Assessment of trace elements in sera of patients undergoing renal dialysis

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ABSTRACT

الأهداف: لتقييم مستوى النحاس، الزنك، والحديد، والرصاص لمرضى الفشل الكلوي والخاضعين لعملية الديلزة.

الطريقة: تمت دراسة 42 مريض يعانون من الفشل الكلوي الخاضعين للديلزة الدموية مع 18 مريض خاضعين للديلزة البريتونية. كما تمت المقارنة مع 18 متطوع من الأشخاص الأصحاء تمت هذه الدراسة بين (سبتمبر 2011م – اكتوبر 2012م) في مستشفى الملك خالد الجامعي بالرياض. حيث تم قياس مستوى النحاس، الرصاص، الزنك بواسطة جهاز الامتصاص الذري (Atomic Absorption spectrophotometery). وتم قياس مستوى الحديد بطريقة تحديد الطيف الضوئي (spectrophotometric determination).

النتائج: لقد وجد أن معدل مستوى النحاس في دم مرضى الديلزة الدموية (20.5nmol/L؛95%[CI]17.52–22.39؛Interquartile range [IQR]16.40–24.20) أعلى من مستوى النحاس في دم الأشخاص الأصحاء (14.30nmol/L؛95% CI 9.72–16.91؛IQR 9.70–17) ومن مستواه أيضاً في دم مرضى الديلزة البريتوانية (15.60nmol/L؛95% CI 14.17–16.66؛ IQR 14.10–16.70) بينما لم يكن هناك اختلاف بين متوسط مستوى الزنك في مرضى الديلزة الدموية والبريتوانية (9.50 nmol/L؛95% CI 7.83–12.09؛ IQR 7.00–14.40) والتي كانت أقل من مستواها في دم الأشخاص الأصحاء (13.20 nmol/L؛95% CI 10.65–15.22؛ IQR 10.58–15.35؛ p=0.03). كما وجد أن نسبة النحاس/ الزنك لدى مرضى الديلزة الدموية كانت 2.4بينما نسبتها لدى مرضى الديلزة البريتوانية كانت 2.5 وعند الأشخاص الأصحاء كانت النسبة 0.88. كما وجد أن متوسط مستوى الحديد في دم مرضى الديلزة الدموية (10mmol/L؛95% CI 8.03–11.96؛IQR 7–14.50؛ p=0.003) والبريتوانية (10mmol/L؛95% CI 6.56–14.43؛IQR 5.50–15؛ p=0.03) كان أقل من معدلة في دم الأشخاص الأصحاء. كما وجد أن نسبة مستوى الرصاص في دم المرضى الديلزة البريتوانية (0.11µmol/L؛95% CI 0.02–0.14؛IQR 0.02–0.14) أقل من مرضى الديلزة الدموية (0.18µmol/؛95% CI 0.15–0.21؛IQR 0.13–0.25؛ p=0.005) والأشخاص الأصحاء (0.15µmol/L؛95% CI 0.07–0.24؛ IQR 0.06–0.25؛ p=0.04).

الخاتمة: الإختلافات الملاحظة في معدل مستوى المعادن النادرة لدى مرضى الفشل الكلوي والخاضعين لعملية الديلزة تظهر الحاجة إلى دراسة أسباب هذه الإختلافات والمراقبة الدائمة لهؤلاء المرضى لتجنب الأمراض المصاحبة لعملية الديلزة.

Objectives: To assess the serum levels of copper, zinc, iron, and lead in patients on maintenance dialysis.

Methods: This cross-sectional study performed at King Khalid University Hospital, Riyadh, Saudi Arabia between September 2011 and October 2012 included 42 patients with end stage renal disease on hemodialysis (HD), 18 patients on peritoneal dialysis (PD), and 18 normal controls. Serum copper, zinc, and lead levels were determined by atomic absorption spectrophotometry, and serum iron was determined by spectrophotometric determination.

Results: The median serum copper level in HD patients (20.5 nmol/L; 95% confidence interval [CI]: 17.52-22.39; interquartile range [IQR]: 16.40-24.20) was higher (p=0.001) than the controls (14.30 nmol/L; 95% CI: 9.72-16.91; IQR: 9.70-17), and the PD patients (15.60 nmol/L; 95% CI: 14.17-16.66; IQR: 14.10-16.70). Although no different from PD patients' serum levels of zinc in HD patients (9.50 nmol/L; 95% CI: 7.83-12.09; IQR: 7.00-14.40) were lower than controls (13.20 nmol/L; 95% CI: 10.65-15.22; IQR: 10.58-15.35; p=0.03). Copper/zinc ratio in HD patients was 2.4, 2.5 in PD patients, and 0.88 in controls. The serum iron levels in HD patients (10 mmol/L; 95% CI: 8.03-11.96; IQR: 7-14.50; p=0.003), and PD patients (10 mmol/L; 95% CI 6.56-14.43; IQR 5.50-15; p=0.03) were lower than controls. Serum lead levels in PD patients (0.11 µmol/L; 95% CI: 0.02-0.14; IQR: 0.02-0.14) were lower than HD patients (0.18 µmol/; 95% CI: 0.15-0.21; IQR: 0.13-0.25; p=0.005), and controls (0.15 µmol/L; 95% CI: 0.07-0.24; IQR: 0.06-0.25; p=0.04).

Conclusion: Alterations in serum trace elements emphasize the need for monitoring trace elements in patients receiving maintenance dialysis.

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Alterations in trace element levels in patients receiving long-term renal replacement therapy have been implicated in immune dysfunction and increased oxidative stress.[1] Renal dialysis is primarily directed to removal of uremic toxins by manipulating the contents of dialysate, whereas the lack of monitoring of trace elements may result in their deficiency, or accumulation in patients with end stage renal disease (ESRD) undergoing renal dialysis.[2,3] An excess of harmful trace elements such as lead, cadmium, and copper, and deficiency of essential trace elements such as zinc and selenium are known for their adverse effects not only in the general population, but also in patients undergoing renal dialysis, particularly hemodialysis (HD).[4,5] Although both HD and peritoneal dialysis (PD) offer similar benefits to patients with ESRD, there is, however, evidence that both the treatment procedures differ with regards to the occurrence of adverse events and clinical outcomes.[6] This dichotomy may partly be explained by differential accumulation, or depletion of trace elements associated with the 2 treatment modalities. Copper may accumulate in patients receiving HD, whereas patients undergoing PD usually have normal serum copper levels.[7] Iron levels appear to be higher in HD patients, whereas patients on PD usually have normal serum levels of iron.[8] Mercury also tends to be higher in patients receiving HD, whereas little is known of serum mercury levels in patients undergoing PD.[8] Despite these differences, both HD and PD have been shown to be associated with less than the normal serum levels of both selenium and zinc.[7,8] Low levels of selenium, zinc, and iron in patients receiving HD and not PD have been shown to be associated with increased oxidative stress and exhibit a positive correlation with low percentages of CD3 and CD4 that may contribute to immune dysfunction in HD patients.[1] This may be evident from the fact that patients on PD exhibit better immune responsiveness compared with patients on HD.[9] It is therefore possible that differences in trace elements by interfering with immune regulation may be predisposing HD patients to infections more frequently compared with those on PD. In the backdrop of existing data, this study was performed to assess the serum levels of trace elements in patients receiving HD and PD at King Khalid University Hospital, Riyadh, Saudi Arabia.

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Methods. This cross-sectional study was approved by the Institutional Review Board of the College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA). All consenting patients regularly attending the Nephrology Department at King Khalid University Hospital, Riyadh, KSA for renal dialysis were enrolled in the study between September 2011 and October 2012. All the patients with ESRD either on HD or PD were being dialyzed for less than 2 years duration (range 2-23 months) except for one patient on HD who was receiving HD for 6 years. All patients on HD were being dialyzed using high flux filters for 4-hour sessions 3 times a week for a total duration of 12 hours per week. Of the 18 patients on PD, 12 patients were being treated by automated peritoneal dialysis (APD) for 8-10 hours, whereas 6 patients were being treated by continuous ambulatory peritoneal dialysis (CAPD) with 4 exchanges of 4-6 hours of dwelling time daily. Out of the total (60) ESRD patients, 27 (45%; HD n=21, PD n=6) had diabetes mellitus, 9 (15%; HD n=7, PD n=2) were infected with hepatitis C virus, and 5 (8.3%; HD n=4, PD n=1) patients had evidence of hepatitis B infection. Fifty-four (90%; HD n=44, PD n=10) patients had hypertension and were receiving anti-hypertensive therapy at the time of collection of blood samples. None of the patients or the healthy controls either in the past or at the time of enrollment in the study was a smoker.

Measurement of trace elements. From patients and controls, 5ml venous blood was collected in trace element free BD royal blue top plain tubes (Becton Dickinson, San Jose, CA, USA) for copper, and zinc estimation, BD royal blue top EDTA containing tubes for lead measurement, and plain Vacutainer for iron assessment. Specimen from each HD patient was collected before the dialysis session. The blood sample was allowed to clot, and serum was collected after centrifugation at 3000 rpm/min for 5 minutes at room temperature. Copper, zinc, and lead concentrations were determined by Graphite Furnace System using atomic absorption spectrophotometry by Perkin Elmer, Norwalk, CT, USA. Serum levels of iron were measured by bichromatic endpoint technique that involved spectrophotometric determination using Dimension RXL Max system (Siemens Healthcare Diagnostics Inc. Newark, DE, USA) analyzer.

Statistical analysis. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 19. For each group of patients and controls, data were represented by median value with interquartile range (IQR). Frequency histograms were used to determine the

data distribution. Mann Whitney U test was applied for comparison between groups, as the data were not normally distributed with a few outliers. A $p<0.05$ was considered significant.

Results. Patients group comprised of 42 patients with ESRD on HD, and 18 patients on PD. A group of 18 normal healthy individuals were also enrolled in the study as a control group. **Table 1** describes the characteristics of patients receiving renal dialysis and the normal healthy controls. The HD group included 24 females and 18 males (mean age 60.63 ± 19.23 years), the 18 PD group included 11 females and 7 males (mean age 67 ± 17.66 years), and the group comprising normal healthy individuals had 9 females and 9 males (mean age 54.23 ± 11.3 years) **Figure 1** describes serum copper levels in ESRD patients and normal healthy individuals. The median copper level in HD patients was 20.5 nmol/L with 95% confidence interval (CI) 17.52-22.39; IQR 16.40-24.20, which was significantly higher (p=0.001) than the median copper level of 14.30 nmol/L (95% CI 9.72-16.91; IQR 9.70-17) in normal healthy individuals. The median level of copper in PD patients was 15.60 nmol/L (95% CI 14.17-16.66; IQR 14.10-16.70), which was not statistically different from the normal healthy individuals. Copper levels in ESRD

Table 1 - Characteristics of patients with end stage renal disease undergoing renal dialysis (n=60) and controls (n=18).

Characteristic	Hemodialysis	Peritoneal dialysis	Controls
Number	42	18	18
Males	18	7	09
Females	24	11	09
Mean age, years	60.63 ± 19.23	67 ± 17.66	54.23 ± 11.3
Duration of dialysis	<2 years except one patient	<2 years	-
Dialysis	High flux filters	APD (12), CAPD (6)	-
Frequency of dialysis	3 x 4 hours session/week	APD; 8-10 hours/day CAPD; 4 x 4-6 hours dwelling time/ day	-
Diabetes mellitus	21	06	-
Hepatitis C infection	07	02	-
Hepatitis B infection	04	01	-
Hypertension	44	10	-

APD - automated peritoneal dialysis, CAPD - continuous ambulatory peritoneal dialysis

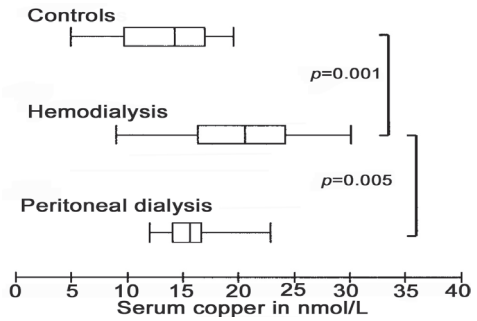

Figure 1 - Comparison of serum levels of copper in 42 patients receiving hemodialysis, 18 patients on peritoneal dialysis, and 18 normal controls.

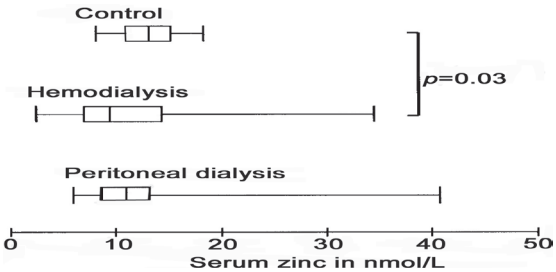

Figure 2 - Comparison of serum zinc levels in 42 patients on hemodialysis, 18 patients on peritoneal dialysis, and 18 normal controls.

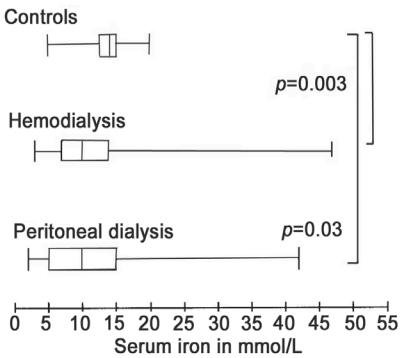

Figure 3 - Comparison of serum iron levels in 42 patients receiving hemodialysis, 18 patients on peritoneal dialysis, and 18 normal controls.

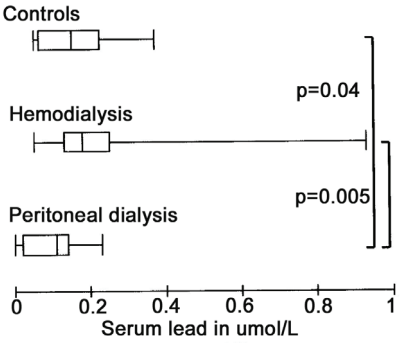

Figure 4 - Comparison of serum levels of lead in 42 patients on hemodialysis,18 patients on peritoneal dialysis, and 18 normal controls.

patients on HD were however, significantly higher than the patients being treated with PD (p=0.005). **Figure 2** shows comparison of zinc levels in ESRD patients and normal controls. The median serum level of zinc in patients on HD was 9.50 nmol/L (95% CI 7.83-12.09; IQR 7-14.40), in patients on PD was 11.01 nmol/L (95% CI 9.03-12.56; IQR 8.73-13.20) and in normal controls it was 13.20 nmol/L (95% CI 10.65-15.22; IQR 10.58-15.35). Comparative analysis revealed that patients on HD had significantly lower serum zinc levels compared with the normal controls (p=0.03), whereas no difference was observed between HD patients and PD patients or between PD and normal controls. The copper/zinc ratio in HD patients was 2.4, median 2.1 (IQR 1.2-2.9), in PD patients was 2.5, median 1.5 (IQR 1.14-2.09), and in normal healthy controls it was 0.88, median 0.82 (IQR 0.66-1.05). **Figure 3** shows data comparing serum iron levels in ESRD patients on HD and PD, and in the normal healthy individuals. The median serum iron level in HD patients was 10 mmol/L (95% CI 8.03-11.96; IQR 7-14.50), in PD patients was 10 mmol/L (95% CI 6.56-14.43; IQR 5.50-15), and in normal healthy individuals it was 16.50 mmol/L (95% CI 12.89-20; IQR 13-20). Serum iron levels both in HD patients (p=0.003) and PD patients (p=0.03) were significantly lower than the normal healthy controls, whereas no statistical difference in serum iron levels was observed between HD patients and PD patients with ESRD. **Figure 4** describes data for serum levels of lead in ESRD patients undergoing HD or PD, and normal healthy individuals. The median serum level of lead in HD patients was 0.18 µmol/L (95% CI 0.15-0.21; IQR 0.13-0.25), in PD patients was 0.110 µmol/L (95% CI 0.02-0.14; IQR 0.02-0.14), and in normal healthy individuals was 0.15 µmol/L (95% CI 0.07-0.24; IQR 0.06-0.25). Whereas no difference was observed between serum lead levels between HD patients and normal healthy controls, the serum levels of lead in PD patients were significantly lower than the normal healthy controls (p=0.04) and HD patients (p=0.005).

Precision, accuracy, and imprecision of the assay were calculated by EP Evaluator computer software. The total assay variation according to analytical claim for zinc and copper assay was up to 15%, and the total variations of the results in the present study for zinc and copper were 14.1% and 9.8%. Inter and intra-assay variations for copper were 6.5% and 5.8%. Similarly, inter and intra-assay variations for zinc were 5.6% and 5.5%. For lead the analytical claim for total variation was 20%, whereas the observed total variation in the study was 4%, with inter-assay variation of 2.4%, and intra-assay variation of 2.2%. The claimed total analytical variation for iron was 1.6%, and the total variation of iron assay in this study was 0.7%, with inter-assay (0.6%) and intra-assay (0.5%) variations.

Discussion. Elevated serum copper levels were detected in ESRD patients on HD in the present study. Data regarding serum copper levels in ESRD patients either on HD or PD are conflicting. Whereas a number of studies have reported high levels of serum copper in ESRD patients on long term HD in the past,[10,11] there is however, evidence suggesting no difference in serum copper levels between patients on HD and healthy individuals.[3] Similarly serum copper levels in the present study in ESRD patients on PD were no different than the normal controls, but were remarkably lower than the HD patients. Although these findings are in agreement with previously reported normal serum levels of copper in PD patients,[3] high levels of serum copper however, have also been reported in PD patients.[12] Little is known about the factors contributing to the elevated copper levels in patients receiving long term dialysis. A study investigating the kinetics of metal cations has however reported a continuous increase in the concentration of plasma copper levels during prolonged HD.[13] On the contrary, monitoring of serum copper and zinc levels in patients receiving PD has revealed that these patients can not only absorb zinc, but there is also a tendency to lose significant amounts of copper.[14]

Despite no difference between the serum zinc levels between the normal controls and the PD patients, HD patients were found to have significantly low levels of zinc in the present study. Consistent with the findings of this study, low serum levels of zinc have been reported in patients being treated by long term HD.[15,16] Zinc deficiency has been implicated in a number of nonspecific conditions frequently observed in HD patients such as anorexia,[17] reduced cognitive functions,[18] dysgeusia,[19] and reduced ability to handle oxidative stress.[20] A higher incidence of infections in HD has also been linked with increased predisposition due to zinc deficiency in these patients. This is evident from the fact that zinc repletion has been shown to decrease the risk of systemic infections in patients on maintenance HD.[21] The immune protection provided by zinc could be due to zinc being a potent anti-oxidant and an anti-inflammatory agent in addition to its role in regulation of T and B lymphocyte functions.[22,23]

Both HD and PD patients in the present study had a higher copper/zinc ratio compared with the normal controls. An elevated copper/zinc ratio has been linked to nutritional abnormalities, inflammation, oxidative stress, and immune abnormalities[10,12] that

may contribute to adverse outcomes in patients on maintenance dialysis. The exact mechanism mediating copper and zinc homeostasis is not known, there is however, evidence associating zinc supplementation with improvement in proteins catabolic rate in HD patients.[24] Similarly restoration of serum zinc levels in HD patients has been shown to decrease the elevated levels of C reactive protein (CRP) a biomarker of inflammation in HD patients.[25] Similarly a high copper/zinc ratio in patients receiving PD has also been linked to high levels of CRP and alterations in the percentages of T and B lymphocytes.[12] Collectively, these data indicate that a higher copper/zinc ratio in HD and PD patients is detrimental and may serve as a marker for increased likelihood of adverse outcome in ESRD patients on maintenance dialysis who may benefit from zinc supplementation.

The ESRD patients either being treated by HD or PD had significantly lower serum iron levels compared with the normal health controls. These findings were consistent with the previously reported low serum iron levels in HD patients that were shown to positively correlate with CD3 and CD4 lymphocyte percentages.[1,26] Low levels of serum iron have also been reported in PD patients, and iron supplementation despite inducing improvement in several parameters such as transferring saturation, serum ferritin level, and hematocrit made no difference in the incidence of catheter infections and peritonitis.[27] Hepcidin a small defensin like peptide produced by hepatocytes is believed to control serum iron levels by regulating iron loss from intracellular stores.[28] Removal of prohepcidin a precursor of Hepcidin in ultrafiltrate in HD patients and particularly in peritoneal effluent of PD patients,[29] may be a contributing factor to low serum iron levels in both HD and PD patients suffering from ESRD. In addition, repeated and excessive blood loss from the access cannulation site using an arteriovenous graft in HD sessions has recently been implicated in the causation of anemia in patients on long term dialysis.[30] Moreover, vitamin D insufficiency among patients on maintenance dialysis has not only been linked with anemia and erythropoietin resistance, but has also been shown to contribute to resistance against erythropoiesis-stimulating agents thus interfering with erythropoietin responsiveness.[31] Collectively, these observations indicate that the cause of anemia in patients receiving renal dialysis is multifactorial.

There was no difference in serum lead levels between the normal healthy controls and the HD patients; however, significantly low serum lead levels were detected in PD patients. In contrast to the findings of the present study, a meta-analysis of a large number of studies shows that serum lead levels in HD patients tend to be higher than the normal controls.[5] High serum levels of lead and increased copper/zinc ratio have been shown to correlate with carotid artery intima-media thickness in HD patients; thus, predisposing these patients to carotid artery atherosclerosis.[32] Moreover, high serum lead levels in PD patients have been shown to be associated with higher mortality rates.[33] Lead nephropathy, and interstitial nephritis thought to be due to persistent exposure to high lead levels is now believed to be caused by sustained exposure to low lead levels as well, particularly in patients with preexisting hypertension, diabetes, or chronic kidney disease.[36] It is therefore, imperative that serum lead levels should be monitored in patients either on HD or PD to avoid lead toxicity and the associated morbidity in ESRD patients.

In conclusion, this study was limited by lack of age matched consenting controls, the alterations in serum levels of trace elements detected in the present study however emphasize the need for continuous monitoring of trace elements in patients receiving prolonged maintenance dialysis. Moreover, the differences observed in the serum levels of trace elements between HD and PD patients could be due to the difference in the treatment modalities, and may contribute to predisposition to the unfavorable outcomes associated with each procedure. Further investigations to validate the findings of the present study and serial evaluations of trace elements in follow up studies are recommended.

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