ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Recovery of serum metallothionein level after hemodialysis in patients with end stage renal failure

Sazan D. Saeed, Ansam N. Al-Hasani, Marwan S. Al-Nimer[*]

Department of Pharmacology and Toxicology, College of Pharmacy, Hawler Medical University, Erbil-Iraq

ABSTRACT

Metallothioneins (MTs) are intracellular metal-protein which not alone have inactivated heavy metals but also have traced elements while improving the antioxidant status. Serum levels of some heavy metals and trace elements showed toxic levels in the end-stage renal failure. This study is aimed to determine the serum level of metallothionein in patients with chronic kidney disease (CKD) and to show the effects of hemodialysis on its level. This cross-sectional study included 125 patients with CKD managed with regular hemodialysis. Estimated glomerular filtration rate (eGFR) was calculated by using the Cockroft and Gault equation adjusted to the body surface area ($ml/min/m^2$), and 4 variable modified diet in renal disease (4-v MDRD) equation ($ml/min/1.73m^2$). The mean $\pm$ SD of serum metallothionein was significantly less than the corresponding level of healthy subjects (1113.4$\pm$289.5 pg/ml versus 1536.0$\pm$341.4 pg/ml). Hemodialysis improves the serum level of metallothionein to attain 1437.6$\pm$406.1 pg/ml. There is a non-significant correlation between serum metallothionein with the eGFR and hemodialysis did not produce a significant effect on the correlation between serum metallothionein with eGFR. We conclude that serum metallothionein level is a useful prognostic marker for CKD and hemodialysis plays a role in the recovery of serum metallothionein level.

[*]Corresponding Author

Name: Marwan S. Al-Nimer
Phone: +9647703866579
Email: marrwan@pha.hmu.edu.iq

INTRODUCTION

Metallothioneins (MTs) are intracellular metal-protein, which contained 20 cysteine out of 61-68 amino acids in mammalian (Alexander *et al.*, 1987; Romero-Isart and Vašák, 2002). There are four MT isoforms in the mammalian, in addition, there are another 13 MTs-like proteins (Wong and Ho, 2012). These isoforms were categorized according to their site in the soft tissue, brain, heart, kidney, reproductive organs, and in the stratified squamous cell epithelium (Moffatt and Denizeau, 1997; Moffatt and Seguin, 1998). The primary function of MTs is to detoxify heavy metals and stabilize the essential metal ion in the body (Vašák, 2005; Klaassen *et al.*, 2009). With respect to its physiological chelating property, MTs have high affinity to bind zinc metal, and it gets replaced by copper or cadmium in pathological conditions leading to an excess of copper or cadmium (Shaw *et al.*, 1991). Zinc-binding MTs served as an antioxidant against free radicals as it regulates the synthesis of glutathione (Kojima-Yuasa *et al.*, 2005). Patients with chronic hemodialysis have significantly low levels of serum zinc, copper and manganese while the serum levels of

cadmium, cobalt and magnesium were significantly increased than corresponding values of healthy people (Kaya *et al.*, 2012). Moreover, the serum levels of zinc and selenium were significantly reduced in children with chronic kidney disease (CKD) managed with hemodialysis compared with healthy children or patients not subjected to hemodialysis (Esmaeili and Rakhshanizadeh, 2019).

Long term adverse clinical outcome of chronic hemodialysis was associated with a low concentration of zinc and significantly high concentrations of arsenic, mercury, and lead (Tonelli *et al.*, 2018). Moreover, the risk of death was associated with high levels of serum copper and cadmium (Tonelli *et al.*, 2018). Our hypothesis that in the established CKD, there is an alteration in the trace elements alterations and the status of antioxidants which changed after hemodialysis. Therefore, serum levels of MTs could be served as a marker of the effects of hemodialysis against these alterations. This study aimed to measure the immediate effect of hemodialysis on the serum levels of MTs protein in chronic hemodialysis patients with established CKD.

MATERIALS AND METHODS

This cross-sectional observational study was approved by the local Scientific Committee at the Ethical board in the Hawler Medical University (No. 9-2664) according to the guidelines of the Helsinki. According to the guidelines, any intervention used in this study does not carry any risk or harm to the patient, and the patient can refuse intervention at any time. The patients signed a consent form at a time of entry into the study. All participants' rights were protected according to the Helsinki Declaration.

Setting

This cross-sectional study was done in the Department of Pharmacology and Toxicology at the Hawler Medical University in Kurdistan region-Iraq from June to December 2018. Patients under regular hemodialysis regimen were collected from the Unit of Dialysis at the Rizgary Teaching Hospital in Erbil-Iraq. Eligible patients were both genders of whatever age. The criteria of inclusion were known cases of CKD of whatever etiological managed with regular regimens of hemodialysis. Patients with chronic renal failure due to terminal illness, intoxication with heavy metals, abnormal electrolytes levels, and complicated pregnancy were excluded from the study.

Sample size estimation

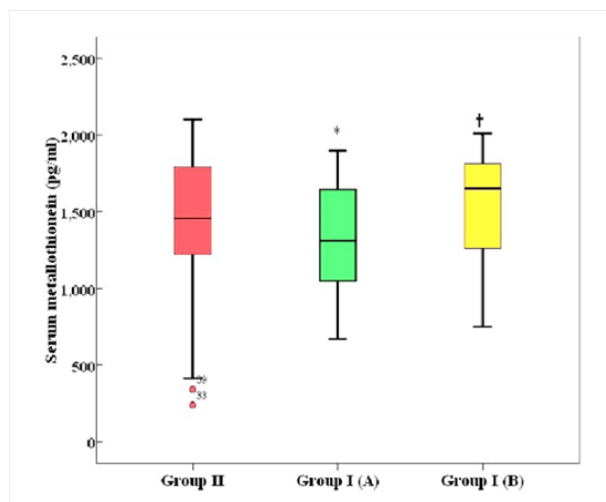The sample size was calculated by using a margin of

Figure 1: Serum level of metallothionein in healthy subjects and chronic kidney disease patients

errors (α = 0.05, β = 0.2), two tails and 95% confidence interval. Therefore, the sample size is equal to 1 + 2C Í (Standard deviation/difference between means)^2, where C represents the Constant value that derived from the statistical tables and it equals to 7.85 when the 1-β =0.8 and α=0.05.

A total number of 125 patients (66 men and 59 women) who were diagnosed as chronic kidney patients were enrolled in the study. The patients were grouped into Group IA: indicating the data of the patients before hemodialysis and group IB indicating the data of the patients after the hemodialysis. Group II represented the data of healthy subjects.

Clinical assessment

The authors interviewed each patient and obtained a demographic and illness history. Anthropometric measurements, including height (m) and weight (kg) were recorded.

Venous blood samples drew from the patients, the sera separated by centrifugation (3,000 RPM for 15 minutes) for determination of the BUN, serum creatinine and metallothionein. Estimated glomerular filtration rate (eGFR) was calculated by using the Cockroft and Gault equation adjusted to the body surface area (ml/min/m^2), and 4 variable modified diet in renal disease (4-v MDRD) equation (ml/min/1.73m^2). Serum blood nitrogen urea (BNU), creatinine, and electrolytes were determined in the laboratory of the hospital as a routine investigation. The serum metallothionein level was measured by using the ELISA technology according to the instruction of the manufacturer.

Statistical analysis

Table 1: Characteristics of the participants enrolled in the study

Determinants	Results
Gender (Female : Male)	59:66
Age (year)	55.8±12.9
Residency	
Rural	32(25.6)
Urban	92(73.6)
Refugee	1(0.8)
Ethnicity	
Kurd	107(65.6)
Arab	18(14.4)
Family history of renal diseases	19(15.2)
Causes of renal failure	
Renal	54
Extra-renal	77
No identified cause	04

The results are expressed as number (percentage) and mean ± SD

Table 2: Effect of hemodialysis on the renal function tests

Determinants	Before dialysis (n=125)	After dialysis (n=125)	p-value
Blood urea nitrogen (mg/dl)	140.2±55.2	60.6±32.5	<0.001
Serum creatinine (mg/dl)	6.14±2.53	3.27±1.61	<0.001
Estimated glomerular filtration rate Cockraft and Gault): (ml/min)	13.53±6.17	33.92±27.02	<0.001
Estimated glomerular filtration rate 4-v MDRD (ml/min/1.73m2)	8.74±4.12	24.56±18.04	<0.001

The results are expressed as mean± SD. P value calculated by using paired student t-test (two-tailed)

Statistical analyses were performed using SPSS version 20.0 and Excel 2003 program for Windows.

The results were provided number, percentage and as mean ±SD. Two-tailed independent two-sample was used to compare between healthy subjects and patients with CKD, and two-tailed paired t-test was used to show the effect of hemodialysis. Simple (Pearson) correlation test was used to show the correlation between serum metallothionein and the estimated glomerular filtration rate. The differences were considered statistically significant when $p \leq 0.05$.

RESULTS

Table 1 shows the characteristics of the participants included in the study. The majority of patients were residents in the urban areas which are three folds of the patients lived in the rural area. Most of the patients were of Kurd ethnicity, and a family history of chronic kidney disease was observed in 15.2% of patients. Extra-renal causes that related to systemic diseases are the etiological factors of chronic renal disease in this study.

Hemodialysis produced a significant decrease in blood indices of renal function and improved the glomerular filtration (Table 2).

Blood urea nitrogen and serum creatinine declined by 56.8% and 46.7% of the baseline levels, respectively. Estimated glomerular filtration rate by using the Cockroft and Gault, and 4-vMDRD equations, improved by 2.5 and 2.8 folds of the baseline rate (before hemodialysis), respectively. Patients with CKD had a significant lower serum metallothionein level compared with the corresponding level of healthy subjects (Figure 1).

In Figure 1 shows, Group I (A): Chronic kidney disease before hemodialysis, Group I (B): Chronic kidney disease after hemodialysis, and Group: Healthy subjects. *p<0.001 compared with Group II, †P<0.001 compared with Group I (A).

Hemodialysis elevates the level of metallothionein level, which approximates the serum level of healthy
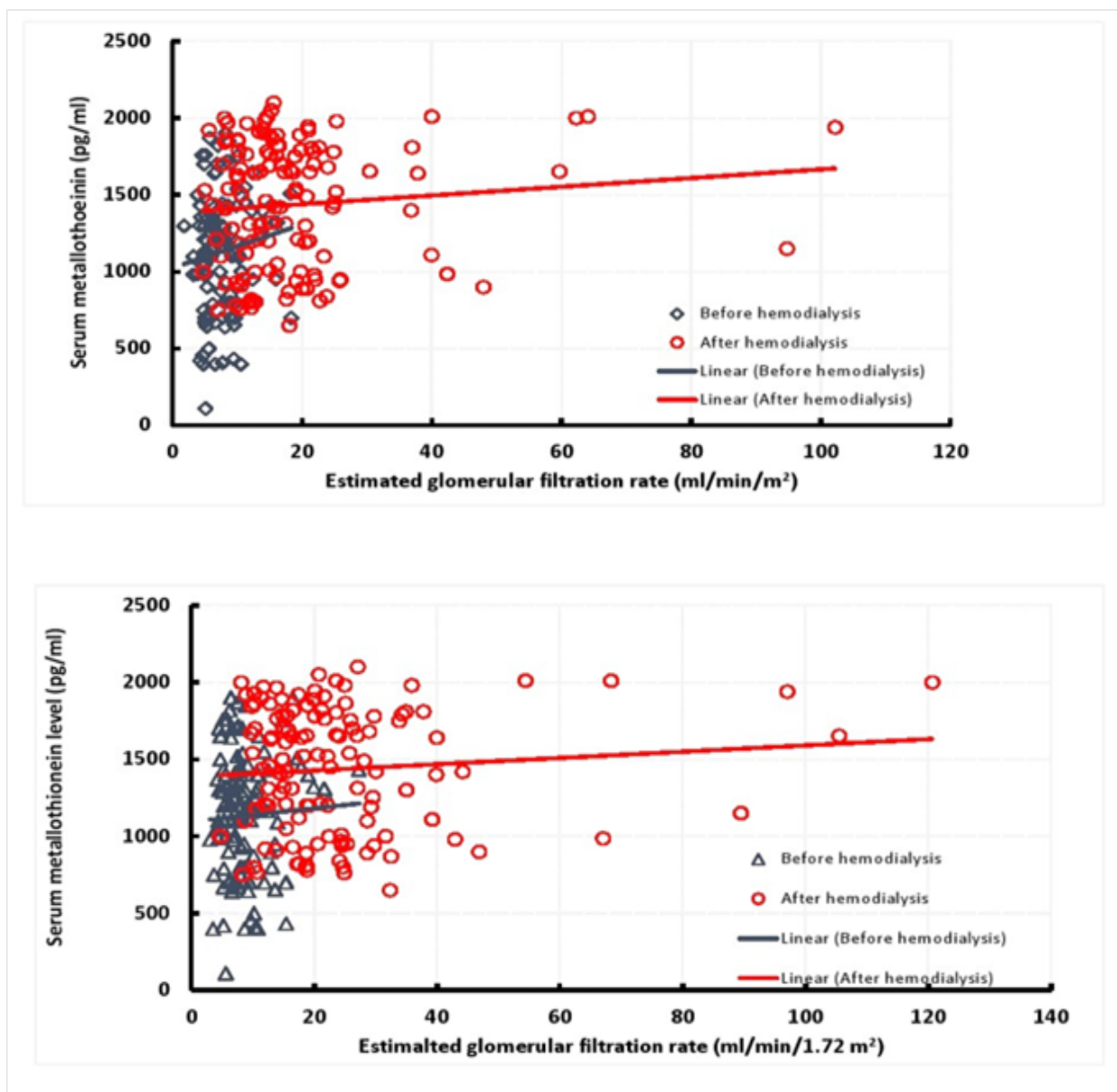
Figure 2: Effect of hemodialysis on the correlation between serum metallothionen and estimated glomerular filtration rate

subjects. Hemodialysis is not producing a significant correlation between the serum metallothionein and the estimated glomerular filtration rate (Figure 2).

Figure 2 shows, (Before dialysis r=0.117, p=0.194; after dialysis r=0.101, p=0.262) and by 4 variable modified diet in renal disease (4-v MDRD) equation [below figure]] (Before dialysis r=0.046, p=0.610; after dialysis r=0.091, p=0.313).

DICUSSION

The results of this study show chronic kidney disease patients have significant low serum metallothionein levels which do not significantly correlate with estimated glomerular filtration rate. In addition, hemodialysis improves the serum level of metallothionein with a non-significant correlation with estimated glomerular filtration rate. Low serum metallothionein levels which were observed in this study may be due to the generation of the free radicals as the oxidative stress that played a role in the pathogenesis of chronic kidney disease (Ziedan and Bhandari, 2019). Moreover, certain serum trace elements were increased in chronic kidney disease and contributed to the generation of free radicals (Esmaeili and Rakhshanizadeh, 2019; Zeng et al., 2019). Therefore, low serum level of metallothionein and increased levels of circulating heavy metals or trace elements aggravate the nephrotoxicity (Zeng et al., 2019; Hao et al., 2015). Immunohis-

tochemistry study revealed that low score of tubular expression of metallothionein indicates a poor renal out cone in patients with nephritis (Faurschou et al., 2008). Moreover, expression of tubular metallothionein increases with advanced age, which has a form of protective effect against the oxidative stress syndrome as the reactive oxygen species are increased with ageing (Leierer et al., 2016). Hemodialysis significantly improves the serum levels of metallothionein irrespective of the improvement of the renal function indices which indicates the following explanations:

a. Hemodialysis improves the binding of metallothionein with heavy metals and trace elements. Metallothionein inactivates the toxic effects of circulating heavy metals which are available in an ionized form by conjugation and thereby clears it from the body (Lentini et al., 2017)

b. Hemodialysis removed the trace elements, heavy metals and other nutrients from the circulation (Joyce et al., 2018). Therefore, higher serum level of metallothionein is the unbound form which up-regulated in response to kidney disease.

Therefore, high serum level of metallothionein indicating that patients with chronic kidney disease were recovered from the toxic effects of the heavy metals and trace elements, and from oxidative stress.

Limitations of the results of this study, including the determination of heavy metals, trace elements, and the status of antioxidant to prove the explanation of high serum levels of metallothionein.

CONCLUSIONS

We conclude that metallothionein is a useful prognostic marker for chronic kidney disease and hemodialysis plays a role in the recovery of the metallothionein.

ACKNOWLEDGEMENT

Conflict of interest

The authors declare no conflicts of interest. They have no financial, personal or professional relationships with other people or organizations in connection with evaluated manuscripts. There has been no direct financial interest in the subject matter or materials discussed in the manuscript that could inappropriately influence the work submitted.

Funding

Author's contribution

All authors must contribute to the manuscript and approve its final version.

Conflict of interest

There are no conflict of interest.

REFERENCES

Alexander, R., Noble, R. A., Kagi, R. I. 1987. Fossil resin biomarkers and their application in oil to source-rock correlation, gippsland basin, australia. *The APPEA Journal*, 27(1):63–63.

Esmaeili, M., Rakhshanizadeh, F. 2019. Serum Trace Elements in Children with End-Stage Renal Disease. *Journal of Renal Nutrition*, 29(1):48–54.

Faurschou, M., Penkowa, M., Andersen, C., Starklint, H., Jacobsen, S. 2008. The renal metallothionein expression profile is altered in human lupus nephritis. *Arthritis Research & Therapy*, 10(4):R76–R76.

Hao, Y., Huang, J., Gu, Y., Liu, C., Li, H., Liu, J., Li, R. 2015. Metallothionein deficiency aggravates depleted uranium-induced nephrotoxicity. *Toxicology and Applied Pharmacology*, 287(3):306–315.

Joyce, T., Brown, F. C., Wallace, D., Reid, C. J. D., Sinha, M. D. 2018. Trace element and vitamin concentrations in paediatric dialysis patients. *Pediatric Nephrology*, 33(1):159–165.

Kaya, Y., Ari, E., Demir, H., Gecit, I., Beytur, A., Kaspar, C. 2012. Serum cadmium levels are independently associated with endothelial function in hemodialysis patients. *International Urology and Nephrology*, 44(5):1487–1492.

Klaassen, C. D., Liu, J., Diwan, B. A. 2009. Metallothionein protection of cadmium toxicity. *Toxicology and Applied Pharmacology*, 238(3):215–220.

Kojima-Yuasa, A., Umeda, K., Ohkita, T., Kennedy, D. O., Nishiguchi, S., Matsui-Yuasa, I. 2005. Role of reactive oxygen species in zinc deficiency-induced hepatic stellate cell activation. *Free Radical Biology and Medicine*, 39(5):631–640.

Leierer, J., Rudnicki, M., Braniff, S.-J., Perco, P., Koppelstaetter, C., Mühlberger, I., Eder, S., Kerschbaum, J., Schwarzer, C., Schroll, A., Weiss, G., Schneeberger, S., Wagner, S., Königsrainer, A., Böhmig, G. A., Mayer, G. 2016. Metallothioneins and renal ageing. *Nephrology Dialysis Transplantation*, 31(9):1444–1452.

Lentini, P., Zanoli, L., Granata, A., Signorelli, S. S., Castellino, P., Dellaquila, R. 2017. Kidney and

heavy metals - The role of environmental exposure. *Molecular Medicine Reports*, 15(5):3413–3419.

Moffatt, P., Denizeau, F. 1997. Metallothionein in Physiological and Physiopathological Processes. *Drug Metabolism Reviews*, 29(1-2):261–307.

Moffatt, P., Seguin, C. 1998. Expression of the Gene Encoding Metallothionein-3 in Organs of the Reproductive System. *DNA and Cell Biology*, 17(6):501–510.

Romero-Isart, N., Vašák, M. 2002. Advances in the structure and chemistry of metallothioneins. *Journal of Inorganic Biochemistry*, 88(3-4):388–396.

Shaw, C. F., Savas, M. M., Petering, D. H. 1991. Ligand substitution and sulfhydryl reactivity of metallothionein. *Methods in Enzymology*, pages 401–414.

Tonelli, M., Wiebe, N., Bello, A., Field, C. J., Gill, J. S., Hemmelgarn, B. R., Holmes, D. T., Jindal, K., Klarenbach, S. W., Manns, B. J., Thadhani, R., Kinniburgh, D. 2018. Concentrations of Trace Elements and Clinical Outcomes in Hemodialysis Patients. *Clinical Journal of the American Society of Nephrology*, 13(6):907–915.

Vašák, M. 2005. Advances in metallothionein structure and functions. *Journal of Trace Elements in Medicine and Biology*, 19(1):13–17.

Wong, C. P., Ho, E. 2012. Zinc and its role in age-related inflammation and immune dysfunction. *Molecular Nutrition & Food Research*, 56(1):77–87.

Zeng, L., Ai, C., Zhang, J., Zheng, J. 2019. Essential element Cu and non-essential element Hg exposures have different toxicological effects in the liver of large yellow croaker. *Marine Pollution Bulletin*, 139:6–13.

Ziedan, A., Bhandari, S. 2019. Protocol and baseline data for a prospective open-label explorative randomized single-center comparative study to determine the effects of various intravenous iron preparations on markers of oxidative stress and kidney injury in chronic kidney disease (IRON-CKD). *Trials*, 20(1):194–194.