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Omnia G. Refaat

Nutrition and Food Science Department, Faculty of Home Economics, Helwan University.

N.M. Rabeh

Nutrition and Food Science Department, Faculty of Home Economics, Helwan University.

A.A. Helmy

Consultant Urologist - Egypt air, Medical services Company.

O.A. Abdel-Alim

Nutrition and Food Science Department, Faculty of Home Economics, Helwan University.

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البريد الإلكتروني للمجلة E-mail JSROSE@foe.zu.edu.eg

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Abstract:

The potential effect of zinc supplementation on kidney functions, hematological parameters, parathyroid hormone and minerals was investigated in hemodialysis patients. Thirty patients in the age between 35 to 60 years who were on regular hemodialysis (HD) for more than three months, three times per week for 4-6 hours in each session received zinc supplement daily 250 mg (zinc citrate) /day (100 mg elemental zinc) for two months. Blood samples were collected at zero time, and at the end of this study, before hemodialysis from each patient. The results indicated that, supplementing hemodialysis patients with zinc caused a highly significant ($P<0.01$) decrease in the levels of serum urea, creatinine, uric acid, phosphorus and potassium. Significant ($P<0.05$) decrease was recorded in levels of white blood cells (WBC) and TIBC, compared to patient's status before zinc supplementation. On the other hand, zinc supplementation caused a highly significant ($p<0.01$) increase in the levels of mean corpuscular hemoglobin concentration (MCHC), calcium and zinc and significant ($P<0.05$) increase in the levels of hemoglobin (Hb). The obtained results suggested that supplementing hemodialysis patients with zinc may cause improvement in kidney functions and hematological parameters.

Key words: Zn, supplementation, hemodialysis patients, kidney functions, hematological parameters, parathyroid hormone, minerals.

Introduction:

Patients with end stage renal diseases that require long-term dialysis are a public health concern worldwide. Despite dialysis treatment, these patients still have high morbidity and mortality rates (**Al Wakeel *et al.*, 2002)**. The major contributing risk factors include wasting, an oxidant-antioxidant imbalance, progressive inflammation, impaired immune responsiveness, and infection **(Guo *et al.*, 2011)**.

At the end stage of renal disease patients, different factors affect serum concentrations of trace elements like increased oral intake, failure of renal excretion, degree of renal failure, use of medications, contamination of dialysate, quality of water used for dialysis and metabolic alterations associated with renal failure **Krachler and Wirnsberger (2000) and Miura *et al.* (2002)**.

Trace element deficiencies (more commonly for zinc, iron and possibly selenium) occur in hemodialysis (HD) patients **(Kalantar and Kopple, 2003 and Guo *et al.*, 2011)**. Zinc is an essential trace element, required for the action of more than 200 metallo enzymes and plays an important role in polymeric organization of macromolecules (like DNA and RNA), protein synthesis and cell division. Zinc plays many significant roles in metabolism (**Smith and Akinbamizo, 2000)**. Also, Zn is an anti-oxidant, has anti-inflammatory properties, and regulates innate and adaptive immune responses, which makes it crucial for resistance to infection **(Guo *et al.*, 2011)**.

Zinc deficiency is more common in many disease states such as patients undertaking hemodialysis **(Parsad, 2001)**. Zn deficient status is associated with immune system disturbances, poor nutritional status, atherosclerosis, high rates of hospitalization due to infections (**Yang *et al.*, 2012)**, some of the uremic symptoms such as anorexia, hypogeusia, sexual dysfunction or decreased immunologic function (**Cabral et al., 2005)**.

Bhogade *et al.* (2013) revealed that serum copper and zinc concentrations in hemodialysis patients are distinctly decreased compared to that of healthy controls. Abnormalities of trace elements are primarily the result of uremia, and they may be further exacerbated by the dialysis procedure. **Guo *et al.* (2013)** reported that Zn supplementation ameliorates abnormally high plasma aluminum concentrations and oxidative stress and improves selenium status in long-term dialysis patients. Also, Zinc supplementation for 2 months improved the serum levels of zinc, antioxidant status, and lipid peroxidation in HD patients **(Mazani *et al.*, 2013)**.

This study was designed to investigate the effect of oral zinc supplementation on kidney functions, hematological parameters, minerals status of hemodialysis patients.

Materials and Methods:

Materials:

Thirty patients in the age of 35 to 60 years who were on regular hemodialysis for more than three months, three times per week for 4-6 hours in each session selected from outpatients HD unit of Egypt Air Hospital, Cairo, Egypt. The patients diagnosed on the basis of detailed clinical history, clinical examination and other relevant biochemical investigations. The patients suffering from other diseases, such as inflammatory diseases and hepatic or respiratory diseases excluded from study.

Zinc citrate capsules were obtained from Amoon Company for Chemicals, Cairo, Egypt, as a gift for encouragement the scientific research.

Methods:

- Dietary assessment: 24 hours recall was done for one day before hemodialysis for all studied patients.
- The patients received zinc supplement daily after hemodialysis as zinc citrate (250 mg/day (100 mg elemental zinc) for two months according to **Ghaemmaghami *et al.* (2010).**

Chemical analysis:

Blood samples were collected at zero time and at the end of the study, before hemodialysis from each patient. The serum was separated within 2 hours after blood withdrawal, by centrifugation at 3000 rpm for 15 minutes. The serum samples were kept frozen at -20°C until determination the following parameters:

- Serum urea, uric acid and creatinine were determined according to **Kaplan (1984)**, **Patton and Crouch (1977)** and **Murry (1984)**, respectively.
- Complete blood count including: hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), red blood cell (RBC) count were measured using automated hematology analyzer (Sysmex, Kobe, Japan). Serum iron, total iron binding capacity (TIBC), ferritin were determined calorimetrically and enzymatically, using sigma diagnostics iron, ferritin and TIBC reagents, (sigma diagnostics, st. Louis, MI, USA), creatinine protein **(AOAC, 1990),** and Transferin saturation (%) was calculated using the following equation: Transferring
  saturation (%) = (Serum iron concentration÷TIBC)× 100 according to **Lee and Nieman (1996).**
- Parathyroid hormone (PTH) was determined by using radioimmunoassay (RIA) kit (M-44-68) **(Yalow and Berson, 1993).**
- Electrolytes: Phosphorus **(Goodwin, 1970)**, calcium **(Gindler and King, 1972)**, potassium, sodium and Zinc was determined by an atomic absorption spectrophotometer **(Shenkin *et al.*, 2006)**.

Statistical analysis:

Data was analyzed using SPSS version 18. Parameters before and after zn supplementation were compared by a paired t-test. A p-value of 0.05 and 0.01 were considered statistically significant (**SPSS, 1986)**.

Results and discussion:

The present study was designed to investigate the effect of zinc supplementation on kidney functions, serum electrolytes and complete blood count in hemodialysis patients.

Dietary assessment:

The results in Table (1) showed that the hemodialysis patients obtained excessive percentage of protein, sodium, potassium, phosphorus, vitamin c and iron, compared with the RDA; with mean values 111.64 ± 5.73, 143.22 ± 14.58, 153.13 ± 10.31, 128.77 ± 6.22, 181.26 ± 21.09 and

135.76 ± 8.50, respectively. On the other hand, they obtained low percentage of energy, carbohydrates, fats, calcium, vitamin B_1 and zinc with mean values 89.16 ± 3.46, 85.30 ± 4.99, 89.85 ± 7.68, 43.51 ± 3.22, 37.53 ± 2.22 and 58.16 ± 5.59, respectively.

Table (1): Percentage of daily dietary intakes for hemodialysis patients compared to RDA.

Nutrients	**Mean ± SE (%)**
Energy	89.16 ± 3.46
Protein	111.64 ± 5.73
Carbohydrates	85.30 ± 4.99
Fats	89.85 ± 7.68
Sodium	143.22 ± 14.58
Potassium	153.13 ± 10.31
Calcium	43.51 ± 3.22
Phosphorus	128.77 ± 6.22
Vitamin B1	37.53 ± 2.22
Vitamin C	181.26 ± 21.09
Iron	135.76 ± 8.50
Zinc	58.16 ± 5.59

The obtained results were in the line with **(Rucker *et al.*, 2010)** who indicated that recommended dietary intake from zinc is 15 mg/day. Our results weren't in agreement with the recommendation dietary allowances that investigated those hemodialysis patients should receive daily energy as 30-40 kcal/kg **(Stenvinkel *et al.*, 2000 and Kalantar *et al.*, 2003).** Dietary protein should be adjusted at least 1.2 g/kg/day in hemodialysis patients as indicated **(Kopple, 2001; Kalantar *et al.*, 2003; Locatelli *et al.*, 2005 and Mahan *et al.*, 2012).** Carbohydrates should provided 60-65% of daily energy from carbohydrates **(Kopple and Massery, 2004).**

Total energy from fat should not exceed 25 to 30%. It should be reduced saturated fat content of the diet and increased unsaturated fat content **(Nissenson *et al.*, 2008).** Controlling sodium and fluid intake are important components of the HD diet. Water and sodium intake in hemodialysis patients are adjusted according to the amount of urine, fluid balance and blood pressure. With hemodialysis, potassium restriction is often necessary, but the measure of restriction depends on residual renal function **(Stark *et al.*, 2011).** Recommended dietary intake from phosphorus, calcium, iron, vitamin C, vitamin B1 (thiamin) for hemodialysis patients are 800-1000 mg/day, <1000 mg/day, 10-18 mg/d, 75–100 mg/day and 1.1–1.2 mg/day, respectively **(Fouque and Guebre, 2007; Nissenson *et al.*, 2008 and Rucker *et al.*, 2010).**

The results in Table (2) showed a highly significant (P<0.01) decrease in the levels of serum urea, creatinine and uric acid after supplementing patients with zinc citrate capsules, compared to before zinc supplementation.

The current results were in agreement with **El-Ashmony *et al.* (2012)** who mentioned that, zinc supplementation for 8 weeks caused significant reduction in blood urea nitrogen (BUN) and serum creatinine (p<0.001). Meanwhile, no significant changes occurred in both parameters in placebo group after treatment compared to those before treatment (p > 0.05). This trial showed significant improvement in renal functions as measured by serum creatinine and BUN. Similar results were observed by **Garg and Bakris (2002)**. While, **Khadim *et al.* (2006)** reported no significant effect of zinc on renal functions as measured by serum as well as urine creatinine. Furthermore, zinc may have renoprotective effect via its antioxidant property **(Parham *et al.*, 2008)**.

Table (2): Effect of zinc supplementation on kidney functions in hemodialysis patients.

Parameters		Before zn supplementation	After zn supplementation	T	Sig.
Urea	(mg/dl)	141.18 ± 5.67	109.21± 3.38	6.12	0.000**
Creatinine		9.88 ± 0.39	8.84 ± 0.36	3.36	0.002**
Uric acid		7.11 ± 0.25	6.10 ± 0.25	3.35	0.002**

Data expressed as Means ± SE. **: highly significant differences at ($p<0.01$).

The results in Table (3) indicated that zinc supplementation caused significant ($P<0.05$) increase in the levels of Hb and highly significant ($p<0.01$) increase in the levels of MCHC after supplementing hemodialysis patients with zinc compared to before zinc supplementation. The data in the same table showed significant ($P<0.05$) decrease in levels of white blood cells (WBC) and TIBC after zinc supplementation compared to before zinc supplementation. On the other hand, there is no significant differences in levels of HCT, RBC, iron, ferritin, transferrin and PTH before and after zinc supplementation.

Contradiction to this results, **Abo-Ghanema *et al.* (2016)** revealed that, there is a significant increase ($p<0.05$) in RBCs number in the zinc citrate and zinc sulfate group as compared with thioacetamide (TAA) treated group**.** Also, the results of Abo-Ghanema's study showed that, there is significant increase ($p< 0.05$) in HCT values in zinc citrate treated groups as compared with control group. But our results were in accordance with Abo-Ghanema's study that stablished that, Hb values showed insignificant increase in zinc citrate and zinc sulfate groups respectively compared with the control group. WBCs values significantly decreased ($p<0.05$) in combined groups as compared with the control group, while it showed a non-significant decreased in zinc citrate and zinc sulfate treated groups as compared with the control group.

In addition, these results were in a harmony with **Donmez *et al.* (2002**) who reported that zinc supplementation did not affect erythrocyte values in chicks, also **Ajayi, (2008)** found that there is no significance difference in red cell volume of zinc deficient rats and the control. **Kobayashi *et al.* (2015)** revealed non significant differences in the levels of PTH in oral polaprezinc therapy group (n=35) or control group (n=35, no supplementation). Patients on polaprezinc treatment (administered at 150 mg, containing zinc at 34 mg/day) and controls were monitored for 12 months.

Table (3): Effect of zinc supplementation on serum haematological parameters in hemodialysis patients.

Parameters		Before zn supp.	After zn supp.	T	Sig.
Hb	**(gm/dL)**	10.70±0.18	11.36±0.26	-2.41	0.022*
Hct	**(%)**	34.00±0.67	33.93±0.75	0.09	0.922(n.s)
MCHC	**(gm/dL)**	31.35±0.17	33.40±0.14	-13.14	0.000**
RBC	**($x10^{12}$/L)**	3.86±0.07	3.72±0.08	1.47	0.151(n.s)
WBC	**($x10^{3}$/µL)**	8.55±0.37	8.00±0.39	2.08	0.045*
Iron	**(µg/dL)**	70.48±4.90	75.57±4.48	-1.12	0.269(n.s)
TIBC		192.69±4.68	182.18±4.50	2.19	0.035*
Ferritin	**(ng/mL)**	509.31±40.07	535.69±49.60	-0.69	0.493(n.s)
Transferrin	**(%)**	42.19±3.00	42.92±3.27	-0.23	0.814(n.s)
PTH	**(pg/mL)**	67.38±3.34	62.20±4.35	1.004	0.323(n.s)

Data expressed as Means ± SE. **: highly significant differences at (p<0.01)

*: significant at (P<0.05). n.s: non significant.

The results in Table (4) showed that supplementing hemodialysis patients with zinc citrate capsules caused highly significant (P<0.01) decrease in the levels of serum phosphorus and serum potassium compared to before zinc supplementation. In addition, the results illustrated: highly significant (P<0.01) increase in the levels of serum calcium and serum zinc after zinc supplementation compared to before. But, there is no significant differences in the level of serum sodium before and after zinc supplementation.

The obtained results were in accordance with **El-Shazly *et al.* (2015)** who found that, no significant difference between two groups (group I, received zinc sulfate supplementation, and group II, received placebo (cornstarch capsules) twice daily for 90 days) at the beginning of the study was observed. Serum zinc mean levels were 53.2±8.15 in group I and 55.45±9.1 µg/dL in group II, which were in the lower than normal range of serum zinc in normal children of the same age and sex (63.8–110 µg/dL) **(Johnsen, 1987)**. After 90 days of study, the mean serum zinc level had highly significantly increased from 53.2±8.15 µg/dL to 90.75±12.2 µg/dL ($P<0.001$) while it remained unchanged in the control group. **Hargunow *et al.* (2000) and Ghaemmaghami *et al.* (2010)** also investigated, the initial mean serum zinc concentrations in both the control group (77±4 µg/dL) and the zinc-supplemented experimental group (76±3 µg/dL) were in the lower limit of the normal range (70-110 µg/dL)**.** At the end of the intervention, the mean zinc concentrations in the supplemented group had increased from 76±3 µg/dL to 102±4 µg/dL, which was statistically significant ($p < 0.05$).

In this context, **(Mahdaviroshan *et al.*, 2013)** investigated that there were no significant differences in calcium and zinc concentrations at baseline between the 2 groups, an intervention group (n = 30) receiving 1 capsule of zinc sulfate 220 mg (containing 50 mg elemental zinc) each day, and a placebo group (n = 30) receiving a placebo containing starch. After 60 days, the group receiving zinc sulfate supplements had a significantly higher serum zinc concentration than at baseline ($P<0.05$). In contrast, there was non significant difference in serum calcium concentration. Also, **Kobayashi *et al.* (2015)** revealed that no significant differences were noted in the serum calcium or phosphate levels in the two groups (oral polaprezinc therapy group (n = 35) or control group (n = 35, no supplementation). Patients on polaprezinc treatment (administered at 150 mg, containing zinc at 34 mg/day) and controls were monitored for 12 months.

Table (4): Effect of zinc supplementation on serum minerals in hemodialysis patients.

Parameters		Before zn suppl.	After zn supp.	T	Sig.
Phosphorus	**(mg/dl)**	6.69±0.39	4.23±0.33	8.30	0.000**
Calcium		7.56±0.11	8.00±0.11	-4.87	0.000**
Potassium	**(mmol/l)**	5.52±0.17	4.63±0.15	4.10	0.000**
Sodium		138.54±0.50	138.54±0.48	0.00	1.000(n.s)
Zinc	**(µg/dl)**	39.72±2.09	97.93±4.77	-10.99	0.000**

Data expressed as Means ± SE. **: highly significant differences at ($p<0.01$) n.s: non significant.

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تأثيـر التدعيـم بالزنـك على المرضـى المعاشـين على الإسـتصفاء الدمـوى

أمنية جلال رفعت[١]– نعيم محمد رابح[١] – أنور أحمد حلمى[٢] – أسامة أبو العلا عبد العليم[١]

١– قسم التغذية وعلوم الأطعمة – كلية الاقتصاد المنزلى – جامعة حلوان.

٢– استشارى جراحة المسالك البولية– شركة مصر للطيران للخدمات الطبية.

الملخص العربى:

تم دراسة تأثير التدعيم بالزنك على وظائف الكلى وصورة الدم الكاملة وهرمون الغدة الجاردرقية والأملاح المعدنية الموجودة فى الدم فى المرضى المعاشين على الاستصفاء الدموى. تمت الدراسة على ٣٠ مريض تتراوح أعمارهم مابين ٣٥ الى ٦٠ سنة يقومون بالغسيل الكلوي المنتظم لأكثر من ثلاثة أشهر، ثلاث مرات أسبوعيا ، لمدة ٤–٦ ساعات في كل جلسة – تم اعطاء المرضى كبسولات الزنك (٢٥٠ ملجم سترات الزنك / اليوم (يحتوي علي ١٠٠ ملجم من الزنك) بعد الغسيل الكلوى لمدة شهرين وتم جمع عينات الدم عند بداية ونهاية الدراسة قبل الغسيل الكلوي من كل مريض على حدى. وأظهرت النتائج أن تدعيم المرضى المعاشين على الاستصفاء الدموى بعنصر الزنك يؤدى الى انخفاض معنوى كبير ($P<0.01$) فى مستويات اليوريا والكرياتينين وحمض اليوريك وكذلك الفوسفور والبوتاسيوم فى السيرم. كذلك أوضحت النتائج وجود انخفاض معنوى ($P<0.05$) فى مستوى كرات الدم البيضاء و وإجمالي قدرة ربط الحديد. من ناحية أخرى، فإن التدعيم بالزنك أدى إلى ارتفاع معنوي كبير ($P<0.01$) فى مستوي متوسط تركيز الهيموجلوبين وعنصرى الكالسيوم والزنك فى الدم وأدت كذلك إلى ارتفاع معنوى ($P<0.05$) فى مستوى الهيموجلوبين فى الدم. الخلاصة: تدعيم المرضى المعاشين على الاستصفاء الدموى بالزنك يؤدى الى تحسن فى وظائف الكلى وصورة الدم الكاملة ومستوى الزنك فى الدم.