca<sup>++</sup>: 1.5 mmol/L; Mg<sup>++</sup>: 0.5 mmol/L; Cl<sup>-</sup>: 109 mmol/L; CH<sub>3</sub>CO<sub>2</sub><sup>-</sup>: 3 mmol/L; HCO<sub>3</sub><sup>-</sup>: 35 mmol/L. The clearance of urea evaluated by the ratio Kt/V was 1.2. The exclusion criteria were infection, gastrointestinal and liver diseases, congestive heart failure, cancer, psychiatric illness, pregnancy, use of immunosuppressants, corticoids, estrogens, or contraceptives, as well as active smoking and alcoholism. The patients were randomly divided into two groups; 43 HD patients received one oral capsule containing 220 mg of Zn sulfate (100 mg of elemental Zn) and 34 HD patients received one oral capsule of similar appearance containing 220 mg of maltodextrin. Capsules were taken daily after dinner for 60 consecutive days. Randomization was performed while stratifying on gender, 5-year age class, and duration of HD. Predialysis blood samples were obtained from all patients after an overnight fast at inclusion (day 1) and after 60 days of supplementation (day 61). Blood samples of hCys were collected in tubes and placed on ice. Serum Zn and Cu concentrations were determined by atomic absorption spectrophotometry (Perkin Elmer, Waltham, USA). Serum hCys was measured by immunology method using the Architect CI8200 auto analyzer (Abbott Diagnostics, Chicago, USA). Serum GSH and CAT activity were assessed by spectrophotometry method. Serum TB was measured by colorimetric method. The study protocol was approved by the Ethics Committee of Rabta Hospital and all patients gave their informed and signed consent to participate in the study. A total of 37 HD patients (17 in the supplemented group and 20 in the placebo group) completed the trial (Figure 1).

### Statistical analysis

Statistical analyses were carried out using the software package SPSS 22.0 for Windows (SPSS Inc, Chicago, USA). Depending on the distribution of variables, data were reported as mean ± Standard Deviation (SD) or median (interquartile range). Between groups, comparisons were made using an independent t-test or Mann-Whitney test, as appropriate. Within groups, comparisons of variables before and after supplementation were performed using paired t-test or Wilcoxon rank test, as appropriate.

A *p* - value < 0.05 based on two-sided calculation was considered significant.

## Results

Baseline clinical and biochemical characteristics were comparable in Zn and placebo groups (Table 1).

After two months of supplementation, serum Zn concentration increased ( $124 \pm 46.4 \mu\text{g/dl}$ ) ( $p = 0.002$ ) and serum Cu to Zn ratio decreased significantly in the Zn supplemented group ( $0.93 \pm 0.47$ ) ( $p = 0.01$ ) but remained unchanged in the placebo group. After supplementation, we found a significant increase in serum GSH ( $0.25 (0.72)$ ) ( $p = 0.005$ ) and TB ( $5.71 \pm 1.58 \text{ mg/L}$ ) ( $p = 0.024$ ). Serum hCys was significantly decreased ( $16.61 \pm 4.69 \mu\text{M/L}$ ) ( $p = 0.000$ ) and also CAT activity ( $0.021 (0.1)$ ) ( $p = 0.02$ ) and thus after zinc supplementation.

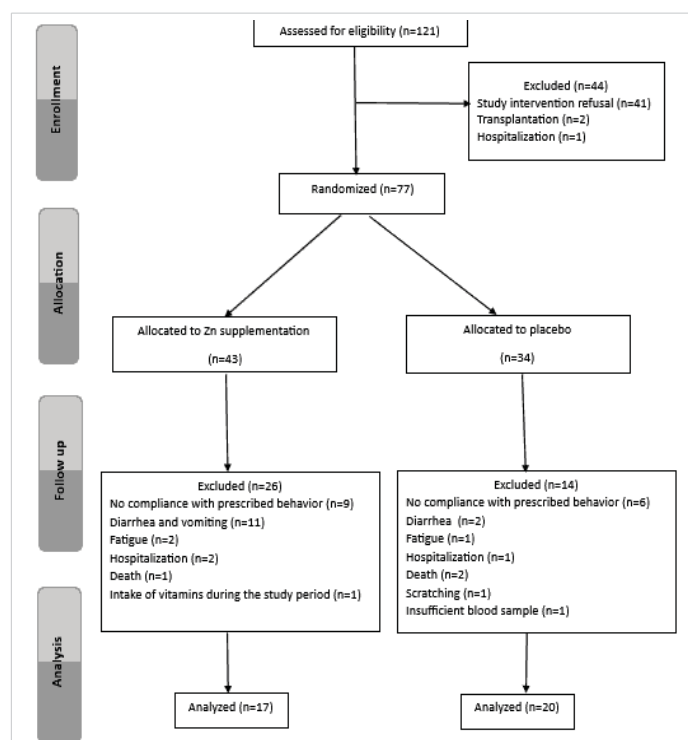


Figure 1: Flow chart for the process of hemodialysis patients selection.

Table 1: Baseline clinical and biochemical characteristics in zinc-supplemented and placebo groups of hemodialysis patients.

	Zinc group (n = 17)	Placebo group (n = 20)	<i>p</i> value
Mean age, years	52.9 ± 13.5	53.6 ± 15.8	0.89
Male gender, %	70.6	55.0	0.33
Duration of HD, years	6.65 ± 3.93	6.55 ± 3.73	0.93
Body mass index, Kg/m <sup>2</sup>	25.2 ± 4.34	26.4 ± 5.74	0.53
Serum creatinine, mmol/L	967 ± 264	896 ± 361	0.50
Serum zinc, μg/dL	76.8 ± 16.6	81.2 ± 16.8	0.42
Zinc deficiency *, %	64.7	55.0	0.55
Serum copper, μg/dL	116 ± 33.2	117 ± 28.1	0.91
Serum copper to zinc ratio	1.52 ± 0.38	1.47 ± 0.35	0.62
Serum hCys, μM/L	21.07 ± 6.07	20.80 ± 8.04	0.90
Serum GSH, U/mg of protein	0.15 (0.23)	0.12 (0.59)	0.51
CAT activity, U/mg of protein	0.038 (0.15)	0.045 (0.01)	0.17
Serum TB, mg/L	4.76 ± 1.36	5.05 ± 1.76	0.62

Values are expressed as mean ± SD or median (interquartile range); hCys: Homocysteine; GSH: Glutathione; CAT: Catalase; TB: Total Bilirubin; \*, serum Zn < 80 μg/dL.

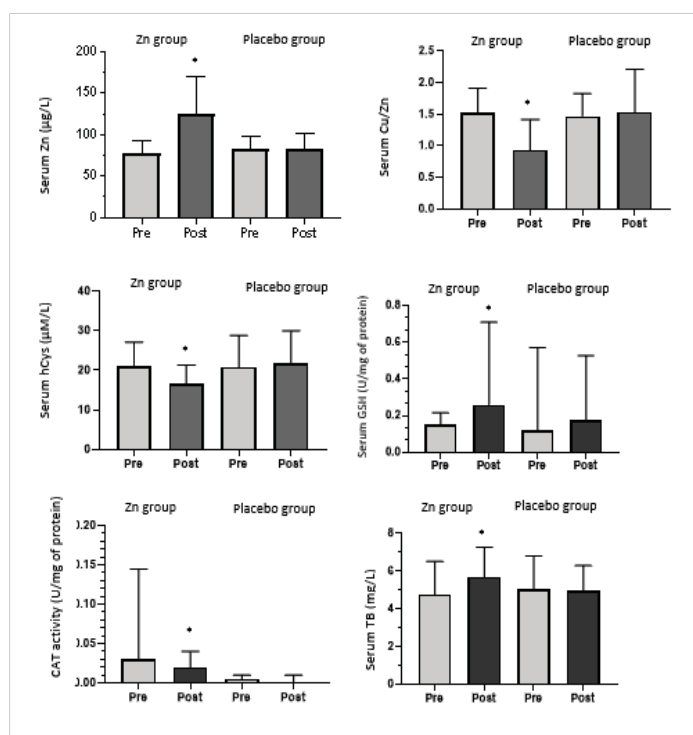
Figure 2 shows variations in zinc, Copper zinc ratio, homocysteine, glutathione, catalase activity, and total bilirubin under supplementation in zinc and placebo groups.

## Discussion

Zinc deficiency is common in HD patients [9]. It has been reported in up to 50% of these patients [10-12]. The present study indicated that 59.5% of Tunisian HD patients have decreased Zn levels. Literature suggests that hypozincemia may be caused by Zn removal during HD treatment, reduced gastrointestinal absorption [8], low dietary intake, and increased urinary excretion [13] as well as protein restriction [14], increased expression of intracellular metallothioneins [15], multi-infections [10] and metabolic acidosis [16].

Zn supplementation in the present study significantly increased serum Zn and decreased serum Cu to Zn ratio, which is consistent with literature data [8]. The improvement in Zn status after Zn supplementation is expected and comprehensible. The competition between the two trace elements at the phases of intestinal absorption and cellular trafficking may explain the decrease in serum Cu after Zn supplementation. Indeed, Zn and Cu share the same enterocyte membranes' transporters and intracellular trafficking proteins [17,18]. A high Cu reflects oxidative stress, increased inflammation, and immune dysfunction [18-20]. Thus, its reduction in HD patients is considered beneficial, which supports the usefulness of Zn supplementation in these patients.

The study also showed a decrease in serum hCys following



**Figure 2:** Variations in zinc, Copper to zinc ratio, homocysteine, glutathione, catalase activity and total bilirubin under supplementation in zinc and placebo groups.

Zn supplementation. hCys is a pro-oxidant component. It is a sulfur-containing amino acid metabolized through transsulfuration and transmethylation pathways. It is reported that hyperhomocysteinemia is an independent risk factor for cardiovascular mortality and morbidity in ESRD patients [21,22]. The beneficial effect of zinc supplementation on hCys levels could be explained by the fact that zinc contributes to the intestinal absorption of dietary folate. In fact, pteroylglutamatehydrolase, an enzyme responsible for the hydrolysis of dietary folate before its absorption, is zinc-dependent [23,24]. Zinc contributes also to the activity of BHMT (betaine-homocysteine methyltransferase), which is involved in the remethylation of homocysteine to methionine [14].

According to the results of our study, Zn supplementation increased serum GSH. GSH is a tripeptide composed of glutamate, cysteine, and glycine, that is synthesized from two sequential reactions catalyzed by glutamate-cysteine ligase and glutathione synthetase. The increase of serum GSH after supplementation could be explained in part by the fact that zinc induces the synthesis of glutathione by increasing the expression of glutamate cysteine ligase which catalyzes the formation of gamma-glutamylcysteine ( $\gamma$ -GC) from glutamate and cysteine [25].

To our knowledge, no previous studies have investigated the effect of zinc supplementation on CAT activity in HD patients. CAT is an important antioxidant enzyme. It allows the transformation of hydrogen peroxide into water and oxygen. Its activity requires the presence of iron. Its serum decrease following zinc supplementation could be linked to the decrease in the bioavailability of iron in relation to iron-zinc antagonism [26].

This study, to our knowledge, is the first that evaluates the effect of Zn supplementation on serum total bilirubin in HD patients. Low STB levels are associated with increased oxidative stress and may be a risk factor for Cardiovascular Diseases (CVD) [27]. High STB levels were associated with reduced risk of cardiovascular disease and mortality in dialysis patients [28].

Bilirubin has been known to have antioxidant properties [29,30], whether conjugated, unconjugated, free, or protein-bound [31]. Bilirubin directly inhibited NADPH oxidase activity and suppressed superoxide generation in vascular endothelial cells and renal tubular cells [32]. Bilirubin has been reported also to act as an oxidant scavenger [32]. After Zn supplementation, STB was significantly increased. Its serum increase following zinc supplementation could be explained by the fact that biliverdin reductase, an enzyme that catalyzes the reduction of biliverdin to bilirubin, is a Zn metalloprotein [33].

There are potential limitations to this study. First, our study was limited by a small sample, resulting in a low



statistical power. In fact, a number of patients in both Zn and placebo groups dropped out from the trial for lack of compliance with the study design or for adverse health effects. Second, HD patients were supplemented without taking into account their baseline serum Zn concentrations. This might explain some side effects that occurred in some patients. Further, trials with large sample sizes in Zn-deficient patients are needed to confirm our results.

## Conclusion

Zinc supplementation improved oxidative status in our HD patients. It increased serum anti-oxidative factors such as Zn, GSH, and bilirubin and decreased serum oxidative factors such as copper to zinc ratio, hCys, and CAT activity. The study results suggest that zinc supplementation may be a useful tool for stress oxidative therapeutic strategy in HD patients which may have implications for cardiovascular complications and propose monitoring of serum zinc concentration in this population.

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