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Determination Uranium and its Isotopes in Biological Samples of Smokers

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Abstract

This study was conducted in the Najaf Governorate- Iraq, to analyze biological samples from smokers and non-smokers. The samples, including blood serum, urine, hair, and nails, were used as biomarkers to determine uranium concentrations (U_C) and isotopes (^{238}U, ^{235}U, and ^{234}U). Using a natural exposure method, the nuclear track detector (CR-39, UK) was utilized to measure these Uc in the samples. Seventy-five samples of blood serum, urine, hair, and nails were collected for smokers of healthy people and fifty samples for non-smokers of healthy people in five age groups. This study was based on age and smoking to compare the results and determine their effects on the Uc. The results show that the average values of Uc in blood serum, urine, hair, and nails for smokers were 0.180±0.042 ppb, 0.759±0.024 ppb, 0.912±0.085 ppm, and 0.934±0.091 ppm, respectively. While, the average values of Uc in blood serum, urine, hair, and nails for non-smokers were 0.110±0.014 ppb, 0.157±0.023 ppb, 0.736±0.032 ppm, and 0.756±0.024 ppm, respectively. The results and comparisons indicate that all Uc depend on the variables on which this study was based (age and smoking). By comparing the Uc for all biological samples for smokers and non-smokers, the value of P-value was highly significant statistically which was <0.001. According to the results of the study samples, the mean values of U_C, ^{238}U, ^{235}U, and ^{234}U for biological samples in smokers were higher than in non-smokers. Thus, it may be said that cigarette smoking is used as a biomarker of the presence of Uc.

Keywords: Biological samples, CR-39, Smoking, Uranium, and Uranium isotopes.

Introduction

Exposure to many chemical and physical variables in the environment may pose a significant risk to human health. Humans are regularly exposed to many substances originating from external sources, such as food, tobacco smoke, air pollution, or ionizing radiation [1,2]. Ionizing radiation is a very hazardous

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kind of environmental contamination that is imperceptible to the senses and can only be detected with specialist devices and equipment operated by skilled professionals[3]. Radionuclides, such as uranium, may enter the human body via the consumption of meat, fish, plants, vegetables, cigarettes, soil, water, and inhalation of air. External exposure to radionuclides occurs when humans come into contact with soil, leading to a rise in radiation concentration in the blood[4,5]. Uranium is a very dangerous radioactive element that may be digested and absorbed into the gut, then carried into the blood stream upon entering the human body. It is a naturally occurring terrestrial element with three isotopes (^{238}U, ^{235}U, ^{234}U) that mostly emits alpha particles, with minor emissions of beta and gamma particles[6]. The radioactive and toxicological activity of uranium and its isotopes make them the most significant source of contamination, posing a hazard to both humans and the environment[7]. Uranium (U) is an element that occurs naturally and is typically present in the earth's crust with an average concentration of 0.0004%. The element has three naturally occurring isotopes: ^{234}U (0.006% mass fraction), ^{235}U (0.72% mass fraction), and ^{238}U (99.3% mass fraction)[8]. According to the World Health Organization (WHO), the acceptable amount of uranium that people may consume daily is 0.6 µg/kg of body weight[9]. Healthy individuals typically eliminate a daily amount of uranium in their urine ranging from 0.01 µg to 0.4 µg, which is contingent upon their food consumption [8]. Tobacco is a plant belonging to the genus Nicotiana, and its leaves are mostly used for smoking. Tobacco may be consumed in many forms and configurations, such as cigarettes, cigars, pipes, and rolling tobacco. Cigarettes dominate the market, representing over 90% of all tobacco products[10]. Tobacco smoke, when burned, produces a very intricate combination of particulate matter (5%) and gases (95%), including more than 8000 distinct chemicals[11]. Tobacco and tobacco products contain chemical and organic compounds and naturally occurring radioactive elements known as radionuclides. These radionuclides include uranium and thorium isotopes (^{234}U, ^{238}U, ^{228}Th, ^{230}Th, and ^{232}Th), along with the byproducts of their decay chains such as radium-226 (^{226}Ra), lead-210 (^{210}Pb), and polonium-210 (^{210}Po)[12]. Quantifying the presence of uranium in biological samples, such as blood, urine, hair, nails, etc., provides a reliable assessment of the number of heavy elements present in certain bodily tissues. Uranium is abundant in nature, occurring in a diverse range of solid, liquid, and gaseous compounds. Uranium rapidly reacts with other elements to create compounds such as uranium oxide, silicates, carbonates, and hydroxides[10]. Research is conducted to determine the quantities of natural radionuclides consumed, ensuring that persons are not exposed to excessive amounts. Urinary excretion is often used as a metric, however, scalp hair has also been considered a potential biomonitor for certain radionuclides[13]. Prior studies have shown a correlation between smoking and elevated levels of uranium in the blood, urine, hair, and nails of smokers. This is concerning because uranium poses a significant risk to human health. Further research has confirmed that smoking cigarettes possesses toxic, geotactic, carcinogenic, lethal, and hazardous properties that are detrimental to one's well-being[14–17]. The impact of uranium on health status and smoking habits, as well as its presence in blood serum, urine, hair, and nails, remains mostly unexplored and understudied in the majority of Iraqi cities. This study seeks to detect uranium isotopes in healthy smokers from Najaf Governorate in central Iraq by using the (CR-39) nuclear track detector.

Materials and Methods

Sample collection

In 2023, the current investigation was carried out in the Al-Najaf Governorate of Iraq, including a sample of 75 randomly picked and identified as smokers. A control group of 50 individuals who did not smoke was included. The participants were distributed throughout various age groups, as shown in Table 1. Their ages span from 21 to 70 years. They were categorized into five categories based on age ranges: 21-30 years, 31-40 years, 41-50 years, 51-60 years, and 61-70 years. The inclusion criteria for smokers in this study were persons without a documented history of drug abuse, who smoke at least five

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cigarettes per day, and who have no occupational exposure to heavy metals or other toxic compounds. In contrast, the persons who did not smoke and were of comparable age to the group of smokers had no previous exposure to cigarette smoking or drug misuse. This research excluded those with pre-existing diseases such as anemia, hypertension, renal disease, diabetes, digestive problems, or cardiovascular disease, as well as those receiving treatments for metal toxicity and taking medicine supplements. Written permission was obtained from all participants in this research. Various statistics and information were collected, including age, employment, marital status, education level, history of cigarette smoking, smoking duration, kind of cigarettes smoked, and average daily cigarette intake. We took great care in selecting biological samples for smokers who used the same type of cigarette. By carefully choosing donors with comparable lifestyles, it has been verified that the biological samples of the donors were exposed to uranium and its isotopes under investigation, in addition to cigarette smoke[14, 17]. Also, participants in Table 1 (smokers and non-smokers) were taken from most areas of Najaf Governorate. It was also ensured that all people had lived in the area since birth.

Table 1. Data of samples in the current research

No.	Age range (year)	No. samples	
		Smokers	Non-smokers
1	21-30	15	10
2	31-40	15	10
3	41-50	15	10
4	51-60	15	10
5	61-70	15	10
	Total	75	50

Sample preparation

For this investigation, we collected four biological samples (blood serum, urine, hair, and nails) from 125 healthy participants (both smokers and non-smokers) aged between 21 and 70. Every participant was given an exclusive code, and samples were prepared for measuring uranium isotopes in the following manner[18,19]:

Blood serum samples: These participants were required to visit the laboratory, where venous blood samples were obtained from the elbow vein. Specifically, 5-6 ml of blood was collected and stored in gel tubes—a trained phlebotomist conducted venipuncture in a pathological analysis laboratory in Najaf Governorate. Subsequently, the blood samples were allowed to clot for 5-10 min to facilitate serum separation from the blood during the subsequent centrifugation process. The centrifuge, a device designed to separate particles based on their mass, was employed to accomplish this task. The device is configured to operate at a rotational speed of 4000 revolutions per minute for 3-5 min, during which the blood is effectively separated from the serum. Each sample was assigned a distinct identification code labeled with the participant's name. Subsequently, the serum samples acquired were transferred into pristine, sterile, hermetically sealed (Eppendorf tubes), each with a volume of 1 ml, and subsequently stored in a freezer to prepare for the measurement process[18].

Urine samples: Urine samples were collected from the same individuals after cleaning special containers using detergents and distilled water to ensure they were completely clean and free of any impurities or contamination. Urine samples of 5-6 ml were collected from smoking and non-smoking participants. After that, the urine samples were placed in (Eppendorf tubes) after writing the symbols on them and transferred to the refrigerator for cooling until the analysis began[19].

Hair samples: The same individuals clipped about 0.5 g of hair from the scalp of varying lengths from 3–6 cm using scissors. The samples were stored directly in zipped polythene bags, after which the hair samples were placed in a glass beaker containing a detergent solution to remove any external impurities such as dirt, fat, and sweat, and finally washed with distilled water without changing the contents of the samples. The samples are then left for a period to dry. Next, the scalp hair was cut into small pieces, mixed thoroughly, and put in plastic containers bearing the symbol and number of each relevant sample[20].

Nail samples: To get nail samples, participants were initially instructed to meticulously cleanse their hands and feet using pine water, followed by drying with a sterile towel to eliminate any potential exterior contaminants. Next, they trimmed nails on both fingers and toes using a pair of nail clippers. Continuing this procedure multiple times is advisable to obtain a quantifiable quantity of about 0.5 g. The specimens were preserved within airtight, zipped polythene bags. Subsequently, each specimen was immersed in a detergent solution for a

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designated duration to facilitate the detachment of adhered particulate matter, after which it was thoroughly rinsed with an ample quantity of distilled water. Thus, any impurities or dirt were removed at this stage. Then, samples were left to dry, cut into small pieces, and finally placed in clean plastic containers bearing the code and number of each sample[20].

Irradiation method

After the samples were collected and prepared, 1 ml of blood serum, urine, and 0.5 g of hair and nails were placed in sterile plastic tubes. These tubes had a size of 10 ml, a length of 9.5 cm, and a diameter of 1.5 cm. The tubes were equipped with a (CR-39) detector, which had a thickness of 1 mm and an area of 1×1 cm^2 (from Track Analysis Systems Ltd, UK). Columbia Resin No. 39, or CR-39, Plastic Nuclear Track Detector (PNTD) is sensitive to protons of energy ≤ 14 MeV, alpha particles of energy ≤ 100 MeV, and heavy ions of all energies. These tubes were stored in the Nuclear and Environmental Laboratory of the College of Science at the University of Kufa for at least three months. This process is illustrated in Fig 1. The current investigation used the Long-term irradiation approach[20].

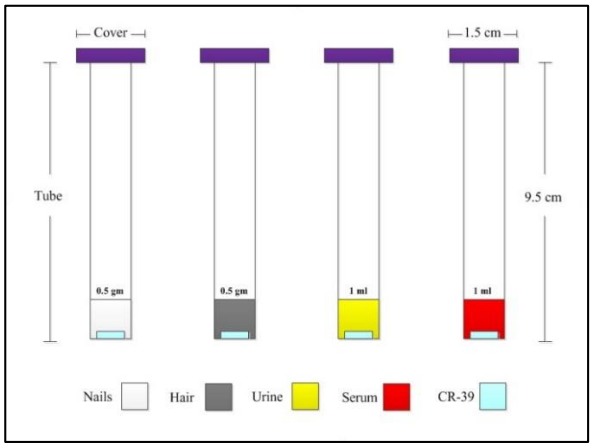

Figure 1. Plastic tubes for measuring uranium concentrations using CR-39 detector.

After the exposure period of 3 months, the CR-39 detectors were removed from the plastic containers and treated with a chemical etching process using NaOH (6.25 N) in a water bath (HH-420, Germany) at a temperature of 98°C for 1 hour. Afterward, the detectors were purified by using distilled water. Ultimately, the number of tracks was determined by using an optical microscope (Novel, China) (magnification 400×) equipped with a digital eyepiece and a known field of view[21].

Calculation of Uranium Concentration

In the present study, uranium concentrations (U_C) in blood serum and urine samples were determined according to the calibration curve that is shown in Fig 2 as following[22].

$$U_c\ (ppb) = \frac{(\rho + 12.5)}{18.6} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

While, uranium concentrations (U_C) in hair and nail samples were determined according to the calibration curve that is shown in Fig 3 as following[23].

$$U_c\ (ppm) = \frac{(\rho + 57.294)}{98.361} \ldots\ldots\ldots\ldots\ldots\ldots 2$$

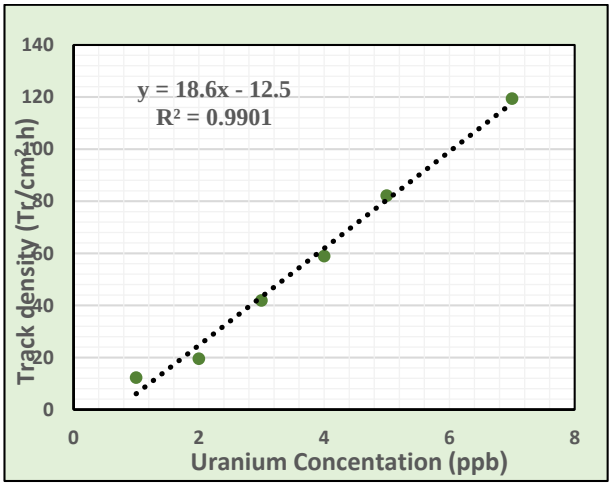

Figure 2. Calibration curve for standard uranium (ppb)[22].

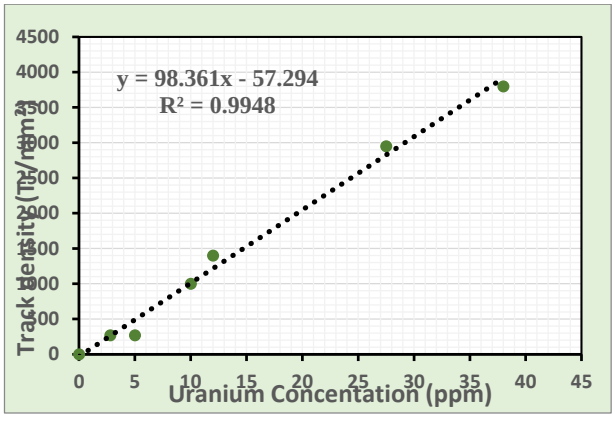

Figure 3. Calibration curve for standard uranium (ppm)[23].

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Calculation of uranium isotopes

Uranium isotopes in all biological samples under study were calculated using Eq 3[24]:

$$A\left(\frac{Bq}{L\ or\ kg}\right) = UC\left(\frac{mg}{L\ or\ kg}\right) \times I.A.M(\%) \times S.P.A.\left(\frac{Bq}{mg}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots 3$$

Where, *A* is the specific activity (Bq/L or kg), *I.A.M* is the isotopic affluence (%) by a mass fraction, and *S.P.A* is the specific activity. The values the specific activity and their mass fraction as shown and illustrated in Table 2[25].

Table 2. Radioactive properties of natural uranium isotopes[25]

Isotope	Specific activity for Uranium (Bq/mg)	Mass fraction (%)
^{238}U	12.44	99.2745
^{235}U	80	0.72
^{234}U	230700	0.0055

Statistical Analysis

The statistical analyses of the data were conducted using IBM SPSS Statistics software, especially (version 27 for the Windows) operating system. First, the independent sample t-test was used to compare the groups of smokers and non-smokers for all parameters of the blood serum, urine, hair, and nail samples. On the other hand, the Single-factor Analysis of Variance (ANOVA) method was employed to conduct a comparative analysis among several groups of smokers, which were stratified based on age.

Results and Discussion

The results of uranium concentrations (U_C) and it is isotopes (^{223}U, ^{225}U, and ^{234}U) in blood serum, urine, hair, and nail samples for smokers and non-smokers are shown in Table 3. The mean values of U_C in blood serum, urine, hair, and nail samples of smokers were 0.180±0.042 ppb, 0.759±0.024 ppb, 0.912±0.085 ppm, and 0.934±0.091 ppm, respectively. While, the mean values of UC in blood serum, urine, hair, and nail samples of non-smokers were 0.110±0.014 ppb, 0.157±0.023 ppb, 0.736±0.032 ppm, and 0.756±0.024 ppm, respectively. The mean values of uranium isotope ^{238}U in blood serum, urine, hair, and nail samples of smokers were 2.224±0.522 mBq/L, 9.383±0.306 mBq/L, 11.269±1.061 Bq/kg, and 11.543±1.126 Bq/kg, respectively. While, the mean values of uranium isotope ^{238}U in blood serum, urine, hair, and nail samples of non-smokers were 1.361±0.181 mBq/L, 1.950±0.284 mBq/L, 9.098±0.404 Bq/kg, and 9.344±0.296 Bq/kg, respectively. The mean values of uranium isotope ^{235}U in blood serum, urine, hair, and nail samples of smokers were 0.103±0.024 mBq/L, 0.437±0.014 mBq/L, 0.525±0.049 Bq/kg, and 0.538±0.052 Bq/kg, respectively. While, the mean values of uranium isotope ^{235}U in blood serum, urine, hair, and nail samples of non-smokers were 0.063±0.008 mBq/L, 0.090±0.013 mBq/L, 0.424±0.018 Bq/kg, and 0.435±0.013 Bq/kg, respectively. The mean values of uranium isotope ^{234}U in blood serum, urine, hair, and nail samples of smokers were 2.285±0.537 mBq/L, 9.640±0.315 mBq/L, 11.578±1.090 Bq/kg, and 11.860±1.156 Bq/kg, respectively. While, the mean values of uranium isotope ^{234}U in blood serum, urine, hair, and nail samples of non-smokers were 1.398±0.185 mBq/L, 2.003±0.292 mBq/L, 9.348±0.415 Bq/kg, and 9.601±0.304 Bq/kg, respectively. Independent sample t-test is used to compare both smokers and non-smoker groups for all the parameters for blood serum, urine, hair, and nail samples; as can be seen from Table 3, there is a highly significant difference between the smokers and non-smoker groups for U_C, ^{238}U, ^{235}U, and ^{234}U since the p-value is less than 0.01. Generally, the mean of uranium concentrations and isotopes for all biological samples of Al-Najaf governorate in smokers' samples was higher than the mean of non-smokers.

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Table 3. Comparison between smokers and non-smokers groups for Uranium Concentrations and Isotopes for Serum, Urine, Hair and Nails samples

Radioactivity	Biological sample	Groups	N	Mean ± SD	P value
U_C (ppb or ppm)	Serum	Smoker	75	0.180±0.042	<0.001
		Non-smoker	50	0.110±0.014	HS
	Urine	Smoker	75	0.759±0.024	<0.001
		Non-smoker	50	0.157±0.023	HS
	Hair	Smoker	75	0.912±0.085	<0.001
		Non-smoker	50	0.736±0.032	HS
	Nails	Smoker	75	0.934±0.091	<0.001
		Non-smoker	50	0.756±0.024	HS
^{238}U (mBq/L or Bq/kg)	Serum	Smoker	75	2.224±0.522	<0.001
		Non-smoker	50	1.361±0.181	HS
	Urine	Smoker	75	9.383±0.306	<0.001
		Non-smoker	50	1.950±0.284	HS
	Hair	Smoker	75	11.269±1.061	<0.001
		Non-smoker	50	9.098±0.404	HS
	Nails	Smoker	75	11.543±1.126	<0.001
		Non-smoker	50	9.344±0.296	HS
^{235}U (mBq/L or Bq/kg)	Serum	Smoker	75	0.103±0.024	<0.001
		Non-smoker	50	0.063±0.008	HS
	Urine	Smoker	75	0.437±0.014	<0.001
		Non-smoker	50	0.090±0.013	HS
	Hair	Smoker	75	0.525±0.049	<0.001
		Non-smoker	50	0.424±0.018	HS
	Nails	Smoker	75	0.538±0.052	<0.001
		Non-smoker	50	0.435±0.013	HS
^{234}U (mBq/L or Bq/kg)	Serum	Smoker	75	2.285±0.537	<0.001
		Non-smoker	50	1.398±0.185	HS
	Urine	Smoker	75	9.640±0.315	<0.001
		Non-smoker	50	2.003±0.292	HS
	Hair	Smoker	75	11.578±1.090	<0.001
		Non-smoker	50	9.348±0.415	HS
	Nails	Smoker	75	11.860±1.156	<0.001
		Non-smoker	50	9.601±0.304	HS

HS: High significant difference between groups (p value <0.01).
S: Significant difference between groups (p value <0.05).

The results of U_C, as well as the specific activity of ^{238}U, ^{235}U, and ^{234}U in blood serum, urine, hair, and nail samples at five age groups (21-30, 31-40, 41-50, 51-60, and 61-70 years) for smokers and non-smokers, were shown in (Tables 4 and 5), respectively. Using five intervals, the study categorized the results of uranium concentrations and isotopes in Tables 4 and 5 in all samples based on age. The findings revealed that the highest levels of uranium concentrations and isotopes were seen in the age interval of 61-70 years, while the lowest levels were found in the age interval of 21-30 years. Statistically, the results for U_C, ^{238}U, ^{235}U, and ^{234}U in all biological samples according to smoking and age showed that the differences in all results Tables 4 and 5 by smoking and age indicate high significant differences at significant levels of LSD (0.05) and P-value (0.01).

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Table 4. Comparison between different ages for Uranium Concentrations and Isotopes in smokers for Serum, Urine, Hair and Nails samples

Radioactivity	Age (years)	N	Serum		Urine		Hair		Nails	
			Mean ± SD	$LSD_{0.05}$ P value	Mean ± SD	$LSD_{0.05}$ P value	Mean ± SD	$LSD_{0.05}$ P value	Mean ± SD	$LSD_{0.05}$P value
U_C (ppb or ppm)	21-30	15	0.130±0.016	<0.001 HS	0.728±0.006	<0.001 HS	0.800±0.017	<0.001 HS	0.812±0.037	<0.001 HS
	31-40	15	0.150±0.019		0.742±0.013		0.856±0.032		0.874±0.030	
	41-50	15	0.178±0.028		0.759±0.012		0.917±0.027		0.930±0.015	
	51-60	15	0.200±0.008		0.774±0.006		0.969±0.047		1.007±0.038	
	61-70	15	0.240±0.002		0.793±0.006		1.019±0.047		1.049±0.014	
^{238}U (mBq/L or Bq/kg)	21-30	15	1.610±0.205	<0.001 HS	8.999±0.083	<0.001 HS	9.886±0.210	<0.001 HS	10.033±0.463	<0.001 HS
	31-40	15	1.861±0.242		9.169±0.161		10.573±0.399		10.795±0.375	
	41-50	15	2.205±0.354		9.381±0.149		11.331±0.338		11.491±0.190	
	51-60	15	2.478±0.105		9.562±0.078		11.968±0.592		12.436±0.478	
	61-70	15	2.967±0.029		9.801±0.085		12.585±0.582		12.961±0.175	
^{235}U (mBq/L or Bq/kg)	21-30	15	0.075±0.009	<0.001 HS	0.419±0.003	<0.001 HS	0.461±0.009	<0.001 HS	0.468±0.021	<0.001 HS
	31-40	15	0.086±0.011		0.427±0.007		0.493±0.018		0.503±0.017	
	41-50	15	0.102±0.016		0.437±0.006		0.528±0.015		0.536±0.008	
	51-60	15	0.115±0.004		0.446±0.003		0.558±0.027		0.580±0.022	
	61-70	15	0.138±0.001		0.457±0.004		0.587±0.027		0.604±0.008	
^{234}U (mBq/L or Bq/kg)	21-30	15	1.654±0.210	<0.001 HS	9.246±0.085	<0.001 HS	10.157±0.216	<0.001 HS	10.309±0.476	<0.001 HS
	31-40	15	1.912±0.249		9.420±0.165		10.863±0.410		11.091±0.385	
	41-50	15	2.266±0.364		9.638±0.153		11.642±0.347		11.806±0.195	
	51-60	15	2.546±0.108		9.825±0.080		12.296±0.608		12.777±0.491	
	61-70	15	3.048±0.030		10.070±0.088		12.930±0.598		13.316±0.180	

HS: High significant difference between groups (p value <0.01).
S: Significant difference between groups (p value <0.05).

Table 5. Comparison between different ages for Uranium Concentrations and Isotopes in non-smokers for Serum, Urine, Hair and Nails samples

Radioactivity	Age (years)	N	Serum		Urine		Hair		Nails	
			Mean ± SD	$LSD_{0.05}$ P value	Mean ± SD	$LSD_{0.05}$ P value	Mean ± SD	$LSD_{0.05}$ P value	Mean ± SD	$LSD_{0.05}$P value
U_C (ppb or ppm)	21-30	15	0.100±0.010	0.002 HS	0.133±0.011	<0.001 HS	0.696±0.016	<0.001 HS	0.727±0.020	<0.001 HS
	31-40	15	0.102±0.005		0.143±0.010		0.715±0.011		0.741±0.014	
	41-50	15	0.110±0.005		0.159±0.010		0.745±0.008		0.762±0.016	
	51-60	15	0.118±0.014		0.166±0.015		0.752±0.012		0.769±0.006	
	61-70	15	0.120±0.020		0.186±0.021		0.773±0.032		0.782±0.008	
^{238}U (mBq/L or Bq/kg)	21-30	15	1.238±0.131	0.002 HS	1.652±0.140	<0.001 HS	8.603±0.201	<0.001 HS	8.984±0.246	<0.001 HS
	31-40	15	1.260±0.065		1.774±0.123		8.832±0.140		9.159±0.173	
	41-50	15	1.364±0.068		1.967±0.124		9.210±0.104		9.411±0.208	
	51-60	15	1.459±0.184		2.055±0.186		9.296±0.157		9.504±0.076	
	61-70	15	1.483±0.251		2.302±0.270		9.551±0.397		9.662±0.100	
^{235}U (mBq/L or Bq/kg)	21-30	15	0.057±0.005	0.002 HS	0.077±0.006	<0.001 HS	0.401±0.009	<0.001 HS	0.418±0.011	<0.001 HS
	31-40	15	0.058±0.002		0.082±0.005		0.411±0.006		0.427±0.008	
	41-50	15	0.063±0.003		0.091±0.005		0.429±0.005		0.439±0.009	
	51-60	15	0.067±0.008		0.095±0.008		0.433±0.007		0.443±0.003	
	61-70	15	0.069±0.011		0.107±0.012		0.445±0.018		0.450±0.004	
^{234}U	21-30	15	1.272±0.135	0.002 HS	1.697±0.143	<0.001 HS	8.839±0.207	<0.001 HS	9.231±0.253	<0.001 HS
	31-40	15	1.295±0.067		1.823±0.127		9.074±0.144		9.411±0.178	

2024, 21(12 Suppl.): 4105-4117
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P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

(mBq/L or Bq/kg)	41-50	15	1.402±0.070	2.021±0.127	9.463±0.107	9.669±0.214
	51-60	15	1.499±0.188	2.111±0.191	9.551±0.161	9.765±0.078
	61-70	15	1.523±0.258	2.365±0.278	9.813±0.408	9.927±0.103

HS: High significant difference between groups (p value <0.01)
S: Significant difference between groups (p value <0.05).

Uranium is a naturally occurring metal that may be found in minute quantities in many components of our environment, including soil, water, rocks, and living organisms. Every individual has trace amounts of naturally occurring uranium throughout their bodies. In its natural state, Uranium exhibits radioactivity but at a very low level, resulting in a similarly low level of radiotoxicity. Nevertheless, being a heavy metal, it demonstrates chemical toxicity comparable to lead, with its chemical toxicity being a more significant problem than its radiotoxicity. The deadly dosage of uranium is normally several grams (g), but the average quantity found in the adult male body is usually a few tens of milligrams (mg)[25]. Uranium is present everywhere in the environment. It is a dense metallic substance that is eliminated from the body via the bloodstream, urine, hair, and nails. Tobacco includes trace amounts of radioactive isotopes from the uranium and thorium series, which are known to be carcinogenic. The use of tobacco and its products amplifies the internal absorption and exposure to natural radionuclides, resulting in an elevated radiation dosage[26]. This study aimed to study the effects of tobacco smoking on increasing uranium concentrations in four types of biological samples (blood serum, urine, hair, and nails). Therefore, it was found that the mean uranium concentrations and the specific activity of uranium isotopes in the smoker group were higher than that of the non-smokers individuals. Independent sample T-test confirmed statistically a high significant difference in uranium between the smokers individuals and the non-smokers group ($P < 0.01$). The mean concentration of uranium in the group of smokers is greater than that in those who do not smoke. The uranium content in the blood serum of the smokers group is 61% higher than that of the non-smokers group. Furthermore, the findings indicated that the uranium levels in the blood serum of both smokers and non-smokers were below the acceptable threshold set by the International Commission on Radiological Protection (ICRP) of 0.115 ppm (115 ppb)[27]. The uranium concentrations in this study are comparatively lower than those reported by other studies[7,14,28]. The uranium content in the urine samples of the smokers group is 21% higher than the concentration in the urine samples of the non-smokers group. These findings suggest that smokers exhibit elevated levels of urine uranium compared to non-smokers as a consequence of the tobacco-derived uranium entering the body via smoking. On the other hand, the results of uranium concentrations in urine samples are about 65% higher than the ICRP reference value of urinary uranium 0.5 ppb (µg/l)[29] as revealed in Table 3. While the results of the uranium concentration in the non-smokers group were less than the permissible limit from the ICRP 0.5 ppb. Accordingly, this illustrates that tobacco smoking is increasingly seriously polluted by uranium in urine samples that study in the present study. The levels of urine uranium excretion vary across various nations. The current detection values are greater than those seen in persons from the USA[30], Germany[31], India[32], and Jordan[33], but lower than those observed in Finland[34]. Compared to physiological fluids like blood, urine, or other attainable tissues, nails, and hair have diverse uses and advantages. Hair and nails have many qualities that make them suitable tissues for lab experiments, including painless removal, sample collection, transportation, and good stability for temperature. Hair and nails serve as excretory organs, where trace elements present in the blood are accumulated. Consequently, the substances present in hair may serve as an indicator of uranium intake by inhalation. The uranium levels in the hair and nails of both smokers and non-smokers were analyzed to evaluate the likelihood of uranium intake via smoking. Thus, in the current investigation, hair and nails were used as potential biomarkers for measuring uranium levels in individuals who smoke. The uranium content in the hair and nail samples of the smokers' group is 81% higher than the concentration seen in the non-smokers' group. The increased concentration is likely due to the individual's smoking habit. However, given the depletion of uranium during the ashing procedures of tobacco leaves, it is proposed that uranium be breathed by smoking. In addition, there are no universally acknowledged set criteria for measuring uranium levels in hair and nails. The quantities of uranium in hair and nails may vary

2024, 21(12 Suppl.): 4105-4117
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Baghdad Science Journal

significantly across individuals and geographic locations. This variation is mostly influenced by dietary variables since most uranium in our bodies is derived from the food we consume. Generally, it is difficult to definitively attribute the concentration disparity only to uranium absorption via smoking since it is also recognized that human hair and nails accumulate airborne particles from the surrounding environment. The levels of urinary uranium excretion in other countries are different and it is found that the uranium concentrations in hair samples of the present study are lower than those of individuals from Blakan[35], Brazil[36], Finland[34], and France[37]. While, the results of uranium concentrations in nail samples were lower than in other countries such as Finland[37], Sweden[38], and Serbia[39]. Overall, the variations in uranium concentrations observed in the biological samples of this study can be attributed to differences in analytical methods used to measure uranium levels, variations in sample collection and preparation methods, regional disparities, and fundamental demographic differences influenced by factors such as nutrition, environment, and the use of cosmetics and pharmaceuticals. The results of the present study have shown that uranium concentrations in attainable tissues, nails, and hair were higher than in blood serum and urine. This is because, in addition to the effect of cigarettes, there is another effect which is external pollutants such as pharmaceuticals or cosmetics, causing an increase in the concentrations of uranium. When comparing the values of the specific activity of uranium isotopes with the permissible limit of uranium concentrations in the natural limit, it can be seen that 100% of the investigated samples are less than the permissible limit value. Natural uranium is found in natural uranium water, and it has a typical level of 25.4 Bq/mg[40] for natural uranium, which contains three forms of uranium isotope ^{238}U, ^{235}U, and ^{234}U. Where the uranium concentrations in all biological samples of smokers and non-smokers were found to increase with the increasing of life years, that means; there is a cumulative relationship between the age and the uranium concentration for smokers and non-smokers, as shown in Tables 4 and 5. These results show that the majority of smokers and non-smokers exposed to high contents of uranium by ingestion and inhalation from the atmosphere are contaminated due to military and human activities. This discovery highlights the importance of uranium excretion as one gets older since it is influenced by the amount of uranium consumed during infancy, assuming a consistent level of consumption. The ICRP uranium model and other research suggest that the amount of uranium in the body increases as a person ages[7,28,41]. Following the conclusion of research conducted in the Al-Najaf governorate, comprising a sample of seventy-five individuals of various age groups who habitually smoke a minimum of five cigarettes each day. The findings of this research demonstrate that biological samples are a very effective method for evaluating individuals and groups for potential uranium exposure resulting from smoking. This study corroborates the results of previous researchers who have arrived at the same conclusions.

It is possible to find the relationship between the results of studied uranium concentrations and isotopes in four biological samples for smokers and non-smokers in the present study using the correlation test utilizing the Pearson coefficient for all biological samples of smokers and non-smokers used. Table 6 displays the correlation matrix for the results of the studied uranium concentrations U_C with biological samples. From Table 6, the Pearson correlation in smokers' samples shows that the uranium concentrations between blood serum and urine have a very strong positive, between blood serum with hair and nails have a strong positive, between urine with hair and nails has a strong positive, and between hair with nails has a moderate positive. According to the P values for uranium concentrations between all biological samples for smokers, samples have high significance with a P-value < 0.01, so the results are statistically significant. Also, from Table 6, the Pearson correlation in non-smokers' samples shows that the uranium concentrations between all biological samples in the present study (blood serum, urine, hair, and nails) have a moderate positive. And high significance with a P-value < 0.01, so the results are statistically significant.

2024, 21(12 Suppl.): 4105-4117
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Baghdad Science Journal

Table 6. Pearson correlation UC between variables for smokers and non-smokers groups for all sample (Serum, Urine, Hair and Nails)

Variables		Correlation	Serum	Urine	Hair	Nails
Smoker	Serum	Pearson Correlation	1	0.837**	0.802**	0.847**
		P value		0.000	0.000	0.000
	Urine	Pearson Correlation	0.837**	1	0.844**	0.892**
		P value	0.000		0.000	0.000
	Hair	Pearson Correlation	0.802**	0.844**	1	0.847**
		P value	0.000	0.000		0.000
	Nails	Pearson Correlation	0.847**	0.892**	0.847**	1
		P value	0.000	0.000	0.000	
Non-smoker	Serum	Pearson Correlation	1	0.254	0.601**	0.452**
		P value		0.076	0.000	0.001
	Urine	Pearson Correlation	0.254	1	0.574**	0.625**
		P value	0.076		0.000	0.000
	Hair	Pearson Correlation	0.601**	0.574**	1	0.716**
		P value	0.000	0.000		0.000
	Nails	Pearson Correlation	0.452**	0.625**	0.716**	1
		P value	0.001	0.000	0.000	

****Correlation is significant at the 0.01 level (2-tailed).**

Conclusion

In the current study, uranium concentrations and it is isotopes such as (^{238}U, ^{235}U, and ^{234}U) concentrations were assessed in the biological samples (blood serum, urine, hair, and nails) of smokers and non-smokers of different ages collected from Al-Najaf governorate, Iraq. The mean uranium concentrations in smokers' samples in the present study were higher than those of non-smokers. Also, the mean of uranium concentrations in all samples of smokers and non-smokers was increased with an increase in age range. The trend of uranium concentrations in biological samples in two groups (smokers and non-smokers) are as follows: nails >hair urine> blood serum. The results of the studied uranium concentrations in urine samples for smoker's samples were higher than the world average according to the ICRP value and other literature reviews. While, the uranium concentrations in other samples (blood serum, hair, and nails) in smokers' samples in the present study were lower than those in uranium in different countries. Moreover, the mean of natural uranium isotopes in all samples of smokers and non-smokers were within the world limit. Conversely, the mean of uranium concentrations in all smoker and non-smoker samples increased with an increase in age range. Statistically, the results obtained from all samples (blood serum, urine, hair, and nails) according to smoking and age indicates a positive correlation between these factors and uranium concentrations. The findings unequivocally established that the uranium detected in the biological samples can be attributed to the act of smoking, hence establishing a direct connection to the origin of the exposure. Blood serum, urine, hair, and nails may be used as very effective indicators of occupational or environmental uranium exposure, offering valuable insights into its origin. However, the use of hair and nails for bioassay is appealing due to its efficacy in measuring bio concentration. Additionally, these samples may be conveniently preserved and the concentration obtained represents a comprehensive value. In general, these differences can be attributed to the use of various analytical methods in measuring the levels of uranium concentrations, in the method of collecting and preparing samples, or regional differences, or basic demographic differences that are affected by nutrition, the environment, and the use of cosmetics and pharmaceuticals.

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Baghdad Science Journal

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Kufa (Application No: MEC-70).

Authors' Contribution Statement

All authors contributed equally in the design and conception of the study. A. A. A. carried out the experiment. M. H. O. wrote the manuscript and A. A. A. helped supervise the project; all authors reviewed the manuscript and approved the final manuscript.

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تقدير اليورانيوم ونظائره في العينات البيولوجية للمدخنين

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[6]قسم المعلومات والهندسة الكهربائية والرياضيات التطبيقية، امبرا مختبر البيئة المحيطة والإشعاع، جامعة ساليرنو، طريق جيوفاني باولو الثاني، 132، I-84084 فيشيانو، إيطاليا. I-84084 فيشيانو، إيطاليا.

الخلاصة

أجريت هذه الدراسة في محافظة النجف بالعراق لتحليل عينات بيولوجية من المدخنين وغير المدخنين. تم استخدام العينات، بما في ذلك مصل الدم والبول والشعر والأظافر، كمؤشرات حيوية لتحديد تركيزات اليورانيوم (U_C) ونظائره (^{238}U، ^{235}U، و^{234}U). وباستخدام طريقة التعرض الطبيعي، تم استخدام كاشف المسار النووي (CR-39، المملكة المتحدة) لقياس تركيزات اليورانيوم في العينات. تم جمع خمسة وسبعين عينة من مصل الدم والبول والشعر والأظافر للمدخنين من الأصحاء وخمسين عينة لغير المدخنين من الأصحاء في خمس فئات عمرية لجميع العينات. اعتمدت هذه الدراسة على العمر والتدخين لمقارنة النتائج وتحديد تأثيرها على تراكيز اليورانيوم. أظهرت النتائج أن متوسط قيم تراكيز اليورانيوم في مصل الدم والبول والشعر والأظافر لدى المدخنين كان 0.180±0.042 جزء في المليون، 0.759±0.024 جزء في المليون، 0.912±0.085 جزء في المليون، و0.934±0.091 جزء في المليون، على التوالي. بينما بلغ متوسط قيم تراكيز اليورانيوم في مصل الدم والبول والشعر والأظافر لدى غير المدخنين 0.110±0.014 جزء في المليون، 0.157±0.023 جزء في المليون، 0.736±0.032 جزء في المليون، و0.756±0.024 جزء في المليون، على التوالي. وتشير النتائج والمقارنات إلى أن جميع تراكيز اليورانيوم تعتمد على المتغيرات التي بنيت عليها هذه الدراسة (العمر والتدخين). وبمقارنة تراكيز اليورانيوم لجميع العينات البيولوجية للمدخنين وغير المدخنين، كانت قيمة P ذات دلالة إحصائية عالية حيث كانت أقل من 0.001. ووفقا لنتائج عينات الدراسة، فإن متوسط قيم U_C، ^{238}U، ^{235}U، و^{234}U للعينات البيولوجية لدى المدخنين كانت أعلى منها لدى غير المدخنين. وبالتالي، يمكن القول أن تدخين السجائر يستخدم كمؤشر حيوي لوجود تركيزات اليورانيوم.

الكلمات المفتاحية: العينات البيولوجية، CR-39، التدخين، اليورانيوم، ونظائر اليورانيوم.