Study and measurements of the uranium and amorphous crystals concentrations in urine samples of breast cancer female patients

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Abstract

In this work, Kinetic Phosphorescence Analyzer (KPA) has been used to measure the concentrations of uranium (UC) and Amorphous crystals (AMO) in urine samples of breast cancer patients in Baghdad. Additionally, a relation between UC and AMO with respect to patient's age has been deduced and studied.

Forty one urine samples of patients and five for healthy were taken from females lived in different residential area of Baghdad. The measured maximum UC value for urine samples of patients was 2.35 ± 0.053, the minimum value was 0.86 ± 0.034 µg/L, and an overall average was 1.6 ± 0.027 µg/L while the average UC for healthy females was 1.03 ± 0.020 µg/L.

From these results, AMO concentrations were found for all breast cancer patients although those patients had not suffered from any kidney function. While all healthy women that tested against uranium and AMO, have been shown zero AMO. Further, UC increases with aging of patients and more incidence of breast cancer of females between the ages 40-59 years. Whereas the value of average AMO independents on the age of patients and also independents on the average value of UC, so the average of AMO is about "+++" for all patients' groups.

Key words

Uranium, Amorphous Crystals, Breast Cancer, KPA.

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قياس ودراسه تراكيز اليورانيوم والبلورات العشوائية في عينات الأدرار لمريضات سرطان الثدي

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الخلاصة

لقد تم في هذه الدراسة استخدام محلل الليزر الفوسفورمتري المستحث في ايجاد تراكيز اليورانيوم (UC) وتراكيز البلورات العشوائية (AMO) في عينات ادرار اخذت من نساء مصابات بسرطان الثدي وجميعهن من محافظة بغداد. بالأضافة لذلك فقد تم ايجاد ودراسة العلاقة بين الزياده في UC في الادرار مع تقدم السن ومع زياده تركيز AMO في الكلى. أن عدد العينات المدروسة 46 عينه, منها 41 عينه لنساء مصابات بسرطان الثدي وخمس عينات لنساء سليمات. لقد كانت القيمة العظمى المقاسة لتركيز اليورانيوم 2.35 ± 0.053 والقيمة الصغرى 0.86 ± 0.034 µg/L ومعدل جميع القياسات 1.6 ± 0.027 µg/L، بينما كان معدل تركيز اليورانيوم المقاس للأدرار النساء السليمات هو1.03 ± 0.020 µg/L. وقد وجد بأن تركيز اليورانيوم يزداد مع تقدم السن. اما بالنسبه لل AMO فكان لجميع المريضات ،والتي لا يعانين من اي تلكأ في وظائف الكلى، بمعدل "+++", بينما كانت جميع النساء السليمات التي تم اختبار عينات ادرارهن ليس لديهن AMO اي أن AMO لا يعتمد على تركيز اليورانيوم وكذلك لا يعتمد على العمر.

Introduction

Nuclear radiation has negative effects on living cell. This important field has been studied by many researchers, since the early days of nuclear industry. In Volume 2 of the Encyclopedia of Occupational Health, under uranium alloys and compounds, page 2238, it reads: "Uranium poisoning is characterized by generalized health impairment. The element and its compounds produce changes in the kidneys, liver, lungs and cardiovascular, nervous and haemopoietic systems, and cause disorders of protein and carbohydrate metabolism.......". One of the challenges for the possible exposure to uranium is the present of naturally occurring levels of uranium in collected bioassay samples [1]. Fluctuation in background levels gives a positive determination of possible low level exposure to manmade radioisotopes become difficult. Chronic and acute exposure to people who are not routinely monitored, the optimum window for collecting urine samples may be missed [2]. An example is the recent exposure of civilians to Depleted uranium from military ammunition in Kosovo and Kuwait [3]. In Iraq, upon both Gulf Wars I and II, Depleted uranium has been used extensively in war ordinance; it caused bad effects on Iraqi's publics.

Uranium excretion in urine is proportional to the uranium level in the body. This is why renal uranium excretion is used in this study to detect incorporated uranium. Measurements of uranium excretion in urine, in contrast to feces, provide a reliable basis for detection uranium. International Committee of Radiological Protection (ICRP) Publication 69 [4] provides the biokinetic model for systemic uranium. In combination with the ICRP respiratory tract model publication [5] 66, it is now possible to predict uranium excretions in urine for different types of exposure within periods of time after exposure.

There are several factors that led to increment in UC in the Iraqi environment. First of them is the Gulf wars, were depleted uranium (DU) was widely used. There is no dispute of the fact that at least 320 tons of DU was "lost" in the first Gulf war only. Much of that was converted at high temperature into an aerosol, that is, minute insoluble particles of uranium oxide, UO_2 or UO_3, in a mist or fog. Secondly, the anarchy and local upraises, which took place immediately after the second Gulf war in areas, where many nuclear facilities are located, such as the Iraqi Atomic Agency in Al-Twaetha site in Baghdad, the Al – Ramahi factory for phosphate fertilizer production in Idaei site in Nineveh governorate, among other sites [6]. Other factors resulted from daily life activities which increased the total intake of Uranium [7]. In general UC in the drinking water represents the main part of the UC in urine. The systemic availability of Uranium after oral intake is very low. Depending on the type and solubility of the corresponding Uranium compound, only about 0.2 – 2 %, at best 6 % [8] are absorbed from the gastrointestinal tract and thus become systemically available. The rest is not absorbed and is excreted in stool after a few days. Gwiazda et al. [9] showed that experience with Gulf War veterans indicates that a 24 hours urine collection analysis show the most promise of detecting Uranium contamination seven or eight years after exposure. However, since this test only measures the amount of Uranium which has been circulating in the blood or kidneys within one or two weeks prior to the testing time, rather than testing the true body burden, it cannot be directly used to reconstruct the received dose. Nevertheless, this seems to be the best diagnostic tool at this time, eight years after the exposure.

The increment in cancer cases in Iraqi civilians, which is noted and registered by

Iraqi Cancer Registry Center (ICRC) [10], can be attributed to many reasons, but in Iraq the rise in UC especially if one considers cancer statics before and after Gulf wars. In addition, the increment of cancer cases among solders participated in the Gulf and Kosovo wars was noticed and studied by many monitoring centers and researchers [10].

On the other hand, the breast cancer can occur in males and females, but the incidence rate in males is very low and equal to 0.01 % from total cases according to ICRC [10].

Kidney is a main filter in the human body which consists of kidney tubules and the major function of it, is to filtrate and purity for the entering substances to the body which came from the digestive system. So, in some cases these substances are contained compounds which are poisonous and harmful to the body, therefore, the kidney will filter and extract it out. There are several kinds of crystals substances may be precipitated in kidneys that can be divided into:

1- Uric acid crystal
2- Calcium Oxalate
3- Triple Phosphate
4- Amorphous crystals (Urate and Phosphate)

The major important of these crystals is the amorphous crystals (AMO). According to the PH number in the urine, one can be classified it into two types; Urate and Phosphate. The increasing of AMO in urine leads to cause tumors in several parts of the body. However, the presence of other kind of crystals with AMO can made stones in the urinary tubules. The measurement unit of AMO in urine is "+" which represents some amount of AMO and if this amount duplicated one can say that the patients has ++ of AMO and so on.

Nevertheless, one can classify Uranium, according to its solubility, into three types: fast, median, and slow [8]. DU is slowing soluble; therefore, it can be deposited in kidneys as AMO, which may represent the main reason of all kinds of cancers.

However, the need for a sensitive, fast, and accurate method for the determination of UC is particularly felt in the environmental, geological, and bioassay fields. Some analytical methods [11, 12] used for Uranium detection require extensive pretreatment of samples such as surface and ground water, sea water, ores, and urine, thus limiting the application of these techniques as routine methods. Phosphorimetry is a sensitive and selective analytical technique, with low detection limits and can be used for routine measurements [13].

The aim of this work is to measure and study the relationship between AMO and UC in urine samples for women suffering from breast cancer. Uranium concentrations were measured using KPA after calibration with low standard concentrations of Uranium according to [14].

Methodology

Forty one samples of twenty four hours urine of patients suffering from breast cancer and five samples of healthy women were collected with different ages living in different sites of Baghdad city. However, and in order to highlight about the correlation between the uranium concentration, the age, and the AMO, all the studied patients have been selected with breast cancer by taking into consideration several things;

- Women: Female's patients with breast cancer have been taken only, because the probability of infected male with breast cancer very low.
- Pregnancy: When collected the samples, pregnant women were excluded because low incidence of pregnancy-associated breast cancer. In fact, historically, the

incidence is estimate 1 in 3000 pregnancies [15]. Based on the National Cancer Institute's Surveillance, Epidemiology, and End Results Program Cancer Statistics Review and rates from 2001 to 2003, that 12.67% of women will develop breast cancer during their lifetime [16]. This lifetime risk translates into one in eight women. This incidence will only increase as more women delay childbearing until later in life (the fact that pregnancy-associated breast cancer is age-related, and women who have their first term pregnancy after the age of 30 years have a two to three times higher risk of developing breast carcinoma than women who have their first pregnancy before the age of 20 years) [16].

- Smoking: The smokers are subjected to alphas radiation in the bronchial epithelium from three sources: (1) from indoor radon and thoron decay products inhaled between cigarettes, (2) from ^{214}Po, ^{212}Po and ^{212}Bi in large mainstream smoke particles and (3) from ^{210}Po which grows from decay of ^{210}Pb-enriched particles that persist at bronchial bifurcations [17]. 50 % of ^{210}Po present in tobacco was transferred into the smoke and the other 50 % remained in the ash and butt. Further, one pack-a-day, smoker inhaled 24 mBq d^{-1} of ^{210}Po through smoking and the annual inhalation was 8.8 Bq. Therefore, the samples were not taken from infected smokers, because cigarettes will be the second source of the radionuclides in patient's body.
- Drinking water: All infected patients with breast cancer were selected that drank water from the tap only. This is because the mean concentration of uranium in the human body comes from the ingestion of drinking water, and the tap water contains uranium concentration more than other types of water [18].
- Education: All urine samples have been taken by take into consideration the educational levels of the individuals due to the effects on their awareness of the health and knowledge of the main source of diseases and epidemics.

However, the kinetic Phosphorimetry measurements were performed using the kinetic phosphorescence analyzer KPA-11 [13]. KPA-11 uses pulsed laser excitation and gated detection for the determination of UC. KPA-11 needs proper preparation of urine samples because unprocessed urine cannot be analyzed without pretreatment except at levels well above 20 mg/L. The recipe for preparing and sampling the urine samples of refs [13, 14] was used. Besides, the same references (refs [13, 14]) were depended in calculating UC. To calibrate the KPA -11, Uranium standard solutions were prepared using Uranium Octoxide (U3O8). Firstly, a stock standard solution of 1000 mg/l (1000 ppm) was prepared by dissolving 117.9 mg of U3O8 in 100 ml of 0.82 M nitric acid (HNO3) in volumetric flask. To construct the calibration curve for KPA-11 analysis, series of calibration standard were prepared to cover a wide range of UC, which may be expected in urine samples. UCs in the series of standards were 0.05, 0.08, 0.1, 0.3, 0.5, 0.7, 1, 3, 5, 7, 9 and 10 μg/L. This set of standards was used to construct the calibration curves for the low range of UC.

On other hand, the AMO analysis was carried by taking 3 ml of urine sample then put in a centrifuge for three minutes at 500 rpm (round per minutes), which separates the sample into two layers; top and bottom. The main content of the bottom layer was AMO which was placed on a slide and viewed with light microscope with 40x magnification to determine the AMO concentrations.

Results and discussion

Forty six urine samples have been measured and can be classified into two groups; the first group included 41 samples of breast cancer female's patients and the second group included 5 samples of healthy females with different ages. All individuals live in Baghdad with age's range 27-63 years. The age, uranium concentration in samples, and AMO for patients were shown in Table 1. UC in urine samples for patients ranging from 0.86±0.034 to 2.35±0.053 µg/L with an overall average 1.6±0.027 µg/L while the average UC in urine for healthy females was 1.03 µg/L.

These results might indicated the presence of uranium radiation sources, which it was found in similar studies (but for chest cancer) that presence of these sources causes exposure of population to uranium contamination [19] according to geographical location of their residency. Despite the fact that all cancer patients drinking tap water, but the results of uranium concentration in the urine of cancer patients indicated the possibility that the pollution is the cause of the disease.

However, the results demonstrated that AMO was found for all cancer patients although those patients had not suffered from any kidney function. While all healthy women that tested against uranium and AMO, have been shown zero AMO (as shown in Table 2). Fig. 1 illustrated the AMO as a function of UC. From this figure one cannot be followed any behavior for AMO with UC. Therefore, the patients classified according to their age into five groups (as shown in Table 3) and for each group the results were averaged. According to the results of this table, the highest of uranium concentration was 2.053 µg/L for age period sixties while the lowest value is 0.86 µg/L for age period of twenties, that’s mean UC increases with aging of patients and more incidence of breast cancer of females between the ages 40-59 years as illustrated in Fig. 2. In addition, the value of average AMO independents on the age of patients and also independents on the average of UC, So the average of AMO is about "+++ " for all patients' groups. Fig. 3 represents the average uranium concentrations as a function of age period for all groups. This figure clearly illustrates that UC (as an average) increases with increasing the age (as age period). An attempt to find roughly a relation between UC in human body and the age period might be approximated in a way, by adopting the twenties ages by "20y" and thirties ages by "30y" and so on for other studied ages. The results of this attempt are shown in Fig. 3, which clearly illustrates those UCs (as an average) increases with the increasing the age (as age period).

Approximated rough results for the average UC (µg/L) with the age period (t) might be found from the fitted equation:

$$average\ UC\ (\mu g/L\ ) = 0.1143t^{0.7022} \quad (1)$$

where (t) is age's period in year.

The above attempted might represent a quick rough approximated method to find the concentrations of uranium from the person age compared to the concentrations of uranium in urine samples, which is usually might be taken a long time period in the usual ways.

Furthermore, the normal UC in the urine depend (mainly) on UC in drinking water. This varies from one location to another, according to ICRP biokinetic model for uranium, the continuous uptake of uranium with drinking water of 1µg/L at a consumption rate of 1.4 L/d results in an ultimate uranium level in urine of approximately 0.2 µg/L for adults. United Nation Scientific Committee on the Effects of Atomic Radiation(UNSCEAR) [20] uses a reference value for uranium in drinking water of 10mBq 238U/kg. In other words, 0.81 µg/L and uranium level in the urine was approximately to 0.16 μg/L according to the biokinetic model for adults. In a Germany study on European bottled mineral waters, WHO [21] recommendation was 2μg/L for drinking water, 20% of the samples was found at the highest UC at 232 μg/L of drinking water. This maximum value observed would lead to UC in urine of up to 4.64μg/L.

Table 1: Uranium concentration (UC), amorphous crystals (AMO) and the age of breast cancer female patients.

Sample No	Age (y)	UC (μg/L)	AMO (+)
1	27	0.86 ± 0.034	3
2	33	1.36 ± 0.017	3
3	35	1.60 ± 0.024	3
4	36	1.43 ± 0.019	2
5	38	1.43 ± 0.022	5
6	41	1.53 ± 0.021	3
7	41	1.53 ± 0.021	3
8	42	0.91 ± 0.026	3
9	42	1.15 ± 0.013	4
10	43	2.33 ± 0.034	3
11	43	1.56 ± 0.022	4
12	44	1.58 ± 0.023	4
13	45	1.02 ± 0.063	4
14	45	1.34 ± 0.021	4
15	45	1.60 ± 0.023	2
16	45	1.62 ± 0.024	4
17	47	1.63 ± 0.024	2
18	49	2.16 ± 0.023	2
19	50	1.25 ± 0.024	4
20	50	1.36 ± 0.031	3
21	50	1.85 ± 0.030	1
22	51	1.70 ± 0.026	3
23	52	1.71 ± 0.026	3
24	53	1.75 ± 0.027	4
25	53	1.73 ± 0.027	4
26	53	1.73 ± 0.027	3

Sample No	Age (y)	UC (µg/L)	AMO (+)
27	55	0.98 ± 0.016	1
28	55	1.16 ± 0.010	4
29	55	1.68 ± 0.019	3
30	55	1.76 ± 0.028	3
31	56	1.77 ± 0.028	3
32	56	1.77 ± 0.028	2
33	57	1.00 ± 0.052	4
34	57	2.15 ± 0.035	4
35	57	1.79 ± 0.028	5
36	58	2.05 ± 0.034	2
37	59	1.81 ± 0.029	3
38	60	2.15 ± 0.033	3
39	62	2.35 ± 0.053	3
40	62	1.85 ± 0.030	4
41	63	1.86 ± 0.031	3
Average	49.93	1.6 ± 0.027	3.21

Table 2: Uranium concentration (UC), amorphous crystals (AMO) and the age of healthy women.

Sample No	Age (y)	UC (µg/L) KPA	AMO (+)
1	32	1.01 ± 0.021	0
2	34	1.05 ± 0.019	0
3	28	1.13 ± 0.023	0
4	42	0.99 ± 0.02	0
5	48	0.98 ± 0.018	0
Average	36.8	1.03 ± 0.0202	0

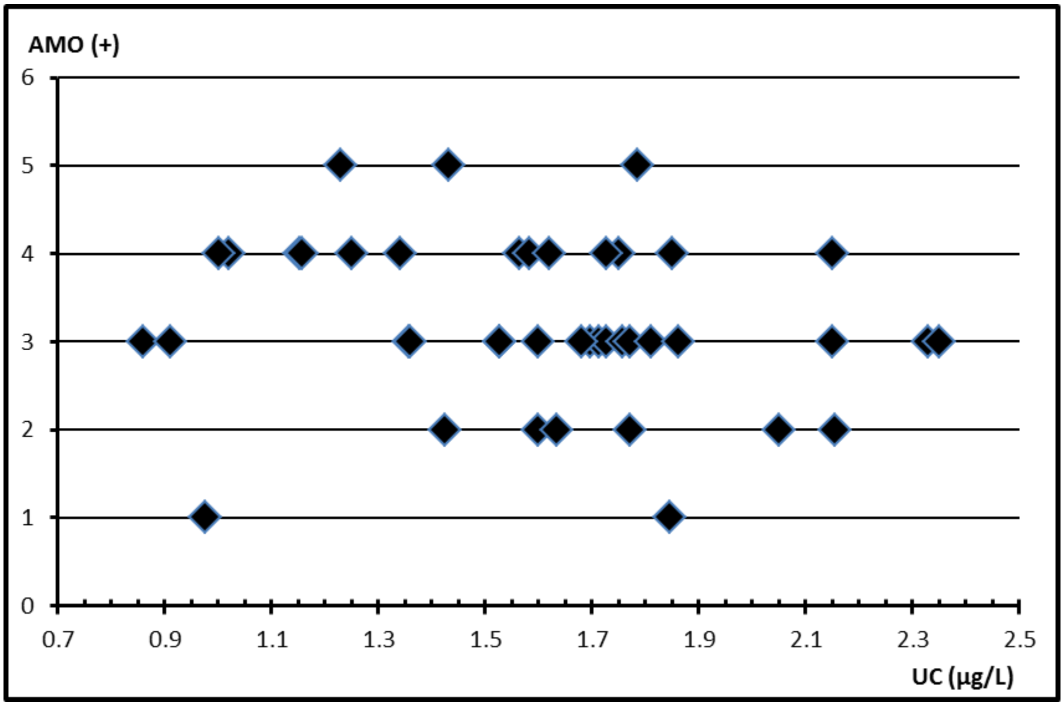

Fig. 1: AMO as a function of uranium concentration for women had breast cancer.

Table 3: Average UC ,average AMO, age period and age iteration for urine samples of female with breast cancer.

Age period (t) (year)	**Iteration of Age**	**Average UC (µg/L)**	**Err. of UC (µg/L)**	**Average AMO (+)**
Twenties 20-29	1	0.86	0.034	3
Thirties 30-39	4	1.454	0.021	3.25
Forties 40-49	13	1.534	0.026	3.23
Fifties 50-59	19	1.63	0.028	3.1
Sixties 60-69	4	2.053	0.036	3.25

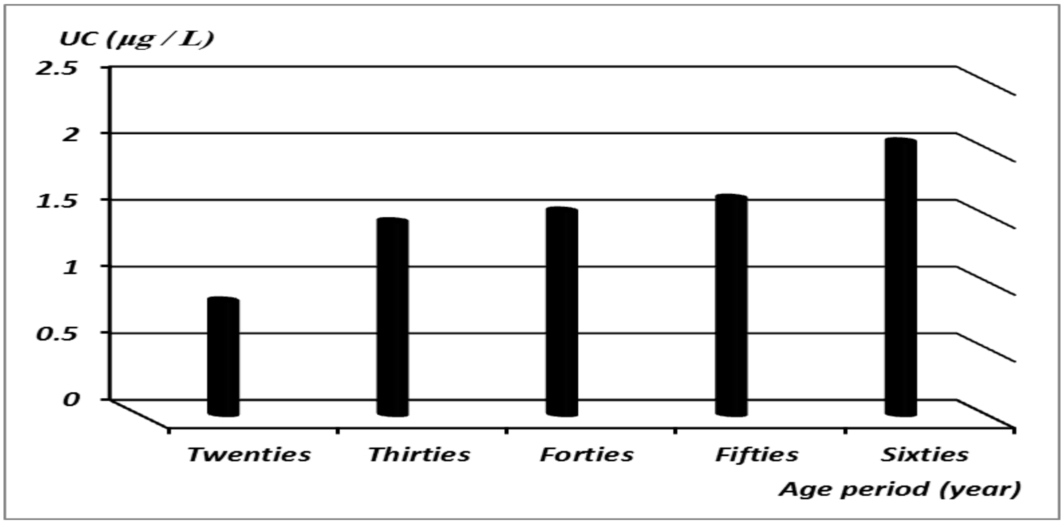

Fig. 2: Relation between UC and age period.

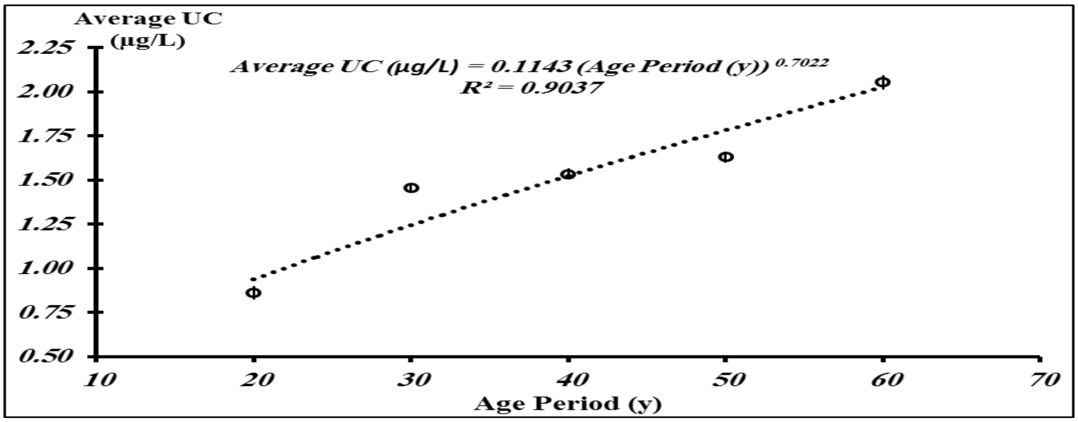

Fig. 3: The average UC as a function of age period.

In comparison to Germany current drinking water, 1.5 µg/L uranium concentrations lead to excretion of 0.3 µg/L of uranium in the urine [1]. However, from above discussion one can note that the allowed variance range between the values of UC in drinking water and urine is about 0.33 (5/15).

The results of the average UC in the drinking water in Baghdad [18] and our results of UC in urine are 1.95 and 1.6 µg/L respectively, i.e. the variance range between the values of uranium concentration in drinking water and urine is about 1.2. Therefore, one can say with good confidence that the urine samples of our study case (females suffer from breast cancer) contaminated with uranium.

Conclusions

This work suggested some reliable conclusions, which can be mentioned as follow:

- All cases of patients present AMO with high concentration.
- The overall average UC for female suffer from breast cancer had high value comparing with the limits of international agencies values and comparing with the results of healthy women.
- For all cases, there was a relation between AMO and Uranium concentration, i.e. for all patients the average AMO concentration was "+++", while for healthy women was "0".

- The behavior of the average UC with respect to the age period of the patients can be roughly deduced from the fitting equation (1).

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