

Assessment of Oxidative DNA Damages in Radiography Staff via Evaluation of Its Urinary Biomarker (8-hydroxy2-deoxyguanosine)

Abstract

Background: Studies have shown that one of the most important complications of exposure to ionizing radiation is the emergence of cancer tumors, as a result of oxidative DNA. Since different radiography groups have high rate of exposure to ionizing radiation, examining the susceptibility rate of cancer in these groups is of prime importance. Therefore, the present study was conducted to measure the level of 8-hydroxy2-deoxyguanosine (8-OHdG) in the radiographers' urine as a biomarker of oxidative damage while comparing it with the nonradiography staff. **Methods:** Samples of two groups were selected for this case-control study, wherein 35 subjects were selected from different radiography groups (including nuclear medicine, radiology, radiotherapy, and CT scan) while the other 35 subjects were staffs who had no exposure to radiation. Later, urine samples were collected at the end of the working shift to determine the 8-OHdG concentration. The samples were obtained via SPE (solid-phase extraction) method. Subsequently, the 8-OHdG concentration was measured by the GC-MS analyzer. **Results:** The results confirmed that, the average concentration of 8-OHdG in the radiographers' urine (253.4 ± 31.2 ng/mg of creatinine) had a significant difference as compared to the nonradiographers' urine (141.1 ± 21.9 ng/mg of creatinine) ($P = 0.004$). **Conclusions:** In conclusion, due to elimination of interfering factors, ionizing radiation affects the increase in 8-OHdG levels and acts as a potential biomarker for the damaged oxidative DNA.

Keywords: 8-hydroxy2-deoxyguanosine, oxidative DNA, radiography staff

Introduction

Oxidative stress was first introduced in 1991 as an imbalance between oxidants and antioxidants in favor of the former. Oxidative stress can induce different reactive oxygen species (ROS) and their frequency in the human body, which can further impose permanent changes in the structure and function of biomolecules. DNA has frequently been studied under oxidative stress conditions and the oxidative DNA damage caused by different ROS has also been investigated. This material can react and cause different damages in the DNA molecule and is found extensively in the human body. One of the frequently studied damages on DNA is about 8-hydroxy2-deoxyguanosine (8-OHdG).^[1]

One of the main modified alkaline products of DNA is 8-OHdG. Formation of 8-OHdG in serum, leukocytes, and urine is often measured to investigate the level of oxidative stress in humans. This compound

is a known carcinogen which is conjugated to thymidine, and G: C \rightarrow T: A conversion occurs. Oxidative stress is thought to be associated with tumor formation.^[2] Therefore, determining the level of 8-OHdG can determine the individual's susceptibility to develop tumor thus resulting in the emergence of cancer.^[3] Increasing the base level of DNA oxidation is accompanied by various diseases including diabetes mellitus, cancer, degenerative diseases of the nervous system, and renal terminal diseases. The level of oxidative DNA lesions depends on several factors, including environmental risks and genotoxic factors, smoking, alcohol consumption, intracellular and extracellular metabolism, and exposure to ionizing radiation. Various methods have been developed for quantitative measurement of 8-OHdG in human DNA specimens, which includes HPLC, GC-MS, the chemistry of immunity texture, and the ELISA test.^[4] The most sensitive method is to measure the FPG (the enzyme formamidopyrimidine glycolase DNA) using GC-MS.^[5] Although there are other

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ways to measure the levels of 8-oxoGua and 8-oxodG in human biological fluids such as urine, serum, plasma, and blood; mass spectrometry (MS), electrochemical detection (EC), and ELISA-based methods are used to decompose the DNA waste from the urine.^[6]

As previously mentioned, one of the environmental factor affecting human physiology and oxidative DNA damage is ionizing radiations, which have been investigated and documented sufficiently during the past century post nuclear incidents and inhalation of/or exposure to ionizing radiation. Regardless of the environmental exposure, artificial resources of ionizing radiation are used increasingly. Recently, exposure to the ionizing radiation as a result of medical diagnostic tests and treatment strategies has increased. Radiography and radiotherapy staff are exposed to the cumulative effect of ionizing radiation.^[7]

Moreover, studies have shown that one of the most important indications in the exposure to ionizing radiation is the cancer tumors that occurred due to oxidative DNA damage. Since radiographers, especially those working in diagnostic radiology and radiation therapy, have the highest exposure and ionizing radiation cumulative dose, it is imperative to assess the incidence of cancer in this stratum. Therefore, the present study was conducted to measure the 8-OHdG level in radiographers' urine, as the biomarker of oxidative damage, and compare it with the nonradiography staff.

Methods

In this case-control study, 70 subjects were selected, who were divided into two groups, 35 of them were selected as the case group (the group exposed to different ionizing radiation) among the different radiography staff working in four state hospitals in the city of Isfahan; including the ones in the nuclear medicine (6 people), radiotherapy (8 people), radiology personnel (10 people), and CT-scan personnel (11 people), and 35 nonradiation workers were selected as the control group (the group that had no exposure to ionizing radiation) among the staffs of Isfahan Medical Science University.

After coordinating with the management of the hospitals, informed consent was obtained from each of the participants. Initially, a checklist of participants' demographic information (gender, age, work experience, and type of occupational group) was prepared. The inclusion criteria for the study was investigated via a checklist for the radiographers and the nonradiation workers.

The subjects were excluded from the study in the event of preventing to give urine sample, smoking, consuming tea and coffee during the working shift, consuming alcohol, taking medication even for a few days prior to sampling, the presence of acute and severe illnesses (such as cancer, diabetes, renal terminal diseases, degenerative diseases of the nervous system, high blood pressure, or any other known disease), as well as occupying a second

job exposed to the ionizing radiation in the group of radiographers. Urine samples were taken from the selected personnel at the end of their work shift. The samples were transferred to the laboratory in ice bags. About 2 ml of the sample was isolated from each sample to determine the concentration of creatinine and was sent to the laboratory (Step 2-1=Materials, Step 2-2=Creatinine assay), rest were kept inside a freezer (-80°C) for the testing stages (Step 2-3=Sample preparation, Step 2-4=GCMS analytical method).

Materials

The standard 8-OHdG and derivative (N-methyl -N-(trimethylsilyl) trifluoroacetamide, MSTFA) were purchased from Sigma Co. (St. Louis, MO, USA). Specific solutions of HPLC and GC including methanol and formic acid (98%) were obtained from MERK Company.

Creatinine assay

The concentration of creatinine in the urine was measured in an approved medical laboratory using commercial kit purchased from Sigma diagnostics (St. Louis, MO, USA), based on the Slot method.^[8]

Sample preparation

Preparation and clean-up of urine samples were performed according to a previously described method with some modifications.^[9] In brief, the urine samples were acidified with formic acid (1:10, v/v) and incubated at 4°C for 1 hour. For clean-up of the urine samples SPE cartridges (Oasis® HLB Vac, 60 mg, Waters, USA) were used. About 5 ml methanol and 5 ml of 20 mM formic acid (pH ≈ 2.75) were used for preconditioning of the cartridges. The urine samples were first centrifuged (5000 rpm for 10 min) and then 5 ml of supernatant was loaded in each preconditioned cartridge (approx. 1 ml/min). After that, 5 ml of 20 mM formic acid was passed through the cartridges to flush the cartridges. Finally, 5 ml of 17.5% (v/v) methanol in 20 mM formic acid was added to the cartridge for elution of 8-OHdG. Drying of the cartridge under vacuum after each clean-up step is necessary. Hence, the final collected fractions were dried using vacuum freeze dryer. Derivatization is a key step before GC analysis. About 50 µl derivatization mixture (Acetonitrile/MSTFA, 1:1, v/v) was added to samples and incubated for 1 hour in 80°C and later 2 µl of the derivatized sample was subjected to GC-MS analysis.

GC-MS analytical method

The GC-MS analysis was performed using a quadruple Agilent GC-MS (7890A, Agilent Technologies, CA, USA) coupled to a mass selective detector (5975C inert), the GC was equipped with a split/splitless injector. The MS was operated at the electron impact (EI) mode (70 eV). The carrier gas was helium (99.999%) at the flow rate of 2 mL/min. A DB-5MS column (60 m, 0.25 mm i.d., 0.25

µm film thickness) was used for separation of 8-OHdG. Following were the oven temperature programs: the initial temperature was set at 210°C (5 min holding time), and then increased from 210°C to 300°C at 15°C/min (4 min holding time); the injector, ion source, mass analyze, and the transfer line temperature was set at 320, 230, 150, and 300°C, respectively. Selected ion monitoring (SIM) mode (m/z 207) was applied to gain the highest possible sensitivity for quantification of 8-OHdG.^[10]

Statistical analysis

The data from the demographic checklist as well as the results of 8-OHdG concentration in both groups were analyzed by SPSS software.

Categorical data were expressed in terms of number and percentage while quantitative data were expressed in terms of mean, standard deviation, and range. The normality of continuous quantitative data (age, work experience, and mean concentration of 8-OHdG) was investigated by Kolmogorov-Smirnov test, which showed that the distribution of these variables was a normal distribution.

In addition, the Chi-square test was used to compare the categorical data between the two groups and the independent *t*-test was used to compare the mean of concentration of 8-OHdG in the two groups and further to compare the quantitative data (age and work experience) between the two groups. One-way ANOVA and Tukey post-hoc tests were applied to compare the mean of concentration of 8-OHdG in different groups of radiographers.

Results

Following results were obtained from the study of demographic data of both the groups of radiographers and nonradiation workers (sex, age, and work experience) [Table 1]:

The age group for radiation workers was ranging from 26 to 56 while for the nonradiographers it was from 29 to 55 years. The Chi-square test showed no significant difference in the frequency distribution of gender between the two groups ($P = 0.47$). The independent *t*-test showed that the mean age ($P = 0.59$) and work experience ($P = 0.86$) were not significantly different between the two groups.

By assessing the data obtained from the analysis of urine specimens, the following results were obtained for both the radiographer and nonradiation worker groups:

The independent *t*-test showed that the mean concentration of 8-OHdG in the urine was significantly higher in the group of radiographers than in the group of nonradiation workers ($P = 0.004$) [Table 2].

Further analysis of the data showed that the mean concentration of 8-OHdG in the urine was also found in different groups of radiographers, which was as follows:

Table 1: Demographic data of both groups

Variable	Radiography staff (n=35)	Nonradiation staff (n=35)
Age (year)		
Mean±SD	40.6±8.6	41.6±6.7
Range	26-56	29-55
Sex		
Male	19 (54.3%)	22 (62.9%)
Female	16 (45.7%)	13 (37.1%)
Work experience (year)		
Mean±SD	15.8±8.6	16.1±7.5
Range	3-29	2-30

Table 2: Mean concentration of 8-OHdG in the urine in the two groups

Group	Mean (ng/mg of creatinine)	Standard deviation	P
Radiography staff	253.4	31.2	0.004*
Nonradiation worker	141.1	21.9	

*significant

Table 3: Mean concentration of 8-OHdG in the urine, broken down by the occupational category of radiographers

Category of radiographers	Mean (ng/mg of creatinine)	Standard deviation	P
Nuclear Medicine	427.1	106.5	0.03*
Radiotherapy	189.9	39.7	
CT-Scan	262.04	53.4	
Radiology	190.4	42.5	

*Significant

One-way ANOVA showed that there was a significant difference in the mean concentration of 8-OHdG in the urine between different occupations ($P = 0.03$). The Tukey post-hoc test showed that the mean concentration of urine in people with nuclear medicine occupation was significantly higher than the ones with occupations such as radiotherapy ($P = 0.02$), CT scan ($P = 0.035$), and radiology ($P = 0.02$). However, there were no significant differences between the other occupations ($P > 0.05$). The mean concentration of the substance in the urine between different occupations is summarized in Table 3.

We also compared the mean concentration of 8-OHdG between the participants in each radiation group and the control group with the Tukey post-hoc test. According to the results, there is significant difference between the control group and the nuclear medicine group ($P = 0.001$), the control group and the CT scan group ($P = 0.01$), the control group and the radiotherapy group ($P = 0.04$), and the control group and the radiology group ($P = 0.03$).

Discussion

Different studies have investigated the effect of various factors on the increase of 8-OHdG concentration in the human body.

In an earlier study, Ren *et al.* measured 8-OHdG level in the urine as a biomarker of oxidative DNA damage, which had resulted due to room contamination in old age group. They stated that air contamination could damage human health through unknown mechanisms and concluded that exposure to alternative sources of contamination might speed up aging and increase the risk of oxidative DNA damage.^[11] Kumodor *et al.* measured the levels of 8-OHdG and 8-isoprostane in women, who were exposed to cooking fire smoke. They considered exposure to smoke as a cause of oxidative DNA damage and lipid peroxidation. The results indicated that the level of systemic oxidative stress in women exposed to wood smoke is much higher.^[12] Moreover, Kim *et al.* examined the level of 8-OHdG of urine as a biomarker for oxidative DNA damage in workers exposed to dust and fine particles of boilers. Dispersed ash is the oil residue of a chemical compound that contains potentially carcinogenic metals because of the potential for oxidative damage. They monitored 20 workers (50% smokers) for 5 days. Then, they measured their urinary 8-OHdG levels which was less at the beginning of the working shift as compared to that at the end of the working shift. The corrected linear regression based on the age and status of chronic bronchitis as well as the urinary creatinine level showed that having contact with ash particles caused a rise in urinary 8-OHdG levels. They suggested that young and healthy workers at the boiler industry would experience a long-term risk of oxidative DNA damage through contact with high levels of particulate matter containing metals.^[13]

Furthermore, Kato *et al.* (2016) studied the effect of ionizing radiation on the level of 8-OHdG, the sensitive biomarker for radiation-induced DNA damage in children who had cardiac catheterization. They evaluated ten healthy and nine diseased children. The results were shown within 24–48 hours after the treatment in comparison to the baseline wherein the mean urinary level of 8-OHdG had significantly increased (44.0 vs. 17.3 ng/mg creatinine, $P = 0.0001$).

They concluded that the cumulative radiation present in air had an essential and significant relationship with the 8-OHdG level in the urine after the course of the treatment. They also stated that the 8-OHdG level in the urine could be a useful biomarker to determine the rate of DNA damage in children due to radiation.^[14] In a study to determine the concentration of 8-OHdG in the urine of the radiographers and nonradiation workers using the ELISA method by Rahimipour *et al.*, it was found that the 8-OHdG level in the urine of the group of radiographers was significantly higher than that of the nonradiation workers.^[15]

The results of this study, which was done for the first time in the country by using solid-phase extraction method for data extraction and then analyzed by GC-MS to determine

the 8-OHdG level in the urine, indicated that 8-OHdG concentration in radiographers' urine (with the average of 253.4 ng/mg of creatinine) had significant difference with that of the nonradiographers' urine (with the average of 141.1 ng/mg of creatinine) ($P = 0.004$). The mean concentration of urine in people with nuclear medicine occupation was significantly higher than the ones with occupations such as radiotherapists, CT scan operators, and radiologists.

Since it is difficult to determine 8-OHdG by chromatographic methods (such as expensive required instruments and difficult derivatization procedure), most researchers tend to determine 8-OHdG with the available commercial ELISA kits. However, it is notable that the use of GC-MS method for determination of 8-hydroxy-2-deoxyguanosine is at least ten times better than the ELISA method and there is no possibility for false positive or negative results in GC-MS determination.^[16]

According to the results of the proposed method, the effect of ionizing radiation in increasing the 8-OHdG levels, as oxidative biomarkers has been identified. Evidently, observing the radiation protection principles by the radiographers will lead to less exposure to radiation which have been mentioned as follows:^[17]

1. Reducing the exposure time to radiation
2. Increasing the distance from the source
3. Placing a protective shield between the person and the radiation source
4. Self-protection against radioactive contamination by using appropriate clothing and covers

The dose received by staff workers in the nuclear medicine group is higher than other workers due to their work deputed in the banned area (little distance of the technician from the source of radiation) than the rest of the staff. The higher the distance with the source, the lower the exposure. Any object between the technician and the source of radiation will reduce the amount of exposure, and as a general rule, if the object or matter between the technician and the source of the beam is denser, a better protection will be provided.^[18] Increasing the concentration of 8-OHdG in the urine of the nuclear medicine group indicates that with the higher amount of radiation, the oxidative damage will be more. Regarding the relationship between oxidative stress and cancer, it seems that antioxidants like vitamin E and C and β -carotene are beneficial in preventing cancer. Several studies have also been carried out in this regard.^[19] Also, the effect of exercise on oxidative stress has been investigated in some studies. The results of the research by Rahimi *et al.* showed that oxidative DNA damage in athletes is less than that in nonathletes. This may be due to the history of regular resistance exercises performed by the bodybuilding athletes, and it is possible that antioxidant capacity in the athletes may be developed by regular exercise.^[20]

Conclusion

Regarding the similarity between the two groups of radiographers and nonradiation workers in terms of sex, age, work experience, and elimination of any factors in both the groups which contradicts the inclusion criteria to the study, it can be concluded that ionizing radiations had significant effects on the increased level of 8-OHdG thereby considering it as one of the possible oxidative biomarkers in the body of the radiographers.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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