

Lead Levels in Fingernails of Hemodialysis Patients and Healthy Individuals in Karbala, Iraq: A biomonitoring Study Using ICP OES

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Abstract

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Keywords

Fingernail; Hemodialysis; ICP OES; Biomonitoring; Lead

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Cover Page Footnote

Funding This work was financially supported by the Ministry of Higher Education and Scientific Research and Kerbala University (Official letter No. A/6/336 in 26 January 2022) **Ethics information** This study received ethical approval from the University of Kerbala's Ethics Committee (Official Letter No: 363/6, dated January 26, 2022). Prior to signing the consent form, all participants were fully briefed on the study procedures. Additionally, each subject completed study questionnaires, providing personal details as well as information regarding their health, diet, smoking habits, and lifestyle at the time of sample collection. **Conflicts of interest** There are no conflicts of interest associated with this work. **Authorships contribution statement** Rusul Jaafar: Conceptualization, Methodology, Software, Rana Hameed: Data curation, Writing - original draft, Baker A. Joda: Supervision, Visualization, Validation, Writing - review & editing. **Acknowledgments** We gratefully acknowledge the assistantship from the University of Kerbala, and the Ministry of Science and Technology/ Agricultural Research Department.

RESEARCH PAPER

Lead Levels in Fingernails of Hemodialysis Patients and Healthy Individuals in Karbala, Iraq: A Biomonitoring Study Using ICP OES

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Abstract

The use of non-invasive media to evaluate the association between trace elements and diseases has spread in recent centuries. Fingernail samples were collected from 103 individuals, including both healthy participants and those undergoing hemodialysis, residing in Karbala, Iraq, and ranging in age from 21 to 75 years. The concentrations of lead were determined using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES). Statistical analyses using F-tests and two-tailed t-tests were conducted to examine the effects of health status, gender, smoking status, and their interactions on lead levels in washed fingernails. The concentrations of Pb in washed fingernails of healthy individuals ($11.245 \pm 3.530 \mu\text{g/g}$) and females ($10.519 \pm 3.626 \mu\text{g/g}$) exhibited significantly elevated levels relative to those in hemodialysis patients ($7.769 \pm 1.589 \mu\text{g/g}$) and males ($8.747 \pm 2.563 \mu\text{g/g}$) at ($P \leq 0.05$), respectively. Conversely, the difference between the concentrations of Pb in smokers ($9.059 \pm 2.483 \mu\text{g/g}$) and non-smokers ($9.652 \pm 3.367 \mu\text{g/g}$) was insignificant at $P \leq 0.05$. In summarize, the levels of lead (Pb) in fingernails of 103 individuals in Karbala, Iraq was measured and found significantly higher levels in healthy participants than hemodialysis patients, and in females compared to males, while smoking showed no significant effect.

Keywords: Fingernail, Hemodialysis, ICP OES, Biomonitoring, Lead

1. Introduction

The concentrations of trace elements are homeostatically regulated under normal conditions of healthy individuals [1,2]. In contrast, these elements can play an important role in the onset of diseases depending upon their concentration and metabolisms inside the human body [3,4]. The previous biomonitoring studies offered a comprehensive analysis for trace elements in tissue and fluid samples in many countries or regions, for example, Iraq [5,6], Germany [7–9], Slovenia

[10,11], Belgium [12], France [13,14], Bangladesh [15], the United Kingdom [16], the Czech Republic [17], and Canada [18,19]. In Iraq, the environmental contaminants consist of gaseous emissions, airborne particulate matter, and trace elements such as lead (Pb) [20]. Examples of these pollutants include transportation, industry, oil industries, fossil fuels combustion, domestic and public generators in addition to energy production, heating, brick and cement industry, agriculture fires and dust storms [21]. Long-term exposure to some trace elements has been linked to the beginning of

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various illnesses and disorders, for instance, asthma, diabetes, hypertension, cancer, kidney problems, heart disease, anemia, infertility, and possible birth defects [22–25]. It is well established that lead can enter the human body through multiple pathways, including the respiratory tract (via air and dust), the digestive system (through water, food, and medications), and potentially through skin absorption [26–28], as well as by using paints of toys, playgrounds, goods, and walls [29,30]. Fig. 1 indicates that different biological matrices can be used to assess biomarkers of lead exposure [31].

The use of unconventional biological samples (tissues and fluids) as biomarkers to monitor the concentrations of trace elements has been investigated [32–37]. Most of these studies have been established for various trace elements in biological samples such as fluids (blood, saliva, teardrops, and urine) and tissues (hair and fingernails). In previous years, human scalp hair and fingernail materials were used as useful tools for assessing environmental exposure to numerous contaminants. These media can also be utilized to identify the health state of humans regarding the concentrations of elements [38–40]. Fingernail tissue offers several advantages over blood involving being non-invasive and easy to collect. It may also reflect long-term biological changes, as various trace elements can accumulate in fingernails over a period ranging

from 2 to 18 months [41,42]. It was found that these advantages help to determine the health situation of individuals for a long time due to the materials of nails remaining separated from any metabolic activities inside the body [43,44]. Hence, these materials are known to be used as useful markers to estimate changes in the human body [45,46]. Exposure to high concentrations of Pb can cause several illnesses or health problems, namely hypertension, impaired renal function, fertility, and adverse pregnancy outcomes [47–49]. Hemodialysis is one of the most common methods for the treatment of individuals with renal disease at the end stage. Moreover, it is commonly referred to as the most prevalent form of cardiovascular disease. The main risk that can increase hemodialysis disease is a low nutrition intake which is related to anorexia and dietary restrictions [50]. The deficiencies or excesses of elements can significantly influence the onset of various diseases as discussed in the literature by several researchers [51,52,33,53–56]. There are a wide range of analytical techniques that have been employed for trace element analysis, including atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS). The principal advantages of this method include a wide dynamic range, an autosampler, a

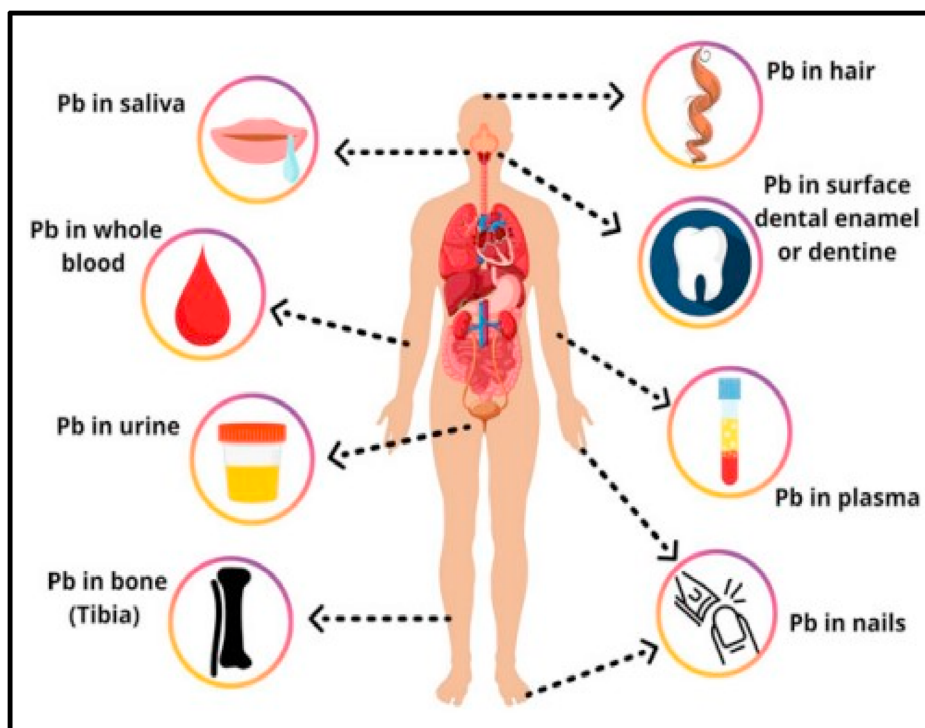


Fig. 1. Biomarkers of lead exposure.

high-throughput sample introduction system, the ability to handle samples containing up to 1% dissolved solids, good detection limits, and the capability to detect most elements of the periodic table [57]. The article aims to investigate the associations between fingernail lead concentrations and health status in patients undergoing hemodialysis.

2. Materials and methods

2.1. Sample collection and preparation

The samples of fingernail ($n = 103$) were collected from all 10 fingers, then cut, and washed using the International Atomic Energy Agency (IAEA) protocol. This method was selected based on the validation data; Fingernail samples were covered with acetone for each tube. The samples were sonicated for 10 min at 35 MHz, room temperature and subsequently centrifuged at 1000 rpm for 5 min. This washing procedure was repeated three times using deionized distilled water (DDW), followed by a final rinse with acetone [58,59]. The washed samples were then dried overnight in an oven at 60 °C and 0.500 ± 0.001 g of sample was digested using Kjeldahl™ tubes using 1 mL of concentrated nitric acid (Aristar® 65%) [60]. The digestion tubes were then placed on a digestion block and heated at 165 ± 10 °C until complete digestion of the fingernail samples was achieved (approximately 30 min). The digested samples were diluted with deionized distilled water (DDW) in polyethylene volumetric flasks to obtain a 100-fold dilution. The resulting solutions were centrifuged at 3000 rpm for 10 min (MSE Mistral 2000, Thermo Life Sciences) and filtered through 0.45 µm Millex membrane filters (MF-Millipore; Millipore, Carrigtwohill, Co. Cork, Ireland). The digested fingernail solutions were transferred to labeled 50 mL Sterilin® centrifuge tubes and stored at 4 °C prior to ICP-OES analysis. In addition to the fingernail samples, reagent blanks, pooled samples, and a certified reference material for human hair (GBW09101; National Research Centre for Certified Reference Materials, China) were processed in parallel using the same wet digestion procedure to assess method precision and accuracy. Table 1 shows the types of study samples.

2.2. Instrumentation

The Shimadzu ICPE- 9000 (Axial view) was used to determine the concentrations of Pb in fingernail samples. The typical operation parameters, namely, RF power (1.2 KW), plasma gas flow (10 L/min), auxiliary gas flow (0.6 L/min), and nebulizer gas flow (0.6 L/min), were used for this instrument.

2.3. Internal standard

An internal standard was used to correct for matrix effects and instrumental drift. Yttrium (Y) (0.5 mg L⁻¹) was added to all samples, blanks and calibration standards and the Pb signal was normalized to the Y signal (ratio of analyte intensity to internal-standard intensity) before applying the calibration curve. The ratio of intensity of Pb/Intensity of Y was determined; this normalized signal was used to build the calibration curve and to calculate Pb concentrations in samples.

2.4. Precision and accuracy

The range of relative standard deviation (%RSD) was utilized to calculate the value of precision, while the range of percentage recovery (%R) was used to determine the value of accuracy. Method precision and potential matrix effects were evaluated by repeated analysis ($n = 10$) of a pooled sample prepared from at least six fingernail samples. Certified reference materials (CRMs) were included in each analytical batch to assess method validity and accuracy. Standard Reference Material GBW 09101 was selected based on its matrix similarity and certified elemental composition. Method accuracy was expressed as mean percentage recovery (%R), standard deviation (SD), relative standard deviation (%RSD), and percentage recovery values are summarized in Table 2. Good levels of precision (4.585% RSD) and accuracy (94.866 %R) were found (Table 2). The detection limit (DL) was found to be (3.581 µg/L) for lead ($\lambda = 220.353$ nm). The instrument limits of detection (LODs) were calculated from at least ten replicate measurements ($n = 10$) performed at concentrations close to the blank level using the following equation ($LOD = X_{bl} + K S_{bl}$, where X_{bl} is the mean blank

Table 1. Study fingernail samples ($n = 103$) collected from Karbala/Iraq.

Factors	Health status		Gender		Smoking activity	
	Healthy	Hemodialysis	Males	Females	Smokers	Non-smokers
Sub-groups	59	44	57	46	24	79
Number						

Table 2. The values of precision, accuracy, and limit of detection for lead in fingernail samples.

Precision Mean \pm SD (%RSD)	Accuracy ($\mu\text{g/g}$)			DL ($\mu\text{g/L}$)
	Measured value Mean \pm SD	Certified value Mean \pm SD	Percentage recovery (%R)	
9.302 \pm 0.426 (4.585)	6.830 \pm 0.111	7.2	94.866	3.581

signal, S_{bl} is the standard deviation (SD) of the blank measurements, and K is a numerical factor selected according to the required confidence level (typically 3) [57]. Furthermore, instrument stability of the ICP-OES was verified using a quality control chart.

2.5. Statistical analysis

Descriptive statistical analyses, including the arithmetic mean, standard deviation (SD), range, and 95% confidence interval, were performed on the concentration data obtained from washed fingernail samples. Statistical significance was assessed using F-tests and two-tailed t-tests to evaluate differences in lead levels between healthy individuals and patients, smokers and non-smokers, and males and females.

3. Results and Discussion

The concentration of lead in fingernail samples varied from one country to another due to several factors, namely nutrition; geographical differences; and the environment [61]. Hence, it is not possible to determine the reference range for lead in human fingernails. Therefore, the lead concentrations ($\mu\text{g/g}$) in washed fingernails were compared with previous studies reported in the literature [61]. Generally, these findings agree with several studies in literature [62] and disagree with other studies (Table 3) [63,64]. Based on a comparison of the range in this study with those available in the literature, it is possible to recommend that factors like age, sex, smoking habits, lifestyle, environmental exposure may affect lead concentrations in fingernails [65].

3.1. The effect of hemodialysis—link to human health

The correlation between health status and lead concentrations has been investigated by using significant tests (F-test and a two-tailed t-test), as shown in Table 4. A significant difference was found between hemodialysis and healthy individuals at $P \leq 0.05$. The main reason is that hemodialysis may play a role in these differences in lead concentrations through the influence of lead

Table 3. The values of mean, standard deviation (Sd), range, median and 95% confidence interval of lead ($\mu\text{g/g}$) for washed fingernails ($n = 103$).

Variable	Concentration ($\mu\text{g/g}$, dry weight)	Literature range ($\mu\text{g/g}$)
Mean \pm Sd	9.761 \pm 3.337	5.50 – 77.5, [62]
Range	4.190 – 16.822	0.27 - 4.75, [63]
Media	9.050	0.10 – 0.83, [64]
95% confidence interval	(9.116-10.405)	

metabolism in the human body. It was observed that the concentrations of Pb are found to be significantly higher in healthy individuals (mean \pm SD: 11.245 \pm 3.530 $\mu\text{g/g}$ of Pb) when compared with hemodialysis patients (mean \pm SD: 7.769 \pm 1.589 $\mu\text{g/g}$ of Pb) at P value of <0.001 . The patients will generally have a reduced exposure concentration because they are probably spending most of their time indoors. The day-to-day activities of healthy and sick people are significantly different. Therefore, sick people are less exposed to potential sources of Pb [66,67]. In addition, the higher levels of lead found in healthy individuals compared to hemodialysis patients are primarily due to the active removal of lead from the blood during dialysis sessions. While healthy kidneys lead slowly and continuously, maintaining a steady state [66]. The patients are on highly restricted diets to control potassium, phosphorus, and fluid intake. This often leads to; (1) reduced intake of lead-containing foods: They may consume less processed foods, certain types of seafood, or foods grown in contaminated soil, which are common sources of lead exposure for the general population, and (2) medical supervision: Their diet is frequently monitored by renal dietitians, potentially leading to overall healthier and less contaminated food choices [68].

3.2. The effect of gender

The gender effect on the concentrations of lead was determined in washed fingernails for male and female samples. F-tests and a two-tailed t-test were employed to assess whether significant differences existed in lead concentrations between males and females at $P \leq 0.05$. The results confirm the role of gender in these significant differences ($t_{(101)} = 3.442$,

Table 4. Lead results of fingernail samples for various groups (healthy and patients), (males and females), and (smokers and non-smokers).

Factor	Concentration ($\mu\text{g/g}$) Mean \pm Standard deviation (range)		Levene's Test for Equality of Variances F-test			t-test for Equality of Means Two-tailed t-test		
			Variance	F_{calc}	Sig.	t_{calc}	df	P
Healthy status	Healthy	Hemodialysis						
	11.245 \pm 3.530 (4.600-16.822)	7.769 \pm 1.589 (4.190-11.600)	EVA	29.091	<0.001	6.083	101	< 0.001
Gender	Males	Females						
	8.747 \pm 2.563 (4.190-16.568)	10.519 \pm 3.626 (4.910-16.652)	EVA	14.958	<0.001	3.442	101	< 0.001
Smoking activity	Smokers	Non-Smokers						
	9.059 \pm 2.483 (4.910-15.800)	9.652 \pm 3.367 (4.190-16.652)	EVA	6.362	0.013	-3.847	101	1.178

EVA (equal variances assumed); df (degrees of freedom); F_{calc} and t_{calc} (calculated values); P (probability), $t_{\text{crit}} = 1.98$ at $P = 0.05$.

$t_{\text{critical}} = 1.98$, $P < 0.001$) for the lead concentrations in fingernails of females (10.519 \pm 3.626 $\mu\text{g/g}$) when compared to males (8.747 \pm 2.563 $\mu\text{g/g}$), as reported in Table 4. These results agree with other findings determined in other studies using scalp hair as a biomarker [69]. The levels of lead in fingernails are a biomarker of longer-term exposure (approximately 3-12 months, reflecting the time it takes for the nail to grow out) [70]. The reasons for gender disparity involve both external behavioral factors and internal biological factors [71]. In the case of external factors, it was found that female may have more frequent contact with lead-containing sources such as (1) cosmetics, which include traditional eyeliners, skin lightening creams, nail polish, and certain hair dyes can contain high levels of lead as well as the direct handling during application can contaminate fingernails [72]; and (2) occupational exposure such as females may have more frequent contact with lead-contaminated house dust arising from lead-based paint, soil tracked indoors, or occupational take-home exposure from a partner [73]. Furthermore, the biological and physiological factors can also play significant role in increasing the levels of lead in females due to the nail matrix, where the nail forms, is vascularized. Trace elements circulating in the blood are incorporated into the growing nail plate. Therefore, factors that lead to higher internal body burden will be reflected in the nail. For example, (1) pre-menopausal women have a much higher prevalence of iron deficiency. This upregulates the divalent metal transporter in the gut, significantly increasing the absorption of dietary lead, leading to a higher overall body burden and more lead available for incorporation into keratinous tissues like nails and hair [74]; (2) bone lead mobilization, where lead is stored in bone. During periods of high bone turnover such as pregnancy, lactation, and especially menopause this stored lead is released back into the bloodstream. This internal release provides a source of lead for incorporation into growing nails

throughout these life stages, meaning a menopausal female's nail lead level reflects both recent exposure and exposure from decades ago; and (3) differences in nail biology and growth, where the rate of nail growth can influence the concentration of elements. If there are hormonal influences on nail growth, for example slower growth in post-menopausal females, it could theoretically lead to a higher concentration of deposited lead per milligram of nail, though this is a less studied and more hypothetical mechanism. Similar results have been reported for lead levels in serum samples, as shown in Table 5.

3.3. The effect of smoking activity

Smoking is known to have a significant role regarding the environmental risk factors which are associated with many diseases, such as heart diseases, kidney, respiratory diseases, and cancers [75]. In general, tobacco materials can provide hazardous amounts of toxic elements to the various organs throughout blood [76,77]. The effect of smoking activity was measured by using statistical tests such as F-test and a two-tailed t-test, as presented in Table 4. The results reveal no notable variation in Pb levels in the fingernails of smokers (3.239 \pm 0.711 $\mu\text{g/g}$ of Pb) compared to non-smokers (9.652 \pm 3.367 $\mu\text{g/g}$ of Pb) ($t_{(101)} = -3.847$, $t_{\text{critical}} = 1.99$, $P < 1.178$). Previous studies have found similar results [78] while the higher concentrations of Pb in smoker's fingernails than those in non-smokers has been reported by another study [79]. The lack of a significant difference is not evidence that smoking doesn't expose people to lead. This may be because of much stronger environmental and dietary signals and persistent issues with external contamination. Therefore, the amount inhaled and absorbed from smoking may be small compared to the total body burden acquired from lifelong dietary and environmental intake [80]. In addition, the levels of lead in fingernails are a

Table 5. Serum elemental levels found in the groups of males and females, (n = 109).

Element (n ₁ , n ₂)	Concentration (µg/mL) Mean ± Sd (range)		F-test			Two-tailed t-test		
	Male	Female	Variance	F	Sig.	t	df	Sig.
Pb (58,51)	3.076 ± 0.755 (1.88-4.932)	3.535 ± 0.751 (1.660-4.898)	Equal variances assumed	1.268	0.263	3.179	107	0.002
			Equal variances not assumed			3.180	105	0.002

complex mixture of lead from all sources over the past year, including a large historical burden stored in bones that is slowly released. The additional contribution from smoking in the last few months may be too small to significantly alter this long-term integrated measure [81]. Moreover, the boiling points of trace elements can significantly influence their concentrations in cigarette smoke, thereby affecting their impact on smoker health [82]. Consequently, lead concentrations in cigarette smoke may be lower than those detected in cigarette filters and ash [74,83]. Similar results have been reported for lead levels in serum samples, as shown in Table 6.

3.4. Interaction effects

3.4.1. Interaction between health status and gender

In statistics, an interaction effect occurs when the effect of one independent variable (e.g., gender) on the dependent variable (Pb concentration) depends on the level of another independent variable (e.g., health status). Fig. 2 shows the interaction effect among (healthy/hemodialysis) and (males/females) in order to investigate whether the difference between hemodialysis and healthy is the same for males and females. It was clarified that the Pb concentrations are increased from males to females in both healthy individuals and hemodialysis patients. However, this increase is similar in the low and high concentrations of lead for healthy individuals and patients, therefore, there is no interaction between the two factors due to the lines are approximately parallel [84].

3.4.2. Interaction between smoking activity and health status

Fig. 3 shows the interaction effect among (healthy/hemodialysis) and (smokers/non-smokers)

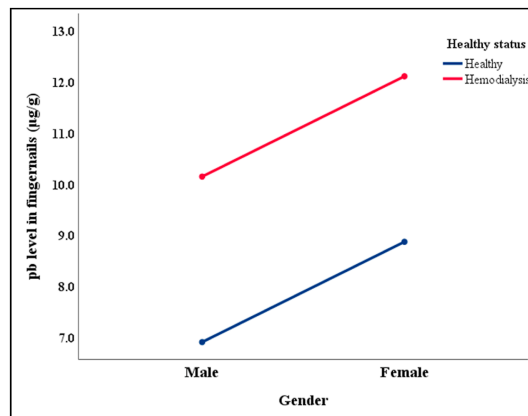


Fig. 2. The interaction effect of both factors (health status and gender) in terms of Pb concentrations in fingernail samples (n = 103).

in order to investigate whether the difference between hemodialysis and health is the same for non-smokers and smokers in terms of Pb concentrations. The Pb concentrations for both smokers and non-smokers are approximately similar for hemodialysis patients and healthy individuals. Comparable Pb concentrations were observed in healthy individuals regardless of smoking status, indicating no significant interaction between these factors [71].

3.4.3. Interaction between smoking activity and gender

Fig. 4 shows the interaction between smoking activity and gender for Pb levels in fingernails. Although, the concentrations of Pb in the smokers and nonsmokers' fingernails are similar for male, there is a big difference for females. In the case of smokers, the Pb mean values were highest for males, while for non-smokers, however, the highest Pb mean values occur for females. Since smokers and non-smokers exhibit distinct gender-based differences in Pb levels, smoking habits must be

Table 6. Serum elemental levels found in the groups of smokers and non-smokers, (n = 109).

Element (n ₁ , n ₂)	Concentration (µg/mL) Mean ± Sd (range)		F-test			Two-tailed t-test		
	Smoker	Non-smoker	Variance	F	Sig.	t	df	Sig.
Pb (24,85)	3.239 ± 0.711 (2.120-4.932)	3.305 ± 0.807 (1.660-4.898)	Equal variances assumed	0.657	0.419	0.361	107	0.718
			Equal variances not assumed			0.388	41	0.700

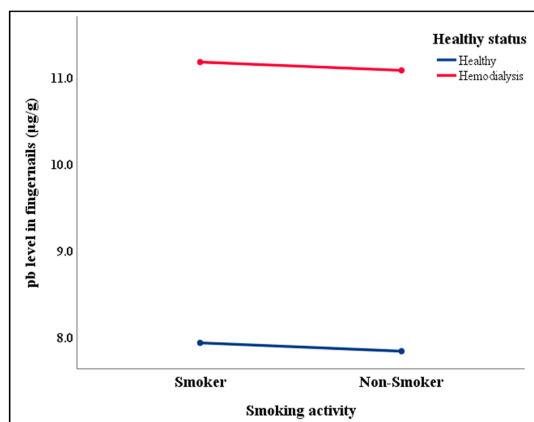


Fig. 3. The interaction effect between smoking activity and health status for Pb concentrations ($\mu\text{g/g}$) in fingernail samples ($n = 103$).

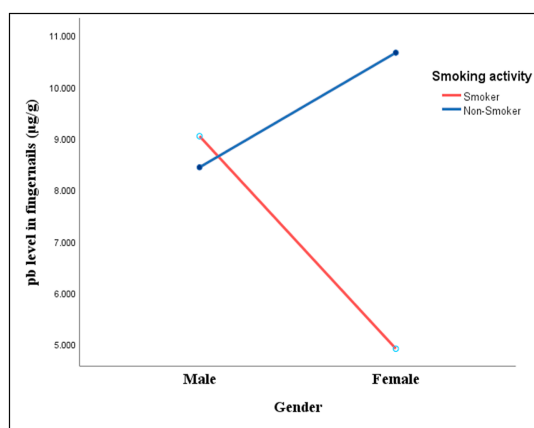


Fig. 4. The interaction effect between smoking activity and gender for Pb concentrations ($\mu\text{g/g}$) in fingernail samples ($n = 103$).

considered when examining the effect of gender. Moreover, this interaction shows that gender-based differences in Pb accumulation depend on smoking behavior.

4. Conclusion

To the best of our knowledge, this study is the first comprehensive investigation demonstrating the use of fingernails as a biomarker of lead levels in the bodies of hemodialysis patients in Iraq. This study provides a preliminary evaluation of lead determination in washed fingernail samples from individuals in Karbala Province, Iraq. Lead concentrations in the washed fingernails of hemodialysis patients were significantly lower than those of healthy individuals ($P \leq 0.05$). The effects of gender, smoking status, and health status on lead levels were assessed using two-tailed t-tests. Significant

effects were found for health status and gender on the levels of lead in washed fingernails, but there is no significant effect that was reported by the smoking activity. The results indicate that health status, gender, and smoking behavior may influence lead levels in the human body. The interaction between the study factors has also been clarified. No significant interactions were observed between health status and smoking status or gender. In contrast, a significant interaction was identified between smoking status and gender. Despite its reliability and precision, ICP OES presents certain limitations for determining lead in fingernail samples. The complex organic matrix of nails can cause matrix effects and spectral interferences that influence analytical accuracy. Complete digestion of keratinized tissue is essential but difficult to achieve, and residual material may affect aerosol formation. Moreover, the method's detection limit is higher than that of ICP-MS, which can be restrictive for samples with very low Pb concentrations. Finally, the technique requires high-purity argon and careful instrument maintenance, contributing to higher operational costs.

Ethics information

This study received ethical approval from the University of Kerbala's Ethics Committee (Official Letter No: 363/6, dated January 26, 2022). Prior to signing the consent form, all participants were fully briefed on the study procedures. Additionally, each subject completed study questionnaires, providing personal details as well as information regarding their health, diet, smoking habits, and lifestyle at the time of sample collection.

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Conflict of interest

There are no conflicts of interest associated with this work.

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