Measurement of uranium concentrations in urine samples of adult healthy groups in Najaf governorate with estimation of urine concentrations of 8-OHdG compound as biomarker for DNA damage

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Abstract. In this study, 88 healthy adults in Al-Najaf governorate of Iraq have been divided into three groups according to the type of exposure to radiation including environmentally uranium exposures n=29 (G₁), occupationally exposures n=27 (G₂), and non-uranium exposures n=32 (G₃). All groups were subjected for estimation of uranium concentration in urine samples using CR-39 SSNTD method beside measurement of urine concentrations of 8-OHdG compound as a biomarker for oxidative DNA damage using Elisa technique. Reactive oxygen species produced under the effect of many causes including ionizing radiation exposure, (OH⁻)free radical induced the formation of 8-OHdG compound from cellular and mitochondrial DNA through attack of C-8 in purine ring of guanine base. The results showed that mean values of uranium concentration were (1.836±0.426 µg/L), (2.02±0.404 µg/L) and (1.755±0.437 µg/L) for group1, group2 and group3, respectively. While mean values for 8-OHdG were (49.810±15.484 ng/ml), (47.717±14.232ng/ml) and (46.769±14.249ng/ml) for the three groups, respectively. No significant statistical differences were recorded between all data obtained. These results suggested the presence of uranium pollution in all groups including the non-exposed group as compared with reference value of (WHO, 2004). Results also demonstrate the presence of DNA oxidative damage in all groups according to concentration levels of 8-OHdG in urine samples. Therefore, the uranium pollution in urine samples of the examined groups indicate the presence of chronic low dose radiation source in Najaf governorate, which might be the cause of DNA damage as reflected by the urinary levels of 8-OHdG compound.

Keywords: uranium concentration, 8-OHdG concentration and urine sample.

1. Introduction

Environmental pollution with many chemical and physical factors can be hazardous to people health. Humans are constantly exposed to many agents derived from exogenous sources such as food, tobacco smoke, air pollution or ionizing radiation [1]. Ionizing radiation is a high energy radiation that produces ionization or electrical charges as it passes through medium or matter [2]. There are three types of atomic radiation of concern to human health namely, α, β and γ. Sources of radiation may be natural (80%) or human made (20%) [3,4]. The most dangerous human made source of ionizing...
radiation is the misuse of forbidden weapons as what happened in Gulf war I and II in Iraq (1990-2003) Which caused depleted uranium pollution in limited areas of Iraq [5]. Exposure of healthy humans to Reactive Oxygen Species (ROS) agents can induce as a situation called oxidative stress [6]. This is characterized as a situation in which the antioxidant protection capacity is overwhelmed by an increase in the generation level of ROS resulting in oxidative damage to cellular macromolecules such as lipids, DNA and proteins [7]. Balance is preserved between endogenous oxidants and various enzymatic and non-enzymatic antioxidants under normal conditions and in all aerobic species [8]. The most important free radical causing damage to basic macromolecules is the hydroxyl radical (OH) which attack nuclear DNA and mitochondrial DNA causing addition of (OH) to c-8 of purine ring in guanine base and produce c-8-OH radical which lead to formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) [9]. This compound is then used as biomarker to examine the oxidative DNA damage, its discovery reported for first time by Kasai and Nishimura in1984 [10]. ROS correspond to super oxide anion (O2•−), singlet oxygen (O2•), hydroxyl radical (OH•), hydrogen peroxide (H2O2), and nitric oxide (NO) are crucial for many physiological processes and usually exist in cell in a balance with biochemical antioxidant like enzymes including catalase, glutathione peroxidase, superoxide dismutase and low molecular weight antioxidants such as (β-caroten and tocopherol) [7]. It is suspected that oxidative stress leads to the onset and progression of many diseases and the natural aging process [11]. ROS are highly reactive and have an extremely short half-life, so their direct determination is normally impractical [12].

Measuring oxidative modified cellular constituent biomarkers like DNA in biological samples offers a promising public health strategy in the field of public health [13]. In pathological conditions such as carcinogenesis, coronary heart disease and diabetes, measurements of urinary 8-hydroxy-2-deoxyguanosine (8-OHdG) have been extensively studied [14,15]. In recent years, (8-OHdG) has been commonly used not only as a biomarker for endogenous DNA damage assessment but also as a risk factor for many diseases including cancer [16]. Also (8-OHdG) has been used to estimate DNA damage after exposure to agents causing cancer such as: (tobacco smoke, asbestos fiber, heavy metals, and polycyclic aromatic hydrocarbons) [17]. Ionizing radiation (IR) has been reported as one cause of oxidative stress and DNA damage [1, 18, 19].

In Al-Najaf governorate of Iraq, there are some uranium concentrations either naturally (Al heera uranium mine) or human made pollution, while other regions in the governorate are supposed to be not polluted. The present study aims to evaluate uranium pollution in urine samples of uranium-exposed and non-exposed samples. Moreover, estimation the urine concentration of (8-OHdG) compound as a biomarker for DNA oxidative damage and its health consequences.

2. Materials and method

2.1. Collection and Preparation of the Samples

2.1.1. Groups of study

To measure the uranium concentrations, 88 urine samples were collected from three different groups of adults in Al-Najaf governorate as described below.

1. Group one (G1): Included 29 healthy people living in expected polluted areas such as the village of Al-Zajri near the uranium mine in Al-Heerradistrict in Al-Manathera, the village of Al-Aama in Al-Abbsiyeh district in Al-Kufa, as well as Al-Ansar neighborhood.

2. Group two (G2): Included 27 healthy people exposed to uranium radiation by virtue of their work, from Al-Sadr Medical City employees, Al-Hakim General Hospital, Consultative Clinic for Chest and Respiratory Diseases, Middle Euphrates Cancer Center.

3. Group three (G3): Included 32 healthy people living in unpolluted areas, from some sporadic areas of the governorate.
2.1.2. Sample Collection

The sample was taken from the adult person containing urine cups (60 ml); and immediately acidified by adding 1 ml of concentrated HCl for each sample to prevent urine sample polymerization, then placed in a cooling box until the bio-laboratory was reached.

Each urine sample divided by two parts in test tubes: first for uranium concentration estimation by SSNTD technique using CR-39 detector, second for 8-OHdG concentration measurement in urine samples by ELISA technique.

2.2. Measurement of Uranium Concentration

A CR-39 detector was made by Pershore Moulding LTD Company UK, with a thickness of 500μm, was put in the first part from urine samples for measuring the Uranium concentrations, for 90 day. Each detector was cut into 1×1 cm\(^2\) an area; the tubes are stored at -80˚C in the deep freezer. At the end of this period, the exposed detectors were etched using sodium hydroxide (NaOH) solution at a temperature of 70º C for 5 hrs. The normality of solution was set at 6.25 in the water bath. These etched detectors were then washed using distilled water. The track density of each CR-39 detector (track/cm\(^2\)) was calculated using an optical microscope (NOVEL, China) with a magnification power of 10×40. The reading of each detector was corrected for the background radiation.

\[
(\rho) = \frac{N}{(A \times t)} \quad \ldots \ldots (1)
\]

Where \(\rho\) = Track density (no./cm\(^2\).h), \(N\) = Average number of tracks, \(A\) = Area of field view (cm\(^2\)), \(t\) = Time of exposure (h).

To calculate the \(U_c\) in the serum sample one could use the following fitting equation [21]:

\[
U_c=(\rho +12.5) / 18.6 \quad \ldots \ldots (2)
\]

2.3. Measurement of 8-Hydroxy-2’-Deoxyguanosine (8-OHdG)

The competitive inhibition enzyme immunoassay technique is employed in this assay. The 8-OHdG-specific antibodies were pre-coated onto a micro plate. Standards and samples were pipetted into the wells with 8-OHdG conjugated with Horseradish peroxidase (HRP). Between 8-OHdG (standards or samples) and HRP conjugated 8-OHdG with the pre-coated 8-OHdG specific antibody, a competitive inhibition reaction was launched. The further 8-OHdG in the samples, the less HRP-conjugated 8-OHdG is bound by the antibodies. A substrate solution was applied to the wells after being washed to eliminate any unbound reagent, and color was produced in comparison to the amount of 8-OHdG in the sample. The production of color was halted and the color intensity was assessed. In urine samples, the 8-OHdG content is determined by comparison with a predetermined normal 8-OHdG curve.

3. Results and Discussion

3.1. Uranium concentration in examined groups

Results of present study are reported in table (1) which shows the mean values of uranium concentrations in urine samples of uranium healthy exposures by environmental contamination (G\(_1\)) uranium healthy exposures by virtue of their occupations (G\(_2\)) and non-uranium exposures healthy people (G\(_3\)) in Al-Najaf governorate were (1.836±0.426) μg/L, (2.020±0.404)μg/L and (1.755±0.487) μg/L respectively, other local studies reported uranium concentration in urine samples of healthy control in Baghdad city (0.464-6.212) μg/L [22], (3.212±0.593) μg/L [23], (1.361) μg/L for male, (0.9533) μg/L for females, (3.196) μg/L, for radiation field workers [24].

The standard value of uranium concentration in urine will be 0.3 μg/L after 30 years of continuous exposure to 15 μg/L uranium concentrations in drinking water [25]. According to standard value, the results of present study suggested the presence of uranium pollution in all of the studied groups including healthy group of non-polluted areas of Al-Najaf governorate (G\(_3\)).

Uranium concentration in all urine samples may reflect the presence of general environmental pollution with ionizing radiation in Najaf governorate. In Iraq, the levels of depleted uranium raised
after misuse of weapons forbidden in Gulf war I and II causing environmental pollution with uranium in limited areas, but no procedures had ever been taken to isolate these contaminated areas all over Iraq to stop and avoid the spreading of this radioactive contamination and its dangerous consequences [26, 27]. The average annual intake of uranium by adults has been estimated to be 460 µg from ingestion and inhalation [28]. Most of α-emitting materials leave the body through feces, small portion of urine will get into blood and the body can get rid of within few days, and the rest can stay in bones, kidney, liver pancreas, spleen, and central nervous system.

If we breathe the dust of α-emitting material, some of it is exhaled and some stay in our lung [29, 30]. The results of present study showed that mean value of uranium concentration in urine of G2 is higher than that of G1 and G3, this might be obtained because this group (G2) exposed to both environmental and artificial ionizing radiation sources. The natural environmental radiation is inevitable. It has been recorded that the annual effective dose produced by artificial sources likes X-Ray machines and other diagnostic systems is nearly equal to the total radiation dose obtained from natural dose [31]. In their effects on matter, natural and artificial radiation sources are identical. The U.S. Nuclear Regulatory Commission (NRC) mandates that its licensees restrict human radiation exposure to 1 mSv per year for individual members of the public and limit occupational radiation exposure to 50 mSv per year for adults employed with hazardous materials above the background level of radiation exposure.

3.2. Concentration of 8-OHdG as biomarker for DNA damage

Table (2) demonstrates the concentrations mean values of 8-OHdG compound as a biomarker for DNA damage in urine sample were (49.810±15.842 ng/ml), (47.717±14.232 ng/ml), (46.769±14.249 ng/ml) in G1, G2 and G3 respectively. A local study reported 8-OHdG concentration in urine samples of healthy people in Baghdad city as (283.96±43.14 ng/ml) [32]. Comparing the results of the present study with the results of non-local same studies on healthy people it was clear that the concentration of 8-OHdG in Iraqi people in much higher than that of other populations where it was (9.08 ng/dl) in Makkah [33], (1.32±0.20 ng/ml) in Turkey [34], (1.53 ng/ml) in Portland [35], (12.29±5.72 ng/ml) in China [36], (8.43 ng/mg creatin) in Japan [7], (15.2±5.71 ng/mg creatin) in Japan [6]. Studies on healthy people recorded that urinary 8-OHdG is associated with arteriosclerosis related factors [7]. Diet also related to urinary 8-OHdG. For fruit intake a significant correlation for an observed decrease in DNA damage level with increasing fruit intake for females [1].

The increased level of 8-OHdG concentration in urine samples of all study groups suggest DNA damage because of oxidative stress. Also, all urine samples were uranium polluted according to mean values estimations of uranium concentrations. Ionizing radiation is one cause of oxidative stress and can damage any part of cell and interfere with many cellular processes and DNA is the most critical biological target. There is no safe level for radiation exposure; any level could be harmful [37]. Ionizing radiation as a toxicant and carcinogen can produce ROS and cause severe oxidative damage such as single and double strand break, oxidized base and DNA protein cross link.

If these damages are not repair correctly, they may lead to gene mutation and cancer [34], oxidative damage of DNA by reactive oxygen and nitrogen species leads to production of 8-hydroxy-2-deoxyguanosine (8-OHdG) which is a specific biomarker for oxidative stress [38]. Normal value of 8-OHdG found to be significantly different in terms of age, gender, smoking and alcohol consumption but not different in term of body mass [7]. In recent years, 8-OHdG compound has been commonly used not only as a biomarker for endogenous DNA damage assessment but also as a risk factor for many diseases, including lung cancer, in many studies [12], ovarian cancer [39] and breast cancer [33]. Measuring oxidative modified cellular constituent biomarkers, including 8-OHdG for DNA damage in biological samples, is a promising strategy in the field of public health. It has been discussed before that a promising future is to incorporate knowledge from genome programs to broaden the scientific frontiers on etiology health risk prediction and prevention of environmental disease risk by developing a reliable biomarker database [40]. ROS produced under the effect of ionizing radiation induce the formation of 8-OHdG compound from cellular and mitochondrial DNA, hydroxyl radical (OH) plays important role in producing 8-OHdG. The 8-OHdG estimated in urine
and blood cells as a biomarker for oxidative DNA damage [16]. The results of G2 showed high level concentration of 8-OHdG and the highest level of uranium concentration in urine samples. It has been reported that occupational exposure to low dose radiation is prevalent with wide-spread applications of ionizing radiation in several industries. While the International Radiological Safety Commission recommends an effective dose limit of 20 mSv for an average of 5 years and the effective dose should not be greater than 50 mSv in any single year [31].

4. Conclusions
The percent study demonstrates that the uranium pollution is presence in urine samples of all the examined groups reflecting the presence of chronic low dose radiation source in Al-Najaf governorate. Moreover, uranium pollution contributes to DNA damaging in all of the scanned groups. The oxidative damage of the DNA means higher probability of many diseases mainly cancer, a point which needs more investigations.

| Table 1. The levels of uranium concentration in urine for three groups |
|--------------------------|--------------------------|--------------------------|
| **Uranium concentration (µg/L)** | G1 | G2 | G3 |
| **Variable** | Range | 0.989-2.977 | 1.471-2.782 | 1.145-3.062 |
| [Mean± STD Deviation] | 1.837±0.427 | 2.021± 0.404 | 1.755±0.487 |
| **P-value** | 0.738 | 0.054 | 0.217 |

![Figure 1. Uranium concentration in group G1, G2, G3.](image-url)
Table 2. The levels of urinary 8-OHdG concentration for three groups

| Variable                      | Range          | G1             | G2             | G3             |
|-------------------------------|----------------|----------------|----------------|----------------|
| Range                         | 33.89-94.46    | 26.15-78.96    | 28.96-90.94    |
| [Mean± STD Deviation]         | 49.810±15.484  | 47.717±14.232  | 46.769±14.249  |
| P-value                       | 0.548          | 0.901          | 0.611          |

Figure 2. Urinary 8-OHdG concentration in group G1, G2, G3.

References
[1] Hofer T, Karlsson HL and Möller L 2006 DNA oxidative damage and strand breaks in young healthy individuals: a gender difference and the role of lifestyle factors *Free Radic. Res.* 40 707-714.
[2] Collin PH 2004 *Dictionary of Environment and Ecology* 5th ed. Bloomsbury Publishing Plc. London.
[3] Hill MK 2004 *Understanding Environmental Pollution (A primer)* 2nd ed. Cambridge University press. UK.
[4] Wright J 2005 *Environmental Chemistry* 2nd ed. Routledge Publishing. London.
[5] Shafik SS and Qaddoori SM 2018 Urinary excretion as a function of uranium concentration in bladder cancer patients using kinetic phosphorimetry analyzer *J. Phys Conf. Series*.
[6] Kimura S, Yamauchi H, Hibino Y, Iwamoto M, Sera K and Ogino K 2006 Evaluation of urinary 8-hydroxydeoxy guanine in healthy Japanese people *Basic Clin. Pharmacol. Toxicol.* 98 496-502.
[7] Sakano N, Wang DH, Takahashi N, Wang B, Sauriasari R, Kanbara S, Sato Y, Takigawa T, Takaki J and Ogino K 2009 Oxidative stress biomarkers and life styles in Japanese healthy people *J. Clin. Biochem. Nutr.* 44 185-195.
[8] Scandalios JG 1997 *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*. New York: Cold Spring Harbor Laboratory Press.
[9] Halliwell B and Gutteridge JMC 1999 *Free Radical in Biology and Medicine*, 3rd ed. Oxford: Oxford University Press.
[10] Kasai H, Hayami H, Yamaizumi Z, Saito H and Nishimura S 1984 Detection and identification of mutagens and carcinogens as their adducts with guanosine derivatives Nucleic Acids Res. 12 21270–2136.

[11] Finkel T and Holbrook NJ 2000 Oxidants, oxidative stress and the biology of ageing. Nature 408 239–247.

[12] Yano T, Shoji F, Baba H, Koga T, Shiraishi T, Orita H and Kohno H 2009 Significance of the urinary 8-OHdG level as an oxidative stress marker in lung cancer patients. Lung Cancer 63 111–114.

[13] Bacchi S, Palumbo P, Di Carlo M and Coppolino MF 2016 Cocaine effects on generation of reactive oxygen species and DNA damage: formation of 8-hydroxydeoxyguanosine in active abusers Int. J. Pharmacol. Toxicol.

[14] Wu LL, Chiou CC, Chang PY and Wu JT 2004 Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics Clin. Chim. Acta. 339 1-9.

[15] Kimura S 2006 Evaluation of Urinary 8-Hydroxydeoxyguanine in Healthy Japanese People : URINARY 8-HYDROXYDEOXYGUANINE AND HEALTHY PEOPLE Basic Clin. Pharmacol. Toxicol. 4.

[16] Valavanidis A, Vlachogianni T and Fiotakis C 2009 8-hydroxy-2- deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. J Environ. Sci. Health Part C 27 120–139.

[17] Droge W 2002 Free radicals in the physiological control of cell function Physiol. Rev. 82 47–95.

[18] Scandalios JG 2001 Molecular Responses to Oxidative STRESS Mol. Anal. Plant Adapt. Environ. 181-208.

[19] Salehi A, Ebrahimpour K, Forouharmajd F and Zarean M 2020 The relationship between collective effective doses of radiation and, urinary concentration of 8- Dihydroxy- 2'-Deoxyguanosine among radiography staff Int. J. Radiat. Res. 18.

[20] Khan F, Williams M and Wilkins A 1984 The physics of radiation therapy Baltimore, USA.

[21] Qaddoori SM and Shafik SS 2018 CR-39 As A Tool For Uranium Concentration Calculation In Bio Assay Sample: Bladder Cancer As Case Study. Res. J. Pharmac. Biol. Chem. Sci. 9 6 228-239.

[22] Fadel KH 2014 Estimation IL-6 level in Patients with Respiratory Diseases That Caused by Bacteria and Other Causative Agents in Some Baghdad Hospital MSc. Thesis, Baghdad University.

[23] Khraibet KI 2015 Estimation of IL-2 level and uranium concentration in a sample of recurrent aborted women in Baghdad and Maysan governorates using ELISA and SSNTDs techniques MSc. Thesis, Baghdad University.

[24] Al-Jubouri EMR 2012 Measuring the concentrations of uranium and thorium in the urine of Cancer patients in the city of Baghdad MSc. Thesis. Dep. Phys. Coll. Sci. Unv. Baghdad. Iraq.

[25] WHO 2004. Guidelines for Drinking Water Quality Third edition, World Health Organization, Geneva (2004).

[26] Al-Hamadany 2011 Radiation pollution in cancer and other diseases using some immunological and clinical parameters. Ph.D. Thesis. Dep. Biol. Coll. Sci. Baghdad Iraq.

[27] Annual report to congress 2007 Federally Sponsored Research on Gulf War Veterans’ Illnesses for 2007. Department of veterans affairs.

[28] World Health Organization, WHO 2001 Depleted Uranium Sources, Exposure and Health Effects.

[29] Fentiman AW, Smith M and Veley RJ 2004 How do radioactive materials move through the environment to people Ohio State University Extension. (2004).

[30] Hassan SF 2006 Determination of Uranium Concentration in Human Blood in Some Governorates of Iraq M.Sc. Al- Nahrain University.
[31] Gao Y, Wang P, Wang Z, Han L, Li J, Tia C, Zhao F, Wang J, Zhao F, Zhang Q and Lyu Y 2019 Serum 8-Hydroxy-20-Deoxyguanosine Level as a Potential Biomarker of Oxidative DNA Damage Induced by Ionizing Radiation in Human Peripheral Blood Int. J.

[32] Al-Rubaie All 2014 Potential Impacts of Uranium Pollution and its Relation to Some Immunological Parameters in a Sample of Iraqi Patients With Lung Cancer PhD. Thesis, College Of Science, University of Baghdad Iraq.

[33] Mohamed NourEldin EE, El-Readi MZ, NourEldein MM, Alfalki AA, Althubiti MA, Kamel HF, Eid SY, Al-Amodi HS and Mirza AA 2019 8-Hydroxy-2′-deoxyguanosine as a Discriminatory Biomarker For Early Detection of Breast Cancer.

[34] Cukurova Z, Cetingok H, Ozturk S, Gedikbasi A, Hergunsel O, Ozturk D, Don B, Cefle K, Palanduz S and Ertem DH 2019 DNA damage effects of inhalation anesthetics in human bronchoalveolar cells Medicine. 98 32. (2019).

[35] Withee ED, Tippens KM, Dehen R, Tibbits D, Hanes D and Zwickey H 2017 Effects of Methylsulfonylmethane (MSM) on exercise-induced oxidative stress, muscle damage, and pain following a halfmarathon: a double-blind, randomized, placebo-controlled trial J. Int. Soc. Sports Nut. 14 24.

[36] Ma Y, Zhang L, Rong S, Qu H, Zhang Y, Chang D, Pan H, and Wang W 2013 Relation between Gastric Cancer and Protein Oxidation, DNA Damage, and Lipid Peroxidation. Oxidative Med. Cell. Longevity.

[37] Vakil C and Harvey L. 2009 Human health implications of the nuclear energy industry Canad. Assoc. Phys. Environ.

[38] An AR, Kim KM, Park HS, Jang KY, Moon WS, Kang MJ, Lee YC, Kim JH, Chae HJ and Chung MJ 2019 Association between Expression of 8-OHdG and Cigarette Smoking in Non-small cell Lung Cancer J. Pathol. Translat. Med. 53 217-224.

[39] Xu X, Wang Y, Guo W, Zhou Y, Lv C, Chen X and Liu K 2013 The significance of the alteration of 8-OHdG in serous ovarian carcinoma J. Ovarian Res. 6 74.

[40] Bonassi S and Au WW 2002 Biomarkers in molecular epidemiology studies for health risk prediction Mut. Res. 511 73- 86.