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In-vitro radiological and toxicological detection in urine samples of cancer patients in Al- Diwaniyah governorate, Iraq

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► ***Original article***

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ABSTRACT

Background: The examination of the urine samples is the preferable procedure to observing the inner exposure to radioactive and toxic pollutants in human body. This study focuses on the uranium concentrations (UC) and toxic metal concentrations (TMC) in urine samples of cancer patients and healthy volunteers from Al-Diwaniyah governorate. ***Materials and Methods***: Fission track technique with CR -39 track detectors and atomic absorption spectrophotometry were used to determine the uranium and toxic metals, respectively. ***Results***: For the patients group the result of UC ranged between 5.04 µg /l to 2.21 µg/l, with the average value was 3.83 µg/l, while the data of UC of the healthy group varied from 3.15 µg/l to 0.49 µg/l, with the average value was 1.91 µg/l. The average values of TMC (Pb, Ni and Cd) in urine samples of cancer patients were 0.342 mg/l, 0.231 mg/l, and 0.075 mg/l respectively, whereas the average values of corresponding elements in urine samples of healthy subjects were 0.233 mg/ l, 0.127 mg/l, and 0.025 mg/l, respectively. ***Conclusion***: The current in vitro investigation shows the pollution in urine samples of the participants through UC and TMC and also finds that the UC and TMC in urine of cancer patients significantly higher than healthy volunteers. The results of (UC) and (TMC) in urine samples of the participants exceeded the recommended limits.

INTRODUCTION

Radiological and toxicological contaminants can cause cancerous disease and other changes in the health of humans and the living world in general, thus substantially influencing the changes in the environment [1-2]. Uranium - heavy element, silver colour - is a radioactive natural element that is commonly distributed in the crust of the earth and it has both radioactive and toxic effects. It occurs in numerous minerals such as phosphate rock and sand [3-5]. The daily consumption from uranium via the human body originates from water, air and food where the latter being the largest source [6]. There are some other pollutants within the surrounding environment such as toxic metals (TM) that are considered as one of the problems that have received widespread around the world. The high impact in the atmosphere and nature of toxic elements justifies the increased interest in their monitoring. Heavy metals such as lead (Pb), nickel (Ni) and cadmium (Cd) are generally toxic to both human body and ecological balance. Lead (Pb) has carcinogenic properties by weakening both the respiratory and suppressing the immune system. This metal is particularly damaging in kids. Nickel (Ni) is concentrated mainly within the spinal cord and brain due to its carcinogenicity and mutability. Cadmium (Cd) often concentrated in the kidney which considered the principle site of Cd accumulation, it also accumulates in the lungs and the heart.

Inside the body, heavy metals are essential in small amounts but will be toxic in larger doses. The main paths of human's exposure to the uranium and toxic metals are during inhalation polluted air, contaminated water, contaminated soil and industrial waste [1, 7, 8]. Human-related emissions of uranium and toxic elements exceed natural resources as a result of the widespread use of these elements in a wide variety of manufacturing processes. Uranium and toxic elements deposited into the bones and other tissues are consequently released out of the bloodstream causing various health troubles, ranging from cancer to kidney failure and other unknown diseases [9, 10]. The assay of urine samples is significant biomarker for evaluating the radiological and toxicological pollutants inside the human body it provide a reliable indicator of internal exposure [11].

The Iraqi environment has been badly affects, especially in the southern part of the radiological and toxic contaminants due to the human and military activities which lead to raise the levels of the environmental contaminants and increase the cases of cancerous disease [1, 12]. Neutron activation technique with CR-39 detector is being useful to estimate uranium contents in biological samples as reported elsewhere [13]. Sensitive and reliable detection of heavy metal ions at trace levels in urine

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samples is a very essential part of research on the environment. For this reason, atomic absorption spectrophotometer (AAS) has been used continuously [14]. This study was the first study carried out in Al-Diwaniyah governorate to determine the uranium contents (UC) and toxic metals content (TMC) in urine samples of cancer patients and healthy volunteers.

MATERIALS AND METHODS

Sample collection

In the current examination 60 sample of urine samples were collected from males and females volunteers from Al-Diwaniyah. The urine samples had been taking from two groups: Cancer patients and healthy individuals. The cancer patients group was collected from numerous hospitals in Al-Diwaniyah. Table 1 shows the type of cancers in patients. Similarly, healthy individuals were collected from different regions of Al-Diwaniyah. We took into consideration that the number of males and females is identical between the study groups in terms of demographic information including age, gender, and smoking habit, health status, and region. Samples were kept in cool boxes at (4 °C), with the code of the sample. The volunteers had no preceding date of vocational exposure for uranium and toxic elements. Simple questionnaire of the volunteers including gender, age, and smoking habit is revealed within table 2.

Table 1. Show the type of cancers patients.

Cancer Type	Cases Number
Leukemia	4
Lung	3
Colon	3
Spinal	1
Bladder	3
Pancreatic	1
Breast	4
Stomach	2
Lymphoma	6
Kidney	1
Bone	1
Prostate	1

Table 2. Display statistical information of Study groups participating in the study.

Information	Patients group	Healthy group
Males number	15	13
Females number	15	17
Age range	3 - 65	4 - 69
Average age/years (males)	34.2	36.6
Average age/years (females)	33.2	31.2
Number of smoking	8	6
Average age/years (Total)	33.7	33.6

Experimental method

Determination of uranium concentration (UC)

In this investigation, the (UC) in urine was done by using the neutron activation technique with alpha detector CR-39: An organic detector with a thickness of 500 µm (Pershor, Moulding Ltd, UK). Initially, two drops of urine with an established extent of 100 µm were left to dry in a square piece of CR-39 detector with size (1×1) cm^2, in a dust-free environment at normal room temperature. Then covered with another piece of the detector on both sides and located in a paraffin wax plate at (5 cm) distance as of Am-Be neutron source with a thermal flounce (3.024 × 10^9 $n.cm^2$). After 7 days of irradiation, the CR-39 detector was etched in sodium hydroxide solutions (NaOH) as reported by Al-Hamzawi *et al.*, 2015 [1] then washed in distilled water and dried. The induced fission track densities were recorded using an optical microscope type (Novel, China) with a magnification of 400x. The (UC) in urine samples then calculated by compare the track densities recorded on CR-39 detectors of the unknown samples with that of the standard samples using equation 1[13]:

$$U_x\ (\mu g/l) = U_s\ \rho_x/\rho_s \qquad (1)$$

Where: U_s, U_x are uranium concentrations in unknown samples and standard samples; ρ_x and ρ_s are fission tracks densities for the unknown samples and standard samples respectively. Ethical committee approval of this study (registration number: 6579 in date 2019.6.26).

Determination of toxic metals concentration (TMC)

In this method, the urine samples with (8 ml) were mineralized in (4 ml) of nitric acid HNO_3 (65 %) in addition to one ml from hydrogen peroxide H_2O_2 (30 %). The urine samples were first digested using heating digested at 200 °C for 1 hour. All urine samples have been cooled at room heat after the digestion process. The samples of urine were completed by distilled water (100 ml) and filtered using filter paper (0.42 µm) which washed with water and acid and dry in advance [15]. The heavy metals (Pb, Ni and Cd) are analyzed using atomic absorption spectrophotometer (AAS) type (AA500, UK) by wavelength (217nm, 232nm and 218.8nm), respectively.

Statistical analysis

The consequences had been acquired of each the urine samples of the study individuals have been statistically analyzed by using the statistically program (SPSS). The significance from the level of possibility (P) was predicted by independent sample *t-test*.

RESULTS

Table 3 summarizes the analytic results of UC in urine samples of the participants. From this table, the highest value obtained of UC in urine samples of

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cancer patients was 5.04±0.18 μg/l it belongs to a male, 51 years old, suffering from colon cancer. However the lowest value obtained was 2.21±0.16 μg/l which belongs to a female, 3 years old, and suffers from leukemia. The average value of UC in urine samples for this group was 3.83±0.16 μg/l. Whilst, the results of UC in urine of healthy volunteers ranged between 3.15±0.20 μg/l for male, 48 years old and 0.49±0.10 μg/l for female child, 6 years old. The average value of UC in urine samples of this group was 1.93±0.14 μg/l.

Table 3. Descriptive statistics of UC (μg/l) in urine samples of the participants.

Statistical values	Cancer patients	Healthy group
No. of subjects	30	30
Maximum	5.04 ± 0.18	3.15 ± 0.20
Minimum	2.21 ± 0.16	0.49 ± 0.10
Mean ± Std. Error	3.83 ± 0.16	1.93 ± 0.14
P – value	P < 0.001	

Table 4 exhibits the mean values of urinary uranium depending on the gender of the volunteers. The average value of UC in urine samples of males and females cancer patients is 3.86±0.17 μg/l and 3.79±0.16 μg/l respectively, whereas the average value of UC in urine samples of males and females healthy individuals group is 2.10±0.15 μg/l and 1.75±0.14 μg/l, respectively.

Table 4. The average value of UC (μg/l) in urine samples as a function of gender.

	Gender	Number of subjects	Mean ± Std. Error	P – value
Patients group	Male	15	3.86 ± 0.17	P < 0.05
	Female	15	3.79 ± 0.16	
Healthy group	Male	13	2.10 ± 0.15	P < 0.01
	Female	17	1.75 ± 0.14	

The mean value of urinary uranium of the study groups as a function of the smoking status is shown in table 5. In the table below the mean value of UC in urine samples of the smokers and non-smokers of cancer patients group are 4.30±0.17 and 3.66±0.16 μg/l respectively, whilst the corresponding values of healthy volunteers are 2.66±0.15 and 1.93±0.14 μg/l, respectively.

Table 5. The average value of UC (μg/l) in urine samples as a function of of smoking status.

	Gender	Number of subjects	Mean ± Std. Error	P – value
Patients group	Smokers	8	4.30 ± 0.17	P < 0.01
	Non-smokers	22	3.66 ± 0.16	
Healthy group	Smokers	6	2.66 ± 0.15	P < 0.01
	Non-smokers	24	1.93 ± 0.14	

On the other side, the results of TMC in human urine samples of patients and healthy volunteers are presented in table 6. In the table below the minimum, maximum and average value of Pb in urine samples of cancer patients were 0.088 mg/l, 0.99 mg/l and 0.342 mg/l, respectively. Concentrations of Ni ranged from 0.114 to 0.416 mg/l with an average value of 0.231 mg/l. Similarly, the concentrations of Cd in urine samples of this group varied from 0.034 to 0.088 mg/l with an average value of 0.075 mg/l. While, the minimum, maximum and average value of Pb in urine samples of healthy volunteers were 0.087 mg/l, 0.46 mg/l and 0.233 mg/l, respectively. The levels of Ni ranged between 0.073 to 0.249 mg/l with an average of 0.127 mg/l. Besides, Cd levels varied from 0.012 to 0.098 mg/l with an average of 0.025 mg/l. As it is shown in the following table 6, the average values of Pb, Ni and Cd in urine samples of the cancer patients were significantly higher than healthy subjects (P<0.01).

Table 6. Descriptive statistics of TMC (mg/l) in urine samples of the participants.

Toxic metal	Statistical values	Patients group N^* = 15	Healthy group N = 15
Pb	Mean ± Std. Error	0.342 ± 0.027	0.233 ± 0.021
	Minimum	0.088 ± 0.041	0.087 ± 0.019
	Maximum	0.99 ± 0.078	0.46 ± 0.028
	P – value	P < 0.01	
Ni	Mean ± Std. Error	0.231 ± 0.014	0.127 ± 0.011
	Minimum	0.114 ± 0.021	0.073 ± 0.017
	Maximum	0.416 ± 0.035	0.249 ± 0.041
	P – value	P < 0.01	
Cd	Mean ± Std. Error	0.075 ± 0.003	0.025 ± 0.002
	Minimum	0.034 ± 0.008	0.012 ± 0.002
	Maximum	0.088 ± 0.006	0.098 ± 0.011
	P – value	P < 0.01	

The analytic results of TMC in urine samples of the study groups depending on the gender of the participants are presented in table 7. In the following table the average values of Pb, Ni and Cd in urine sample of males in the cancer patients group were 0.364 mg/l, 0.240 mg/l and 0.080 mg/l, respectively. The average values of the corresponding elements in urine samples of males in the healthy group are 0.297 mg/l and 0.129 mg/l and 0.032 mg/l, respectively. Based on these average values, the levels of TMC (Pb, Ni and Cd) were more concentrated in urine samples of males compared to females, which may be caused by vocational exposure. This result is agreed with the other investigators [16].

Table 7. The mean value of TMC (mg/l) in urine samples depending on the gender of participants.

Toxic metal	Statistical values	Patients group		Healthy group	
		Male n = 7	Female n = 8	Male n = 8	Female n = 7
Pb	Mean ± Std. Error	0.364 ± 0.026	0.318 ± 0.021	0.297 ± 0.043	0.154 ± 0.048
	P- value	P < 0.05		P < 0.001	
Ni	Mean ± Std. Error	0.240 ± 0.034	0.220 ± 0.045	0.129 ± 0.051	0.125 ± 0.022
	P- value	P < 0.05		P < 0.05	
Cd	Mean ± Std. Error	0.080 ± 0.005	0.069 ± 0.017	0.032 ± 0.007	0.018 ± 0004
	P- value	P < 0.05		P < 0.01	

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A significant relationship was observed between the levels of the excreted elements in urine samples and smoking status of the participants. Concentrations of the studied elements in urine samples of smokers for the study groups were extensively higher than non-smokers as shown in table 8. This finding shows that the smokers have high accumulation of toxic elements than non-smokers. The results of this finding explained to contain the tobacco plant varying amounts of metals [17-19].

Table 8. The mean value of TMC (mg/l) in urine samples of participants as a function of smoking status.

Toxic metal	Statistical values	Patients group		Healthy group	
		Smokers n = 8	Non-smokers n = 7	Smokers n = 5	Non-smokers n = 10
Pb	Mean ± Std. Error	0.428 ± 0.035	0.244 ± 0.028	0.244 ± 0.051	0.224 ± 0.041
	P- value	P < 0.01		P < 0.001	
Ni	Mean ± Std. Error	0.258 ± 0.030	0.199 ± 0.023	0.148 ± 0.022	0.116 ± 0.016
	P- value	P < 0.05		P < 0.01	
Cd	Mean ± Std. Error	0.077 ± 0.002	0.073 ± 0.006	0.039 ± 0.015	0.019 ± 0.001
	P- value	P < 0.01		P < 0.01	

DISCUSSION

According to the obtained results, the mean level of urinary uranium of cancer patient group was 2 times higher than those of the healthy individuals. Independent sample test that is confirmed statistically difference in urinary uranium between healthy individuals and cancer patients group. These results show that the most cancer patients exposed to high levels of uranium by inhalation air, ingestion of food and water polluted with uranium which come as of military and human activities in Al-Diwaniyah governorate, south of Iraq. On other hand, the outcomes of the present study are about threefold to sevenfold higher than ICRP references value of urinary uranium 0.5 µg/l [20].

The outcomes demonstrate that the average of UC intended for males within the study groups are larger than females. Males ingested more uranium through food and water, therefore, the adult male showed a higher urine volume than female. This result comes to confirm the results of other researchers [11].

The mean values of UC of smokers of patients group and healthy volunteers are higher than those non-smokers. This is due to people who smoke a higher dose of uranium than non-smokers. Many studies reported high levels of alpha emitters and uranium in different organs of the human body of smokers [1, 21].

Table 9 illustrates a comparison of the current investigation with the finding of the different countries. The finding of the current investigation showed that the urinary uranium of healthy volunteers was higher than those of Germany and USA while lower than Finland.

Table 9. UC (µg/l) in urine samples for different countries.

Country	Uranium content	References
Germany	0.023 ± 0.018	(22)
Finland	2.64	(23)
USA	0.035	(24)
Iraq Cancer patients Healthy subjects	3.83 ± 0.16 1.93 ± 0.14	This study

On other hand, the increase factor of the average value of TMC (Pb, Ni and Cd) in urine samples between cancer patients and healthy subjects was 1.48, 1.81 and 3 times. This investigation suggests an association between the environmental contamination of TMC in the study area and cancerous illnesses. Some studies have shown that there is a contamination of toxic elements in the environment of Al-Diwaniyah governorate as a result of the military and human activities [12, 25].

The average values of TMC in urine sample of all the participants were 0.286 mg/l, 0.179 mg/l and 0.05 mg/l respectively. Based on these findings the level of Pb in urine sample was higher than Ni and Cd. The obtained average values of (Pb, Ni and Cd) were higher than the acceptable limits 0.15 mg/l, 0.025 mg/l and 0.02 mg/l respectively as reported by Horng *et al.*, 2002 [26]. The comparison between the levels of TMC in urine samples of the current in vitro evaluation with other researchers is presented in the following table 10. The figures of this examination are higher than those of persons from UK, Germany, and China and lower than those of Spain and Egypt. The current research was the first study carried out involving UC and TMC in human urine samples of individuals from Al-Diwaniyah.

Table 10. Toxic elements concentrations in urine samples for different countries.

Country	Pb (mg/l)	Ni (mg/l)	Cd (mg/l)	References
Egypt	17.2	NA	1.94	(15)
Germany	0.0013	NA	0.00016	(27)
UK	0.012	0.0008	0.0003	(28)
China	0.080	0.013	0.0039	(29)
Spain	22.28	1.67	0.25	(30]
Iraq Cancer patients Healthy group	0.342 0.233	0.231 0.127	0.075 0.025	This study

CONCLUSION

The obtained outcomes indicate that the contents of UC and TMC in urine of patients were significantly higher than healthy subjects; this result confirms the link between the radiological and toxicological contaminations with the cancerous diseases of the study region. The levels of UC and TMC in urine samples of the participants exceeded the acceptable limits.

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Compliance with ethical standards: I confirm that this manuscript entitled "*In-vitro* radiological and toxicological detection in urine samples of cancer patients in Al- Diwaniyah governorate, Iraq" has not been published elsewhere in part or in entirety, and is not under consideration by another journal. The authors have approved the manuscript and agree with submission to your respected journal.

Conflicts of interests: I confirm that this manuscript has not been published or presented elsewhere in part or in entirety, and is not under consideration by another journal. The authors have approved the manuscript and agree with submission to your esteemed journal. There are no conflicts of interest to declare.

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