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Increased DNA Damage Of Radiology Personnel Chronically Exposed To Low Levels Of Ionizing Radiation

Sürekli Olarak Düşük Dozlarda İyonize Radyasyona Maruz Kalan Radyoloji Personelinin DNA Hasarındaki Artış

Vehbi Atahan Toğay[1], Funda Yıldırım Baş[2], Dilek Aşcı Çelik[1], Nurten Özçelik[1], Gülçin Yavuz Türel[1], Mustafa Calapoğlu[3], Pınar Aslan Koşar[1]

[1]Süleyman Demirel University, Faculty of Medicine, Department of Medical Biology, Isparta, Turkey.
[2]Suleyman Demirel University, Faculty of Medicine, Department of Family Medicine, Isparta, Turkey.
[3]Suleyman Demirel University, Faculty of Science and Art, Department of Biochemistry, Isparta, Turkey.

Abstract

Objective: People working in the hospital units such as Radiology and Nuclear Medicine are subject to higher doses of ionizing radiation than people working in other professions. We examined the association between DNA damage and ionizing radiation exposure in the personnel working in university hospital and considered different variables such as smoking, working years, gender, age.

Material-Method: 48 radiation exposed personnel, aged between 20-50 years old, working in radiological units within the Süleyman Demirel University Research - Application Hospital and 51 individuals, aged between 18 and 57, who do not work in hospital, constitute our research group. Lymphocytes isolated from blood samples taken from the participants were evaluated with the comet method for DNA damage. Tail DNA percentage parameter, obtained through Open Comet program, was chosen to assess DNA damage and the results were evaluated by the One-Way Anova statistical test.

Results: The results, obtained from statistical comparison of tail DNA percentage parameter, indicate that even the low dose radiation caused DNA damage and age, gender, smoking habits and working years did not show any significant differences except for dosimetry value. Increasing dosimetry value resulted in increased DNA damage.

Conclusions: This work supports the previous results of biomonitoring of radiology workers chronically exposed to ionizing radiation. This means ionizing radiation is still an important DNA damaging agent despite many improvements such as exposed time reduction, working conditions and technology.

Keywords: Ionizing Radiation, Comet Assay, DNA Damage, Radiology Personnel .

Özet

Amaç: Hastanelerin radyoloji veya nükleer tıp birimlerinde çalışanlar, diğer meslek gruplarına göre daha yüksek dozlarda iyonize radyasyona maruz kalmaktadır. Çalışmamızda radyoloji personelinin DNA hasarı ile iyonize radyasyona maruziyetleri arasındaki ilişki incelenmiş ve sigara içme alışkanlığı, cinsiyet, çalışma yılı ile yaş gibi farklı parametreler de hesaba katılmıştır.

Materyal-Metot: Süleyman Demirel Üniversitesi Araştırma ve Uygulama Hastanesi bünyesinde yer alan radyolojik birimlerde çalışan yaşları 20 ila 50 arasında 48 personel ile hastane personeli olmayan yaşları 18 ila 57 arasında 51 birey çalışma grubumuzu oluşturmaktadır. Bireylerden alınan kan örneklerinden izole edilen lenfositler DNA hasarı açısından komet metodu ile değerlendirilmiştir. Open Comet programı aracılığı ile elde edilen Kuyruk DNA yüzdesi parametresi DNA hasarını göstermesi için seçilmiş ve sonuçlar tek yönlü anova istatistik testi ile değerlendirilmiştir.

Bulgular: Kuyruk DNA yüzdesi parametresinin istatistiksel olarak karşılaştırılması sonucunda elde edilen veriler düşük doz radyasyonun bile DNA hasarına sebep olduğunu ve dozimetre değerinin önemli bir değişken olduğunu göstermektedir. Dozimetre değeri arttıkça daha yüksek DNA hasarı tespit edilmiştir. Yaş, cinsiyet, sigara içme alışkanlığı ve çalışma yılı ise anlamlı bir farklılığa sebep olmamıştır.

Sonuç: Sonuçlar, radyoloji personelini konu alan benzer çalışmalarda bildirilen biyo-izleme verileri ile paralellik göstermektedir. Bu durum, gelişen teknoloji ve çalışma koşullarının geliştirilmesi gibi önemli iyileştirmelere rağmen iyonize radyasyonun radyolojik birimlerde çalışan personel için halen önemli bir DNA hasar etkeni olduğunu ortaya koymaktadır.

Anahtar kelimeler: DNA Hasarı, İyonize Radyasyon, Komet Metodu, Radyoloji Personeli.

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ORCID: VAT 0000-0003-4722-3845, FYB 0000-0001-6852-3180, DAÇ0000-0002-2914-4695, NÖ 0000-0003-2326-6090, GYT0000-0001-9481-4475, MC0000-0002-6539-1335, PAK0000-0003-2602-5145

Yazışma Adresi / Corresponding: Vehbi Atahan Toğay,
Suleyman Demirel University, Faculty of Medicine, Departmant of Medical Biology, Isparta, Turkey.
Tel: +90 246 211 32 85
E-posta / E-mail: atahantogay@sdu.edu.tr

Introduction

Occupational life has a direct relation with health. Work environments harbor various health and safety hazards. These hazards may rise occupational diseases and accidents (1). One of the risks in work environments is ionizing radiation (Gamma Rays, X Rays etc.). Personnel in radiology departments are workers who chronically exposed to low level of ionizing radiation (IR). The annual dose limit should be 20 mSv per year over 5 year period according to The International Commission on Radiological Protection (ICRP) and International Atomic Energy Agency (IAEA) (2-4). Additionally, it is mentioned that the radiation exposure dose of workers should be kept as low as reasonably achievable (ALARA principle) (5). Although the radiation exposure remains below of 20 millisievert (mSv) in many hospital units, it is obvious that there is a higher risk for workers (6). Various measures are taken, such as wearing a dosimeter, to minimize the risk of exposure. However, dosimeters may be insufficient to show actual exposure due to reasons such as improper use (6).

Upon exposure, IR may lead to DNA damage, and increase the frequency of mutations. It can directly create single and double strand breaks and also indirectly cause both oxidative base modifications and DNA chain breaks by increasing the interaction between hydroxyl radicals and the DNA (7). Many research indicated that long-term exposure to low-dose ionizing radiation, even below the permitted levels, could result in increased oxidative stress, which may lead to DNA damage and mutagenicity (8). DNA damage plays an important role in the development of diseases such as cancer, cardiovascular diseases, immune disorders, degenerative diseases and aging. Therefore DNA damage and its consequences are quite important in terms of health. (9). It has been shown that regardless of the cell type, when a diploid mammalian cell is exposed to 1 Gray (Gy) of IR, 1000 single strand breaks and 30 double strand breaks occur (10, 11). In order to prevent this, radiation exposure should be effectively monitored. Beyond the classical monitoring with dosimeter, non-classical methods such as monitoring the DNA damage provide high accuracy and early information thanks to sensitive methods such as the comet assay.

"Comet assay" or "Single Cell Gel Electrophoresis" is a sensitive, reliable and rapid method that can detect this DNA damage. (12-14). In the recent years, utilization of Comet Assay has been considered in bio-monitoring and several studies have been carried out in this regard. When standardized and validated, the comet assay can provide invaluable information in the areas of hazard identification and risk assessment of environmental and occupational exposure (15). The Comet assay measures changes in genomic stability and is one of the most reliable biomarkers to indicate early biological effects, and therefore accepted by various governmental and some regulatory agencies such as "Registration, Evaluation, Authorisation and Restriction of Chemicals Substances programme of the European Commission" (REACH) (16). In addition to measuring DNA damage, the assay can be used to monitor the cellular or in vitro repair of strand breaks or oxidized bases. It also has applications in assessing the antioxidant status of cells (17). Therefore, alkaline comet assay is a rapid and sensitive technique and is suitable for in vivo human biomonitoring, and in this case in cases of exposure to IR. In the study, we aimed to detect DNA damages in personal whom occupationally exposed to IR via the comet assay. Effects of donor age, gender, smoking and years of exposure were also evaluated. This research aimed to reveal the current situation and provide preliminary data for more detailed research, as well as aimed to hospital staff and authorities access to data about to take better measures for hospital personnel.

Material and Methods

Study Design

The study included 48 radiation exposed personnel who have been working in University Hospital and handling the diagnostic machines for more than a year and the 51 control who have not underwent any radiological examination within the last six months. Totally 99 individuals (50 m and 49 f), aged between 18-57 y, were included. The study group comprised of 29 m and 19 f; and the control group comprised of 23 m and 28 f. Mean age of the exposed personnel was 36.16±6.63 y, and mean age in control group was 32.33±7.89 y. All the participants were healthy volunteers who had been given detailed information about the study and the consent form were taken from all. Age, gender, smoking state, years of radiation exposure and dosimetry values for personnel were recorded for each participant. All volunteers lived in the same city. This study were approved by local responsible committee on human experimentation (03.09.2014–135) and has been performed according to the ethical standards. All of the IR exposure levels obtained from workers' dosimeters was below the legally permitted levels. The average of the last 6 months dosimetry values was taken and divided into two groups as <0.1 mSv (monthly) or ≥0.1 mSv (monthly) to compare DNA damage within groups.

Blood samples was taken into heparinized tubes, cooled and analyzed within 2 h. Equalization of timing was achieved for each person prior to blood drawing. Mononuclear blood cells were used to demonstrate DNA damage. Two slides were prepared for every sample, and 50 cells on average were photographed for each under Zeiss Imager A1 fluorescence microscope. Photographed samples were evaluated using Open Comet (18). In this study, we used alkali comet assay and the application steps are described below.

Application Of Comet Assay

Blood samples were mixed 1:1 with Histopaque-1077 (Sigma-Aldrich Co. LLC.) in a separate microcentrifuge tube, and centrifuged at 2000 rpm for 20 min. After centrifugation, leukocytes were mixed 1:1 with phosphate buffer salt (PBS) and centrifuged at 2500 rpm for 10 min. The supernatant was removed, and diluted with 25-50 µL PBS, depending on the density of the remaining cells. Approximately 20 µL cell suspension was mixed with 100 µL 0.6% low melting point agarose (LMA, Fisher Scientific Company LLC.) for

embedding on slides, which were coated with 1.0% Normal Melting Point Agarose (NMA, Serva Electrophoresis GmbH). After the agarose gel solidified, the slides were kept in lysis solution in dark and cold for 90 min in order to lyse cellular and nuclear membranes. After the lysis step, slides were transferred to the electrophoresis tank, electrophoresis buffer was added, and tank was kept in dark and cold (+4°C, pH>13) for 30 min. Electrophoresis was then performed at 25 V (1.02 V/cm) for 25 min. Slides were rinsed with neutralization buffer twice, each lasting 5 min. Throughout the study, all samples were analyzed within 24 h.

After the procedure, samples were stained with ethidium bromide, and were photographed under Zeiss Imager A1 fluorescence microscope, using Zeiss Axiocam Icc 1 camera. Photographed samples were automatically evaluated using Open Comet, an open source code visual evaluation program

Statistical Analysis

According to the relevant literature (12), tail DNA percentage parameter (TDNAP, Tail DNA%) was chosen to assess DNA damage. The results were evaluated by the SPSS v20 (Armonk, NY: IBM) packet program by the One-Way Anova test. $p<0.05$ was considered statistically significant.

Results

In comparison of TDNAP values, between exposed and control groups, significant differences were detected ($p<0.001$). DNA damage of the exposed group is about 2 times higher than control group.

Association among age, gender, smoking habit, working years, dosimetry values and the DNA damage of exposed group were also evaluated. DNA damage did not show any association with age, gender, smoking habit and working years ($p>0.05$) except dosimetry values ($p<0.05$). Accordingly, increasing dosimetry value, resulted in increased DNA damage even at low doses. Results are shown in Table 1 and Table 2 and demonstrated in Figure 1 and Figure 2.

Table 1. Mean tail DNA percentage values of the groups

Groups	Means of Tail DNA Percentage
Control (1)	3.73±0.10
Exposed Personnel (2)	6.64±0.25*

(Mean±Std. Err.)*: Statistically significant ($p<0.001$)

Table 2. Mean tail DNA percentage values of the exposed group according to dosimeter value

Groups	Means of Tail DNA Percentage
<0.1 mSv (1)	5.97±0.23
≥0.1 mSv (2)	7.66±0.54*

(Mean±Std. Err.)*: Statistically significant ($p<0.05$)

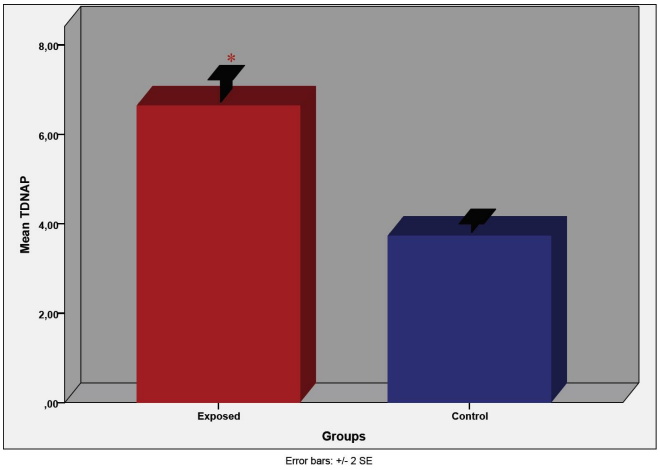

Figure 1. Demonstration of the comparison of the groups in the study*: Statistically significant difference ($p<0.001$)

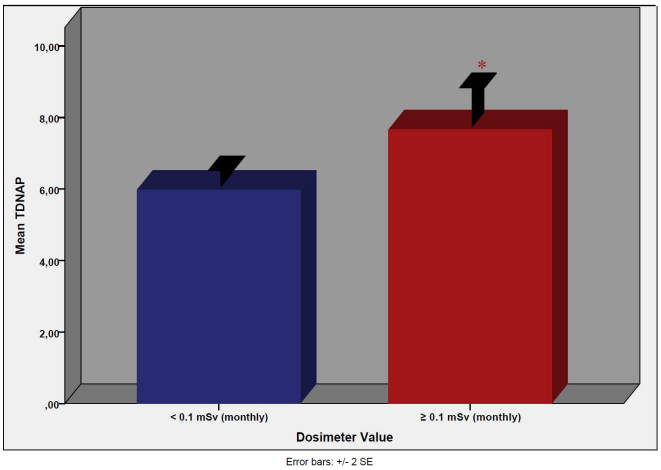

Figure 2. Demonstration of the comparison of individuals in the exposed group according to dosimeter value*: Statistically significant difference ($p<0.05$)

Discussion

Each passing day, we experience greater rate of radiation exposure due to the advances in technology. The amount of artificial radiation created by humans corresponds to 15% of the total radiation exposure, and approximately 96-99% of this is caused by medical applications (19). It can be hypothesized that personnel in radiological units would carry greater DNA damage because more frequent exposing to IRs. Exposure to ionizing radiation results in the immediate formation of free radicals. The subsequent metabolic alterations in multiple intracellular processes following irradiation are due to the initial oxidative damage caused by reactive oxygen and nitrogen species (8). DNA damage can therefore be considered as an expected result. For example, Bedir et al. found that increasing doses of radiation exposure led to increased DNA damage (20). Supporting this hypothesis, in our study, we found statistically significant difference in DNA damage between exposed and control groups. Age, gender, smoking habit and working years were found not significant. Conversely, increase in DNA damage was observed when dosimeter value increases, as expected. Similar to our results,

Wang et al., states that a clear dose-response relationship with DNA double-strand breaks using the comet assay was found at different times after irradiation (21). Vellingiri et al. reported that hospital workers exposed to radiation had greater DNA and chromosomal damage (22); Ündeger et al. found increased DNA damage in technicians who were working in hospital and being exposed to radiation (23); and Wojewodzka et al. reported increased DNA damage in those who were subject to occupational exposure to radiation (24). Bozkurt et al. investigated sister chromatid exchanges in 16 nuclear medicine physicians who occupationally exposed to low doses of I-131 and Tc-99m and found that statistically significant higher difference in SCE frequencies which indicates the possibility of genotoxicity (25). Differently, Erol et al. found that SCE frequencies are not significantly affected in invasive cardiology laboratory workers who are occupationally exposed to ionizing radiation, although some degree of reversible chromosomal aberrations appear after exposure and disappear at the end of two month non-exposure period (26). In similar studies to our present study, Garaj-Vrhovac and Kopjar (13) and Martinez et al. (27) assessed DNA damage in radiology personnel who were exposed to IR, and found significantly increased DNA damage compared to the control group. Garaj-Vrhovac and Kopjar did not find any association of smoking habit or gender with the damage. Our results and the related literature indicate that radiation workers carry greater risk towards IR exposure and its adverse effects. Besides DNA damage, many other studies reported chromosome aberrations (CA) and sister chromatid exchanges (SCE). Zakeri and Hirobe used CA analysis, cytokinesis-block micronucleus (MN) assay biological indicators of ionizing radiation exposure in different types of radiology personnel. Occupational dosimetry records were also collected by the researchers and they found significantly higher frequencies of CAs and MN in all exposed groups than in the controls (6). Dias et al. also found significantly higher MN formation in the occupationally IR exposed group and Bozkurt et al. found statistically significant difference between SCE frequencies of occupationally IR exposed and non-exposed groups (25, 28).

In their study with mice and rats, Ueno et al. found that radiation caused varying degrees of injury in different organs, and they stated that repair of the damage might also be at varying speeds (29). This suggests that DNA damage may occur with different rates at different tissues and organs. Therefore, it would be better if more specific studies were done to evaluate difference between different tissues and radiation sources. This well-established risk should be considered thoroughly by both the employees and the employers, and all necessary measures should be taken.

Although the mutagenic and carcinogenic effects of smoking are well-established, Hoffmann and Speit stated that neither they nor other researchers could completely reveal this effect using comet assay. They compared individuals who smoked more than 20 cigarettes a day with those who never smoked in detail using several methods. Despite their efforts, they could not find a significant effect of smoking on DNA damage (30). In a meta-analysis including 38 studies, Hoffmann et al. stated that smoking had effects on DNA damage but there were contradicting study results, and they proposed that this might have been related to not using computer software for assessment of DNA damage, and also to limited sample size (31). Similar to previous studies, in our study, we found no additional DNA damage between comparison of values of smokers and non-smokers. Of course, radiation may be masking the effects of smoking. Smoking certainly has well-known adverse effects but in order to interpret the results better, more comprehensive studies may be beneficial in order to demonstrate additional DNA damage effects of smoking.

Conclusion

Consequently, IR certainly have an effect on DNA of radiology personnel even despite various precautions. This findings supports the previously reported data. Sufficient precautions could still not be taken on behalf of health professionals whom working to make our lives healthier. Making the work environment healthier and safer place, of course, has great significance, but worker's healthiness in a work environment is not only important for worker but also for the other people that the personnel serves. Reducing the risks in work environment would return as increased quality of the health care (1). For exposed personnel, who can conveniently be categorized in high risk group in terms of health, all necessary personal and institutional measures should be taken without any monetary doubt. Additionally, due to the existence of many studies showing that DNA damage occurs even at low dose radiation exposure, we think that it should be a legal obligation to monitor the DNA damage of the personnel working in radiation-related works as a standard.

As a result, there is need for appropriate equipment, continuous observation of health, employment of sufficient number of personnel and periodic audit of work pattern and output to minimize radiation exposure (32).

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