# Assessment of Some Trace Elements: Copper, Zinc and Magnesium and Their Impact on CD3 and CD4 Levels in Children on Chronic Hemodialysis

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**Abstract: Background:** Infection stills a major cause of morbidity and mortality in end-stage renal disease (ESRD) patients. The susceptibility of ESRD patients to infections is typically linked to the immunodeficient state associated with uremia. Changes in essential trace elements may affect the immune system of children on hemodialysis (HD). **Aim:** To assess the relationships between some trace elements (zinc, copper and magnesium) content and some markers of immune status (CD3, CD4) in children on hemodialysis. This would help better understanding of mechanisms (factors) of immune dysfunction in these patients aiming at development of new therapeutic strategies that improve morbidity and immortality. **Methods:** The present study included 20 Children with ESRD on regular HD, they were 11 females (55.00%) and 9 males (45.00%) and their ages ranged 4-15 y (mean 8.91±3.46). They were compared with 20 age and sex matched apparently healthy children as control group. The trace elements; zinc, copper and magnesium and CD3and CD4 as well as complete blood picture, serum urea, creatinine and albumin were measured. **Results:** Significantly decreased zinc level, significantly decreased percentages of CD3 and CD4 T lymphocytes and increased levels of urea, creatinine, phosphorus and copper were observed in hemodialysis patients in comparison to control group. hemodialysis patients had also low levels of albumin and hemoglobin. **Conclusions:** Hemodialysis patients are at risk for deficiency of essential trace elements and excess of toxic trace elements, both of which are potentially harmful as it cause suppression of immune function in ESRD patients as evidenced by decreased percentages of CD3 and CD4 lymphocytes, so continuous evaluation of trace elements is important in chronic hemodialysis patients.

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**Key words:** Immune system, trace elements, hemodialysis.

## 1. Introduction

Substances that have lower concentrations in dialysate than in blood tend to be removed by dialysis. Although this is appropriate in the case of uremic toxins, it may lead to depletion of biologically essential substances. Besides the potential for ongoing removal of trace elements by dialysis, hemodialysis patients are at risk for low dietary intake of such substances due to uremia-related anorexia and dietary restrictions ***(Tonelli et al., 2009).***

Uremia-related immune dysfunction is a complex pathological condition in which chronic activation of immune system and immune suppression coexist. Understanding the mechanisms behind the immune dysfunction that is peculiar to ESRD represent an important target for intervention aiming to reduce morbidity and mortality in these patients (***Hauser et al., 2007).***

Long-term dialysis leads to significant changes in the concentrations of some trace elements and increased susceptibility to oxidative stress, inflammation, and reduced immune regulation of T lymphocytes ***(Guo et al., 2011).*** Efficient immune function also depends on the status of trace elements and inadequate levels of these elements may lead to depression of the immune response ***(Maggini et al., 2007).*** Hemodialysis patients are exposed to very high volumes (>300 liters/week) of dialysate. Therefore, even minute levels of toxic substances in source water could lead to tiny concentration gradients between blood and dialysate, which in turn could lead to clinically relevant toxicity. Substances present in dialysate but not in blood will tend to accumulate in the patient, and the lack of renal clearance in hemodialysis patients might theoretically lead to toxicity of ingested trace elements even when they are not present in dialysate. Thus, hemodialysis patients are at theoretical risk for both deficiency and accumulation of trace elements, depending on dietary intake, removal by dialysis, the composition of the source water used for hemodialysis, and residual kidney function ***(Zima et al., 1999)***. We performed this study to compare copper, zinc and magnesium status between hemodialysis patients and healthy controls and their impact on CD3, and CD4 lymphocytes.

**Aim of The Work** The aim of this work was to assess the relationship between some trace elements (zinc, copper and magnesium) content and some markers of immune status (CD3, CD4) in children on hemodialysis. This would help better understanding of the underlying mechanisms of immune dysfunction in these patients aiming at development of new therapeutic strategies that improve morbidity and immortality.

**2. Subjects and Methods**

This prospective cross sectional study was carried out on 40 children 20 of them with end stage renal disease (ESRD) as defined by Kidney Disease Outcome Quality Initiative ***(KDOQI, 2008)*** and undergoing hemodialysis (HD) with dialysis prescription of 12 hours per week (3 sessions of 4 hours each). Dialysis was carried out on Fresenius (4008.B and 4008S) and dialysators were from synthetic polysulphone, using low flux hemodialyser membranes with vascular accesses through arteriovenous fistula and central venous catheter. Hemodialysis was performed using bicarbonate buffered dialysate. They were selected from the renal dialysis unit in pediatric department in Al-zahraa university hospital between May 2012 and August 2012. Their ages ranged from 4 to 15 years and duration of HD was 7.2 ± 0.9 years. Twenty apparently healthy children with matched age and sex were taken as control group. An informed verbal and written agreement received from all parents of the patients and control group before getting them involved in the study.

**All studied participants were subjected to the following:** Full history and complete general examination with anthropometric measurements including weight (kg) and height (m). BMI was calculated according to the following formula: weight in kilograms divided by height in meters square. Patients with associated liver diseases or systemic infection or received immune suppressant drugs or supplementation with antioxidant vitamins or Zinc (Zn) were excluded from the study.

Laboratory investigations: Five ml of venous blood samples were drawn in the morning after an overnight fast of 12 hours before the dialysis session. The samples were divided into two portions:

- Two ml were placed into a vacutainer tube containing EDTA for complete blood picture using automated cell counter model Sysmex Kx 21N hematology analyzer (Sysmex Corporation of Japan).
- Three ml were placed into a tube with no anticoagulant, centrifuged within 30 min of collection and serum was separated and used to assess:

(1) Serum albumin, urea and creatinine were determined using Cobas C-311 autoanalyzer. Reagents were supplied by Roche Diagnostics (Roche Diagnostics, Indianapolis, IN, USA).

(2) Measurement of:

- Serum Zn (kits supplied by Quimica Clinica Aplicada [QCN], Spain, Ref. no. 992814, Lot. no. v40679),
- Serum Cu (kits supplied by Biovendor research and diagnostic products, European Union, Ref. no. 1081, Lot no. RD/2232),
- Serum CD3 (kits supplied by Glory science Co. LTD, Texas, USA, Ref. no. DRE 64359, Lot no. 20120515 ) and
- Serum CD4 (kits supplied by Glory science Co. LTD, Texas, USA, Ref. no. DRE 72208, Lot. No. 201208).

All were measured by ELISA using a SLT Spectra ELISA reader (SLT Lab Instruments, Salzburg, Austria).

**Statistical analysis:** Data were entered to SPSS version 17 and quantitative data were presented as mean, standard deviations and range while the qualitative data were presented as number and percentages. The comparison between two groups with qualitative data were done by using Chi-square test while the comparison between two groups with quantitative data were done by using Independent sample t-test. The comparison between more than two groups was done by using one way analysis of variance (ANOVA). Pearson correlation coefficient was used to assess the relation between the studied parameters. P-value was considered significant at $< 0.05$.

**3. Results:**

The results of this work showed that: significant decrease in hemoglobin, albumin, CD3 and CD4 in dialysis patients in comparison to control. Significant increase in urea, creatinine and copper in dialysis patient in comparison to control was determined.

**Table (1):** Comparison between patient and control groups regarding demographic data.

| | Control (n=20) mean ± SD (range) | Patients (n=20) mean ± SD (range) | *P*-value | Significance |
|---|---|---|---|---|
| **Age (years)** | 10.91 ± 3.46 (4 - 15) | 12.35 ± 3.54 (5 - 18) | 0.331 | NS |
| **Sex (M:F )** | 9/11 | 9/11 | 1.0 | NS |
| **BMI (kg/m$^2$)** | 17.52 ± 2.55 (14.2 - 26.1) | 16.8 ± 2.99 (12.9 - 26.2) | 0.418 | NS |
| **Height (cm)** | 129.025±15.560 (99 – 160) | 118.800±13.037 (101 – 144) | 0.030 | S |
| **Weight (kg)** | 30.716±13.560 (22 – 45) | 35.390±15.027 (22 – 47) | 0.308 | NS |

BMI; body mass index.

**Table (2): Comparison between patient and control groups regarding laboratory data.**

| | Control (n=20) mean ± SD (range) | Patients (n=20) mean ± SD (range) | *P*-value | Significance |
|---|---|---|---|---|
| **HB (g/dL)** | 12.22 ± 0.98 (10.3 - 14.1) | 9.34 ± 2.09 (5.9 - 13.5) | 0.000* | S |
| **Albumin (gldL)** | 4.11 ± 0.33 (3.4 - 4.5) | 3.86 ± 0.35 (3.3 - 4.3) | 0.024* | S |
| **Urea (mg/dL)** | 18.25 ± 5.98 (14 – 33) | 205 ± 64.79 (107 – 380) | 0.000* | S |
| **Creatinine (mg/dL)** | 0.40 ± 0.13 (0.2 – 0.6) | 8.53±2.32 (4 - 12.5) | 0.000* | S |

HB; hemoglobin.

**Table (3): Comparison between patient and control groups regarding CD3, CD4, Zn, Cu and Mg**

| | Controls | Patients | Test | *P*-value | Sig. |
|---|---|---|---|---|---|
| **CD3** (ng /mL) Mean±SD | 7.13±1.97 | 0.49±0.22 | 14.983 | 0.000 | HS |
| Range | 4 - 10.5 | 0.2 - 0.88 | | | |
| **CD4** (ng /mL) Mean±SD | 10.73±6.53 | 0.43±0.18 | 7.050 | 0.000 | HS |
| Range | 4.17 - 28.38 | 0.2 - 0.85 | | | |
| **Zn** (µg/dL) Mean±SD | 85.31±10.80 | 75.96±12.21 | -2.565 | 0.014 | S |
| Range | 57 – 105 | 55 - 167 | | | |
| **Cu** (µg/dL) Mean±SD | 128.40±40.47 | 278.25±86.26 | 7.033 | 0.000 | HS |
| Range | 86 – 229 | 115 – 462 | | | |
| **Magnesium** (mg/dL) Mean±SD | 2.2±0.32 | 2.92±0.58 | 4.861 | 0.001 | HS |
| Range | 1.83 – 3.56 | 1.98 – 3.88 | | | |

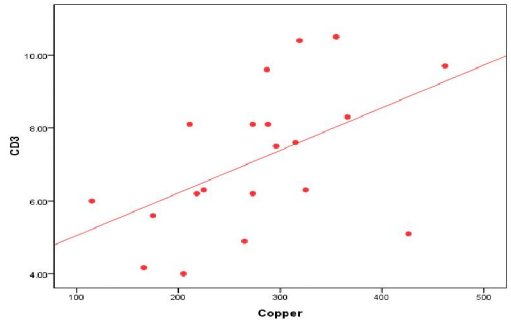


**Fig (1):** Correlation between Serum CD3 and serum copper

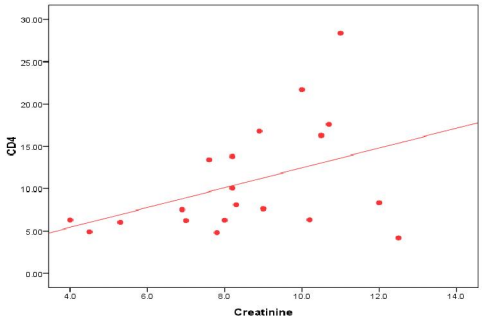


**Fig (2):** Correlation between Serum CD4 and serum creatinine.

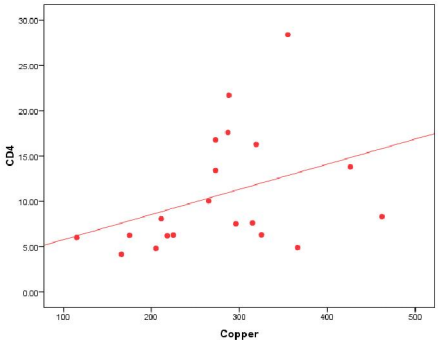


**Fig (3):** Correlation between Serum CD4 and serum copper

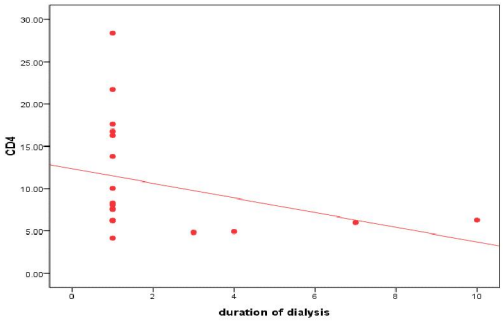


**Fig (4):** Correlation between Serum CD4 and duration of dialysis

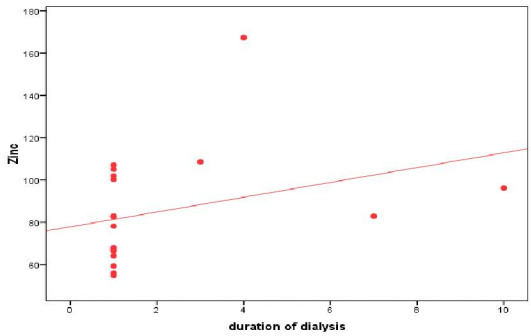


**Fig (5):** Correlation between Serum Zinc and duration of dialysis

## 4. Discussion

Hemodialysis is associated with considerable morbidity and mortality due to accelerated cardiovascular disease and infection ***(Zima et al., 1998 & 1999).*** Because trace elements are components of many enzymes and proteins and they have many functions, their changes may have more profound effects on children who are during growth and developmental period ***(Esfahani et al., 2006)***.

Hemodialysis patients are at theoretical risk for both deficiency and accumulation of trace elements, depending on dietary intake, removal by dialysis, the composition of the source water used for hemodialysis, and residual kidney function. Or due to dialysate contamination/ depletion may also disturb trace element concentration in dialysis patients ***(D'Haese and de Broe, 1996).***

Average blood levels of biologically important trace elements were substantially different in hemodialysis patient, trace element status influences the risk of adverse clinical outcomes ***(Tonelli et al., 2009),*** in order to prevent some complications in chronic HD patients, it is very important to regulate levels of trace elements ***(Hosokawa et al., 1990 and Sen et al., 1991)***.

Copper (Cu) is an essential trace element that is required for a number of enzymes. Cu has vital roles in hemoglobin synthesis and immune function. Excess blood Cu, particularly the free fraction, may lead to tissue injury apparently due to its pro-oxidant effects and the depletion of anti-oxidant reserves ***(Maggini et al., 2007)***.

Our results showed significant increase in serum copper level in dialysis patients in comparison to control and this could be explained by, dialysis treatment because in dialysis treatment, Cu is accumulated in the blood and plasma/serum of patients resulting in an elevated Cu levels ***(Salvadeo, 1979; Wallaeys et al., 1986; Lin et al., 1996; Krachler et al., 1997; Alarcon et al., 2006).*** Also ***Navarro-Alarcon et al. (2006) and Guo et al. (2011)*** explain the increased plasma Cu levels seen in HD patients may be due to the release of Cu during inflammatory tissue damage. Serum cu even increased more in postdialysis values than predialysis concentrations ***(Krachlera and Wirnsberger, 2000).*** On the other hand, ***Weissgarten et al. (2001) and Anees et al. (2011)*** reported that Cu was significantly deficient in hemodialysis patients and ***Shouman et al. (2009)*** who found no difference in Cu concentrations between patients and controls.

Zinc (Zn) is a dietary metal required for the healthy functioning of the body. It is one of the most important trace elements in the body and it is essential as a catalytic, structural, and regulatory ion. It is involved in homeostasis, in immune responses, in oxidative stress, in apoptosis and in ageing**.** Zinc is second to iron as the most abundant trace element in the body ***(Salwen, 2011)***. On the cellular level, the functions of zinc can be broadly divided into three categories: catalytic, structural and regulatory. Zinc is a critical component of the catalytic site of hundreds of metalloenzymes, including pancreatic carboxypeptidases, carbonic anhydrase, alkaline phosphatase, RNA polymerases, and alcohol dehydrogenase ***(Tuerk et al., 2009).*** In its structural role, zinc coordinates with certain protein domains; facilitating protein folding and producing structures such as 'zinc fingers'. Zinc is also involved in the structure and stabilization of some enzymes, such as the antioxidant superoxide dismutase ***(Orlova and Orlov, 2011)***.

Zinc deficiency is linked to decreased immunity leading to increased infection susceptibility frequently encountered in uremic patients ***(Hirano et al., 2008).***

Our results revealed significant decrease in zinc level in dialysis patients as compared to control group this could be explained by Zn removal during hemodialysis, decreased gastrointestinal absorption of Zn, inadequate Zn intake, and reduced Zn-binding proteins in these patients. Increased expressions of intracellular metallothioneins following oxidative damage can sequester plasma Zn, and up-regulation of Zn-importing proteins by pro inflammatory cytokines can also reduce the plasma concentrations of Zn ***(Liuzzi et al., 2005; Haase and Rink, 2009; Guo et al., 2011).***

This results agreed with ***Cabral et al. (2005), Alarcon et al. (2006), Hsieh et al. (2006), and Marcello Tonelli (2009),*** *also* ***Sahin et al. (2009) and Youssef et al. (2012)*** founded low zinc and level and the later recommend supplementation of Zn for patients on regular hemodialysis specially those with iron deficiency. But a different study done by ***Kozielec et al. (1994)*** revealed high zinc level in blood serum in 10-14 years old group**,** ***Huang et al. (2000),*** and ***Padovese et al. (1992)*** where they found normal serum zinc concentration**.** ***Kaminska et al. (1994) and Koca***

***et al. (2010)*** also found non-significant differences between HD groups and the control group. This can be attributed to the fact that explained by ***El-Habib et al. (2012)*** that Zn in the blood is complexed with albumin forming a large particle unable to pass through the pores of dialysis membranes.

Toxins in uremic patients may cause defects in cell-mediated immunity, and alteration of cellular nutrients in HD patients ***(Sayarlioglu et al., 2006).*** Patients on HD seem to be unable to restore suppressor T cell activity ***(Grzegorzewska et al., 2001).***

T-cell damage by increased oxidative stress in end-stage renal disease (ESRD) patients undergoing chronic hemodialysis (HD) led to the increased T-cell apoptosis and the alteration of surface markers and Th1/Th2 ratio in CD4+ T lymphocytes. On the other hand, the activation of the T-cell receptor/CD3 complex leads to the immediate activation of transcription factors that regulate a variety of activation-associated genes of cytokines and surface receptors for coordinating the immune response and regulating transcriptional induction of several cytokines and T-cell activation-induced proteins ***(Macian et al., 2005)***.

In uremia, the immune system shows signs of activation coexisting with signs of deficiency, furthermore, most studies in adults report a normal ratio of T-helper (CD4) and T-suppressor (CD8) cells ***(Deenitchina et al., 1995; Cohen et al., 1997).*** Remarkably little is known about the immune system in children with chronic renal failure, on dialysis ***(Cameron et al., 1995).*** In children with chronic renal failure, total lymphocytes, B cells, cytotoxic T cells, NK cells, and monocytes are reduced in number before and after dialysis start ***(Antonia et al., 2000)***.

***Hisano et al. (1992) and Szczepanska et al. (2005)*** described a normal percentage of CD4, CD8, and NK, and they explain T cell depletion in uremic patients mostly from uremia-related toxicities. Various abnormalities in T and B cell function have been well documented in long-term dialysis patients ***(de Cal et al., 2008). Schollmeyer (1988)*** discuss that HD may cause impairment of the immune response.

Our results revealed lower percentages of CD3 and CD4 helper T cells in hemodialysis patients than normal control subjects, these abnormalities in T cell function is due to long-term dialysis, these may be due to Zn deficiency which lead to downregulation of T helper cells ***(Prasad et al., 2007).*** Efficient immune function depends on the status of some micronutrients and an inadequate status may lead to depressed immune responses. Zn is an immune regulator with multiple functional activities for immune effector cells, such as natural killer cell activity, and chemotaxis of neutrophils and monocytes ***(Kent et al., 2000).*** Disturbances in Zn homeostasis can lead to a shift in the Th1/Th2 balance towards a Th2 response. In hemodialysis patients with Zn deficiency, the percentages of circulating CD3 and CD4 T lymphocytes are significantly lower than in controls; plasma Zn concentrations are correlated with %CD3 and %CD4 T cells, and CD4/CD8 ratios ***(Guo et al., 2010)***. Also anemia and hypoalbuminemia which lead to malnutrition .and malnutrition is also known to cause significant impairment of immune responses ***(Sayarlioglu et al., 2006)***. Together with long term dialysis all these factors may explain decreased CD3 and CD4 levels among our study. Another explanation was that in end-stage renal disease (ESRD) patients undergoing chronic hemodialysis (HD), the activation of leukocytes with non-biological materials of the extracorporeal system can further produce burst amounts of reactive oxygen species (ROS), such as O2−•, H2O2 and HOCl, which impair erythrocytes, immune cells and endothelial cells and subsequently lead to anemia and resistance to erythropoietin therapy ***(Huang et al., 2006),*** cardiovascular diseases ***(Muller et al., 2004),*** malnutrition , inflammation , immune-deficiency and high mortality ***(Nitta et al., 2002).***

As regard anthropometric measurements our results revealed significant decrease in height for age in hemodialysis patients in comparison to control. This could be explained by zinc deficiency as Zinc appears to be an important linear-growth–limiting micronutrient. The effect of zinc on growth was mediated by its effect on Insulin-like Growth Factor-I (IGF-I) levels. Zinc deficiency reduces IGF-I production and may also decrease cellular IGF-I responsiveness. Zinc supplementation was found to increase basal levels of IGF-I, Insulin-like Growth Factor Binding Protein-3 (IGFBP-3), alkaline phosphatase, and osteocalcin, without changing GH levels or increasing subjects' sensitivity to exogenous GH ***(Gat-Yablonski et al., 2009).***

BMI is a simple, cheap and easy to calculate standardized measure of body size. It is widely used to define body size and a grading of overweight and obesity ***(Hall and Cole, 2006).*** In chronic hemodialysis patients normal growth was achieved and was associated with normal pubertal growth spurt. These findings suggest that the combination of increased dialysis and adequate nutrition can promote normal growth in children treated with long-term hemodialysis ***Tom et al. (1999)*** and this may discuss why our results revealed non-significant difference between patients and control group as regards BMI ( $p>0.005$). More attention should be paid to patients who are underweight instead of overweight ***(Renée de Mutsert et al., 2007).***

Anemia is a common finding in a vast majority of maintenance hemodialysis patients. The

main reason for anemia in these patients is inadequate erythropoiesis caused by functional erythropoietin deficiency. Another reason of anemia in hemodialysis patients is reduced survival time of RBCs ***(Komidori et al., 1985).*** This coincide with our results as there was highly significant decrease of Hb ($p$<0.001) in patient group compared to control group, this was also agree with ***Azhir et al. (2007)***, ***Jander et al. (2012) and El-Wakeel et al. (2013)*** ; and they contributed it to several factors as erythropoietin deficiency, decrease erythrocyte survival and increase blood loss.

Mean level of albumin among our patients ranged (3.86±0.35, $p$<0.05) which is significantly diminished which agree with ***(Chand et al., 2005).*** National Kidney Foundation Dialysis Outcomes Quality Initiative practice guidelines recommend serum albumin ≥4.0 g/dl for adults who are on hemodialysis**.** ***Amaral et al. (2008)*** assumed that patients who are able to achieve serum albumin>or = 4.0/3.7 g/dl may have the lowest mortality risk. An important interaction between anemia and hypoalbuminemia is founded, ***Madore et al. (1997)*** assumes that albumin deficiency was the most closely associated with low hemoglobin concentration in HD patients." The authors postulated that visceral protein deficiency manifested as hypoalbuminemia might contribute to ineffective erythropoiesis and subsequent anemia. ***Agarwal (2008)*** demonstrated that serum albumin concentration was an important predictor of both baseline hemoglobin and erythropoietin sensitivity. They suggested that both serum albumin and hemoglobin may be markers of overall health status, with declines in both markers occurring with episodes of infection, increased inflammation, oxidative stress, and malnutrition , some investigations suggest a positive association between blood Zn concentrations and albumin, hematocrit and prealbumin, which suggests that better nutritional status is a reason for higher Zn status in dialysis patients ***(Grzegorzewska and Leander, 2001; Alarcon et al., 2006).*** In addition, Zn promotes insulin-like growth factor-1 (IGF-1), which plays a role in the regulation of hematopoiesis and osteoclastogenesis ***(Yamaguchi et al., 2010).*** Thus, Zn status also seems to be related to hemodialysis bone disease and anemia. Among our patients there was statistically significantly elevated serum creatinine level, which coincide with ***Fassinger et al. (2010), and Anees et al. (2011)*** who assumes that creatinin as a marker for kidney dysfunction, its increase is due to the loss of functional tissue and/or the entire nephron in kidney failure, which leads to decreased filtration of creatinine and abnormal survival of small serum proteins resulting in an increase of their serum levels.

Serum magnesium concentration is a significant predictor of mortality in maintenance hemodialysis patients , Hypomagnesemia is significantly associated with the presence of vascular calcification of the hand arteries, These results suggest that higher serum magnesium concentrations may play an important protective role in the development of vascular calcification in hemodialysis patients, and that magnesium concentration of dialysis fluid may be reconsidered in view of preventing vascular calcification in hemodialysis patients *(Ishimura et al., 2007).*

Our results revealed significantly higher level of magnesium among studied group and this fortunately is beneficial to our patients as patients with mildly elevated serum magnesium levels could have a survival advantage over those with lower magnesium levels *(Massy and Drueke, 2012).* Low serum magnesium may be an independent risk factor for premature death in CKD patients.

**Conclusion:**

Hemodialysis patients are at risk for deficiency of some essential trace elements and excess of toxic trace elements, both of which can affect the immune function in ESRD patients as evidenced by decreased percentages of CD3 and CD4 lymphocytes, so continuous evaluation of trace elements is important in chronic hemodialysis patients.

**References:**


1. ***Agarwal R, Davis JL and Smith L:*** *Serum albumin is strongly associated with erythropoietin sensitivity in hemodialysis patients.* Clin J Am Soc Nephrol 2008; 3(1)*:* 98– *104.*
2. **Alarcon MM, Pérez RA, Garcia LH, *et al.*:** Longitudinal study of serum zinc and copper levels in hemodialysis patients and their relation to biochemical markers. Biol Trace Elem Res 2006; 113(3):209–22.
3. **Amaral S, Hwang W, Fivush B, *et al.*** Serum albumin level and risk for mortality and hospitalization in adolescents on hemodialysis. Clin J Am Soc Nephrol 2008; 3(3): 759.
4. **Anees M, Mumtaz A, Frooqi S, *et al.*** Serum trace elements (Aluminum, Copper, Zinc) in hemodialysis patients. Biomedica 2011; 27: 106-110.
5. **Antonia HM, Out BTA, Schröder CH, *et al.*:** Characteristics of peripheral and peritoneal white blood cells in children with chronic renal failure, dialyzed or not", in Peritoneal Dialysis International 2000; 20: 748–756.
6. **Azhir A, Nasiri J and Gheisari A:** Prevalence and severity of anemia in pediatric hemodialysis patients Saudi Med J 2007; 28(2): 249-53.
7. **Cabral PC, Diniz ADS and De Arruda IKJ.** Vitamin A and zinc status in patients on maintenance hemodialysis. Nephrology 2005; 10(5): 459 - 463.

8. **Cameron JS:** Host defences in continuous ambulatory peritoneal dialysis and the genesis of peritonitis. Pediatr Nephrol 1995; 9:647–62.
9. **Chand DH, Brier M and Strife CF:** Comparison of vascular access type in pediatric hemodialysis patients with respect to urea clearance, anemia management, and serum albumin concentration." In Am J Kidney Dis 2005; 45(2): 303-8.
10. **Cheng SM, Yang SP, Ho LJ.** Irbesartan inhibits human T-lymphocyte activation through downregulation of activator protein-1. Br J Pharmacol 2004; 142: 933–942
11. **Cohen G, Haag-Weber M and Horl WH:** Immune dysfunction in uremia. Kidney Int. 1997; 52(62): S79–S82.
12. **De Cal M, Cruz DN, Corradi V, *et al.*** Nutritional status and immune functions in maintenance hemodialysis patients. Hindawi Publishing Corporation, Mediators Inflamm 2006; 20264: 1–4.
13. **Deenitchina SS, Ando T, Okuda S, *et al.*:** Cellular immunity in hemodialysis patients: a quantitative analysis of immune cell subsets by flow cytometry. Am J Nephro 1995; 15:57–65.
14. **D'Haese PC and De Broe ME:** Adequacy of dialysis: trace elements in dialysis fluids**.** *Nephrol Dial Transplant* 1996*;* **11**(2)**:**92-97.
15. **EL-Habibi EM, Bakr MA and Kamal N.** Impact of Hemodialysis on Certian Trace Elements Among Patients Suffering from End Stage Renal Disease. J Am Sci 2012; 8(12):827-833.
16. **El-Khawaga OY and El-sayed IH:** Evaluation of trace elements and antioxidants in pre and post hemodialysis of chronic renal failure patients. I.J.S.N; 2012; 3(3): 617-620.
17. **Esfahani ST, Hamidian MR, Madani A, *et al.*:** Serum zinc and copper levels in children with chronic renal failure." In: Pediatr Nephrol 2006; 21 (8):1153-6
18. **El Wakeel M, Ali RM, El Kassas GM, *et al.*:** Retinol binding protein-4 in children on chronic hemodialysis. Journal of Applied Science Research 2013; 9(1): 519-526.
19. **Fassinger N, Imam A and Klurfeld DM:** Serum retinol, retinol-binding protein and transthyretin in children receiving dialysis. J Ren Nutr 2010; 20(1):17-22.
20. **Gat-Yablonski G, Yackobovitch-Gavan M and Phillip M (2009):** Nutrition and bone growth in pediatrics. Endocrinol Metab Clin N Am; 38:565–586.
21. **Grzegorzewska AE and Leander M.** Lymphocyte subsets in the course of continuous ambulatory peritoneal dialysis. Adv Perit Dial 2001; 17:10–14.
22. **Guo CH, Chen PC, Yeh MW et al.,:** Cu/Zn ratios are associated with nutritional status, oxidative stress inflammation and immune abnormalities in patients on peritoneal dialysis. Clin Brochem 2011; 44(4): 275-280.
23. **Hall DM and Cole TJ:** What use in the BMI? In: Arch Dis Child 2006; 91(4): 283-286**Hauser AB, Stinghen AE, Kato S, *et al.*** Characteristics and causes of immune dysfunction related to uremia and dialysis. In: Perit Dial Int 2008; 28(3): S183-S187.
24. **Hirano T, Murakami M, Fukada T, *et al.*:** Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule. Adv. Immunol 2008; 97:149–176.
25. **Hisano S, Miyazaki C, Hatae K, *et al.*** Immune status of children on continuous peritoneal dialysis. Pediatr Nephrol 1992; 6:179–81.
26. **Hosokawa S, Oyamaguchi A and Yoshida O:** Trace Elements and Complications in Patients Undergoing Chronic Hemodialysis**"** in: Nephron 1990; 55(4): 375–379.
27. **Hsieh Y, Shin W, Lee L, *et al.*** Long-term changes in trace elements in patients undergoing chronic hemodialysis. Biol Trav Elem Res 2006; 109: 115-121.
28. **Huang JW, Hung KY, Lee SH, *et al.*:** Trace elements in blood and dialysate among continuous ambulatory peritoneal dialysis patients: A prospective, multicenter collaborative study. Dial Transplant 2000; 29: 62–8.
29. **Huang KC, Yang CC, Hsu SP *et al.*** Electrolyzed-reduced water reduced hemodialysis-induced erythrocyte impairment in end-stage renal disease patients. Kidney Int 2006; 70: 391–398
30. **Jander A, Wierciński R, Bałasz-Chmielewska I, *et al.*:** Anaemia treatment in chronically dialysed children: a multicentre nationwide observational study. Scand J Urol Nephrol 2012; 46 (5): 375-80.
31. **Kaminska-G, Grzeszczak W, Jedryczko A, *et al.*:** Influence of long-term hemodialysis on serum trace elements concentration in patients with chronic renal failure. Przegl Lek 1994, 51(1): 9-14 .
32. **Koca T, Berber A, Koca HB, *et al.*:** Effects of hemodialysis period on levels of blood trace elements and oxidative stress. Clin Exp Nephrol 2010; 14(5): 463–468.
33. **Komidori K, Kamada T, Yamashita T, *et al.*:** Erythrocyte membrane fluidity decreased in uremic hemodialyzed patients. Nephron 1985; 40 (2): 185-8.
34. **Kozielec T, Kaszczyk-Kaczmarek K, Kotkowiak L, *et al.*:** The level of calcium, magnesium, zinc and copper in blood serum in children and young people between 5 and 18 years of age. Przegl Lek 1994; 51(9): 401-5.
35. **Krachler M, Wirnsberger G and Irgolic KJ:** Trace element status of hemodialyzed patients. Biol Trace Elem Res 1997; 58:209–221.
36. **Krachler M and Wirnsberger GH:** Long-Term Changes of Plasma Trace Element Concentrations in Chronic Hemodialysis Patients in Blood Purif 2000; 18: 138–143.
37. **Lin TH, Chen JG, Liaw JM, *et al.*** Trace elements and lipid peroxidation in uremic patients on

hemodialysis. Biol Trace Elem Res 1996; 51(3):277-283.
38. **Macian F.** NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol 2005; 5: 472–484.
39. ***Madore F, Lowrie EG, Brugnara C, et al.:*** *Anemia in hemodialysis patients: Variables affecting this outcome predictor*. J Am Soc Nephrol 1997; 8(12)*:* 1921– *1929.*
40. **Maggini S, Wintergerst ES, Beveridge S.*et al.,*** Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Br J Nutr 2007; 98: (S1) 29-35.
41. **Muller C, Eisenbrand G, Gradinger M *et al.*** Effects of hemodialysis, dialyser type and iron infusion on oxidative stress in uremic patients. Free Radic Res 2004; 38: 1093–1100
42. **Nitta K, Akiba T, Kawashima A *et al.*** Characterization of TH1/TH2 profile in uremic patients. Nephron 2002; 91: 492–495.
43. **Orlova M and Orlov A :** Role of Zinc in an Organism and Its Influence on Processes Leading to Apoptosis. British Journal of Medicine & Medical Research2011; 4: 239-305.
44. **Padovese P, Gallieni M, Brancaccio D, *et al.*:** Trace elements in dialysis fluids and assessment of the exposure of patients on regular hemodialysis, hemofiltration and continuous ambulatory peritoneal dialysis. Nephron 1992; 61:442–448.
45. **Prasad AS.** Zinc: Mechanisms of Host Defense. J Nutr 2007; 137(5):1345-1349.
46. **Renée de Mutsert, Snijder MB, Femke VD, *et al.*:** Association between Body Mass Index and Mortality Is Similar in the Hemodialysis Population and the General Population at High Age and Equal Duration of Follow-Up 2007 American Society of Nephrology. *JASN* 2007; 18(3): 967-974.
47. **Sahin HH, Uyanik FF, Inanç NN, *et al.*:** Serum zinc, plasma ghrelin, leptin levels, selected biochemical parameters and nutritional status in malnourished hemodialysis patients. Biol Trace Elem Res 2009; 127(3):191–9.
48. **Salvadeo A, Minoia C, Segagni S, *et al.*** Trace metal changes in dialysis fluid and blood of patients on hemodialysis. Int J Artif Organs 1979; 2: 17–21.
49. **Salwen M (2011):** Vitamins and trace elements, McPherson: Henry's Clinical Diagnosis and Management by Laboratory Methods, 22nd ed.:417-428.
50. **Schollmeyer P and Bozkurt F.** The immune status of the uremic patient: Hemodialysis vs CAPD. Clin Nephrol 1988; 30(l):37–40.
51. **Sen S, Bor N, Colakoglu M, *et al.*:** Clearance of zinc and copper during hemodialysis. Journal of Islamic Academy of Sciences 1991, 4(3): 265-267.
52. **Shouman MG, Ibrahim HY, Salam EEE, *et al.*:** Trace Elements in Pediatric Hemodialysis Patients. Res. J. Medicine & Med Sci 2009; 4(2): 435- 440.
53. **Szczepanska M, Szprynger K and Mazur B:** Expression of surface antigens on peripheral blood t-cells in children on maintenance hemodialysis with the use of vitamin e-coated dialysis membrane. Oxford Journal, Dialysis – Nutrition, Malnutrition, Oxidative Stress, Inflammation 2005; 313:124-125.
54. **Tom A, McCauley L, Bell L, *et al.*:** Growth during maintenance hemodialysis: Impact of enhanced nutrition and clearance: The Journal of Pediatrics. 1999; 134 (4): 464–471.
55. **Tonelli M**, **Wiebe N**, **Hemmelgarn B**, ***et al.*** Trace elements in hemodialysis patients: a systematic review and meta-analysis. *IN; BMC Medicine* 2009, **7**:25.
56. **Tuerk J and Fazel N (2009):** Zinc deficiency. Gastroenterology; 25: 136–143.
57. **Wallaeys B, Cornelis R, Mees L, *et al.*** Trace elements in serum, packed cells, and dialysate of CAPD patients. Kidney Int 1986; 30(4): 599–604.
58. **Weissgarten J, Berman S, Bilchinsky M, *et al.*** Total Cell-Associated Zn and Cu and Proliferative responsiveness of Peripheral Blood Mononuclear Cells from Patients on Chronic Hemodialysis. Metabolism 2001, 50 (3): 270-276.
59. **Youssef DM, Noseer AI, Abdallah AM, *et al.*:** Evaluation of serum zinc and copper in children with chronic kidney disease in journal of pediatric Biochemistry 2012, 2(1): 57-60.
60. **Zima T, Mestek O, Nemecek K, *et al.*:** Trace elements in hemodialysis and continuous ambulatory peritoneal dialysis patients**.** *Blood Purif* 1998; **16:**253-260.
61. **Zima T, Tesar V, Mestek O, *et al.*: Trace elements in end-stage renal disease. 2. Clinical implication of trace elements.** *Blood Purif* 1999; **17:**187-198.

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