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Assessment the Effects of Heavy Elements on Some Hematological Parameter in CKD Patients Undergoing Hemodialysis in Thi-Qar Province / Iraq

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***Abstract*— The present study aimed to assess the level of heavy elements (HEs) (Pb, Cd, Cu and Zn) in blood serum and their effects upon selected parameters (WBC, RBC, Hb and PLT.) in patients with chronic kidney disease (CKD) undergoing hemodialysis (HD) in Thi-Qar province Iraq. The study was from Sept 2022 until July 2023. Fifty samples of blood were taken from patients with CKD, whereas 25 samples were taken from healthy people (males and females as a control group. The patients and the control group ranged from (17-76) years. All the patients were submitted to the HD for (1-15) yeas in Al-Hussain Hospital in the mentioned province. The results showed that the sex ratio of the samples was more than 70% for males and 30% females respectively, and the high number of the patients with CKD was 48% for the advance age group (57-76). The mean concentration of (Cd, Cu, Zn and Pb) in the blood serum of patients pre HD were 0.065±0.018, 0.014±0.006, 0.016±0.008 and 1.041±0.38 mg/L respectively, Pb and Cd recorded the higher mean concentration, while Cu and Zn recorded lower concentration compared to the control group .The mean count of (WBC, RBC, Hb and PLT) were decreased , recorded 2.78±0.79, 3.06±0-54, 8.54±1.43 and 164.4±51.8 respectively , it concluded from the present study that the CKD affected the hematological parameters directly while HEs affected indirectly.**

***Keywords*—Heavy elements, Chronic kidney disease (CKD), Complete blood count (CBC), Hemodialysis (HD), Thi-Qar province.**

I. INTRODUCTION

The high levels in the use of heavy elements (HEs) in the past time led to an increase in the elimination of toxic metals in the environment Yang and Rose [1]. The HEs are concerned because of their many effects and their concentration stimulated toxic illnesses and effects the life forms Damien *et al.*, [2]. HEs that possess an atomic number of more than 20 or a density four times the density of water is called HEs Aliasgharpour [3]. HEs are two types, fist: have vital role such as Cu, Zn, Fe and Mn, while the second type has no biological role such as Hg, Pb and Cd Aliasgharpour [3] and Prashanth *et. al*, [4].

The toxicity of HEs become interesting in the human body especially at the last five decades, because of this the large products have been disposed of as industrial waste and it is un biodegradable, there resistance make them remaining long time in the environment. High levels of these elements are still present in the environment, which results in chronic exposure in the general population Sabath and Robles-Osorio [5]. The HEs enter the human body through many pathways such as inhalation, ingestion and through broken skin Kishna and Mohan [6]. Accumulation of HEs means the net between uptake and intake of these elements Kishna and Mohan [6]. Most of the HEs are carcinogenic, teratogenic, and mutagenic and pose a threat to human health and the environment Farombi *et al.*, [7].

Exposure to several HEs specially Pb, Cd and Hg is affected upon the hematological profile with characteristic pancytopenia, mainly aplastic anemia and an increased risk of developing cancer (acute myeloblastic leukemia), also red blood cells (RBCs) expose to some morphological effects such as microcytosis and inclusion Chielle *et al.*, [8]. Formation of hematological components begins in the bone marrow, and they continue to mature in the peripheral blood tissue, therefore, they are affected by many harmful compounds that cause damage to the bone marrow and peripheral blood tissue Jha *et al.*, [9] , as shown in figure 1.

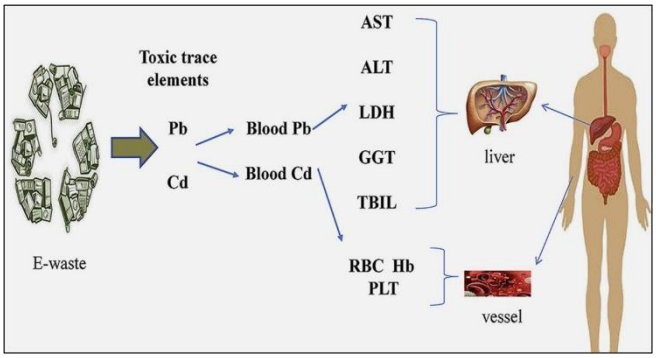

Fig 1. The effects of toxic elements on human hematological and hepatic functions by Chen *et al.*, [10].

The one of the major health problems worldwide is chronic kidney disease (CKD). The morbidity and mortality which are related with CKD is often of great concern in developing nations Nitta *et al.*, [11]. The most common causes of renal failure are infections, autoimmune diseases, diabetes and other endocrine disorders, cancer, and toxic

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chemicals, which progressively deteriorate kidney function Chielle *et al.*, [12]. Hence, renal failure can be a result of complications associated with other severe medical conditions. CKD is created from a wide range of physiological processes changed by the continuing decline in glomerular filtration rate (GFR) Hamer and El Nahas [13] and Jha *et. al.* [14]. Hematological parameters have shown various altered particularly erythrocytes indices, are most affected, giving rise to anemia. As CKD progresses, the changes in hematological parameters become more pronounced Babitt and Lin [15].

The decreased production of erythropoietin due to the kidney dysfunctions which is considered the most common reasons for anemia associated with CKD, also another causes of anemia: the iron deficiency resulting from the frequent phlebotomy, gastrointestinal bleeding, blood remaining in the tubes and dialyzer of the hemodialysis device, acute and chronic inflammatory conditions, as well as severe secondary hyperparathyroidism, and the short life of RBCs. Altering in the bones and minerals metabolism, like hyperphosphatasemia, and hypo-or hypercalcemia, are mostly observed in chronic kidney failure patients Mahmood *et. al.* [16].

There is not any study related to this subject has been done in Thi–Qar province, so the aim of this work is to determine the effects of HEs (Pb, Cd, Cu and Zn) upon some hematological parameters in CKD patients.

II. METHOD

This research involved 50 sample of patients with chronic renal failure, males, and females, with an age ranging from (17–76) years, which they were present at the hemodialysis units in the Al-Hussain hospital, the period of study was started at Sept 2022 until –July 2023. All the patients undergo hemodialysis from (1-15) years. Twenty-five healthy people (males and females) were chosen as a control group. The blood samples were collected during the study period from patients in the mentioned hospital undergo hemodialysis and healthy persons. The personal information was obtained from each participant (patient and healthy) through the special questionnaire containing the following items: age, sex, occupational data, smoking, chronic disease, and duration of renal failure (Verbal consent was obtained from each participant without mentioned to his name).

A. Collection blood sample

Three milliliter of blood was collected from CKD patients pre hemodialysis sessions, About [2 milliliter] of the blood sample was putted in the tube and separated to obtain blood serum for the estimating of HEs and the rest of blood (1 ml) putted in EDTA test tube and to ensure that complete anticoagulation of the blood gentle mixing was done immediately, for hematological test (WBCs, RBCs ,Hb and PLTs) , the test tubes of blood serum were transferred in an ice box until the lab is reached . The separated serum was kept in the refrigerator at a temperature of - 20 ºC, and then the required analysis was carried out on the preserved serum.

B. Prepration and Serum Digestion

About 2 ml of blood samples were centrifuged (6000 rpm/min.) for fifteen moments. to get the serum which was kept at -20 ˚C. The digestion of serum was done according to method described in Ji and Ren [17] with little modification where two ml of HNO_3 (70%) was added to 1 ml of $HCLO_4$ (70%) and 1 ml of serum in a pyrex tube was also added. After that heating of this mixture by water path on a hot plate at 160 °C at 1 H., then reducing its temperature and in the end completed to10 milliliter by 30% HCL, plastic (polypropylene) vessels acid-washed were used for saving and storing serum samples.

C. Apartuses

Flame Atomic Absorption Spectrophotometer (FAAS. - AA7000 shemadzu / Japan) was used to estimation of Pb, Cd, Cu and Se elements in the blood serum, and this was done according to the manufacturer's procedure. Hematological parameters were measured by Hematological analyzer device (Genex / USA).

D. Statistical Analysis

The data of this study were statistically analyzed by using the statistical package of social science version 26 SPSS, based on the use of an independent sample t test to compare mean ± SD between patients and control group at p. value < 0.05 and 0.01.

III. RESSULTS AND DISCUSSION

About 50 patients undergoing hemodialysis were used for the study, among the mentioned patients were 35 (70%) males and 15 (30%) were females. Their ages ranged from 17-76. High percentage of patients were observed in the age category 57-76 (48%) followed then 37-56 year (38%) and 17-36 year (14%), as shown in the table I.

TABLE I. NUMBERS OF SEX AND THE AGE GROUP OF PATIENTS

Sex	No. of patients	%
Male	35	70
Female	15	30
Total	50	100 %
Age / year	No. of patients	%
17 – 36	7	14
37 – 56	19	38
57 – 76	24	48

The results of the study table II observed that the Pb and Cd recorded high mean concentration (1.04 ± 0.38 and 0.065 ± 0.018) mg/L respectively in the patients' blood serum pre-HD with significant differences when it compared with control group, these results concert with other studies Statarug [18] and Pocsi *et. al.* [19]. Aggarwal *et. al.* [20]. which they demonstrate that the cadmium was observed higher concentration than the healthy adults. There are more than 4000 a chemical substance in the form of elements, molecules and gases found in the environment these are responsible for their toxic effects on the kidney

especially Cd and Pb, the effect of these elements increases with the incidence of chronic diseases and the duration of exposure. This is consistent with the results of Bernard *et. al.* [21] which showed that Cd directly affect the bone mineralization and leads to loss of calcium from the bone and increased renal excretion, as well as having a direct effect on renal tubular transport of calcium, also continuous exposure to Cd causes glomerular damage and leading to increases albumin secretion and a progressive decline in glomerular filtration rate (GFR), eventually causing the end-stage renal failure.

The cumulative of kidney damage can result from chronic Cd - exposure, also other diseases like proteinuria and deteriorated of renal function can result from this case Mendley *et. al.* [22]. The nephrotoxicity created from the accumulation of Cd in the renal cortex Egan *et. al.* [23] the concentration of Pb in the blood may be cause chronic renal illness Orr and Bridges [24], a correlated between blood lead level and the chronic renal dysfunction was showed by Tonelli *et. al.,* [25]. The results also observed that the mean concentration of Cu and Zn in patients undergo HD were lower as compared to control group this consistent with a study conducted by Orr and Bridges [24]. the results of these investigations showed a decrease in the mean concentration of Cu and Zn, this is due to the endogenous toxicities , impaired or cessation kidney function , dietary restriction and therapeutic measures (HD) , also this decrease in the mean concentration of the mentioned elements may be because of Zn and Se were made as cofactors of the antioxidant enzymes and they are continuously used to create the antioxidant enzymes which play important role in the detoxification of the ROS Tonelli *et. al.* [25] and Reena *et. al* . [26].

TABLE II. MEAN CONCENTRATION ± SD (MG/L) OF HES IN THE STUDY SAMPLES

HEs	Pre-HD No.. 50	Control No. 25	p. value
	Mean ± SD (mg/l)		
Cd	0.065 ± 0.018^{a}	0.050 ± 0.015^{c}	0.002^{**}
Cu	0.014 ± 0.006^{c}	0.108 ± 0.044^{a}	$< 0.001^{**}$
Zn	0.016 ± 0.008^{c}	0.037 ± 0.005^{a}	$< 0.001^{**}$
Pb	1.04 ± 0.38^{a}	0.71 ± 0.27^{b}	$< 0.001^{**}$

- The similar letters refer to insignificant differences between two comparison means, while different letters refer to a significant difference between two comparison means within the same column.
- The p. value marked with a star (*) refer to a significant difference at the 0.05 probability level, and the two stars (**) refer to a significant difference at the 0.01 probability level, while the values without a star refer to that there were insignificant differences, as in the other tables.

The results of the table III recorded a significant decrease in the mean complete blood count pre the HD compared to the control group. A low level of RBCs and Hb was observed in chronic kidney failure patients , this may be due to the majority of erythropoietin is synthesized in the juxta glomerular apparatus except 10% in liver and other organs , therefore the reducing in the creating of erythropoietin from the renal cause erythropoiesis inhibition Fishbane [27]. as well as, iron deficiency considered a cause of anemia, the iron is an important element for Hb generation and the shortage of iron produce from the reduction in the ferritin that protein which responsible for the iron storage , as well as produce from the decline iron absorption and malnutrition Kadhim *et. al* .[28].

The mean count of WBCs in this study was reduced with significant differences compared to control group, the reducing in WBCs in patients with CKD undergoing to HD is because of the complement activation which affected neutrophil aggregation and adherence to endothelial surface. Many studies are consistent with the results of present such as Singh and Bhatta [29].

The mean count of platelets was decreased due to two reasons, first : increase in the breakdown of platelets , which might be drug induced as in many clinical settings the anticoagulant medication (Heparin) used for HD which creates immune tolerance resulting in production of antibodies which causes damage of platelets , second : impaired erythropoietin secretion leads to a decrease in platelet count because of platelets levels can affected by erythropoietin levels , also the high similarity between erythropoietin and thrombopoietin , erythropoietin act as the main humoral regulator and play important role in platelet mass Habib [30].

Exposure to some chemicals confuses the hematological structure by reducing the number of RBCs Wahab [31]. The results of table III recorded decrease in the mean count of (WBC, RBC, Hb and PLT) in patients, which may be due to the toxic effects resulting from the occupational and environmental exposure Kim *et al.,* [32] which occurs through the emission of industrial factories, smoking, also through food and water contaminated Langman and Kapur [33]. The half-life period of lead is about 27–36 day in blood, 30–40 day in tissue and in the bone nearly about 104 days, the half-life period of Cd ranged from 15–30 year, also liver is considered the storage organ of the mentioned element Bernard [34]. The bind between Pb and iron (Fe), will interferes with many cells function based on Fe, like Hb formation, which create hypochromic anemia. High concentrations of lead in the blood lead to impaired cognitive development and anemia Sharifi *et al.,* [35]. The existence of Pb in the blood circulation reflects the balance between uptake and accumulation of it in the tissues Sharifi *et al.,* [35]. The main effect on the hematopoietic process is known oxidative stress Matović *et. al.,* [36]. There was a significant correlation (negative) between RBCs and Hb, when Pb and Cd levels were below 10 g/dL. Firouzkouhi *et al.* [37]. Some HEs such as Cd and Pb can inhibit the formation of Hb or affected erythrocytes directly Lee *et al.,* [38].

TABLE III. CBC MEAN ± S. D IN THE STUDY SAMPLES

HEs	Pre-HD No.50	Control No. 25	p. value
	Mean ± SD (mg/l)		
WBC$*10^{3}$/µL	2.78 ± 0.79^{b}	6.55 ± 1.84^{a}	$< 0.001^{**}$
RBC $*10^{6}$/µL	3.06 ± 0.59^{b}	4.61 ± 0.47^{a}	$< 0.001^{**}$
Hb g/dl	8.54 ± 1.43^{b}	13.1 ± 1.26^{a}	$< 0.001^{**}$
PLT $*10^{3}$/µL	164.4 ± 51.8^{b}	256.4 ± 68.8^{a}	$< 0.001^{**}$

IV. CONCLUSION

In conclusion, the present study showed that the percentage of CKD patients in males was more than in the females, also incidence rate with CKD was in the advanced age (57-76) years. The levels of the toxic elements Pb and Cd were higher than the levels of vital elements Zn and Cu in the serum of the patients' blood is due to inefficient kidney function and patients' exposure to the studied elements. Reducing the levels of hematological parameters in the patients basically refers to the incidence of CKD and with less degree of the HEs exposure.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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