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SERUM TRACE ELEMENTS (ALUMINIUM, COPPER, ZINC) IN HEMODIALYSIS PATIENTS

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ABSTRACT

Introduction: Trace element is defined as one that makes up less than 0.01% of body's mass. Those present at ug/dl in body fluids and at mg/kg in tissues are referred to as trace elements and those found at ng/dl in body fluids or ug/kg in tissues are referred to as ultra – trace elements. Although these elements constitute a relatively small amount of total body tissues these are essential for many vital processes.

Objective: To determine the serum level of trace elements i.e Aluminum (Al), Zinc (Zn) and Copper (Cu) in haemodialysis (HD) patients.

Methods: Fifty patients of End Stage Renal disease (ESRD) on (HD) for more than three months from dialysis center of Shalamar Hospital Lahore were included in the study. Patients of acute renal failure, duration less than three months were excluded from the study. Fifteen subjects age and gender matched having normal kidney functions were included as controls. Demographic data was collected in a separate form having age, gender, cause of ESRD, duration of dialysis and viral markers.

Results: The major causes of ESRD were diabetic and hypertensive nephropathy. The mean age was 46.10 ± 16.29 years and predominantly patients were from middle class. Mean duration of dialysis was 24.87 ± 22.1 months and majority of the patients 40 (74.1%) were on twice weekly dialysis. Mean serum Al level was 65.44 ± 33.41 µg/L as compared to controls (13.20 ± 6.155) which was statistically significant ($p < 0.001$). Mean serum Zn level was 59.17 ± 32.51 µg/L and Cu level was 35.35 ± 31.52 µg/L which were significantly ($p < 0.001$) lower than controls (107.53 ± 13.32, 107.00+11.85 µg/L). Duration of the dialysis had negative correlation with serum Zn level. It was significantly ($p< 0.02$) low (44.78 ± 33.061 µg/L) when duration of dialysis was more than 30 months as compared to duration less than 30 months (66.36 ± 30.174 µg/L).

Conclusion: Serum levels of trace elements were significantly different in ESRD patients. Majority of patients had high Al levels and decreased Cu and Zn level. High serum Al levels in patients were due to intake of Al based phosphate binders. Decreased Zn and Cu levels were due to poor intake and dietary restriction. Duration of the dialysis had negative correlation with serum Zn level.

Key Words: Trace Metals, ESRD, Hemodialysis, Aluminum, Zinc and Copper.

INTRODUCTION

Trace element is defined as one that makes up less than 0.01% of body's mass. Those present at *u*g/dl in body fluids and at mg/kg in tissues are referred to as *trace elements* and those found at *n*g/dl in body fluids or *u*g/kg in tissues are referred to as *ultra – trace elements*. Although these elements constitute a relatively small amount of total body tissues these are essential for many vital processes. Trace elements play an important role in the structure of proteins, enzymes and complex carbohydrates to participate in biochemical reactions.[1-2] A number of studies have reported that patients of chronic kidney disease (CKD) on HD have disturbed levels of trace elements.[3-4] Contamination of the dialysis fluids and inability to remove these by dialysis lead to accumulation of the trace elements to toxic levels in the body.[5] In ESRD 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.[6-8] Serious, acute as well as chronic toxicities and deficiencies of trace elements have been reported.[9-11] To prevent these complications in chronic haemodialysis patients, it is imperative to regulate the levels of trace elements by adequate water treatment before dialysis. In Pakistan, there is no data available in this aspect so this study was conducted

to measure the levels of trace elements in HD patients.

METHODS

Fifty patients of ESRD, on regular HD for more than three months were included in the study from dialysis centre of Shalamar Hospital, Lahore from June to August 2009. Written consent was taken from patients for inclusion in the study. Fifteen subjects, age and gender matched, having normal kidney functions (urea and creatinine) were included as controls. Patients on dialysis for less than three months, acute renal failure and pregnancy were excluded from the study. The samples were drawn for routine haematological (haemoglobin, haematocrit, MCV, MCH and MCHC), biochemical parameters (urea, creatinine, albumin). Sample for trace elements was collected in 2% nitric acid treated tubes at the start of dialysis from arterio venous fistula. The trace elements were measured on the absorption of primary radiation by the analyte atom in their ground state. The measured absorbance signal constitutes a measure of concentration of respective element in the analysed sample (Instrument CONTRA 700). Ethical approval for the study was taken from the ethical review committee.

Statistical Analysis

The data was entered and analysed using SPSS 16.0 (Statistical Package for Social Sciences). Mean ± SD was given for normally distributed quantitative variables. Frequencies and percentages were given for qualitative variables. Two – independent sample t test was applied to observe group mean differences. Pearson correlation was applied to observe correlation between quantitative variables. A *p*-value of < 0.05 was considered statistically significant.

Table 1: *Lab Parameters of patients and controls.*

Parameter	*Patients= 50 (Mean ± SD)*	*Controls=15 (Mean ± SD)*	*p-Value*
Urea (mg/dl)	115.57 ± 36.71	24.27 ± 6.19	< 0.001*
Creatinine (mg/dl)	10.18 ± 2.97	0.78 ± 0.108	< 0.001*
Albumin (g/dl)	2.93 ± 0.688	4.17 ± 0.438	< 0.001*
Uric acid (mg/dl)	7.86 ± 1.85	4.06 ± 0.850	< 0.001*
Hemoglobin (g/dl)	9.681 ± 2.306	13.62 ± 1.063	< 0.001*
Hematocrite (%)	29.95 ± 7.82	40.19 ± 5.93	< 0.001*
MCV (fL)	94.71 ± 15.84	83.47 ± 7.10	0.01
MCHC (g/dl)	32.68 ± 3.30	34.53 ± 2.93	0.05

*Statically Significant Value (p < 0.05)

RESULTS

Fifty patients of ESRD on HD were included in this study. There were 27 (53.7%) females and 23 (46.3%) males in the patient group. The age range of the patients was from 15 to 77 years with a mean of 46.10 ± 16.29 years. The patients predominantly were from families with moderate socioeconomic status and had poor hygienic and household facilities. Duration of dialysis was 24.87 ± 22.09 months. Frequency of dialysis was once / week in one (1.85%), twice weekly in 40 (74.1%) and thrice weekly in 13 (24.1%) patients. Majority of the patients n = 46 (85.2%) had permanent access for dialysis. Major causes of ESRD were diabetes mellitus in 25 (50%), hypertension in 17 (33.3%), chronic glomerulonephritis 4 (9.2%) and unknown in 4 (7.5%). Lab parameters of dialysis patients and controls are shown in Table 1. Highly statistically significant differences were observed between serum levels of Al, Zn and Cu in patients on HD and control subjects as shown in figure No. 1. Mean serum Al levels in HD patients was 65.44 ± 33.41 ug/L as compared to controls 13.20 ± 6.155 ug/L which was statistically significant ($p < 0.001$). Mean serum Zn level in HD patients was 59.17 ± 32.51 ug/dl which was significantly ($p < 0.001$) lower than controls 107.53 ± 13.32 ug/dl. Mean serum level of Cu in HD patients was 35.35 ± 31.52 ug/d as compared to controls 107.00 ± 11.85 ug/dl. Thus serum Cu levels in HD patients were significantly decreased as compared to controls ($p < 0.001$). Duration of dialysis has negative correlation with serum Zn level. Patients with duration of HD of less than 30 months has high serum Zn level (66.36 ± 30.174 ug/dl) than patients with duration of more than 30 months (44.78 ± 33.061 ug/dl) which was statistically significant ($p < 0.02$). A strong positive correlation was found between Zn and albumin deficiency (r= .324, p = 0.007).

DISCUSSION

Treatment modalities for ESRD patients are dialysis and renal transplant. As kidneys play an important role in the excretion of these elements, and when its function gets disturbed, there is accumulation of these elements in the body. Renal replacement therapy, performed to maintain healthy life, is the source of accumulation of these metals in the body if water is not being properly pretreated for dialysis. Some metals may be removed from body due to concentration gradient between the blood and dialysate. The type and permeability of dialysis membrane also plays an important role in this context. Problems still

remain even after much improvement in dialysis technique to minimise serious complications caused by changes of these trace elements.[12]

In this study Al levels in dialysis patients is significantly ($p < 0.001$) higher 65.44 ± 33.41 µg/L, as compared to controls 13.20 ± 6.155 ug/L. Our results are in accordance with previous studies except that the degree of elevation is more exaggerated in our study. Lee et al[13] reported mean serum Al concentration 44.3 ± 28.28 ug/L which is statistically higher than the control subjects 22.5 ± 4.5 ug/L ($p < 0.001$). Skarupskiene et al,[14] measured serum Al levels by atomic absorption spectrometer and found that mean serum Al level was significantly higher than controls {(33.1±38.9 and 24.5±45.9 ug/L) ($p<0.001$)}. Different studies have shown variable levels due to the use of different types of specimens and different types of analytical techniques.[15]

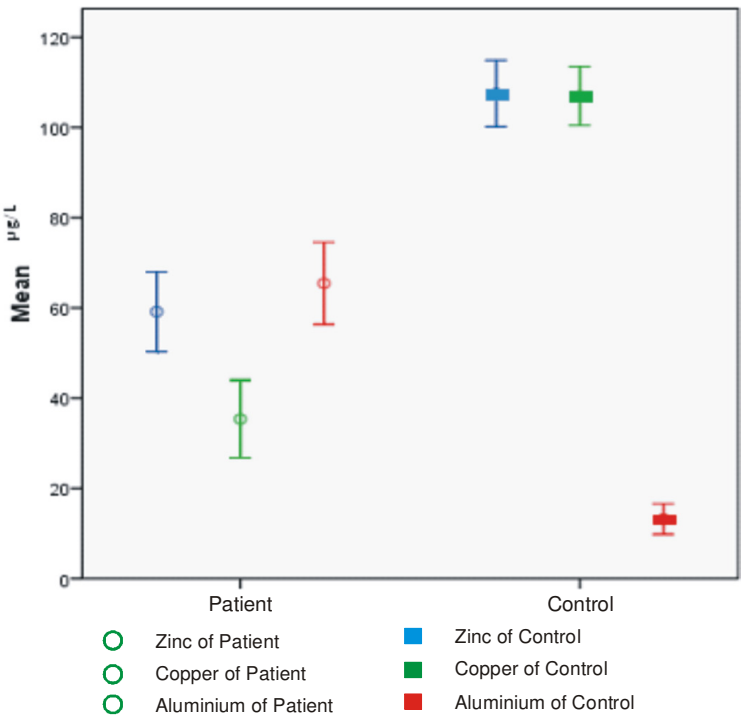

Figure 1: *Trace Metals in Haemodialysis Patients and Control Groups.*

In our dialysis center, maintenance of Reverse Osmosis (RO) plant is done on regular basis and quality of the product water is checked on quarterly basis. We upgrade the quality of water and maintain RO plant in the light of the recommendation of lab report regarding quality of water. During the study, Al level was not detectable in the product water and it was less than the Association for Advancements of Medical Instrumentation (AAMI) standard i.e < 0.01 ppm. Hence, in our patients, dailysis solution was not responsible for elevated serum Al levels.

The second major cause of high Al levels in HD patients is the use of Al containing drugs e.g. aluminium hydroxide. In patients with CKD there is decreased excretion of phosphate which causes increase calcium phosphorus product due to hyperparathyroidism and renal bone disease. Routinely calcium based phosphate binders are used to control this complication. But in certain circumstances, when this product is high then non calcium based phosphate binders are recommended for use which are usually Al hydroxide and sevelmer (Cap. Renagel). The cost of sevelmer is very high and increases the burden on the treatment which is about 470 US dollar. Moreover, Sevelmer is not easily available in Pakistan, as it is not registered for sale. The alternate of this is Al hydroxide which is cheap and easily available in market. Therefore most of the patients are bound to take this drug and develop high level because large amounts of Al are absorbed in the gastrointestinal tract. Although we have not checked that how many patients took this drug at that time so there is need to do a study to correlate the intake of this drug with serum Al level. Molony et al[16] made same observation that Al based phosphate binders result in accumulation of Al in dialysis patients. S – H Lee et al[17] observed different serum Al levels for patients who were taking Al based phosphate binders (54.71 ± 26.70 µg/L) than non Al based phosphate binders (41.15 ± 28.03 µg/L) which were statistically significant $p < 0.001$. Another reason for Al accumulation in HD patients is the decreased excretion of Al in urine.[18-19]

In contrast to trace elements' accumulation, removal of trace elements during dialysis may contribute to the relative deficiency of particular essential elements. Zn deficiency may be present in dialysis patients.[20-21] This leads to decreased appetite, loose motions, negative nitrogen balance, skin manifestations and impotency.[22] Hence to avoid these problems in dialysis patients, Zn supplementation is recommended according to K/DOQI clinical practice guideline[23]. In this study, we found levels of Zn and Cu to be decreased in dialysis patients. Our results are in accordance with previous studies in which they documented significantly lower Zn levels in HD patients when compared to healthy controls. Sen et al,[19] reported that serum Zn and Cu levels in patients maintained on HD had serum Zn levels 60.69 ± 1.21 ug/dl which was significantly lower ($p < 0.05$)

than the normal controls 93.2 ± 1.8 ug/dl. Tetiker et al[3] found serum Zn levels (83.6 ± 13.1 ug/dl) in HD patients that was significantly lower ($p < 0.02$) than control (92.9 ± 11.5 ug/dl).

Low concentration of plasma Zn levels in HD patients may possibly be because of restricted food intake. All the patients on HD were receiving the diet with limited protein content. This diet according to the food analysis tables provides only 10.7 mg of Zn while the advisable optimal daily requirement of Zn for human is 15 mg. In Pakistan, there is late referral of dialysis patients to nephrologist.[24] Most of the patients before referring to nephrologist are being treated by non renal physicians who restrict protein intake (major source of Zn) right from the start of the renal failure. Similar observation was made by Sen et al.[19] According to them, rich sources of Zn like meat, fish, cheese, chicken, nuts, and almond were restricted in dialysis patients. It is therefore obvious that dietary restriction appears as a primary cause of Zn deficiency in our patients. Loss of appetite, impaired absorption by GI tract, lower serum albumin levels and increased excretion by faecal or urinary pathway may also contribute to this deficiency. In addition, a third possible cause of decreased serum Zn and Cu levels in these patients may be excessive loss of trace elements in the dialysate. During efficient water purification process, the ultra – low dialysate level of a number of trace elements may lead to deficiency.[25]

The results of study conducted by Sen et al[19] however, indicates that HD didn't significantly influence ($p < 0.05$) serum Zn levels of these patients. This was perhaps due to the fact that Zn in the blood is complexed with albumin forming a large particle unable to pass through the pores of dialysis membranes. Therefore the Zn and Cu deficiency observed in patients with ESRD appears to be due to nutritional reasons and because of poor gastrointestinal absorption. Tonelli et al[15] pointed out that Zn supplementation should be routinely used to correct deficiency in dialysis patients which significantly reduces the risk of infections and mortality. One relatively small (n = 265) study published in Lithuania,[14] showed a significant association between lower serum Zn levels in HD patients and the risk of infections. HD patients with infectious complications had significantly lower serum levels of Zn 821.9 ± 389.5 ug/L as compared to patients without complications 905 ± 346.6 ug/L, $p < 0.005$ hence infectious complications were associated with Zn deficiency ($p < 0.01$).[26]

Serum levels of total protein and especially of albumin in patients with ESRD are usually low. In the present study, serum albumin levels were significantly ($p < 0.001$) lower as compared to controls. In our patients, Zn levels were reduced in parallel with serum albumin and haemoglobin ($p < 0.05$), both of which are used as markers for nutritional assessment of dialysis patients. Therefore low serum Zn level was indicative of malnutrition. Our results are in agreement with the study of Kiziltas et al,[18] who reported a significant positive linear relationship between serum levels of albumin and Zn in total HD patients ($r = 0.400$; $p = 0.028$). Albumin acts as the main carrier of Zn in plasma so the use of albumin might be a suitable choice in determining Zn deficiency.[17] In our study serum Zn level has negative correlation with the duration of dialysis. Similar observation was made by Shouman et al[27] that serum Zn level and dialysis duration ($r = 0.410$, $P < 0.05$) has negative correlation. But Zima *et al* found that as duration of dialysis increased, the zinc and cadmium levels were increased.

Cu plays an essential role in the function of some enzymes like lysyl oxidase which helps in maintaining the integrity of connective tissue in the heart and blood vessels. It also plays a part in bone formation and teeth.[28] Clinical characteristics of Cu deficiency are leucopaenia, hypochromic microcytic anaemia and osteopenia. In a present study, serum Cu level is significantly low as compared to the healthy controls. Similar observation was made by Sen et al.[19] A rich source of the Cu is organ meat, and legumes. When the patient develops CKD, the diet containing these foods are restricted which leads to deficiency of these trace elements in the patients on HD. However other studies have reported different results. Shouman et al,[27] studied thirty three paediatric HD patients and there was no difference in the level of the Cu in HD patients and control subjects. Alarcon et al,[29] studied serum Cu level in 48 HD patients and it was significantly increased as compared to controls ($p < 0.005$).

In *conclusion* majority of patients should high Al levels and decreased Cu and Zn level. High serum Al level in patients was mostly due to intake of Al based phosphate binders. Decreased Zn and Cu levels were due to poor intake and dietary restriction. Duration of the dialysis had negative correlation with serum Zn level.

Recommendations

- Measurement of the trace elements should be done before starting dialysis and at regular intervals during dialysis.
- Al based phosphate binders should be used very cautiously to avoid its toxicity and non Al based phosphate binders need to be used.

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REFERENCES

1. Zima T, Tesar V, Mestek O, Nemecek K. Trace elements in End-stage renal disease. 2. Clinical implications of trace elements. Blood purification. 1999; 17: 187-198.
2. Mumtaz M, Siddique A, Mukhtar N, Mehboob T. Status of trace elements level in blood samples of different age population of Karachi (Pakistan). Tr. J. of medical sciences. 1999; 29: 697-699.
3. Tetiker T, Paydas S, Yuregir G, Sagliker Y. Trace elements alterations in chronic hemodialysis patients with chronic renal failure and proteinuria. Journal of Islamic Academy of Sciences. 1993; 6: 1, 33-35.
4. Vanholder R, Cornelis R, Dhondt A, Lameire N. The role of trace elements in uraemic toxicity. Nephrol Dial Transplant. 2002; 17 [Suppl 2]: 2-8.
5. Sandstead H.Harold. Trace elements in uremia and hemodialysis. American journal of clinical nutrition. 1980; 33: pp. 1501-1508.
6. Miura Y, Nakai K, Suwabe A, Sera K. Trace elements in renal disease and hemodialysis. Nuclear instruments and methods in physics research. 2002; 189: 443-449.
7. Krachler M, Wirnsberger G H. Long-term changes of plasma trace element concentrations in chronic hemodialysis patients. Blood Purif. 2000; 18: 138-143.
8. Miura Y, Nakai K, Sera K, Sato M. Trace elements in sera from patients with renal disease. Nucl. Inst. and Meth. in Phys. Res. 1999; 150: 18-21.
9. Covic A, Gusbeth-Tatomir P. Trace elements in end-stage renal disease – unfamiliar territory to be revealed. BMC Nephrology. 2009; 10: 12.
10. D'Haese P C, Broe M E. Adequacy of dialysis: trace elements in dialysis fluids. Nephrol Dial Transplant. 1996; 11 [suppl 2]: 92-97.
11. Esfahani S T, Hamidian M R, Madani A, Ataei N, Mohseni P, Roudbari M et al. Serum trace elements in children on maintenance hemodialysis. Acta Medica Iranica. 2007; 45 (5): 351-354.
12. Krachler M, Wirnsberger G H. Long-term changes of plasma trace element concentrations in chronic hemodialysis patients. Blood Purif. 2000; 18: 138-143.
13. Lee S H, Huang J W, Hung K Y, Leu L J, Kan Y T, Yang C S et al. Trace metals abormalities in hemodialysis patients: relationship with medications. Artificial Organs. 2000; 24 (11): 841-844.
14. Skarupskiene I, Kuzminskis V, Abdrachmanovas O, Ryselis S, Smalinskiene A, Naginiene R et al. Influence of hemodialysis on changes of trace metals concentrations in blood of patients with end-stage renal failure. Medicina (Kaunas). 2003; 39: 1 131-8.
15. Tonelli M, Weibe N, Hemmelgarn B, Klarenbach S, Field K, Manns B, Thadhani R, Gill J. Trace elements in hemodialysis patients: a systematic review and meta – analysis. BMC medicine. 2009; 7: 25.
16. Molony, D.A., B. Marthy, Accumulation of metals and minerals from phosphate binders. Blood Purif. 2005; 23 (Supple): 2-9.
17. S-H Lee, J – W Huang, K – Y Hung, L – J Leu, Y – T Kan, C – S Yang, D C Wu, C – L Huang, P – Y Chen, J – S Chen and W – Y Chen. Trace Metals' Abnormalities in Hemodialysis Patients: Relationship with Medications Artificial Organs. 2001; 24 (11): 841–844.
18. Kiziltas H, Ekin S, Erkoc R. Trace element status of chronic renal patients undergoing hemodialysis. Bio Trace Elem Res. 2008; 124: 103-109.
19. Sen S, Bor N, Colakoglu M, Gultekin A. Clearance of zinc and copper during hemodialysis. Journal of Islamic academy of sciences. 1991; 4: 3, 265-267.
20. Sprenger KG, Bundschu D, Lewis K, Spohn B, Schmitz J, Franz P. Improvement of uremic neuropathy and hypogeusia by dialysate zinc supplementation: a double-blind study. Kidney Int1983; 24: S315–S318.
21. Bozalioglu S, Ozkan Y, Turan M, Simsek B. Prevalence of zinc deficiency and immune response in short – term hemodialysis. Journal of trace elements in medicine and biology. 2005; 18: 243-249.
22. F Locatelli, D Fouque, Olof H, Tilman B. Drüeke, Jorge B. Cannata – Andía, W H. Hörl and E Ritz Nutritional status in dialysis patients: a European consensus. 2002; Nephrol Dial Transplant 17: 563-572.
23. National Kidney Foundation. K/DOQI Clinical practice guidelines for nutrition in children with chronic kidney disease. AJKD. 2008: (Supple 1).
24. M Anees, A Mumtaz, M Nazeer, M Ibrahim, SM Rizwan and Tahira K, Referral pattern of hemodialysis patients to nephrologist. JCPSP 2007; 17 (11): 671-674.
25. Dvornik S, Cuk M, Racki S, Zaputovic L. Serum zinc concentrations in the maintenance hemodialysis patients. Coll Antropol. 2006; 30 (1): 125-9.
26. Senft V, Krizek M, Motan J, Racek J. Problems with trace elements in hemodialysis. Evaluation of serum levels. Cas Lek Cesk. 1999; 19; 138 (8):245-8.
27. Shouman M.G., Ibrahim H.Y., Salama E.E.E., El-Khayat Z., Ashour M. Trace Elements in Pediatric Hemodialysis Patients. Research Journal of Medicine and Medical Sciences 2009; 4 (2): 435-40.
28. Food and Nutrition Board. Institute of Medicine. Copper. Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, D.C.: National Academy Press, 2001: 224-257.
29. Navarro – Alarcon. M, Reyes – Pérez A., Lopez – Garcia H., Palomares – Bayo M., Olalla – Herrera M. and Lopez – Martinez M. C. Longitudinal study of serum zinc and copper levels in hemodialysis patients and their relation to biochemical markers. Biological Trace Element Research 2005; 113 (3): 209-22.