

Study of the Effect of Cadmium Level on Breast Cancer Patients using Human Scalp Hair

¹Ayad Kadhim Fadhil*

¹Chemical Sciences,
Faculty of Sciences, University of Kerbala

Abstract - The technique of natural materials as pointer in follow part thinks about has enhanced in compatibilities of flowed look into ponders. In this examination, human scalp hair was utilized to be a potential biomarker for cadmium in the human body. Models were picked up from breast cancer patients (n=42) and healthy people (n=129) inhabitant in Karbala, Iraq. The level headedness, accuracy and precision of the methodology were ascertained utilizing a "gathered" example for every medium and guaranteed reference materials. The affirmation techniques gave acceptable levels of exactness and accuracy with bring down scope of RSD (6.4%) and reasonable scope of central recuperations (97.60 %), separately. The outcomes demonstrate that the cadmium levels of study test are when all is said in done concurrence with the writing ranges for scalp hair. It was discovered that there was critical distinction for cadmium between the levels of Cadmium in bosom malignancy patients (0.6071 ± 0.54914 mg/kg) and solid people (0.1445 ± 0.11022 mg/kg). The outcomes indicate more elevated amounts of Cd in washed scalp hair for females (0.3629 ± 0.54826 mg/kg) when contrasted with guys (0.1545 ± 0.11898 mg/kg). On other hand the levels of Cd was found in the human scalp hair of smokers (0.4381 ± 0.2504 mg/kg) when contrasted and those of non-smokers (0.1175 ± 0.0201 mg/kg) (at $P < 0.05$). Contingent upon importance rate of relationship coefficient ($r = 0.752$, $P < 0.05$) was shown that there is no a huge connections for cadmium levels between scalp hair and drinking water.

Keywords: *Human scalp hair, Breast cancer; Cadmium.*

INTRODUCTION

In the last few decades, human scalp hair and nails (finger & toe) have commonly been used as a good biomarker in the valuation of exposure to various pollutants in an occupational and/or environmental setting, and in terms of assessing the metabolic state of humans, for essential and toxic trace elements [1, 2, 3]. Hair and nail tissues have several benefits over blood and urine, including: non-invasive materials and easily sampled; possibly represent a long-term growth material; and several heavy metals may collect in hair tissues over a time frame of 2 to 18 months. These advantages may provide useful data in determining the health status of an individual over long periods, as the tissues remain isolated from other metabolic activities in the human body [4, 5]. The use of human hair as an exceptional tool to assess changes in our bodies has received a great deal of attention for a few decades and become successful in different applications [6]. The connection between inorganic elements and human health has long been recognized. This is may be due to human scalp hair is a long-term growing material which may provide beneficial data in determining health status of an individual for long periods and several heavy metals being collected in hair as well [7].

MATERIALS AND METHODS

Study Population

Human scalp hair samples were collected from individuals living in Karbala which is a city in Iraq located about 100 Km south west of Baghdad. The participants were clearly informed of all the study procedures before signing the informed consent form. Generally, volunteers were interviewed at the time of sampling to obtain some general information about their health status, lifestyle, typical diet and smoking habits. In total, 171 samples were collected from individuals in relation to different forms of smoking (none, passive and active), varying in gender (male and female) and age from three groups old.

Sample collection and preparation

Scalp hair

Scalp hair samples were collected from the same site of the head for all individuals, namely, from the back of the head, less than 1 cm from the scalp using acetone/distilled deionised water washed scissors. This pre-treatment was undertaken to prevent contamination introduced by the tool during sample collection. Generally, a sample (mass > 0.5 g) was collected and stored in a polyethylene bag at room temperature until the time of analysis [8, 9]. Hair samples were cut into small pieces (~ 5 mm) using acetone/distilled deionised water washed scissors so as to make the sample more homogenous[9].

Washed and digestion

In brief, the cut scalp hair samples were washed using the sequential washing procedure (acetone-water-water-water-acetone) was utilized in this study [8]. Samples were dried in an oven overnight at 60°C then stored at room temperature in labelled polyethylene bags. The wet digestion method using a KjeldahlTM tube was employed for the complete digestion of washed human scalp hair [10].

Sample analysis

Instrumentation

The new JY 2000 – 2 ICP Atomic Emission Spectrometer (ICP – AES) Horiba Scientific was used in this study. An echelle grating and the charge-coupled device (CCD) were used in the ICP-OES instrument.

Precision and accuracy

The levels of precision and accuracy for the ICP-AES instrument were confirmed by calculation of the relative standard deviation (%RSD) and percentage recoveries (%R) using ten replicate measurements of a "collected" water sample, and certified reference materials (CRMs). In general, good levels of precision were obtained for most elements with perfect value of 6.4% RSD, as shown in table 1. Measured CRM values obtained for the analysis of cadmium by ICP-AES were highly comparative to certified values. Analytical recovery value is 97.60% for cadmium determined, as reported in table 1. The value of limit of detection (LOD) for cadmium under investigation was within expected range for ICP-AES (0.011 mg/kg).

Table 1: Accuracy (%R) and precision (%RSD) levels for human scalp hair CRM NIST SRM® 1643e and pooled human scalp hair samples (n = 10), respectively.				
Element (n = 3)	Elemental level (mg/kg)			
	Accuracy			Precision
	Measured value mean \pm SD	Certified value mean \pm SD	Percentage recovery (%R)	%RSD
Cd	0.122 \pm 0.009	0.125 \pm 0.007	97.60	6.4

SD is standard deviation, RSD and R are relative standard deviation and recovery, respectively (quoted as a % in brackets).

Statistical analysis

Descriptive data analysis (arithmetic mean, standard deviation (SD), range and 95% confidence interval) was performed on concentration values obtained for washed scalp hair. An F-test and a two tailed t-test tests were used to assess the significance of the variations in washed scalp hair Cadmium levels for healthy individuals and breast cancer patients. The Pearson product correlation coefficient (*r*) was determined for cadmium to evaluate if there was any significant correlation between washed scalp hair and drinking water for cadmium.

RESULTS AND DISCUSSION

In total, 171 human scalp hair samples were collected from healthy Iraqi individuals (n=129) and breast cancer patients (n=42) resident in Karbala in order to determine the cadmium levels of scalp hair. This can be used to investigate whether human scalp hair can play a significant role as a biomarker in the assessment of human health and environmental chemical exposure. Cadmium levels (mg/kg dry weight, d.w.) in washed scalp hair for healthy individuals and patients are summarised in Tables 2.

Table 2: Population data for Cadmium levels (mg/kg) in washed scalp hair from individuals resident in Kerbala (Iraq).		
Element	Variable	Concentration (mg/kg, dry weight)
Cd	Lower Quartile	0.08
	Mean \pm SD	0.26 \pm 0.35
	%RSD	73.97
	Upper Quartile	0.31
	Confidence at 0.05%	0.05
	Mean + CI	0.31
	Mean -CI	0.21

SD is standard deviation, RSD is relative standard deviation, CI is confidence interval for mean

Influence of Breast Cancer – Link to Human Health

The concentration of essential trace elements are homeostatically regulated when the health status of individuals is under normal conditions (healthy individuals) [11]. There is accumulating evidence that the metabolism of several trace elements is altered in cancer, and may play significant roles in the pathogenesis and progress of this disease [12]. Many studies have previously discussed the relationship between trace elements and cancer for patients by comparing them with healthy individuals [13, 14]. In this work, the results of healthy individuals and breast cancer patients resident in Kerbala have been compared in order to evaluate whether there are any significant differences in the levels of Cadmium between the two groups, as shown in table 3. This can be used to describe whether breast cancer plays any significant role in these differences by increasing or decreasing the Cadmium levels inside the human body through the effect on the metabolism of Cadmium. In this work, the results of healthy

individuals and breast cancer patients resident in Kerbala have been compared in order to evaluate whether there are any significant differences in the Cadmium levels between the two groups. This can be used to describe whether breast cancer plays any significant role in these differences by increasing or decreasing the Cadmium levels inside the human body through the effect on the metabolism of essential elements. The mean and standard deviation values for Cadmium levels in scalp hair of the healthy and breast cancer populations were compared by using an F-test and a two-tailed t-test, and the results obtained are listed in table 3. Although the levels of Cadmium are higher in breast cancer patients (0.6071 ± 0.54914 mg/kg) than healthy individuals (0.1445 ± 0.11022 mg/kg), but the differences are statistically significant ($P < 0.05$). The box-plots for Cadmium levels in the populations under investigation are summarized in Fig. 1. The mean values and standard deviations of Cd in washed scalp hair of breast cancer patients (only non-smokers) (0.1659 ± 0.20116 mg/kg) and healthy individuals (only non-smokers) (0.1093 ± 0.08456 mg/kg) as shown in table 4. It was found that the levels of Cd were significantly higher in breast cancer patients when compared to those results obtained from healthy individuals. In the light of these results, it can be seen that Cadmium has a pro-angiogenic effect on humans, which may be implicated in Cadmium-induced tumorigenesis. The box-plots for Cadmium levels in the populations under investigation are summarized in fig. 2. Furthermore, the effect of various factors, namely gender, age, smoking activity and drinking water were also investigated in this study. This step was carried out to assess whether factors like gender and age may affect the Cadmium concentrations in washed scalp hair of the individuals under study.

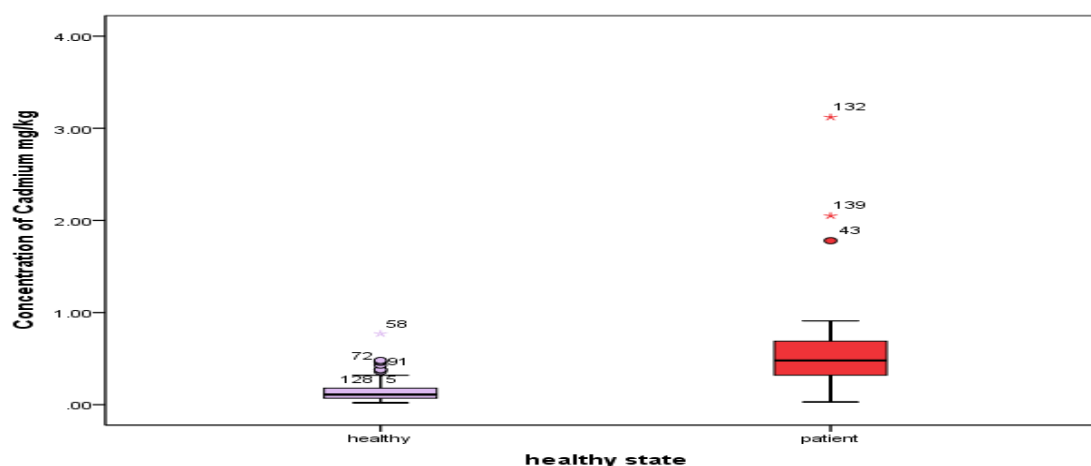


Figure 1: Cadmium levels (mg/kg) in washed scalp hair for healthy n = 129 and breast cancer n = 42, middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

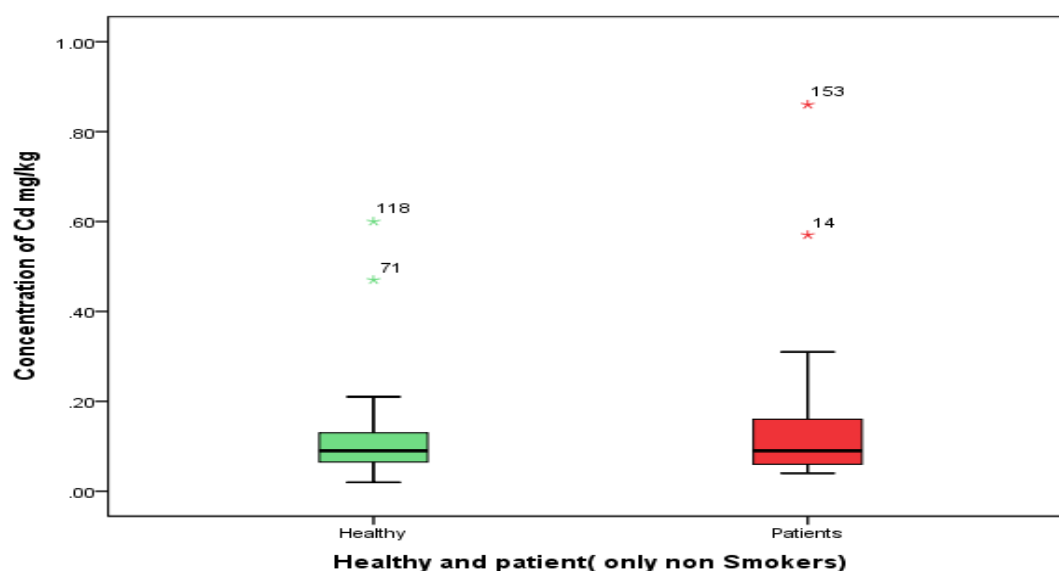


Figure 2: Cadmium levels (mg/kg) in washed scalp hair for healthy only non-smokers n = 75 and breast cancer only non-smokers n = 21, middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

Influence of Gender

Table 5: Cadmium mean and standard deviation values in human scalp hair for males and females from Kerbala, Iraq along with the literature values.

Element	Mean \pm SD (mg/kg)		F – test			Two - tailed t-test			
	Male ($n_1 = 86$)	Female ($n_2 = 85$)	Variance	F_{calc}	Sig.	t_{calc}	df	Sig.	t_{crit}
Cd	0.1545 ± 0.11898	0.3629 ± 0.14826	EVA	24.602	0.000	4.081	169 ⁺	<001	1.974
			UVA			4.060	95 ⁺⁺	<001	

SD is standard deviation, n_1 , n_2 are the number of samples for males and females, respectively, df = degrees of freedom at $n_1 - 1$ and $n_2 - 1$ for F-test, ⁺ degrees of freedom for t-test ($n_1 + n_2 - 2$), ⁺⁺ degrees of freedom for t-test determined, F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $P = 0.05$, Sig. = level of significance, EVA = equal variances assumed, UVA = unequal variances assumed.

The total population from Kerbala ($n = 171$) was divided into two gender groups, males and females. The effect of gender on the levels of Cadmium in washed scalp hair samples was investigated, and the mean and standard deviation (\pm SD) for each gender group are summarised in Table 5. In order to determine whether there is any significant difference that can be attributed to gender, an F-test and a two-tailed t-test were undertaken on the scalp hair data from individuals. The findings show that there is a significant effect of gender on the levels of Cd ($t_{(169)} = 4.081$, $t_{crit} = 1.974$, $P < 0.05$), (where the number in brackets is the number of degrees of freedom and the critical value (t_{crit}) is determined at the probability level of $P = 0.05$). The results show higher levels of Cd in washed scalp hair for females (0.3629 ± 0.14826 mg/kg) when compared to males (0.1545 ± 0.11898 mg/kg). Fig. 3 shows the box-plots for Cd levels in the populations of males and females.

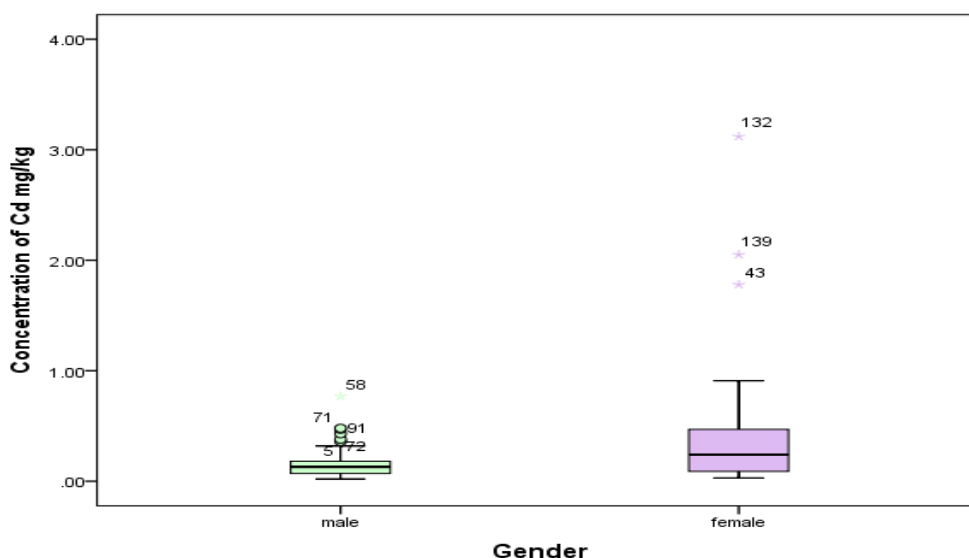


Figure 3: Cadmium levels (mg/kg) in washed scalp hair for males (M) $n = 86$ and female (F) $n = 85$, middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

Influence of Smoking Activity

Smoking is considered to be a major environmental risk factor associated with many serious systemic diseases, including respiratory diseases, heart diseases and cancers [15,16]. Cigarette tobacco which can cause more health problems and disorders [17]. Many studies have investigated the elemental levels of cigarette tobacco and associated health/pollution implications. It has been known for a few decades that tobacco combustion has the potential to deliver dangerous quantities of heavy metals to the blood and various organs [18]. In this study, the population of Kerbala was divided into smokers and non-smokers, as shown in table 5. The influence of smoking activity on the Cd levels in washed scalp hair was examined by using an F-test and a two-tailed t-test, and the results are reported in table 6. It was found that there is a significant effect of smoking activity for Cd in scalp hair ($t_{(169)} = 6.685$, $t_{crit} = 1.974$, $P < 0.001$). In other words, high levels of Cd was found in the human scalp hair of smokers (0.4381 ± 0.4504 mg/kg) when compared with those of non-smokers (0.1175 ± 0.0201 mg/kg). Figure 4 shows the box-plots for Cd levels in the groups of smokers and non-smokers.

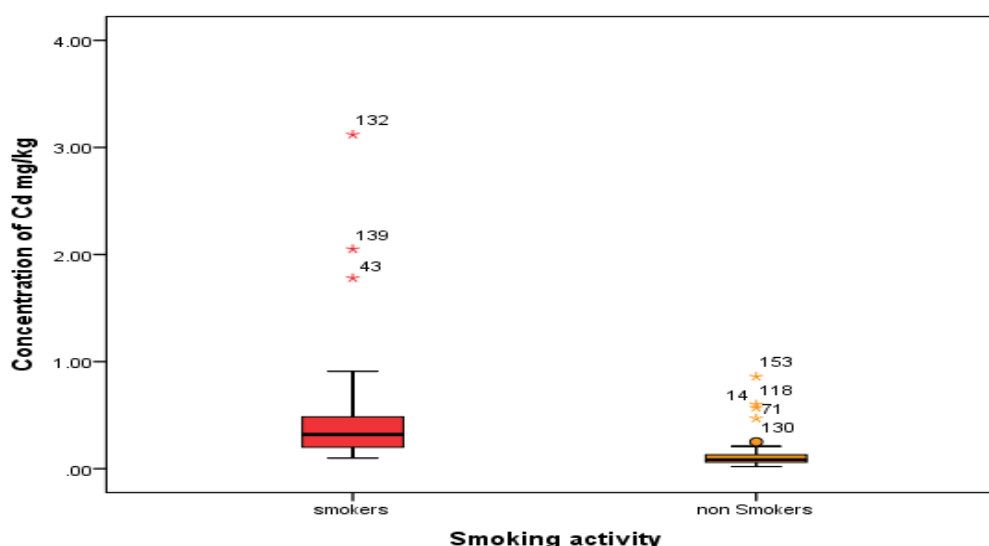


Figure 4: Cadmium levels (mg/kg) in washed scalp hair for smokers (S) $n = 75$ and non-smokers (NS) $n = 96$, middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively.

Influence of Drinking water

According to World Health Organization (WHO) instructors and previous studies, high levels of Cd in water can be a possible cause of adverse effect on human health [19]. In this study, the levels of Cadmium in drinking water (domestic bottled) (0.093 ± 0.128 mg/l) are approximately within the WHO guideline for drinking water. However, the participants could be exposed to Cd by food and environmental sources. Significant exposure to Cadmium occurs through both anthropogenic and natural sources. Occupational exposure to Cadmium is common in the smelting, mining and microelectronic industries and the production of iron and steel [20]. The relationship between the level of Cadmium in drinking water and washed scalp hair was investigated. The strength and direction of this relationship was evaluated using correlation coefficient (r) analysis; the value of r was calculated and then subjected to a significance test. It was no significant correlation is found for Cadmium levels between drinking water and scalp hair ($r = 0.752$, $t_{(43)} = 2.21$, $P < 0.05$, $t_{crit} = 2.01$), as shown in Fig. 5.

Table 6: Cadmium mean and standard deviation values in human scalp hair for smokers and non-smokers from Kerbala, Iraq along with the literature values.

Element	Mean \pm SD (mg/kg)		F – test			Two - tailed t-test			
	Smokers ($n_1 = 75$)	Non-smokers ($n_2 = 96$)	Variance	F_{calc}	Sig.	t_{calc}	df	Sig.	t_{crit}
Cd	0.4381 ± 0.4504	$0.1175 \pm$	EVA	24.40	0.043	6.685	169 ⁺	< 0.001	1.974
		0.020116	UVA	-		6.002	82 ⁺⁺	< 0.001	

SD is standard deviation, n_1 , n_2 are the number of samples for smokers and non-smokers, respectively, df = degrees of freedom at $n_1 - 1$ and $n_2 - 1$ for F-test, ⁺ degrees of freedom for t-test ($n_1 + n_2 - 2$), ⁺⁺ degrees of freedom for t-test determined, F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $P = 0.05$, Sig. = level of significance, EVA = equal variances assumed, UVA = unequal variances assumed.

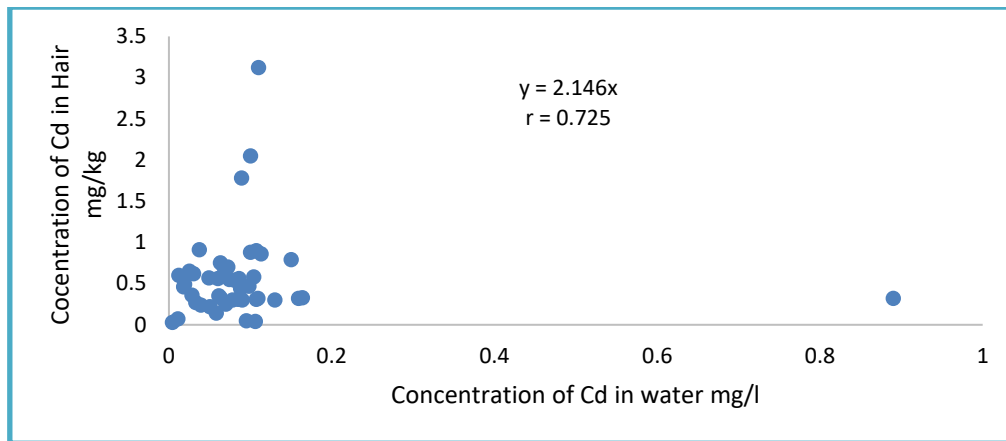


Figure 5: Correlation between Cadmium levels in washed scalp hair and drinking water.

Influence of Age

Additionally, the influence of age was also studied in order to evaluate whether this parameter provide any significant effects on the levels of Cadmium in washed scalp hair along with other factors. The study population was divided into three age groups: < 20 years (n= 44), 20–40 years (n= 71), and > 40 years (n= 56). Mean, standard deviation, and 95% confidence interval for mean of cadmium levels in washed scalp hair for individuals as shown in table 7. One-way ANOVA was used to check whether there were any significant differences exist between the age groups of Cadmium levels at the probability level of $P < 0.05$. The results show that there is a significant difference for Cadmium, as shown in Tables 8. It was found that there is a significant difference ($P < 0.05$) somewhere among the mean values of the levels of Cadmium in washed scalp hair for the three groups, as shown in Table 8. This does not provide which group is different from which other groups. Therefore, the post-hoc test was used to determine whether any significant differences among the groups, as shown in Table 9. The results show that the levels of Cadmium in the age group (over 40 year) are significantly different when compared with the two remaining groups (under 20 and 20 – 40 year) at the $P < 0.05$. On the other hand, there is no any significant difference between the two groups (under 20 and 20 – 40 year), as shown in table 9. In the light of these results, the levels of Cadmium are increased when the age of individual's increase as shown in Fig. 6.

Table 7: Mean, standard deviation, and 95% confidence interval for mean of cadmium levels in washed scalp hair for individuals from Kerbala, Iraq.

Age (year)	n	Mean \pm SD (mg/kg)	95% CI	
			Lower	Upper
Under 20	44	0.0959 \pm 0.05055	0.0416	0.1909
20 to 40	71	0.1706 \pm 0.09680	0.2178	0.2787
Over 40	56	0.4966 \pm 0.52306	0.4343	0.5227

SD = standard deviation, n = number of samples, CI is confidence interval for mean.

Table 8: Analysis of variance ANOVA for Cadmium levels in washed scalp hair for individuals from Kerbala, Iraq.

Source of variance				Sum of Squares	df	Mean Square	F	Sig.
Between Groups				4.887	2	2.444	25.960	0.000
Within Groups				15.813	168	0.094		
Total	20.700	170						

df = degrees of freedom, for between-groups (df_B) = number of groups – 1; within-group (df_W) = $df_T - df_B$; Total number of degrees of freedom (df_T) = number of observations – 1, mean square = (SS/df), F is the calculated value for F-test, $F = MS_B/MS_W$, Sig. is the significance level.

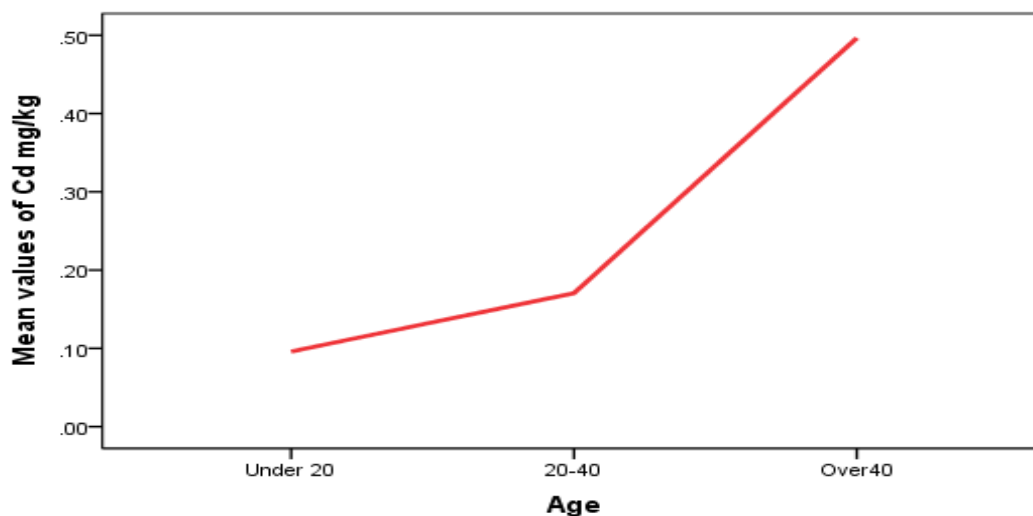


Figure 6: Concentrations of Cadmium in washed scalp hair for < 20 years (n= 44), 20 - 40 years (n= 71) and > 40 years (n= 56) of age (mg /kg).

Factors Influencing Elemental Data (Factorial Analysis)

The mean values of Cadmium levels was categorised according to different parameters (factors) obtained from the questionnaire, as shown in table 10. Two - Way Analysis of covariance (ANCOVA) was used to investigate the effects and interactions of independent variables (factors) on the level of Cadmium (dependent variable) in washed scalp hair for individuals resident in Kerbala (n = 171). The effect for each factor (health status and smoking activity) and covariate (gender and age) was investigated. The *P*-values can be used to determine whether there is a significant effect. If the value of "Sig" for each factor is less than the level of significance ($P < 0.05$), then there is a significant effect for this factor. The effects of health status and smoking activity on the Cadmium levels in washed scalp hair samples are reported in table 10, using analysis of covariance (ANCOVA). In general, significant effect (at $P < 0.05$) is found for Cd in terms of the smoking activity ($F_{(1,165)} = 6.841$, $P < 0.05$). On the other hand, there is no significant effect (at $P < 0.05$) observed in the case of gender ($F_{(1, 165)} = 0.613$).

Table 9: Post Hoc test for Cadmium levels in washed scalp hair using different ages for individuals from Kerbala, Iraq.						
Age/ year		Mean Difference (G1 – G2)	Std. Error	Sig.	95% Confidence Interval	
G1	G2				Lower Bound	Upper Bound
Under 20	20 to 40	0.07465	0.05886	0.206	0.1909	0.0416
	over 40	0.40070*	0.06181	0.000	0.5227	0.2787
20 to 40	under 20	0.07465	0.05886	0.206	0.0416	0.1909
	over 40	0.32604*	0.05483	0.000	0.4343	0.2178
Over 40	under 20	0.40070*	0.06181	0.000	0.2787	0.5227
	20 to 40	0.32604*	0.05483	0.000	0.2178	0.4343

*. The mean difference is significant at the $P = 0.05$, G = group, Std. = standard, and Sig. = significance.

Table 10: Two – way ANCOVA results for Cd in washed scalp hair (n = 171) without gender effect.

Source of Variance	Sum of Squares	df	Mean Squares	F	Sig.*
Corrected Model	8.184a	5	1.637	21.576	0.000
Intercept	11.733	1	11.733	154.669	0.000
Smoking activity	0.519	1	0.519	6.841	0.010
Gender	0.020	1	0.020	0.257	0.613
Age	0.628	2	0.314	4.140	0.018
Healthy state	1.290	1	1.290	17.006	0.000
Error	12.517	165	0.076		
Total	32.094	171			
Corrected Total	20.700	170			

df = degree of freedom, F = is the calculated value for F-test, $F_{critical} = 4.001$, Sig. = level of significance. Mean of squares (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$. * is significant when the value < 0.05.
a. R Squared = 0.395 (Adjusted R Squared = 0.377)

CONCLUSION

The present study is the first full study, to my knowledge to highlight the use of scalp hair tissue as a biomarker for the level of cadmium in the human body. In addition, this study provides a preliminary assessment of the determination of cadmium levels in washed scalp hair for Iraqi individuals in the province of Kerbala, Iraq. The results show that both the aim and main objectives of this study have been achieved. Firstly, an assessment of the cadmium exposure of the inhabitants of Kerbala was carried out, using washed scalp hair as a potential biomarkers. Secondly, this provides evidence that washed scalp hair can potentially be used as a biomarker for determining the health status of an individual. Finally, significant difference was found in the levels of cadmium throughout this study between healthy and breast cancer; females and males; and smokers and non-smokers. These results can confirm that factors like gender, age and smoking activity can affect the elemental levels in the human body.

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