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The relationship between collective effective doses of radiation and urinary concentration of 8-Dihydroxy-2'-Deoxyguanosine among radiography staff

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▸ ***Short Report***

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ABSTRACT

Background: The effect of ionizing radiation of 8-HYDROXY- 2'-DEOXYGUANOSINE (8-OHdG) level in radiographers' urine as the oxidative damage biomarker was investigated in this study and this biomarker was compared with the collective effective doses. ***Materials and Methods:*** The samples were selected into two categories in this cross-sectional study, 35 of whom were from different radiography groups (including nuclear medicine, radiology, radiotherapy, CT scan), and 35 subjects were from the staff who had no exposure to radiation. The results of the film badge were gathered from the hospitals, and the collective effective dose was obtained according to the respective formula. Then, at the end of the work shift, the urine samples were taken to determine the 8-OHdG concentration. The samples were obtained via the SPE (solid-phase extraction) method. After that, the 8-OHdG concentration was read by the GC/MS analyzer. Finally, the data resulted from the 8-OHdG concentration and the collective effective dose of the radiation were analyzed by the SPSS software. ***Result:*** The results showed that the 8-hydroxy-2-deoxy-guanosine concentration in the urine of the radiation workers had significant correlation with the collective effective dose of the radiation the past 6 periods (P=0.009) and also with the collective effective dose of the radiation of the 30 last periods (P=0.009). ***Conclusion:*** Observing the radiation protection principles by radiation workers results in decreased radiation and, in turn, reduces the level of oxidative stress, thus, reducing the potential effects of radiation.

Keywords: *Radiography staff, collective effective dose, 8-Dihydroxy-2'-Deoxyguanosine.*

INTRODUCTION

Ionizing radiation, as a toxicant and carcinogen, can produce reactive oxygen species and cause severe oxidative and DNA damage, such as single- and double-strand breaks. These damages are not repaired correctly, and they may lead to the oxidized bases, and DNA–protein cross-links, chromosomal aberration (CA), cell death, and increased risk of gene mutation and cancer [(1)]. As the product to modify the oxidative guanine that is released in the urine, the level of 8-OHdG is one of the most sensitive biomarkers of DNA oxidative damage [(2)]. 8-hydroxy-2-deoxyguanosine (8-OHdG) is one of the main modified alkaline products of DNA. Formation of 8-OHdG in serum, leukocytes, and urine is often measured to investigate the level of oxidative stress in humans [3]. Therefore, investigating the level of 8-OHdG can determine the individual's susceptibility to developing a tumor and the emergence of cancer [4]. Various methods have been developed for the quantitative measurement of 8-OHdG and 8-oxodG in human DNA specimens, which include HPLC, GC/MS, the chemistry of immunity, and ELISA test [5]. However, the most sensitive method is to measure the FPG (the enzyme formamidopyrimidine glycolase DNA) and GCMS [6].

It is considered that no dose of ionizing radiation exposure is safe. However, once the accurate absorbed dose is estimated, one can be given appropriate medical care and the severe consequences can be minimized [7]. Despite the existence of several accurate dose estimation modalities, but the ICRP Commission introduced the rates of collective dosage to optimize the radiological protection. The radiation collective effective dose is the product of the effective mean dose per each individual in a group or population in the number of exposed individuals. This unit is usually used to obtain a possibility and the generalized estimation of the occurrence of cancer and hereditary problems in a population. It is also used for the rough comparison of the created biological effects due to the various ionizing radiation in a population or a group [8].

Since all of the occupied radiography staff in the radiology, radiation therapy, CT scan and nuclear medicine sections are in contact with the ionizing radiation, this study aimed to determine the level of 8-OHdG via the sensitive method (which was done for the first time in the country by using solid-phase extraction method for data extraction and then analyzing by GC/MS to determining 8-OHdG level in urine) and compare it with the mean collective effective dose to find out about the biological effects of ionizing radiation in order to optimize the radiation protection.

MATERIALS AND METHODS

In this cross-sectional study, 70 subjects were selected in two group, 35 of whom were selected 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 staff (8 people), radiology personnel (10 people) and CT-scan personnel (11 people) as the group exposed to different ionizing radiation, and 35 non-radiation workers were also selected among Isfahan Medical Science University staffs the control group (the group that had no exposure to ionizing radiation). After coordinating with the management of the hospitals, the informed consent with code of ethics IR.MUI.REC.1396.3.293 was obtained from each of the participants. Initially, a checklist of participants' demographic information (gender, age, work experience (30 years of work in Iran), and type of occupational group) was prepared. Results of the film badges of each radiation worker was collected from the related hospitals and collective effective dose was calculated according to the equation 1 [9]. For the group of radiation workers (35 people), the results of effective dose of radiation was collected for the last one year and also the last 30 working day periods of each of the radiographers (Since the film badge monitoring period is usually 2 months, therefore last 6 periods again with the last 1 year and the last 30 periods are the equivalent to the last 5 years) (Each period was considered as 2 months for film badge monitoring, therefore 6 periods equals to one year or 12 months, and 30 periods equals to 5 years or 60 months). Hence, through the relevant computations:

$$S=\sum E_i.N_i \quad (1)$$

Where Ei is the mean collective effective dose in the subgroup with "i" population, and Ni indicates the number of people in the same subgroup.

Experimental procedures

Materials

The standard 8OHdG, 8-hydroxy-2-deoxyguanosine 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 prepared from Merck Company.

Subjects and sampling

The inclusion criteria to the study were investigated via a checklist for the radiographers and the non-radiation 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 in 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 shift work. The samples were transferred to the laboratory on ice bags. 2 ccs were isolated from each sample to determine the concentration of creatinine and sent to the laboratory, and the rest were kept inside a freezer (-80° C) for the testing stages .

Creatinine assay

The concentration of creatinine in the urine was measured in an approved medical laboratory using its commercial kit purchased from Sigma Diagnostics (St. Louis, MO,USA),based on the Slot method [10].

Sample preparation

Preparation and clean-up of urine samples were performed according to a previously described method with some modifications [11]. Briefly, 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. 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 fist centrifuged (5000rmp for 10 min) and then 5 ml of supernatant loaded in each preconditioned cartridge (approx. 1 ml/min). After that, 5 ml of 20 mM formic acid passed through the cartridges to flush the cartridges. Finally, 5 ml of 17.5% (v/v) methanol in 20 mM formic acid add to cartridge for elution of 8OHdG. Drying of cartridge under vacuum after each clean-up step is necessary. The final collected fractions were dried using vacuum freeze dryer. Derivatization is a key step before GC analysis. 50 µl derivatization mixture (Acetonitrile/MSTFA, 1:1, v/v) added to samples and incubated for 1hour in 80˚C. 2 µl of the derivatized sample was subjected to GC–MS analysis.

GC-MS analysis

The GC-MS analysis was performed using a quadruple Agilent GC-MS (7890A, Agilent Technologies, CA, and 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 DP-5MS column (60 m, 0.25 mm i.d., 0.25 µm film thickness) were used for separation of 8OHdG. The oven temperature program included: 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 were 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 8OHdG [12].

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. The categorical data was expressed in terms of number and percentage, and the quantitative data were expressed in terms of mean, standard deviation, and range. The normality of continuous quantitative data (age, work experience, mean concentration of 8-OHdG and collective effective doses) was investigated by the Kolmogorov-Smirnov test, indicating that the distribution (age, work experience, and 8-OHdG

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concentration) was normal, while the distribution of the collective effective dose was not normal. For this purpose, 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 to compare quantitative data (Age & work experience) between these two groups.

RESULTS

The results of demographic data for both groups of the radiographers and non-radiation workers (sex, age, and work experience) are summarized in table.

The chi-square test showed that there was 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.

The rate of the effective dose received by each radiographer was obtained for different time periods according to the results of the film badges existing in each hospital. Two related samples test showed that the mean collective effective dose in the last 5 years periods was significantly more than that in the last one year periods in the group of radiographers (P = 0.001). The mean effective dose of radiation in the last 1 year and the last 5 years periods (mSv) in the group of radiographers. Results of mean collective effective dose (man Sv) is shown in table 2.

Moreover, the collective effective dose between different groups of radiographers were analyzed for different periods. The Kruskal-Wallis test showed that the mean effective dose of radiation in the last one year and the last 5 years as well as the collective effective doses of radiation in the last one year and the last 5 years periods in the nuclear medicine group was significantly higher than the other groups (P=0.001).

The independent t-test analysis of urine specimens, indicated that the mean concentration of 8-hydroxy-2-deoxy-guanosine in urine was significantly higher in the group of radiographers than that in the group of non-radiation workers (P=0.003) (table 3).

Finally, the Spearman correlation coefficient showed that the 8-hydroxy-2-deoxy-guanosine concentration in the urine of the radiation workers had significant relation with the collective effective dose of the radiation the past 6 periods the last one year (P=0.009) and also with the collective effective dose of the radiation of the last 5 years (P = 0.009).

Table 1. Demographic information of study groups.

Variable	Radiography staff (n=35)	non-radiation worker (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
Rang	3-29	2-30

Table 2. The Mean effective dose (mSv) and collective effective dose (manSv) of radiation in the last 6 and the last 30 periods in the group of radiographers.

	effective dose (mSv)		Collective effective dose (manSv)	
	last 6 periods	last 30 periods	last 6 periods	last 30 periods
Mean ± SD	0.77± 0.25	2.67±0.87	5.22±1.29	18.7±4.2
P_1		0.001*		
P_2				0.001*

P1: p- value for comparing between mean effective dose for the last 6 periods and the last 30 periods. P1: p- value for comparing between mean collective effective dose for the last 6 periods and the last 30 periods. * Statistically significant at p < 0.05.

Table3. The Mean concentration of the 8-OHdG in the urine in the 2 groups.

group	Mean±SD (ng/mg of creatinine)	P-value
Radiography staff	259.4± 31.07	0.003*
non-radiation worker	141.1± 21.8	

*Statistically significant at p < 0.05.

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DISCUSSION

Several studies have reported that the concentration of 8-OHdG increases with exposure to low-dose ionizing radiation. The findings of a study showed that levels of 8-OHdG in urine of individuals exposed to ionizing radiation were significantly higher than those who did not have exposure [13-14].

In a study by Rahimipour and his colleagues to determine the concentration of 8-OHdG in the urine of the radiographers and non-radiation workers using the ELISA method, it was found that the 8-OHdG level in the urine of the group of radiographers was significantly higher than that of non-radiation workers [15]. The results of this study showed that the concentration of 8-OHdG in the urine of the group of radiographers (with the average 259.4±31.07 ng / mg of creatinine) was significantly different from that in the group of non-radiation workers (with the average 141.1±21.8 ng/ mg of creatinine) (P=0.003). Regarding the similarity between the two groups of radiographers and non-radiation workers in terms of sex, age, work experience and elimination of any factor in both groups that contradict the inclusion criteria to the study, it can be concluded that as one of the oxidative biomarkers in the body of the radiographers, the ionizing radiation was effective in the increased level of 8-OHdG. It was also found that the 8-OHdG urinary concentration in the nuclear medicine group was higher than the radiotherapy and radiology groups. In a study by YU GaO *et al.*, the difference in serum levels of 8-OHdG was significant between the nuclear medicine and radiology groups, but no significant difference was found between the radiotherapy and radiology groups as well as the radiotherapy and nuclear medicine groups [1]. The difference in the frequency of CA and serum levels of OH-8G in healthy and the radiographers has been determined. A positive association was found between serum levels of 8-OHdG and age, work experience, collective dose and the CA frequency [16]. Sanaa *et al.* obtained a serum level of 8-OHdG for 60 radiographers by ELISA method and compared it with the effective dose results from badge films of the employees. According to their study results, there was a significant correlation between serum levels of 8-OHdG and an effective one-year dose of radiation. (P<0.001) [7].

On the contrary, other researchers believe that serum levels of OH-8G in patients, who have long been exposed to radiation due to radiotherapy are lower than healthy subjects, and there is no relationship between collective dose and serum levels of 8-OHdG due to DNA repair capacity [17-18]. Since the previous studies have often evaluated the one-year collective effective dose of the radiation for comparing with the serum or urine levels of 8-OHdG, this study was conducted to determine the relationship between the collective effective dose of the past year and the past 5 years with the 8-OHdG urinary concentration. It was found that urinary levels of 8-OHdG have a positive correlation with the collective effective dose of radiation for the last one working year and the last 5 working years, and the more the effective dose is, the greater will be the concentration of 8-OHdG in the urine of radiographers. According to the results of this study, the highest rate of the effective dose and the highest rate of the collective effective dose were related to the nuclear medicine group, which also had the highest urine concentration of 8-OHdG among the other radiographers. The dose received by staff working in the nuclear medicine group is higher than other workers due to work in the banned area (little distance between the technician and the source of radiation). It is recommended that to reduce the amount of exposure to radiation, the time spent with the radiation source to be minimized; the longer the distance with the source, the lower will be the rate of exposure to it [19].

CONCLUSION

Observing the radiation protection principles by radiation workers results in decreased radiation and, in turn, reduces the level of oxidative stress, thus, reducing the potential effects of radiation. The occurrence of various

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cancers and hereditary genetic damage is one of the likely effects of ionizing radiation, which increases the likelihood of these effects by increasing the dose.

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