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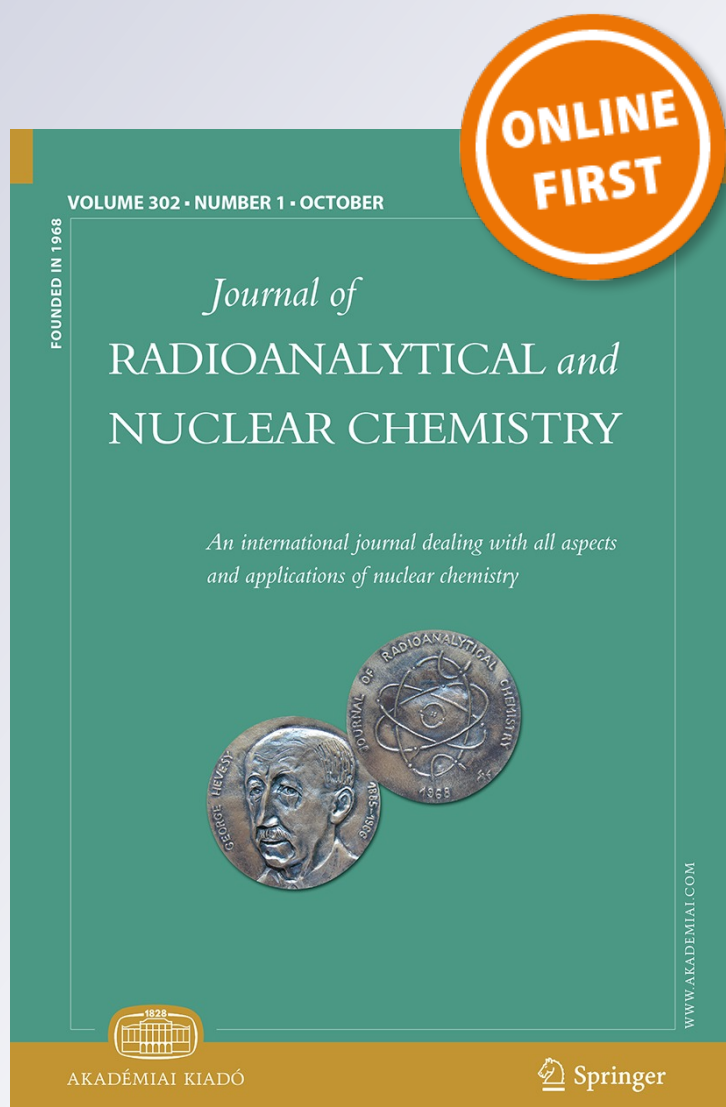
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Concentration of uranium in human cancerous tissues of Southern Iraqi patients using fission track analysis

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Abstract The technique of nuclear fission track analysis with solid state nuclear track detectors CR-39 has been applied to determine concentrations of uranium in cancerous samples of human tissues that excised from patients in the three key southern Iraqi governorates namely, Basrah, Dhi-Qar, and Muthanna. These provinces were the sites of intensive military events during the Gulf Wars in 1991 and 2003. The investigation was based on the study of 24 abnormal samples and 12 normal samples for comparing the results. These samples include four types of soft tissues (kidney, breast, stomach and uterus). The results show that uranium concentrations in the normal tissues ranged between $(1.42\text{--}4.76\ \mu\text{g kg}^{-1})$, whereas in the cancerous tissues ranged between $(3.37\text{--}7.22\ \mu\text{g kg}^{-1})$. The uranium concentrations in the normal tissues were significantly lower than in the abnormal tissues ($P < 0.001$).

Keywords Uranium · Nuclear fission track analysis · Southern Iraq · CR-39 · Human tissues

Introduction

Uranium is naturally occurring radioactive and toxic element, uranium and its compounds are one of the most serious contamination concerns because of its radioactivity and heavy-metal toxicity, which is a threat to human and environmental balance [1–4]. Uranium can enter into the human body mainly through inhalation of dust particles uranium-bearing or ingestion of food and drinking water which is contaminated by uranium [5]. Solubility of uranium is different from one substance to another, but this depends on the nature of the specific composition and the solvent. The function of this solubility is to examine how fast the lungs and the intestines absorb such elements efficiently [6]. Uranium deposited in the kidney, bones and other soft tissues is subsequently released back into the blood stream, which causes several health problems of the human [7–10]. Uranium and its isotopes are used as fuel in nuclear power plants and in the different nuclear and military industries [11]. The first widespread use of uranium weapons by US and NATO forces was during the Gulf War in 1991 against Iraq [12, 13]. Uranium has remained an environmental contamination problem in the exposed areas to uranium weapons in southern Iraq because its increased levels after Gulf Wars in 1991 and 2003 [14, 15]. Epidemiological studies have detected a correlation between uranium and cancer, uranium has carcinogenic possibility [16]. Investigations that have been carried out by the Armed Forces Radiobiology Research Institute in Bethesda pointed out that the normal human cells that are grown in culture dishes might easily be transformed into cancer cells if exposed to uranium [17], also these studies indicated that uranium and radionuclides that emitting alpha-particles cause mutations in DNA and increased chromosomal aberrations [18–22]. It is a vastly accepted base in

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molecular biology that elements which cause damage in DNA can give rise to cancer [23]. Furthermore, the uranium increases cancer risk for individuals exposed because it may increase oxidative stress by increasing free radical formation [24]. The incidence of cancer diseases in Iraq that are registered annually by Iraqi Cancer Board, Ministry of Health has increased significantly after the events of the Gulf Wars [25]. In reported cancers, it is found that some Iraqi southern governorates in Basrah, Muthanna, and Dhi-Qar have witnessed a multiple to quintuple increase in such a disease. Moreover, it has been reported that most of these cases have witnessed a serious damage to the bronchial tubes, lung, skin, bladder as well as the incidence of breast cancer in females and stomach cancer in males [14, 26].

Fission track analysis technique (FTA) with solid state nuclear track detectors (SSNTDs) CR-39 is normally used to determine the trace concentration of uranium in biological samples [1–3, 27]. Fission track analysis technique is quick and accurate with a detection limit of $0.01\text{--}0.1\text{ }\mu\text{g kg}^{-1}$ for uranium determination [28].

The aim of this study is to examine the concentration of uranium in normal and abnormal human tissues using CR-39 nuclear track detector and to evaluate relationship between the concentration of uranium and cancerous tumors.

Material and method

Sample collection

The present investigation was based on the study of 24 abnormal tissues and 12 normal tissues for comparing the results. These samples included four types of soft tissues (kidney, breast, stomach, and uterus) that excised from patients and healthy subjects in the Southern Iraqi governorates Fig. 1. These samples had been collected from the specialized histopathology clinic in the study area. Demographic information such as age, gender, smoking habit, and location about these samples had been collected as shown in Table 1.

Fig. 1 The location of the three governorates involved in the study

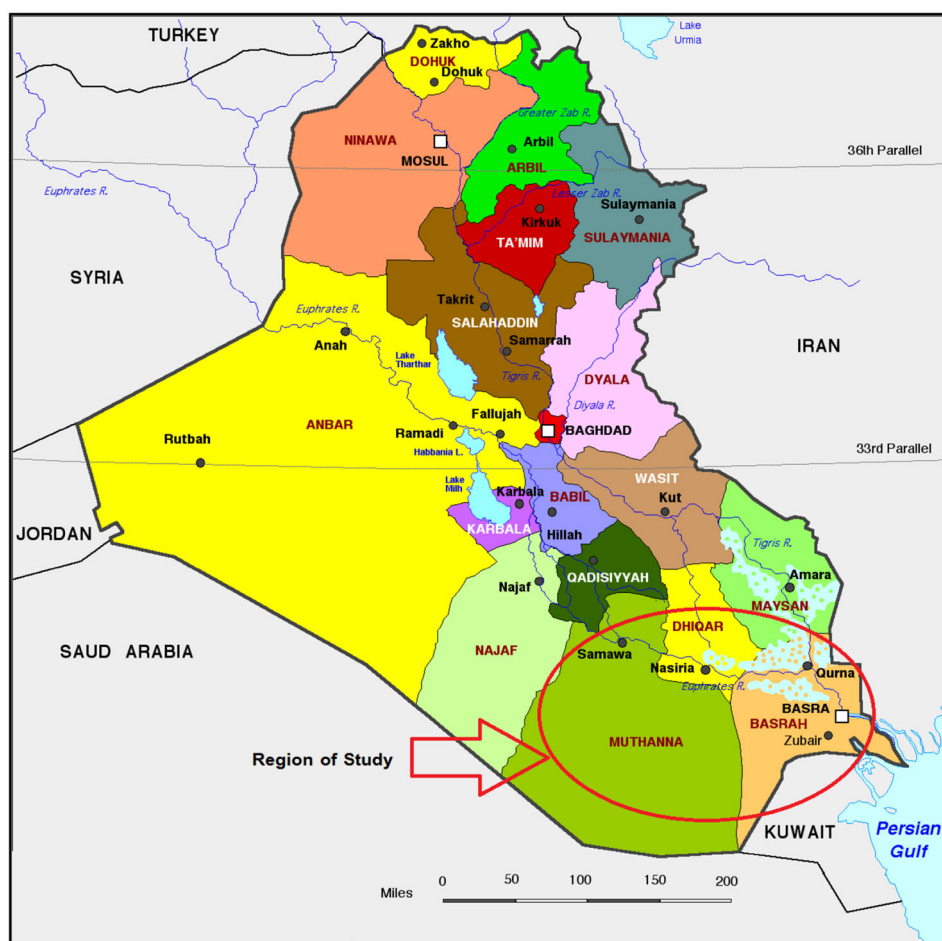


Table 1 Demographic information about the normal and abnormal tissues samples

Sample	Sample no.	Age/gender	Smoking habit	Location
Kidney				
Abnormal	T1	33/M	No	Basrah
	T2	65/F	Yes	Muthanna
	T3	45/F	No	Dhi-Qar
	T4	40/M	Yes	Basrah
	T5	63/M	No	Dhi-Qar
	T6	51/M	Yes	Basrah
Normal	T7	38/M	Yes	Muthanna
	T8	45/F	Yes	Basrah
	T9	43/M	No	Dhi-Qar
Breast				
Abnormal	T10	35/F	No	Dhi-Qar
	T11	57/F	Yes	Muthanna
	T12	30/F	No	Basrah
	T13	19/F	No	Dhi-Qar
	T14	45/F	Yes	Basrah
	T15	52/F	No	Muthanna
Normal	T16	49/F	Yes	Muthanna
	T17	39/F	No	Dhi-Qar
	T18	40/F	No	Basrah
Stomach				
Abnormal	T19	35/M	Yes	Basrah
	T20	62/F	Yes	Muthanna
	T21	60/F	No	Dhi-Qar
	T22	80/M	Yes	Basrah
	T23	45/F	No	Muthanna
	T24	12/M	No	Basrah
Normal	T25	46/F	Yes	Basrah
	T26	40/M	No	Dhi-Qar
	T27	50/M	Yes	Muthanna
Uterus				
Abnormal	T28	35/F	No	Basrah
	T29	58/F	Yes	Dhi-Qar
	T30	34/F	No	Muthanna
	T31	46/F	No	Dhi-Qar
	T32	43/F	Yes	Basrah
	T33	48/F	Yes	Muthanna
Normal	T34	30/F	No	Muthanna
	T35	45/F	Yes	Basrah
	T36	40/F	No	Dhi-Qar

Experimental method

The uranium concentration in cancerous and normal human tissues was determined by using FTA with sensitive plastic nuclear track detector (CR-39, Pershore Moulding Ltd, UK).

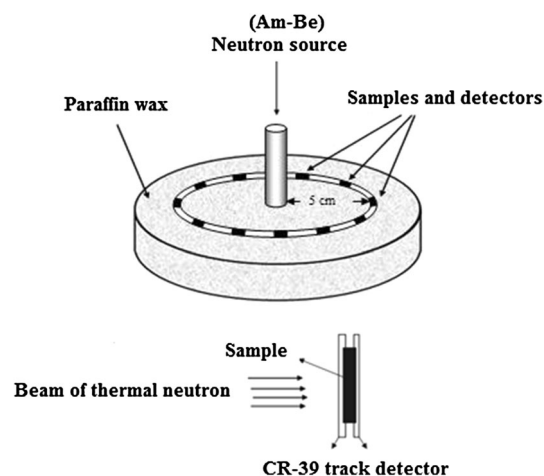


Fig. 2 The irradiation of the samples and detectors to the neutron source

Each sample was washed with distilled water to remove the formalin liquid, and dried by an electric heating incubator, then ashed in an electric oven at 250 °C. The ashed samples were sufficiently grinded by using an agate mortar, and stored in plastic vials. The ashed tissues of (0.5 g) were mixed with 0.1 g of starch (C₆ H₁₀ O₅) which is used as a binder. Thereafter, the blend was compressed into a pellet which has a dimension of 1 cm diameter and 1.5 mm thickness. Both sides of the pellet had been wrapped with CR-39 track detector. In order to cause latent damage to the CR-39 detector due to ²³⁵U (n, f) reaction, the pellets placed in a dish of paraffin wax at a distance of 5 cm from Am-Be neutron source with a thermal fluence equal to (3.024 × 10⁹ n cm⁻²) for 7 days. The setup of the irradiation the samples and detectors to the neutron source is shown in Fig. 2.

After the irradiation, the CR-39 detectors were etched under controlled conditions in NaOH etchant solution as reported elsewhere [1–3]. The fission tracks density was recorded using the optical microscope at a magnification of 400×. The fission track density (ρ) was measured according to the following equation [29]:

$$\text{Track density}(\rho) = \frac{\text{average of total pits (tracks)}}{\text{area of field view}} \quad (1)$$

Calculations

Uranium concentration in the tissues samples was measured by comparison between the tracks density that registered on CR-39 detectors around the samples pellet and that of standard samples pellet by using the following equation:

Table 2 Uranium concentration ($\mu\text{g kg}^{-1}$) in soft tissues of Southern Iraqi subjects

Tissue	Classification	No. of subjects	Min $\pm$ S.D	Max $\pm$ S.D	Mean $\pm$ Std error	<i>P</i> value
Kidney	Abnormal	6	5.83 ± 0.29	7.22 ± 0.26	6.51 ± 0.20	$P < 0.001$
	Normal	3	3.42 ± 0.27	4.76 ± 0.18	4.11 ± 0.38	
Breast	Abnormal	6	4.17 ± 0.18	5.77 ± 0.35	5.04 ± 0.27	$P < 0.01$
	Normal	3	2.64 ± 0.25	3.15 ± 0.29	2.96 ± 0.16	
Stomach	Abnormal	6	4.40 ± 0.14	5.88 ± 0.26	5.22 ± 0.22	$P < 0.01$
	Normal	3	2.53 ± 0.25	3.49 ± 0.30	3.11 ± 0.29	
Uterus	Abnormal	6	3.37 ± 0.16	5.42 ± 0.18	4.61 ± 0.32	$P < 0.01$
	Normal	3	1.42 ± 0.15	3.21 ± 0.22	2.28 ± 0.51	

$$U_x = U_s(\rho_x/\rho_s)(I_s/I_x) \quad (2)$$

where the symbols x and s represents the unknown and the standard, respectively; U is the uranium concentration; (ρ) is the density of the induced fission tracks; I represents the ratio of the isotopic abundance of ^{235}U to ^{238}U . (I_s/I_x) has been taken as unity assuming that the isotopic abundance ratio is the same for the unknown and standard samples. The details of the technique are the same as reported [1–3, 30]. Therefore, the equation becomes

$$U_x = U_s(\rho_x/\rho_s) \quad (3)$$

Statistical analysis

Statistical Package for the Social Sciences (SPSS, version 20) was used to statistically analyze the results of the current study. Independent sample t Test was used to estimate the significance of the probability level (P) for all the results that are obtained from all the samples of the two groups.

Result and discussion

The analytical results obtained from the soft tissue samples (kidney, breast, stomach, and uterus) are involved in this study and are shown in Table 2. For the cancerous kidney tissues, the highest value of uranium content obtained is $7.22 \pm 0.26 \mu\text{g kg}^{-1}$ found in sample T4, whereas the lowest value of uranium concentration obtained is $5.83 \pm 0.29 \mu\text{g kg}^{-1}$ found in sample T3, with the mean value of uranium concentration is $6.51 \pm 0.20 \mu\text{g kg}^{-1}$. The uranium concentration in normal kidney tissues ranged from $3.42 \pm 0.27 \mu\text{g kg}^{-1}$ found in sample T9 to $4.76 \pm 0.18 \mu\text{g kg}^{-1}$ found in sample T8, with the mean value of uranium concentration is $4.11 \pm 0.38 \mu\text{g kg}^{-1}$. The mean value of uranium concentration in the cancerous kidney tissues is higher than those of the normal tissues. Based on these average values, about 158 % increase of uranium concentration was observed in the cancerous kidney tissues and normal tissues.

These uranium concentrations in the abnormal kidney tissues were significantly different ($P < 0.001$) from those in the normal tissues.

The uranium concentration in the cancerous breast tissues ranged from $4.17 \pm 0.18 \mu\text{g kg}^{-1}$ found in sample T13 to $5.77 \pm 0.35 \mu\text{g kg}^{-1}$ found in sample T12, with the average value of uranium concentration is $5.04 \pm 0.27 \mu\text{g kg}^{-1}$. Whereas the uranium concentration in the normal breast tissues varied from $2.64 \pm 0.25 \mu\text{g kg}^{-1}$ which belongs to sample T17 to $3.15 \pm 0.29 \mu\text{g kg}^{-1}$ which belongs to sample T16, with the mean value of uranium concentration is $2.96 \pm 0.16 \mu\text{g kg}^{-1}$. Uranium content in the cancerous breast tissues is significantly higher ($P < 0.01$) than uranium content in the normal tissues with a factor of increase is 1.7.

The maximum value of uranium concentration in the cancerous stomach tissues is $5.88 \pm 0.26 \mu\text{g kg}^{-1}$ found in sample T19, while the minimum value of uranium concentration is $4.40 \pm 0.14 \mu\text{g kg}^{-1}$ found in sample T24. The average value of uranium content in the abnormal tissues is $5.22 \pm 0.22 \mu\text{g kg}^{-1}$. The uranium concentration in the normal stomach tissues ranged from $2.53 \pm 0.25 \mu\text{g kg}^{-1}$ which belongs to sample T26 to $3.49 \pm 0.30 \mu\text{g kg}^{-1}$ found in sample T25, with the mean value of uranium concentration is $3.11 \pm 0.29 \mu\text{g kg}^{-1}$. Average value of uranium concentration in the abnormal stomach tissues is significantly higher ($P < 0.01$) than those of the normal tissues with 168 % increase of uranium concentration was observed in the abnormal stomach tissues and normal tissues.

For the cancerous uterus tissues the uranium concentration varied from $3.37 \pm 0.16 \mu\text{g kg}^{-1}$ which belongs to sample T31 to $5.42 \pm 0.18 \mu\text{g kg}^{-1}$ found in sample T29, with the mean value of uranium concentration is $4.61 \pm 0.32 \mu\text{g kg}^{-1}$. The maximum value of uranium concentration in the normal uterus tissues is $3.21 \pm 0.22 \mu\text{g kg}^{-1}$ found in sample T35, while the minimum value of uranium concentration in normal uterus tissues is $1.42 \pm 0.15 \mu\text{g kg}^{-1}$ found in sample T34. The average value of uranium content in the normal uterus tissues is $2.28 \pm 0.51 \mu\text{g kg}^{-1}$. The mean value of uranium concentration in the abnormal uterus tissues is about twofold higher than

Table 3 Uranium concentration ($\mu\text{g kg}^{-1}$) in the tissues samples as a function of smoking habit

Classification	Smoking habit	No. of subjects	Mean $\pm$ Std error	<i>P</i> value
Abnormal tissues	Smokers	11	5.83 ± 0.22	$P < 0.05$
	Non-smokers	13	4.93 ± 0.25	
Normal tissues	Smokers	6	3.68 ± 0.26	$P < 0.05$
	Non-smokers	6	2.55 ± 0.28	

those of the normal uterus tissues. These results show the uranium concentrations in cancerous soft tissues (kidney, breast, stomach, uterus) are higher than those in normal soft tissues. The average uranium content in human soft tissues in this study is higher than published values with other researchers [31–33]. The cause behind such results can be attributed to the high levels of uranium contamination in the region of southern Iraq as a result of military activities during the Gulf Wars which led to the exposure of individuals to high levels of radioactive pollution [1, 2].

From Table 2, it was found that observed uranium concentrations in kidney were widely scattered and the average uranium concentration ($6.51 \mu\text{g kg}^{-1}$) for abnormal tissues and ($4.11 \mu\text{g kg}^{-1}$) for normal tissues was higher than those in the other abnormal and normal soft tissues, because the kidney is one of the principle sites of uranium deposition in the body [34]. This finding is in agreement with those of other researchers [32, 35]. The degrees of accumulation of uranium in the tissues (kidney, breast, stomach, and uterus) are ranked as follows: kidney > stomach > breast > uterus for normal and abnormal tissues. From these results there is a significant correlation with tissues types ($P < 0.01$).

Table 3 shows the mean value of uranium concentration in abnormal and normal tissues samples as a function of smoking habit. From this table, the mean value of uranium concentration in abnormal tissues and normal tissues of smokers is 5.38 ± 0.22 and $3.68 \pm 0.26 \mu\text{g kg}^{-1}$ respectively, while the mean value of uranium concentration in abnormal and normal tissues samples of non-smokers is 4.93 ± 0.25 and $2.55 \pm 0.28 \mu\text{g kg}^{-1}$ respectively. It is apparent that the mean value of uranium concentration in tissues samples of smokers is higher than those of non-smokers in the abnormal and normal tissues samples with an increase of 120 %. Uranium concentrations in tissues samples of smokers were significantly higher ($P < 0.05$) than uranium concentrations in tissues samples of non-smokers. This suggested that the smokers have the potential of getting the uranium levels greater than the non-smokers. The reason can be attributed to that the uranium content in tobacco enters into the human body through the

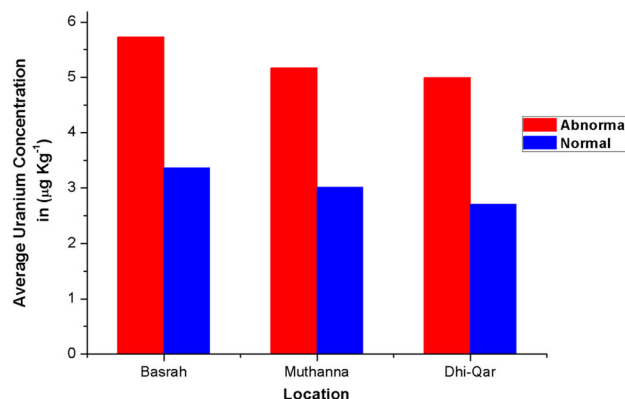


Fig. 3 Average of uranium concentration in the tissues as a function of location

Table 4 Uranium concentration in kidney tissues for different countries

Country	Average uranium concentration ($\mu\text{g kg}^{-1}$)	Reference
Japan	0.34	[32]
USA	0.43 ± 0.26	[33]
Russia	5.30 ± 0.9	[38]
USA	0.90	[39]
Belarus	5.50 ± 0.6	[40]
Iraq		
Abnormal kidney	6.51 ± 0.20	Present work
Normal kidney	4.11 ± 0.38	

smoking, then the uranium transfers to all parts of the body via bloodstream. This explains the high uranium concentrations in tissues of smokers than those of non-smokers. There are some studies that indicate high concentrations of uranium and alpha emitters in different biological samples of smokers that including blood, urine, hair, and tooth [36, 37].

Figure 3 explains the average uranium content in the abnormal and normal tissues samples as a function of residential site. This figure shows that the degrees of accumulation of uranium in abnormal and normal tissues samples are ranked as the follows: Basrah > Muthanna > Dhi-Qar. It is apparent that the average uranium content in the abnormal tissues and normal tissues in Basrah is higher than those in Muthanna and Dhi-Qar.

Table 4 illustrates a comparison of the results in the present work with those by the previous researchers in different countries. In the present investigation study, the average of uranium concentration in cancerous kidney tissues is $6.51 \pm 0.20 \mu\text{g kg}^{-1}$ higher than those of individuals from USA, Japan and Russia. Whilst the average value of uranium concentration in the normal kidney

tissues is $4.11 \pm 0.38 \mu\text{g kg}^{-1}$ lower than those of individuals from Russia and higher than those from USA and Japan.

Conclusion

The uranium level in the abnormal tissues was higher than those of normal tissues. These results mention a direct association between the accumulation of uranium in tissues and malignant tumors. Uranium concentrations in kidney tissues are higher than other soft tissues; this indicates that the kidneys are the critical organ of uranium. Average of uranium concentrations in tissues of smokers is higher than tissues of non-smokers. This study is useful in determining the uranium exposure of the population within the study sites for radiological protection and prevention from extreme exposure.

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