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Radon Concentration in Biological Samples of Smokers and Non-smokers Using Lexan Detector

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ABSTRACT

This study was conducted in the Najaf Governorate, Iraq, to analyze radon concentration in biological samples from smokers and non-smokers. The samples, including blood serum, urine, hair, and nails, were used as biomarkers to determine the presence or absence of radon (^{222}Rn). Using a natural exposure method, the nuclear track detector (Lexan, Belgium) was utilized to measure these radon concentrations 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 radon concentrations. The results show that the average values of radon concentrations (in Bq/m^3) in blood serum, urine, hair, and nails for smokers were 54.7 ± 22.1, 62.9 ± 23.1, 34.7 ± 11.2, and 41.7 ± 15.2, respectively. Meanwhile, the average values of radon concentrations (in Bq/m^3) in blood serum, urine, hair, and nails for non-smokers were 24.2 ± 6.0, 30.0 ± 6.3, 18.7 ± 5.2, and 21.6 ± 6.9 respectively. The results and comparisons indicate that radon concentrations depend on the variables on which this study was based (age and smoking). Smokers and non-smokers had different levels of radon in all biological samples. The P-value was <0.001, which means it was statistically significant. According to the results of the study samples within the study area, the mean values of (^{222}Rn) for biological samples in smokers were higher than in non-smokers.

INTRODUCTION

Researchers have been increasingly focused on the environmental ramifications of radon during the last several decades. One primary issue is the potential health danger it presents, while another problem is its extensive usage as an environmental tracer [1].

One radioactive gas that occurs naturally is radon. The gas is chemically inert and releases alpha particles. This colorless, tasteless, and odorless gas is produced by trace quantities of naturally occurring radioactive uranium, radium, and thorium throughout the Earth's crust [2,3].

The ^{222}Rn isotope is plentiful with a half-life of 3.8 days. Radon undergoes radioactive decay, resulting in the production of 5.49 MeV alpha particles known as radioactive daughters. Radon is present in the earth's crust, soil, air, and groundwater. Additionally, it is capable of dissolving in water [2,4].

Inhaling ^{222}Rn poses a significant concern. The presence of cigarette smoke in the lungs leads to the attachment of radon daughters to lung cells by radioactive particles, resulting in impairment to lung function due to the heightened alpha radiation [5].

Despite a wealth of scientific studies conducted over the last several decades that establish a strong connection between cigarette smoking and a wide range of negative health consequences, a concerning proportion of individuals persist in engaging in this habit. According to WHO, around 1 billion men and 250 million women throughout the globe smoke a combined total of over 15 billion cigarettes per day [6].

Therefore, there are several scenarios when having a precise record of an individual's present and past smoking intensity is crucial. Accurate projections of an individual's risk of acquiring

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smoking-related disorders are contingent upon obtaining this information. This is particularly important in epidemiologic research where smoking may serve as a confounding factor, as well as in clinical trials where smoking might impact drug metabolism [7].

Tobacco smoke comprises of toxic substances that pose a threat to both those who smoke and those who do not. Inhaling even a little amount of tobacco smoke may have detrimental effects on one's health. Cigarettes consist of around 600 components. According to the American Lung Association, the combustion of cigarettes produces over 7,000 compounds. Out of that extensive range of compounds included in tobacco smoke, a minimum of 250 have been identified as detrimental. These include substances such as hydrogen cyanide, carbon monoxide, and ammonia. Out of the 250 identified noxious substances present in tobacco smoke, a minimum of 69 have the potential to induce cancer [8,9].

Studies indicate a growing body of data supporting a multiplicative synergistic relationship between radon and smoking. Estimates suggest that the risk of developing lung cancer might increase up to 18 times when both radon exposure and cigarette smoking are combined [10,11].

After being breathed in, radon gas enters the lung and makes its way to the rest of the body via the blood, which carries it to the organs [12]. Epidemiological studies are often influenced by lifestyle and demographic variables, however, in vitro studies provide the benefit of eliminating confounding factors like smoking and exposure to known or suspected carcinogens [10].

The present study aimed to investigate whether cigarette tobacco is a potential source of radon concentration in the blood, urine, hair, and nails of male smokers and non-smokers in Alnajaf Governorate, Iraq. Therefore, the radon levels from radioactive decay were measured in biological samples using Lexan nuclear track detectors.

MATERIALS AND METHODS

Area of study

Iraq is located within latitude 28°57'0"N to 37°5'0"N and longitude 38°35'0"E to 48°45'0"E (Fig. 1). It covers an area of 437,125.41 km^2, and its boundaries share five adjacent countries [13]. Al-Najaf Province is located in the southwest region of Iraq, as shown in Fig. 1. It spans an area of (28,537) km^2, with latitude ranging from (29°50'00" N-32°21'00" N) and longitude ranging from (42°50'00" E-45°44'00" E). The total area covered is about 28,824 km^2 [14].

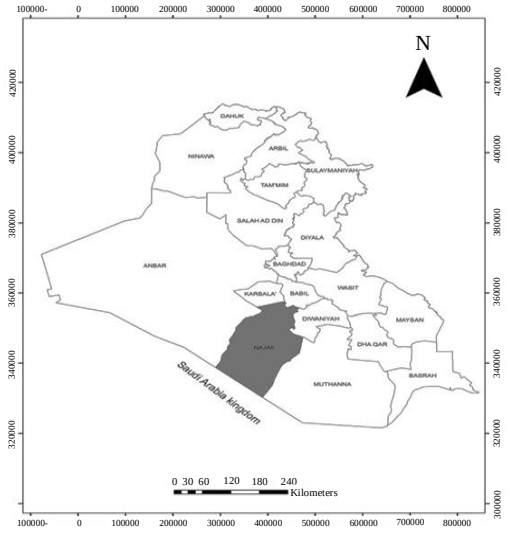

Fig. 1. Al-Najaf governorate area.

METHODOLOGY

Sampling

The present study was conducted in (2023) on male in Iraq's Al-Najaf Governorate on 75 randomly selected smokers and 50 non-smokers as a control group at different age ranges, as shown in Table 1. The selection of participants who smoke was limited to individuals who do not have a documented history of substance misuse, regularly consume a minimum of five cigarettes per day, and lack occupational experience in professions involving heavy metals or other chemical substances. Conversely, the individuals who did not smoke and were of similar age to the group of smokers had no prior experience with cigarette smoking or substance abuse. This study did not include those who had pre-existing conditions such as anemia, hypertension, kidney illness, diabetes, digestive disorders, or cardiovascular disease, as well as those undergoing treatments for metal toxicity and taking medication supplements. All participants in this study provided written consent, and various data and information were gathered, including age, occupation, marital status, education level, history of cigarette smoking, smoking duration, type of cigarettes used, and average daily cigarette consumption. We took great care to select biological samples for smokers who used the same type of cigarette.

Table 1. Data of samples in the present study.

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

In the current work, samples of blood, urine, hair, and nails were taken from healthy people (smokers and non-smokers) participating in this study, amounting to 125 people, and each participant was assigned a unique code. Participants were divided into 75 smokers and 50 non-smokers aged between 21 and 70. Four biological samples were collected from the same individuals and prepared for measuring alpha emitters as follows.

a. Blood serum samples: 5-6 ml of blood was collected and stored in gel tubes. Subsequently, the blood samples were allowed to clot for 5-10 min to facilitate serum separation from the blood during the subsequent centrifugation process. Subsequently, the serum samples acquired were transferred into pristine, sterile, hermetically sealed (Eppendorf tubes), each with a volume of 1 ml, and thereon stored in a freezer to prepare for the measurement process [15].

b. Urine samples: 5-6 ml of urine samples 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 [16].

c. Hair and nail samples: 0.5 g of hair and nails were clipped from the participants and stored directly in zipped polythene bags, after which the samples were placed in a glass beaker containing a detergent solution to remove any external impurities such as dirt, fat, and perspiration, 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 samples (hair and nails) were cut into small pieces, mixed thoroughly, and put in plastic containers bearing the symbol and number of each relevant sample [17,18].

Irradiation method

Sterilized 10 ml plastic tubes were used to contain 1 ml of blood serum, urine, and 0.5 g of hair and nails. A (175μm) thick nuclear Lexan detector, measuring at 1 × 1 cm^2, obtained from polycarbonate (PC), Belgium, was placed on one of the top sides of the tube. It was positioned 1 cm away from the cover and was firmly attached using adhesive tape. A distance of 7.5 cm was maintained between the surface of the sample and the detector for 90 days. As shown in Fig. 2.

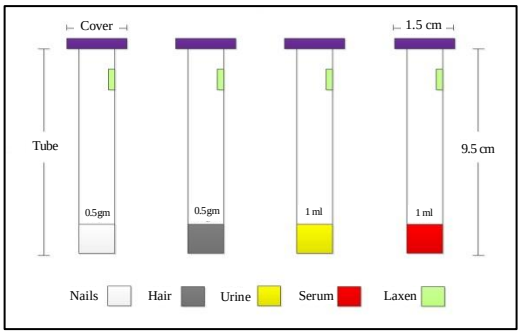

Fig. 2. Plastic tubes for measuring radon concentrations using Lexan detector.

Following the conclusion of the exposure time, the CR-39 detectors were extracted from the plastic containers and subjected to chemical etching using NaOH (6.25 N) in a water bath (HH-420, Germany) at a temperature of 98 °C for 1 hour [19].

The abrasive solution was prepared by dissolving 100 g of sodium hydroxide granules in 400 ml of distilled water using the given equation (1) [20].

$$W = N \times V \times W_{eq} \quad (1)$$

where *W* is the weight of sodium hydroxide (NaOH) granules, *N* is the required standard (6.25N), *V* is the volume of distilled water, W_{eq} is the molecular weight of (NaOH), which is equal to 40.

Subsequently, the detectors were cleansed by employing distilled water. Finally, the number of tracks per cm^2 was counted using an optical microscope (power 400×) connected to a micro camera connected to a personal computer with a known field of view and digital eyepiece [21].

Radon concentration measurement

The density of the tracks (ρ) in biological samples was obtained according to the following relation Eq. (2) [22].

$$(\rho) = \frac{N_{ave.}}{A} \quad (2)$$

where ρ is track density (track /cm^2), *N* is an average of total tracks (track) and *A* is an area of a view field (cm^2).

Radon concentration levels (C_{Rn}) in Bq/m^3 units are calculated by the following relation Eq. (3) [23,24]:

$$C_{Rn} = \frac{\rho}{Kt} \quad (3)$$

where *t* is the exposure time (90 days), and *K* is the calibration factor (0.332 ± 0.061 track/cm^2 per Bq/m^3/day). Lexan detector was calibrated using a ^{226}Ra source (activity = 6600 Bq), which generates ^{222}Rn gas as a radioactive product during the dissolution of the same element. The current investigation employed 0.5, 1, 1.5, 2, 2.5, and 3 day

irradiation for a Lexan detector in a cylindrical container. The values of radon concentrations were measured from a standard source of ^{226}Ra using Air Things device. Fig. 3 shows the curve of the calibration factor (K) for the dosimeters exposed.

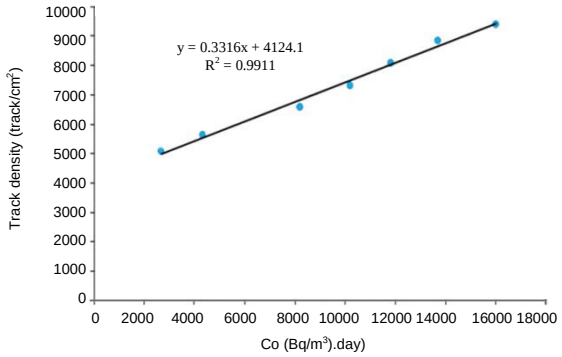

Fig. 3. Calibration curve of radon concentration for biological samples.

Background radon concentrations were measured by placing 4 detectors (Lexan) in 4 empty tubes for 90 days, after which the total number of traces was calculated and subtracted from the number of traces in the biological samples.

Statistical analysis

The statistical analyses of the data were conducted using IBM SPSS Statistics software, version 27 for the Windows operating system. Graphing was also performed as part of the analysis. The analyses encompass the mean, standard deviation, and Pearson correlation coefficients.

RESULTS AND DISCUSSION

The ^{222}Rn concentration results obtained from blood serum, urine, hair, and nail samples for smokers and non-smokers, as shown in Table 2. The mean values of radon concentrations (in Bq/m^3) in blood serum, urine, hair, and nail samples of smokers were 54.7 ± 22.1, 62.9 ± 23.1, 34.7 ± 11.2, and 41.7 ± 15.2, respectively. While, the mean values of radon concentrations (in Bq/m^3) in blood serum, urine, hair, and nail samples of non-smokers were 24.2 ± 6.0, 30.0 ± 6.3, 18.7 ± 5.2, and 21.6 ± 6.9, respectively. Independent sample t-test was 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 2, there is a highly significant difference between the smoker and non-smoker groups for ^{222}Rn, since the p-value is less than 0.01. Generally, the alpha emitters' mean for all Al-Najaf governorate biological samples in smokers samples was higher than that of non-smokers.

Table 2. Comparison between smokers and non-smoker groups for radon for Serum, Urine, Hair, and Nail samples.

Biological sample	Groups	N	Mean ± SD	P value
Serum	Smoker	75	54.7±22.1	<0.001
	Non- smoker	50	24.2±6.0	HS
Urine	Smoker	75	62.9±23.1	<0.001
	Non-smoker	50	30.0±6.3	HS
Hair	Smoker	75	34.7±11.2	<0.001
	Non-smoker	50	18.7±5.2	HS
Nails	Smoker	75	41.7±15.2	<0.001
	Non-smoker	50	21.6±6.9	HS

HS: High significant difference between groups (p value <0.01)

The results of ^{222}Rn concentration obtained from 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 as shown in Tables 3. The study categorized the results of alpha emitters in Table 3 in all samples based on age using five intervals. The findings revealed that the highest levels of alpha emitters 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 ^{222}Rn in all biological samples according to smoking and age showed that the differences in all results (Table 3) by smoking and age indicate high significant differences at significant levels of LSD (0.05) and P-value (0.01).

Table 3. Comparison between different ages for radon in smokers and non-smokers for Serum, Urine, Hair and Nails samples.

Sample	Age (years)	Smokers			Non-smokers		
		N	Mean ± SD	$LSD_{0.05}$ P value	N	Mean ± SD	$LSD_{0.05}$ P value
Serum	21-30	15	29.1±8.8	<0.001 HS	10	19.3±5.2	0.003 HS
	31-40	15	37.9±10.2		10	22.8±3.7	
	41-50	15	55.4±12.8		10	23.5±7.6	
	51-60	15	67.5±11.9		10	26.7±3.6	
	61-70	15	83.4±5.8		10	28.6±5.2	
Urine	21-30	15	34.2±8.5	<0.001 HS	10	26.7±8.0	0.09 NS
	31-40	15	52.8±20.1		10	28.4±6.2	
	41-50	15	63.9±11.7		10	29.2±4.1	
	51-60	15	73.1±12.0		10	32.2±5.8	
	61-70	15	90.7±11.9		10	33.4±5.2	
Hair	21-30	15	23.0±12.3	<0.001 HS	10	14.6±6.1	<0.001 HS
	31-40	15	30.8±9.7		10	15.7±5.8	
	41-50	15	37.3±7.0		10	19.0±2.2	
	51-60	15	38.1±4.4		10	21.6±2.3	
	61-70	15	44.4±8.4		10	22.8±3.3	
Nails	21-30	15	27.1±11.4	<0.001 HS	10	16.3±5.1	0.009 HS
	31-40	15	36.5±18.3		10	19.2±9.2	
	41-50	15	43.4±9.2		10	21.8±5.3	
	51-60	15	46.0±9.8		10	24.7±6.1	
	61-70	15	55.5±9.5		10	25.8±4.2	

HS: High significant difference between groups (p value <0.01)

The study examined the correlation between the levels of radon in serum, urine, hair, and nail samples of individuals categorized as smokers and non-smokers across five age groups (21-30 y, 31-40 y, 41-50 y, 51-60 y, and 61-70 y). This information is presented in Fig. 4, 5, 6, and 7 respectively. Based on these findings, it can be observed that the average concentration of radon in serum, urine, hair, and nail samples among individuals belonging to various age groups who smoke exhibited the highest levels of concentration when compared to non-smokers.

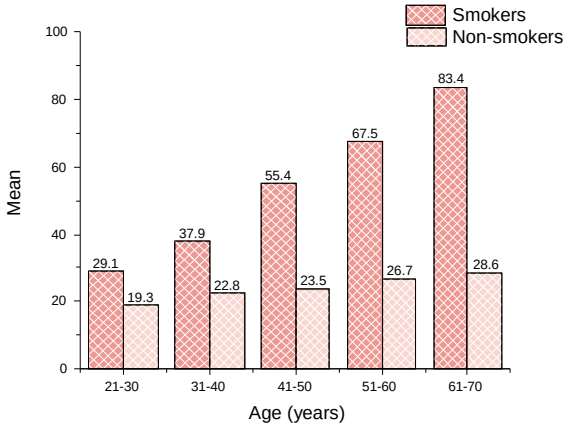

Fig. 4. Relation between smokers and non-smokers for five age groups (21-30y, 31-40y, 41-50y, 51-60y, and 61-70y) on serum.

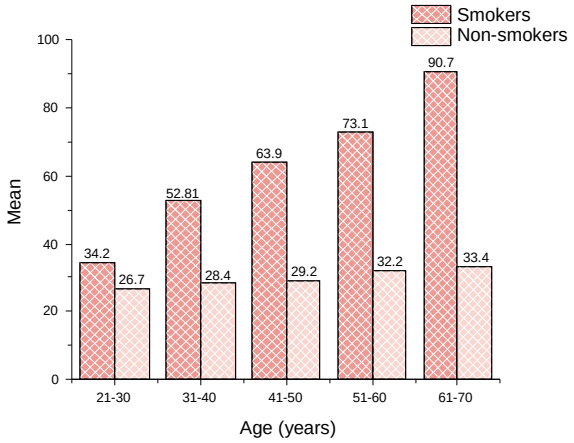

Fig. 5. Relation between smokers and non-smokers for five age groups (21-30y, 31-40y, 41-50y, 51-60y, and 61-70y) on urine.

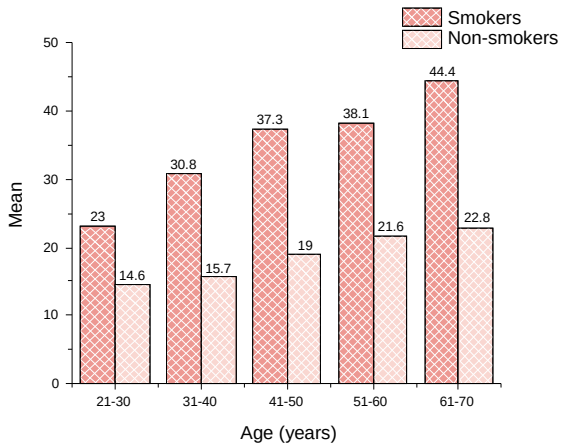

Fig. 6. Relation between smokers and non-smokers for five age groups (21-30y, 31-40y, 41-50y, 51-60y, and 61-70y) on hair.

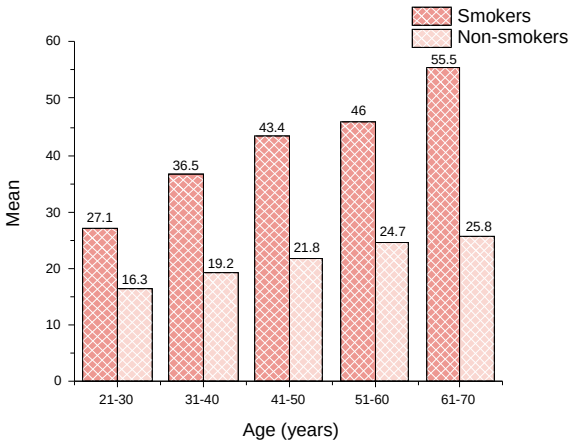

Fig. 7. Relation between smokers and non-smokers for five age groups (21-30y, 31-40y, 41-50y, 51-60y, and 61-70y) on nails.

DISCUSSION

The tobacco plant may absorb naturally existing radium in the soil and from fertilizers through its roots.

Radium undergoes radioactive decay, resulting in the emission of radon gas, which subsequently ascends from the soil surrounding the plants. Radon undergoes radioactive decay, resulting in the formation of the radioactive isotopes ^{210}Pb and ^{210}Po. During the plant's growth, radon emitted from fertilizer, as well as radon decay products naturally present in the soil and rocks nearby, adhere to the adhesive hairs on the underside of tobacco leaves, known as trichomes. Precipitation does not eliminate them. ^{210}Po is a radioactive substance that emits alpha particles and poses the highest level of risk. The global average of allowed radon gas in air has different values according to world organizations such as WHO (100 Bq/m^3) [25] and (200 Bq/m^3) according to ICRP [26], as well as national organizations like EPA (74-148 Bq/m^3) [27] and CFR (39 Bq/m^3) [28].

The primary values of the radon concentrations for smoker samples in the present work in blood serum, urine, hair, and nails were within the global average according to WHO, EPA, and ICRP, except for blood serum, urine, some of hair and nail samples were higher than CFR. Meanwhile, the primary values of the radon concentrations for non-smoker samples in blood serum, urine, hair, and nails were within the global average for all limits by the world organizations.

The ANOVA and independent test results for comparison means of alpha emitters for smoking factor indicate statistically significant differences at 0.01. The results in all samples found that the mean alpha emitters in smokers were higher than in non-smokers. Also, it has been found that the percentage

increase of radon gas in the blood serum is 44.29 %, in urine 47.7 %, in hair 53.9 %, and in nails 51.7 % from non-smokers.

Radon daughters readily adhere to smoke particles in the presence of cigarette smoke. Therefore, smoking amplifies the alpha radiation dosage to a smoker's lungs caused by the natural radon daughters. Smokers who are exposed to radon have a higher likelihood of developing lung cancer compared to individuals who do not smoke. These radioactive components are still present in cigarettes manufactured from this tobacco. Radioactive particles accumulate in the lungs of smokers, persisting as long as the individual continues to smoke. Over a prolonged period, radiation can cause harm to the lungs and increase the risk of developing lung cancer.

Tobacco consumption can heighten individuals' susceptibility to additional carcinogenic substances. Data in Table 2 highlighted that the alpha emitters in biological samples for smokers and non-smokers were written in the following order: urine > blood serum > nail > hair. This is because ingested alpha emitters from cigarette smoking that are not deposited in body tissues are excreted in the urine within a few days. The test can only detect an exposure approximately a week prior and does not provide information about body retention. As with other heavy metals, alpha emitters may be cleared from the circulation and sequestered into tissues after exposure has ceased. The observed outcome can be attributed to the combined influence of radon and cigarette smoking, which may have a synergistic impact. Smokers are expected to have a risk from radon around 25 times higher than non-smokers. While it is true that breathed radon can expose other organs to radiation, the dose delivered to these organs is far lower than the lungs. Furthermore, the results shown in Table 3 indicate that the levels of alpha emitters in biological samples from smokers and non-smokers exhibited a positive correlation with advancing age.

The observed rise in age-related increases among both smokers and non-smokers might be attributed to many factors, including nutritional consumption, particularly throughout youth, which is positively associated with age. Furthermore, when continuously exposed to cigarette smoking, the amount of radon substance increased due to bone turnover would grow in proportion to the duration of exposure and the level of radioactive substances accumulated in the body (skeletal load).

It is possible to find the relationship between the results of studied radon concentrations 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. Table 4 displays the correlation matrix for the results of the studied radon concentrations with biological samples. From Table 4, the Pearson correlation in smokers' samples shows that the radon concentrations between blood serum and urine is very strongly positive, between blood serum with hair and nails is strongly positive, between urine with hair and nails is strongly positive, and between hair with nails is moderately positive. According to the P values for radon concentrations between all biological samples for smokers, the samples have a high significance with a P-value < 0.01, so the results are statistically significant. Also, from Table 4, the Pearson correlation in non-smokers' samples shows that the radon concentrations between all biological samples in the present study (blood serum, urine, hair, and nails) are moderately positive and high significance with a P-value < 0.01, so the results are statistically significant.

Table 4. Pearson correlation ^{222}Rn 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	0.831**	0.692**	0.703**
		P value		0.000	0.000	0.000
	Urine	Pearson Correlation	0.831**	1	0.708**	0.788**
		P value	0.000		0.000	0.000
	Hair	Pearson Correlation	0.692**	0.708**	1	0.642**
		P value	0.000	0.000		0.000
	Nails	Pearson Correlation	0.703**	0.788**	0.642**	1
		P value	0.000	0.000	0.000	
Non-smoker	Serum	Pearson Correlation	1	0.404**	0.428**	0.429**
		P value		0.004	0.002	0.002
	Urine	Pearson Correlation	0.404**	1	0.473**	0.524**
		P value	0.004		0.001	0.000
	Hair	Pearson Correlation	0.428**	0.473**	1	0.582**
		P value	0.002	0.001		0.000
	Nails	Pearson Correlation	0.429**	0.524**	0.582**	1
		P value	0.002	0.000	0.000	

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

When we compared the currently investigated mean values of ^{222}Rn concentrations in biological samples (blood serum, urine, hair, and nails) with the available of the previous studies, as shown in Table 5, we found that the radon concentrations in the present study were the highest.

Table 5. Comparison of the investigated current study with other studies.

Country	Sample	^{222}Rn (Bq/m^3)		Refrence
		Smokers	No-smokers	
Iraq (Babylon)	Blood	4.98	3.59	[29]
Korea	Blood	4.27	1.40	[30]
Iraq (Diwaniyah)	Urine	4.1619	4.0816	[31]
France	Hair	2.2	1.5	[32]
Iraq (Najaf)	Serum	54.7	24.2	Present work
	Urine	62.9	30.0	
	Hair	34.7	18.7	
	Nails	41.7	21.6	

CONCLUSION

In the current study, ^{222}Rn 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 results of the studied radon concentrations for smokers in most samples were higher than the world average according to CFR value. The mean radon concentrations in smokers' samples in the present study were higher than those of non-smokers.

On the other side, the mean of radon concentrations in all samples of smokers and non-smokers was increased with an increase in age range. The trend of radon concentrations in biological samples in two groups (smokers and non-smokers) are as follows: urine > blood serum > nails > hair.

Statistically, the results obtained from all samples (blood serum, urine, hair, and nails) according to smoking and age indicate a positive correlation between these factors and the radon concentrations. Therefore, it concludes that smoking causes an increase in the concentration of ^{222}Rn in biological samples of smokers. So, it recommends smokers to stop smoking to keep their good health.

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AUTHOR CONTRIBUTION

All authors contributed equally in the design and conception of the study. Abdulhussein A. Alkufi carried out the experiment. Mohanad H. Oleiwi wrote the manuscript and Ali Abid Abojassim helped supervise the project; all authors reviewed the manuscript and approved the final manuscript.

FUNDING

The authors received no funding for the study.

DATA AVAILABILITY

The datasets generated and analyzed during the study are available per request from the corresponding author.

CONFLICT OF INTEREST

The authors affirm that there is no conflict of interest.

AVAILABILITY OF DATA AND MATERIALS

The data and supporting information are available within the article.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional University of Kufa (Application No: MEC-70).

HUMAN AND ANIMAL GUIDELINES

All procedures performed in studies involving human participants were in accordance with the 2013 Helsinki Declaration and its later amendments or comparable ethical standards.

CONSENT FOR PUBLICATION

It has been taken informed consent from volunteers for this study.

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