



# Correlation between blood lead level and anemia in hemodialysis patients

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## ABSTRACT

**Introduction:** Studies show that blood lead level (BLL) is higher in hemodialysis patients than in healthy people. Lead can disrupt iron metabolism and hemoglobin synthesis and therefore it is regarded as a cause of anemia.**Objectives:** This study investigated the relationship between BLL and anemia in end-stage renal disease (ESRD) undergoing maintenance hemodialysis**Patients and Methods:** This case-control study was conducted on 70 patients who had received hemodialysis at least for three months. The participants were selected randomly among eligible patients considering the inclusion and exclusion criteria. Based on their hemoglobin levels, the participants were divided into two groups, namely patients with anemia (case) and those without anemia (control).**Results:** The mean age of the participants in the case and control groups were 63.8±11.02 and 55.4±13.34 years, respectively. There were significant differences between two groups regarding hemoglobin, C-reactive protein (CRP) and erythropoietin (Epo) levels ( $P < 0.05$ ). There was a significant correlation between hemoglobin and ferritin levels in the case group and between the length of dialysis and serum iron level in the control group without anemia ( $P < 0.001$ ).**Conclusion:** Our results showed no correlation between BLL and anemia, however the BLL was higher in those undergoing long-term maintenance hemodialysis. Our findings require further investigation with larger studies.

### Implication for health policy/practice/research/medical education:

To investigate the relationship between blood lead level and anemia in end-stage renal disease undergoing maintenance hemodialysis, we conducted a case-control study on 70 patients. No correlation between lead level and anemia was detected, however the blood lead level was higher in those undergoing long-term maintenance hemodialysis.

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## Introduction

End-stage renal disease (ESRD) is the most debilitating condition and results in an increased risk of morbidity and mortality due to cardiovascular events and dialysis associated risk factors, such as oxidative stress, inflammation, anemia and infection (1). Hemodialysis is the most common treatment for ESRD and anemia is a common complication among these patients. Various causes have been reported for anemia among these patients, the main of them is failure of kidneys to synthesize erythropoietin (EPO). EPO, as a hormone with a glycoprotein structure, is mainly synthesized by the

kidneys and slightly produced by liver tissues in adults and causes red blood cell production by stimulating the bone marrow (2). Hemodialysis has a devastating effect on red blood cells and reduces their lifespan too (3). Sometimes iron deficiency can cause anemia even in the presence of sufficient amounts of EPO. Disturbance in iron metabolism is due to inflammatory mediators in renal failure, hemodialysis process, and an increase in oxidative stress (4). On the other hand, kidneys have a significant role in regulating the concentration of trace elements. Renal failure can disturb the levels of the elements like lead, cadmium, mercury and arsenic. Studies have shown

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that blood lead level (BLL) is higher in hemodialysis patients than healthy people (5).

It seems that some secondary reactions related to the poorly dialysis water purification are not respected appropriately, because many of these reactions are regarded as the symptoms of mineral and bone disorders induced by chronic kidney diseases or chronic inflammation unless the patients show acute or sub-acute reactions. The maximum admissible levels of water contaminants in dialysis water have been quantified by the Association for the Advancement of Medical Instrumentation (AAMI). The acceptable lead level in dialysis water is 0.005 mg/L (6).

Lead can disrupt iron metabolism and hemoglobin synthesis. Therefore, lead poisoning is one of the causes of anemia (7).

Hemodialysis patients are exposed to a large amount of water (more than 300 L/wk). As a result, the presence of small amounts of toxic materials in hemodialysis water can cause a gradient between blood and dialysis fluid which eventually results in toxicity-related symptoms. The deficiency of basic elements such as zinc or selenium, and an increase in toxic elements, including lead and arsenic, are hazardous complications that can be fatal for hemodialysis patients (8-10).

Zinc and iron deficiency are the risk factors for lead poisoning (11). Epidemiological studies have shown that long-term exposure to lead is associated with an increased risk of cardiovascular events, cancer and mortality. Moreover, increased BLL is associated with an increase in age-related hypertension and kidney diseases. The harmful effects of this metal have been shown in different studies (12,13). In ESRD patients, the most important factors in determining the concentrations of the trace elements, including lead, are the severity of renal failure and the selected treatment as the renal replacement therapy. Lead poisoning disrupts the functions of many organs which results in several complications such as encephalopathy, anemia, peripheral neuropathy and gout. Studies have shown that increased BLL has significantly and positively correlated with the duration of hemodialysis therapy. Concomitant high BLL and anemia exacerbate the symptoms and finally aggravates disabilities and even causes death of patients. This study designed to evaluate BLL as an effective factor in causing refractory and resistant anemia to EPO and iron replacement therapy.

## Objectives

This study aimed to determine the association of BLL with anemia in patients on maintenance dialysis at a hemodialysis centre in Hamadan, Iran.

## Patients and Methods

### Study design

This case-control study was conducted on 70 hemodialysis patients, who had received hemodialysis for at least three

months during 2017-2018. Information about the study was given to the participants since those who agreed and signed the consent forms enrolled in the study.

The patients were divided into two groups, namely those with anemia (the case group) and those without anemia (the control group) based on their hemoglobin level (a participant with a hemoglobin level of less than 11.5 mg/dL was diagnosed with anemia) (14). The estimated sample size in each group was 35 according to the study by Palaneeswari et al (15).

### Participants

All study patients were recruited from Shahid Beheshti hemodialysis centre in Hamadan. Only patients over 18 years of age who received maintenance hemodialysis for at least three months were enrolled in this study. The exclusion criteria were anemia induced by other underlying reasons such as gastrointestinal bleeding, malignancy, thalassemia and surgery in the last three years, drug abuse (e.g. opium consumption) and infections. Most of patients underwent standard thrice-weekly (at least three hours per session) hemodialysis. The dialysate contained standard ionic components with bicarbonate-based buffer and type of dialyzer was polysulfone membrane. Blood flow and dialysate flow were 230–300 and 500 mL/min, respectively. Dialysis efficacy was measured using serum urea clearance (Kt/V; where k: dialyzer urea clearance; t: total treatment time and V: the total volume within the body that urea is distributed) since the optimal adequacy of dialysis was equal or more than 1.2.

### Laboratory assessments

Prior to hemodialysis, their blood samples were taken and the BLL level was measured using the atomic absorption technique (SpectrAA-200, Variant). The hemoglobin levels of all participants were also measured. The serum iron and ferritin levels were measured in both groups using BioSystem (Spain) and ELISA (Monobind, Germany) kits, respectively. The weekly doses of EPO received by the patients were recorded.

The data collection instrument was a checklist comprising of demographic information, number of hemodialysis sessions, duration of hemodialysis session (hour), hemoglobin level (g/dL), C-reactive protein (CRP) level (mg/L), serum iron level (mcg/dL), ferritin level (ng/mL), lead level (mg/dL), total iron-binding capacity (TIBC) (mcg/dL) and iron saturation percentage and EPO dose (U/kg).

### Ethical issues

The study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee of Hamadan University of Medical Sciences approved this study (#IR.UMSHA.REC.1396.593). Accordingly, written informed consent was taken from all participants before

inclusion in the research. This study was granted by the Hamadan University of Medical Sciences (Grant# 9609145659).

### Statistical analysis

Data were analyzed using the SPSS software (version 21, SPSS Inc., Chicago, IL, USA). Comparison among quantitative variables was analyzed exploiting t-test and Mann–Whitney U-test. Qualitative data was assessed by chi-square test. A *P* value of <0.05 was set significant.

### Results

In this study, 70 patients (35 with anemia and 35 without anemia) undergoing hemodialysis were studied. The mean age of all participants was  $59.64 \pm 12.88$  years. There was a significant difference in the mean age between the case (63.8 years) and control (55.4 years) groups ( $P=0.05$ ). Male participants constituted 48.6% and 54.3% of the case and control groups. In addition, 88.6% and 77.1% of the case and control groups lived in urban area, respectively. Around 74.3% of the case participants and 74.3% of the control participants underwent four hours of hemodialysis and three times per week respectively. There were not any significant “between-group” differences with respect to gender, place of residence, educational attainment, number of dialysis sessions, and duration of dialysis per week ( $P>0.05$ ; Table 1).

We found a significant difference between two groups regarding levels of hemoglobin ( $P=0.001$ ), CRP and EPO

dose ( $P<0.05$ ); whereas, there was not any significant difference between two groups in relation with the serum iron, lead, ferritin and saturated iron and TIBC (Table 2).

The relationships between blood level of lead, iron, ferritin, hemoglobin, and EPO dose and the duration of the dialysis session was investigated. Results showed a significant correlation between the duration of hemodialysis and serum iron ( $P=0.001$ ) and lead levels ( $P<0.05$ ; Table 3).

According to Table 4, a significant correlation between the hemoglobin and ferritin levels in the case group was seen ( $P<0.001$ ). A significant relationship was also observed between duration of the dialysis and serum iron levels in the control group ( $P<0.001$ ; Table 5).

### Discussion

Hemodialysis can be associated with accumulation of toxic elements and profound clinical consequences, such as the increased risk of cardiovascular diseases, immunodeficiency, anemia and bone disease. Anemia is a common complication in dialysis patients while many causes have been reported for its development (3). The lead toxicity affects many organs and causes encephalopathy, anemia, peripheral neuropathy, gout and renal failure. Studies have shown that exposure to lead in the environment is associated with progress of renal failure in patients with and without diabetes (13). This study was conducted to assess the lead level in case and control groups.

**Table 1.** Distribution frequency of the characteristics of the studied patients

Characteristics	With anemia (n=35)	Without anemia (n=35)	<i>P</i> value
Age(year)	$63.8 \pm 11.02$	$55.4 \pm 13.34$	0.005
Gender (%)			
Male	17 (48.6%)	19 (54.3%)	0.406
Female	18 (51.4%)	16 (45.7%)	
Urban area	31 (88.6%)	27 (77.1%)	0.205
Educational levels(high school or more)	10 (28.6%)	10 (28.7%)	0.092
Number of HD sessions (Three times and more)	26 (74.3%)	26(74.3%)	0.991
HD duration (year)	$4.62 \pm 4.00$	$4.58 \pm 4.46$	0.887

HD; hemodialysis.

**Table 2.** Comparison of the investigated variables in the case and control groups

Characteristics	With anemia (case) (n=35)	Without anemia (control) (n=35)	Total	<i>P</i> value
Hemoglobin (g/dL)	$10.09 \pm 0.86$	$12.29 \pm 1.04$	$11.19 \pm 1.45$	0.001
hs-CRP (mg/L)	$8.21 \pm 8.35$	$3.95 \pm 4.75$	$6.08 \pm 7.08$	0.006
Serum iron(mcg/dL)	$118.08 \pm 67.42$	$102.34 \pm 76.14$	$110.21 \pm 71.83$	0.179
Ferritin (ng/mL)	$105.51 \pm 84.70$	$142.02 \pm 176.94$	$123.76 \pm 138.92$	0.751
BLL ( $\mu$ g/dL)	$11.02 \pm 6.13$	$10.87 \pm 2.61$	$10.95 \pm 4.68$	0.401
TS (%)	$33.21 \pm 12.59$	$28.15 \pm 13.95$	$30.68 \pm 13.43$	0.111
EPO dose	$7657.1 \pm 3895.26$	$5791.4 \pm 6227.47$	$6724.28 \pm 5241.09$	0.033

BLL; Blood lead level, Erythropoietin (U/wk), TS; Transferrin saturation.

**Table 3.** Correlation between the investigated variables in hemodialysis patients

Variables	Lead level	Iron level	Ferritin level	Hb level	EPO dose	HD duration
Lead level	1					
Iron level	-0.080	1				
Ferritin level	-0.191	0.075	1			
Hb level	-0.086	-0.219	-0.107	1		
EPO dose	0.083	0.000	-0.149	-0.192	1	
HD duration	<b>0.235*</b>	<b>-0.316*</b>	-0.062	0.082	0.055	1

EPO, erythropoietin; HD, hemodialysis; Hb, hemoglobin. \* *P* value less than 0.05.

**Table 4.** Correlation between the investigated variables in the case group

Variables	Lead level	Iron level	Ferritin level	Hb level	EPO dose	HD duration
Lead level	1					
Iron level	-0.075	1				
Ferritin level	-0.171	-0.167	1			
Hb level	-0.286	-0.266	<b>-0.454*</b>	1		
EPO dose	0.048	-0.092	-0.148	-0.131	1	
HD duration	0.213	-0.088	-0.073	0.253	0.043	1

**Table 5.** Correlation between the investigated variables in the control group

Variables	Lead level	Iron level	Ferritin level	Hb level	EPO dose	HD duration
Lead level	1					
Iron level	-0.084	1				
Ferritin level	-0.231	0.043	1			
Hb level	0.236	0.058	-0.101	1		
EPO dose	0.252	0.075	-0.125	-0.11	1	
HD duration	0.278	<b>-0.554*</b>	-0.083	0.08	0.088	1

EPO; erythropoietin; HD; hemodialysis; Hb; hemoglobin. \* *P* value less than 0.05.

The results showed a significant between-group difference in hemoglobin level, CRP and EPO dose. However, no significant between-group difference was observed in serum iron, lead, ferritin, saturated iron levels and TIBC.

Mean lead level in the case and control groups was 11.02 µg/dL and 10.87 µg/dL respectively, indicating no significant “between-group” difference between them in this regard. However, the mean lead level in hemodialysis patients was higher than the international standard level. Previous studies showed a higher lead level in hemodialysis patients than healthy people (15).

Filler et al reported that the BLL in the hemodialysis group was higher than in normal people (21.1+15.8 µg/L versus 6.35 µg/L) (11). The highest lead level in their study (58 µg/L) was higher than that in the present study (41.6 µg/L) (11). Since the maximum concentration of lead for hemodialysis water based on AAMI must to be 0.005 mg/L, the high serum lead level in the patients can be due to renal failure, reduced kidney function, the inability to get rid of lead from the body and lack of lead removal during dialysis (6).

Recently, the inflammatory processes have been reported

as the main cause of EPO-resistant anemia in hemodialysis patients. The serum CRP levels in hemodialysis patients are 1-5 times higher than in the healthy control (16). CRP is an accurate, reliable, and effective biomarker for early diagnosis of patients vulnerable to cardiac events. It has also been approved as an independent and exact predictor of mortality among hemodialysis patients (17). Kus et al showed that the hs-CRP levels in hemodialysis groups with a hemoglobin level of higher than 12 g/dL were lower than in the group with hemoglobin level lower than 12 g/dL, which is consistent with the present study (18). The between-group comparison showed a higher level of hs-CRP in the case group too. This relationship may suggest that chronic inflammation is the main cause of anemia in hemodialysis patients.

The between-group comparison showed no significant difference in serum iron level, indicating the role of other effective mechanisms in the development of anemia. There were not any significant relationships between the serum iron and lead levels in the case and control groups.

There was also no significant correlation between the ferritin and lead levels in the case and control groups. Ogawa et al showed that the serum ferritin level and iron

saturation percentage in hemodialysis patients to respond to the EPO was lower than the expected level. The ferritin level (<90 ng/mL) and iron saturation level (>20%) are adequate for responding to treatment with EPO (19).

There was a significant relationship between the hemodialysis duration and serum iron level in hemodialysis patients, while, the serum iron level decreased with the prolongation of the hemodialysis sessions. In the present study, the serum lead level increased with prolongation of the hemodialysis sessions. Palaneeswari et al studied 100 hemodialysis patients to measure the serum lead level and its relationship with hemodialysis duration. They found that serum lead level slightly increased with the increases in the duration of hemodialysis session (15).

Huang et al studied 931 patients receiving hemodialysis for at least six months and showed that serum lead level was high in 7% of the patients receiving EPO treatment, whereas, the serum leads level was high in 22% of patients who did not receive EPO treatment (20). In our study, no significant relationship between BLL and EPO dose was detected, which may be attributed to the smaller sample size. In previous studies, long-term lead exposure was associated with production of reactive oxygen species (ROS), decreased nitric oxide availability, inflammation, cell expressing angiotensin II, increased lipopolysaccharide-induced tumor necrosis factor- $\alpha$  factor levels and liver damage. Overproduction of ROS in patients with ESRD has an important role in the development of inflammation that induced long-term complications, including anemia, accelerated atherosclerosis, nutritional disorders in long-term hemodialysis patients (21). Exposure to lead in the environment is an important factor to increase the mortality rate among the general population and in hemodialysis patients. Increased serum lead level in dialysis patients is due to kidney dysfunction and also inadequate lead removal during dialysis. The exposure to lead, even at lower levels, is associated with increased serum lead level and related complications in dialysis patients.

## Conclusion

Although there was not any correlation between the serum lead level and anemia in the present research, it was shown that the serum lead level is higher in those undergoing long-term hemodialysis. In this study, the lead level of inlet water to hemodialysis machines did not measure, and many patients had BLL higher than normal.

Consequently, it is recommended to measure trace elements level periodically and make the required modification based on international standards. It seems that reducing exposure to lead, correcting conditions leading to iron deficiency, finding effective treatments to reduce inflammation, increased anti-oxidative activity, and utilization of chelator therapy can be the most effective therapeutic method for treating anemia in hemodialysis

patients. Further improvement of dialysis-water purity should be conducted to modify the inflammatory status and reduce activation of proinflammatory cytokines with beneficial effect on anemia.

## Limitations of the study

The research limitations were the small sample size and the selection of participants from only one dialysis section. The other limitation of the current study is the lack of measurement of lead levels in water of outlets of reverse osmosis system and inlets of dialysate.

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## Authors' contribution

VS, FAJ and HA contributed to study design, statistical analyses and critical review of the manuscript. VS have been involved in study design, statistical analyses and critical review of the manuscript. PM contributed to data collection, patient sampling and manuscript drafting. Biochemical measurements were performed by FAJ. All authors have read and approved the content of the manuscript and confirmed the accuracy of any part of the work.

## Conflicts of interest

The authors report no conflicts of interest in this study.

## Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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