Journal of Radiation Research and Applied Sciences 9 (2016) 249–258

HOSTED BY

Available online at www.sciencedirect.com

ScienceDirect

Journal of Radiation Research and Applied Sciences

journal homepage: http://www.elsevier.com/locate/jrras

Chromosomal aberrations and oxidative DNA adduct 8-hydroxy-2-deoxyguanosine as biomarkers of radiotoxicity in radiation workers

Sanaa A. El-Benhawy [a,*], *Nadia A. Sadek* [b], *Amal K. Behery* [c], *Noha M. Issa* [c], *Osama K. Ali* [d]

[a] *Radiation Sciences Department, Medical Research Institute, Alexandria University, Alexandria, Egypt*
[b] *Hematology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt*
[c] *Human Genetics Department, Medical Research Institute, Alexandria University, Alexandria, Egypt*
[d] *Department of Radiology and Medical Imaging, University of 6 October, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 17 September 2015
Received in revised form
8 December 2015
Accepted 13 December 2015
Available online 29 December 2015

Keywords:
Ionizing radiation
Chromosomal aberrations
8-OHdG
Radiation workers

ABSTRACT

Background: There are evidences of association between occupational radiation exposure, cytogenetic alterations and the increase in cancer rates. It is known that the probability of carcinogenesis is greater in populations exposed to radiation, since ionizing radiation can raise the frequency of chromosomal aberration and spontaneous mutations.

Objective: Our purpose was to assess the role of chromosomal aberrations and oxidative DNA adduct 8-hydroxy-2-deoxyguanosine (8-OHdG) as biomarkers of radiation injury in individuals occupationally exposed to ionizing radiation.

Subjects: and Methods: Blood samples were collected from 60 radiation workers and 30 healthy volunteers age and sex matched as control group who had never worked in radiation-related jobs. Chromosomal aberrations in peripheral blood lymphocytes were assayed by conventional cytogenetic technique and serum levels of 8-OHdG was measured by enzyme linked immunossorbent assay (ELISA).

Results: The incidence of all types of chromosomal aberrations was significantly higher in all exposed groups than in controls with the highest rate of chromosomal aberrations in the industrial radiographers. Serum 8-OHdG in all radiation workers was significantly higher than in control group. There was a significant higher values among industrial radiographers compared to diagnostic radiologists or radiotherapists. Significantly lower mean corpuscular volume (M.C.V) was found among radiation workers versus the controls reflecting erythrocyte microcytosis.

Conclusions: Scoring of chromosome aberrations such as breaks, fragments and dicentrics is a reliable method to detect previous exposure to ionizing radiation. This type of monitoring may be used as a biological dosimeter instead of physical dosimetry.8-OHdG is a useful oxidative DNA marker among radiation workers and those exposed to environmental carcinogens.

* *Corresponding author*. Present/permanent address: Radiation Sciences Department, Medical Research Institute, Alexandria University, 165 El-Horreya Avenue, El- Hadra, Postal code 21561, Egypt. Tel.: +20 3 4285455; fax: +20 3 4283719.
E-mail address: dr_sanaa_ali13@yahoo.com (S.A. El-Benhawy).

Peer review under responsibility of The Egyptian Society of Radiation Sciences and Applications.
http://dx.doi.org/10.1016/j.jrras.2015.12.004

1. Introduction

Humans are naturally exposed to ionizing radiation from cosmic rays, and artificially through diagnostic procedures, medical treatments or occupationally during work shifts. It is well known that ionizing radiation produces DNA damage through different mechanisms: by loss of bases, single-strand breaks, double strand breaks, and damage to purine and pyrimidine bases. This early damage may lead to chromosomal aberrations and thus to increased risk of mutagenesis and carcinogenesis (Martínez, Coleman, Romero-Talamás, & Frías, 2010). 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. Though several accurate physical dose estimation modalities exist, it is essential to estimate the absorbed dose in biological system taking into account the individual variation in radiation response, so as to plan suitable medical care. Over the last several decades, lots of efforts have been taken to design a rapid and easy biological dosimeter requiring minimum invasive procedures. The metaphase chromosomal aberration assay in human lymphocytes still remains the gold standard for radiation biodosimetry (Agrawala, Adhikari, & Chaudhury, 2010).

Cytogenetic studies in radiation workers have demonstrated an increase in the frequency of chromosomal aberrations in comparison to non-exposed individuals. These chromosomal aberrations are the result of an erroneous repair of the DNA lesions produced by radiation (Ballardi et al., 2007; Kasuba, Rozgaj, & Jazbec, 2008).

Ionizing radiation is a well-established carcinogen due to the resulting oxidative damage, and the molecule most often affected is DNA. Interactions of ionizing radiation with DNA consist of the direct ionization of DNA (direct effect) and its reaction with surrounding water molecules (the indirect effect), followed by DNA destruction by the induced radicals (•OH, e−• and, to much lesser extent, H•) (Karbownik & Reiter, 2000). Generally among nucleic acid components, guanine is the most susceptible DNA target for oxidative reactions mediated by •OH (Shirazi, Ghobadi, & Ghazi-Khansari, 2007). The modified base 8-hydroxydeoxyguanosine (8-OHdG), an oxidative adduct form of deoxyguanosine, is considered a sensitive marker of DNA damage due to a hydroxyl radical attack at the C8 of guanine. Such damage is usually successfully repaired, but if unrepaired, the presence of 8-OHdG in DNA templates may cause the miscoded incorporation of nucleotides in the replicated strand, which may contribute to the development of cancer (Sperati et al., 1999).

The objective of the present study was to assess the role of chromosomal aberrations and oxidative DNA adduct 8-hydroxy-2-deoxyguanosine (8-OHdG) as biomarkers of radiation injury in individuals occupationally exposed to ionizing radiation.

2. Subjects and methods

This study included 60 subjects occupationally exposed to ionizing radiation (radiation workers), their mean age was (35.0 ± 6.67) years. Thirty healthy volunteers age and sex matched who had never worked in radiation-related jobs served as control group, their mean age was (33.53 ± 7.27) years. Radiation workers were divided into three groups according to their job title at the time of blood collection, as follow:

- Radiotherapy group (n = 20) (working on linear accelerator), their mean age and working period was 36.25 ± 6.70 and 11 ± 7.60 years respectively.
- Diagnostic radiology group (n = 20) (using medical diagnostic X-ray machine), their mean age and working period was 31.65 ± 7.58 and 9 ± 6.90 years respectively.
- Industrial radiographers group (n = 20) (using Iridium 192 as a gamma source for radiography), their mean age and working period was 37.10 ± 4.61 and 8.15 ± 4.59 years respectively.

The annual accumulated dose was measured during the person's entire working time using personal dosimeters (film badge and pocket dosimeter). The mean dose was 2.93 ± 1.91 and ranged from 1.5 to 4.5 mSv/year in diagnostic radiology group and 3.13 ± 1.46 and ranged from 1.5 to 6 mSv/year in radiotherapy group. Regarding industrial radiographers group, the mean dose was 5.46 ± 2.35 and ranged from 4 to 13.5 mSv/year.

All subjects were interviewed and completed a questionnaire including demographic data, smoking habit, lifestyles, medical records and radiation exposure history. A written consent for participating in the study was taken according to the declaration of Helsinki and approved by the ethical committee of the Medical Research Institute. The radiation workers were selected from Diagnostic Radiology Department in Medical Research Institute, radiotherapists in Ayadi Al-Mostakbal Oncology Center and industrial radiographers in petroleum sector who followed up in Hematology Department in Medical Research Institute.

None of the study individuals reported alcohol consumption or the presence of known inherited genetic disorders or chronic diseases. None of them received chemotherapeutic drugs or subjected to ionizing radiation for diagnostic or therapeutic purposes in the six months previous to blood collection.

2.1. *Cytogenetic method*

Venous blood samples were collected into heparinised tubes. Lymphocytes cultures were set up within 24 h of sampling according to the conventional method (Sharma & Sharma, 1980). Whole blood cultures were established by placing 0.5 ml of PRMI medium supplemented with 20% fetal calf serum and 1.5% phytohaemagglutinin. Cultures were incubated in the dark at 37 °C for 48 h. Colchicine [0.1 mg/ml] was added for the last 2 h of incubation to arrest the cells at metaphases. Cells were incubated with hypotonic KCl [0.075 M] at 37 °C for 10 min and fixed in 4 changes of cold 3:1 methanol/acetic acid. Slides were prepared by the heat drying technique and were stained with aqueous Giemsa solution. One hundred metaphase were analyzed for every participant.

2.2. Serum 8-OHdG

Circulating levels of 8-hydroxydeoxyguanosine were measured by enzyme linked immunosorbent assay according to the manufacturer's instructions (Enzo Life Sciences, USA). Collect whole blood using established methods. Allow samples to clot at room temperature for 30 min, then centrifuge at 2700 × *g* for 10 min, taking precautions to avoid hemolysis. Transfer the serum to a labeled polypropylene tube. The serum collected is now ready for analysis using the DNA Damage ELISA kit.

2.3. Hematological analysis

Blood samples were collected into EDTA bulbs for complete blood picture analysis (Lewis, Bain, & Bates, 2006).

2.4. Statistical analyses

Statistical analyses were conducted using the statistical soft ware package of SPSS version 17 (SPSS Inc, Chicago, USA). The differences between groups were determined by the two-sided chi-square test and Mann Whitney test. Pearson's correlation coefficients were calculated to evaluate the association between relevant parameters. The influence of age, working period, smoking and gender on chromosomal aberration was tested by regression analysis. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Chromosomal aberrations in all studied groups

Range and mean ± standard deviation (S.D.) of chromosomal aberrations in all studied groups were illustrated in Table 1. The incidence of all types of chromosomal aberrations including gaps%, breaks%, fragments% and dicentric% were significantly higher in all radiation workers than in normal control group ($P_1 = 0.001, P_1 = 0.001, P_1 = 0.001$ and $P_1 = 0.006$ respectively).

As seen in Table 1, the mean values of chromosomal gaps%, chromosomal breaks%, acentric fragments% and dicentric% in radiotherapy, diagnostic radiology and industrial radiographers groups were significantly higher than in control group (Figs. 1 and 2). Furthermore, gaps%, breaks% and dicentric% were significantly higher in industrial radiographers than radiotherapists or diagnostic radiologist while there was no difference among different workers regarding fragments%. Also, there was insignificant difference between radiotherapists or diagnostic radiologist in the incidence of all types of chromosomal aberrations.

3.2. Serum 8-OHdG concentration (ng/ml)

The results of serum 8-OHdG in all studied groups in comparison to control group were illustrated in Table 2 and Fig. 3. The mean values of serum 8-OHdG in all radiation workers

Table 1 – Chromosomal aberrations in all studied groups.

	All radiation workers (n = 60)	Radiotherapy group (n = 20)	Diagnostic radiology group (n = 20)	Industrial radiographers group (n = 20)	Control group (n = 30)
Chromosomal Gaps%					
Range	0.0–7.0	0.0–7.0	1.0–6.0	2.0–7.0	0.0–2.0
Mean. ± SD.	3.55 ± 1.93	2.45 ± 1.96	3.35 ± 1.35	4.85 ± 1.53	1.10 ± 0.55
p_1	0.001*	0.004*	0.001*	0.001*	
p_2		0.001*	0.006*		
p_3		0.078			
Chromosomal Breaks%					
Range	2.0–20.0	2.0–14.0	4.0–18.0	2.0–20.0	0.0–4.0
Mean. ± SD.	8.42 ± 4.33	6.60 ± 3.28	8.20 ± 3.91	10.45 ± 4.91	1.17 ± 0.70
p_1	0.001*	0.001*	0.001*	0.001*	
p_2		0.014*	0.041*		
p_3		0.225			
Acentric Fragments%					
Range	0.0–12.0	0.0–6.0	0.0–8.0	0.0–12.0	0.0–0.0
Mean. ± SD.	3.55 ± 2.42	2.01 ± 3.0	1.94 ± 3.50	3.01 ± 4.0	0.0 ± 0.0
p_1	0.001*	0.001*	0.001*	0.001*	
p_2		0.090	0.621		
p_3		0.147			
Dicentric%					
Range	0.0–5.0	0.0–0.0	0.0–0.0	0.0–5.0	0.0–0.0
Mean. ± SD.	0.50 ± 1.10	0.0 ± 0.0	0.0 ± 0.0	1.50 ± 1.47	0.0 ± 0.0
p_1	0.006*	1.000	1.000	0.001*	
p_2		0.001*	0.001*		
p_3		1.000			

p_1: p value for comparing between control group with each studied group.
p_2: p value for comparing between industrial radiographers with diagnostic radiology and radiotherapy groups.
p_3: p value for comparing between diagnostic radiology and radiotherapy groups.
* Statistically significant at $p \leq 0.05$.

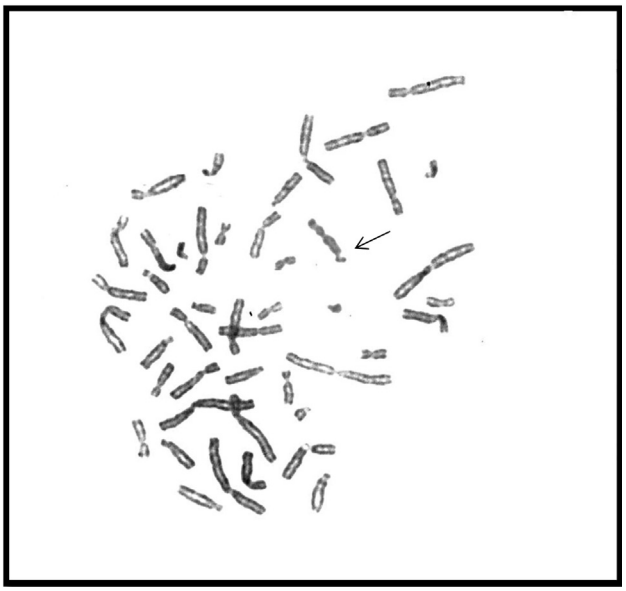

Fig. 1 – A metaphase showing a chromosomal break.

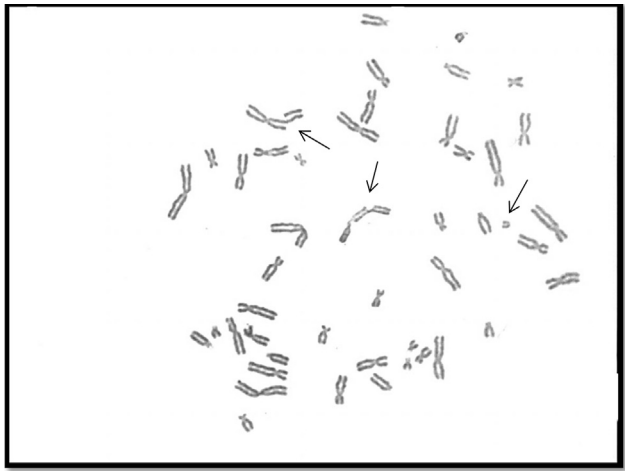

Fig. 2 – A metaphase showing chromosomal breaks and a chromosomal fragment.

was significantly higher than in control group ($P_1 = 0.001$).Also serum 8-OHdG in radiotherapy, diagnostic radiology and industrial radiographers groups was significantly higher than in control group (P_1 = 0.001, 0.001 and 0.001 respectively). Moreover, serum 8-OHdG was significantly higher in industrial radiographers than that in radiotherapy and diagnostic radiology groups (P_2 = 0.009and 0.003 respectively). On the other hand, there was insignificant difference in serum 8-OHdG between radiotherapy and diagnostic radiology groups ($P_3 = 0.625$).

3.3. *Hematological results*

Table 3 showed that, there was insignificant difference in mean values of WBCs, Hb, RDW and M.C.H between all radiation workers and control group (P = 0.729, 0.174, 0.891 and 0.341 respectively). Moreover, insignificant difference was seen between radiation workers groups. On the other hand, the mean values of M.C.V in all radiation workers was significantly lower than in control group ($P_1 = 0.001$). Also the mean values of M.C.V in radiotherapy, diagnostic radiology and industrial radiographers groups was significantly lower than in control group ($P_1 = 0.001$, 0.034 and 0.001 respectively).

Table 4 revealed that, there was insignificant difference in mean Neutrophil/Lymphocytes (N/L) ratio between all radiation workers and control group (P_1 = 0.533). With respect to studied groups, a significantly lower N/L ratio was observed in industrial radiographers compared to diagnostic radiologists, radiotherapists and controls (P_2 = 0.036, P_2 = 0.046 and P_1 = 0.036 respectively). There was insignificant difference between radiotherapy, diagnostic radiology and control groups in mean N/L ratio.

3.4. *Effects of demographic characters on different studied parameters*

3.4.1. *Effect of age*

Table 5, showed a significant positive correlation between age with gaps%, breaks%, dicentric% and serum 8-OHdG concentration (P = 0.010, 0.019, 0.026, 0.017 respectively). While this correlation fail to reach statistical significance in case of fragments% (P = 0.096).

Table 2 – Serum 8-OHdG concentration in all studied groups.

	All radiation workers (n = 60)	Radiotherapy group (n = 20)	Diagnostic radiology group (n = 20)	Industrial radiographers group (n = 20)	Control group (n = 30)
8-OHdG concentration					
Range	1.0–13.50	1.0–13.50	2.0–13.50	2.50–10.0	0.40–2.50
Mean. ± SD.	5.19 ± 2.51	4.69 ± 2.60	4.61 ± 2.62	6.29 ± 2.01	1.50 ± 0.71
p_1	0.001*	0.001*	0.001*	0.001*	
p_2		0.009*	0.003*		
p_3		0.625			

p_1: p value for comparing between control group with each studied group.
p_2: p value for comparing between industrial radiographers with diagnostic radiology and radiotherapy groups.
p_3: p value for comparing between diagnostic radiology and radiotherapy groups.
* Statistically significant at $p \leq 0.05$.

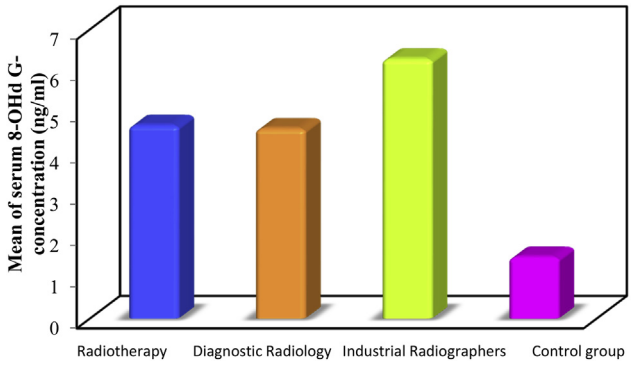

Fig. 3 – Mean values of serum 8-OHdG concentration (ng/ml) in all studied groups.

3.4.2. *Effect of gender*

As shown in Table 6, there was insignificance difference between males and females in mean gaps%, breaks%, fragments %, dicentric% and serum 8-OHdG concentration in all radiation workers (P = 0.265, 0.151, 0.620, 0.167, 0.868 respectively). The same is true for control group (data not shown).

3.4.3. *Effect of smoking*

Table 7 revealed that, the mean gaps% in smokers radiation workers was significantly higher than in non smokers radiation workers (P = 0.007), while there was insignificant difference between smokers and non smokers radiation workers in breaks%, fragments%, dicentric% and serum 8-OHdG concentration (P = 0.098, 0.151, 0.118, 0.374 respectively). In healthy controls, there was insignificant difference between smokers and non smokers in mean gaps%, breaks%, fragments%, dicentric% and serum 8-OHdG concentration (data not shown).

3.4.4. *Effect of accumulated dose per year*

Table 8 showed that, there was significant positive correlation between accumulated dose per year (mSv) with gaps%, breaks %, fragments% and dicentric% (P < 0.000, 0.000, 0.000 and 0.011 respectively). Moreover, the annual accumulated dose was significantly correlated with serum 8-OHdG levels (P < 000).

3.4.5. *Effect of working period*

Table 9 showed that, there was significant positive correlation between working period with gaps%, breaks% and fragments % (P = 0.026, 0.033 and 0.042 respectively), while the

Table 3 – Complete blood picture in all studied groups.

	All radiation workers (n = 60)	Radiotherapy group (n = 20)	Diagnostic radiology group (n = 20)	Industrial radiographers group (n = 20)	Control group (n = 30)
WBCs (10^3/uL)					
Range	2.67–10.74	3.30–10.60	2.67–10.74	3.1–10.50	4.71–10.60
Mean. ± SD.	6.75 ± 1.64	6.92 ± 1.43	6.57 ± 1.91	6.75 ± 1.60	6.70 ± 1.57
p_1	0.729	0.507	0.851	0.744	
p_2		0.839	0.725		
p_3		0.534			
M.C.V. (fl)					
Range	60.30–93.9	60.30–87.0	65–93.9	60.30–83.30	78–93
Mean. ± SD.	77.76 ± 6.95	75.85 ± 6.93	77.12 ± 4.11	74.31 ± 6.22	82.92 ± 3.35
p_1	0.001*	0.001*	0.034*	0.001*	
p_2		0.336	0.147		
p_3		0.510			
Hemoglobin (g/dl)					
Range	10.10–16.41	10.80–16.41	10.90–15.60	10.10–15.80	11.3–15.6
Mean. ± SD.	13.14 ± 1.54	12.79 ± 1.58	13.74 ± 1.13	12.90 ± 1.72	13.58 ± 1.13
p_1	0.174	0.283	0.983	0.418	
p_2		0.996	0.306		
p_3		0.203			
RDW %					
Range	11.10 - 14.50	11.40–14.20	11.10–14.50	11.50–14.20	11–15
Mean. ± SD.	12.54 ± 0.90	12.70 ± 0.91	12.19 ± 0.84	12.74 ± 0.87	12.8 ± 0.6
p_1	0.891	0.851	0.742	0.773	
p_2		0.892	0.460		
p_3		0.761			
M.C.H(pg)					
Range	20.20–34.0	20.20–29.80	24.80–34.0	20.20–29.60	21–35
Mean. ± SD.	27.23 ± 2.63	26.42 ± 2.81	28.47 ± 2.02	26.79 ± 2.64	28.5 ± 1.3
p_1	0.341	0.481	0.673	0.579	
p_2		0.724	0.704		
p_3		0.403			

p_1: p value for comparing between control group with each studied group.
p_2: p value for comparing between industrial radiographers with diagnostic radiology and radiotherapy groups.
p_3: p value for comparing between diagnostic radiology and radiotherapy groups.
*Statistically significant at $p \leq 0.05$.

Table 4 – N/L ratio in all studied groups.

	All radiation workers (n = 60)	Radiotherapy (n = 20)	Diagnostic radiology (n = 20)	Industrial radiographers (n = 20)	Control groups (n = 30)
N/L ratio					
Range	0.60–5.50	0.80–5.50	0.70–5.20	0.60–3.10	1.10–3.50
Mean. ± SD.	1.89 ± 1.08	1.94 ± 1.13	2.30 ± 1.28	1.44 ± 0.56	1.79 ± 0.61
p_1	0.533	0.781	0.355	0.036*	
p_2		0.046*	0.021*		
p_3		0.383			

p_1: p value for comparing between control with each studied group.
p_2: p value for comparing between industrial radiographers with diagnostic radiology and radiotherapy groups.
p_3: p value for comparing between diagnostic radiology and radiotherapy groups.
N/L: Neutrophils/lymphocytes ratio.
* Statistically significant at $p \leq 0.05$.

correlation not reach the statistical significance in case of dicentric% and serum 8-OHdG concentration (P = 0.558 and 0.153 respectively).

3.4.6. The influence of age, working period, dose, smoking status and gender on the frequency of chromosomal aberrations

Multiple regression analysis was applied to estimate the influence of age, working period, dose, gender and smoking on the frequency of chromosomal aberrations. The results showed that age, working period (years), dose (per year) and smoking highly affect the frequency of chromosomal aberrations with coefficient of determination $R^2 = 0.838$, $P < 0.000$. Unlike gender which showed no relation (P = 0.346).

3.5. *Correlation between 8-OHdG concentration with chromosomal aberrations and hematological parameters*

As seen in Table 10 there was insignificant correlation between serum 8-OHdG with WBCs, Hb and M.C.H (P = 0.455, 0.916 and 0.494 respectively). On the other hand, there was a significant negative correlation between serum 8-OHdG and M.C.V (P = 0.005), and a significant positive correlation between 8-OHdG concentration and RDW (%) (P = 0.041). With respect to chromosomal aberration, there was a significant positive correlation between serum 8-OHdG and gaps%, breaks%, fragments% and dicentric% (P = 0.045, 0.001, 0.043 and 0.042 respectively).

Table 5 – Correlation between age with different studied parameters in all radiation workers.

All radiation workers (n = 60)		
		Age (years)
Gaps%	r	0.331
	p	0.010*
Breaks%	r	0.303
	p	0.019*
Fragments%	r	0.217
	p	0.096
Dicentric%	r	0.287
	p	0.026*
8-OHdG concentration (ng/ml)	r	0.307
	p	0.017*

r: Pearson's coefficient.
* Statistically significant at $p \leq 0.05$.

4. Discussion

Hospital workers occupationally exposed to low levels of ionizing radiation exhibit high frequency of chromosomal aberrations in peripheral lymphocytes (Maffei et al., 2004). Ionizing radiation causes detrimental health effects such as cancer and genetic damage (Terzic, Milovanovic, Dotlic, Rakic, & Terzic, 2015). Ionizing radiation induces mutations and cell transformations, predominantly by causing single-strand and double-strand DNA breakage, leading to chromosome instability and carcinogenesis (Eken et al., 2010).

Cytogenetic biomonitoring studies on somatic cells have been proposed as tools to assess the possible genotoxic effects of a hazardous exposure (Maffei et al., 2004). Chromosome aberrations are considered relevant biomarkers for cancer predisposition (Bonassi et al., 2011). It manifests as chromosomal gaps% which is defined as an achromatic region in both

Table 6 – Relation between gender with different studied parameters in all radiation workers.

	All radiation workers		p
	Male	Female	
Gaps%			
Range	0.0–7.0	0.0–7.0	0.265
Mean. ± SD.	3.72 ± 1.92	3.0 ± 1.96	
Breaks%			
Range	2.0–20.0	3.0–14.0	0.151
Mean. ± SD.	8.89 ± 4.51	6.86 ± 3.32	
Fragments%			
Range	0.0–12.0	0.0–6.0	0.620
Mean. ± SD.	3.70 ± 2.53	3.07 ± 2.06	
Dicentric%			
Range	0.0–5.0	0.0–3.0	0.167
Mean. ± SD.	0.59 ± 1.17	0.21 ± 0.80	
8-OHdG concentration			
Range	1.0–13.50	3.0–13.0	0.868
Mean. ± SD.	5.14 ± 2.51	5.36 ± 2.59	

Table 7 – Relation between smoking with different studied parameters in all radiation workers.

	Smoking		p
	No	Yes	
Gaps%			
Range	0.0–7.0	1.0–7.0	0.007*
Mean. ± SD.	2.72 ± 1.95	4.14 ± 1.72	
Breaks%			
Range	2.0–20.0	2.0–20.0	0.098
Mean. ± SD.	7.40 ± 4.23	9.14 ± 4.31	
Fragments%			
Range	0.0–8.0	0.0–12.0	0.151
Mean. ± SD.	2.96 ± 2.11	3.97 ± 2.57	
Dicentric%			
Range.	0.0–3.0	0.0–5.0	0.118
Mean. ± SD.	0.24 ± 0.72	0.69 ± 1.28	
8-OHdG concentration			
Range	1.0–13.50	2.0–13.50	0.374
Mean. ± SD.	4.90 ± 2.57	5.40 ± 2.48	

* Statistically significant at $p \leq 0.05$.

chromatid, the size of which is equal to or smaller than the width of the chromatid, chromosomal breaks% which are an achromatic region in both chromatids, the size of which is more than the width of the chromatid. Acentric fragments% which is two alignment chromatid without an evident centromere and dicentric chromosomes%.

The current study revealed that, the mean values of chromosomal gaps%, chromosomal breaks% fragments% and dicentric% in all radiation workers were significantly higher than in normal control group. On comparing chromosomal aberrations among different workers exposed to ionizing radiation, we found that chromosomal gaps%, chromosomal breaks% and dicentric% were significantly higher in industrial radiographers than radiotherapists or diagnostic radiologist while there was no difference among different workers regarding fragments%. We could explain this discrepancy by the fact that industrial radiographers have to dive under water making it impossible to wear aprons shields or dosimeters.

Table 8 – Correlation between accumulated dose per year (mSv) with chromosomal aberrations and serum 8-OHdG concentration.

Radiation workers (n = 37)		
		Dose (mSv)
Gaps%	r	0.454
	p	0.000*
Breaks%	r	0.557
	p	0.000*
Fragments%	r	0.478
	p	0.000*
Dicentric%	r	0.276
	p	0.011*
8-OHdG concentration (ng/ml)	r	0.520
	p	0.000*

r: Pearson's coefficient.
* Statistically significant at $p \leq 0.05$.

Table 9 – Correlation between working period with chromosomal aberrations and serum 8-OHdG concentration in all radiation workers.

All radiation workers (n = 60)		
		Working period (years)
Gaps%	r	0.496
	p	0.026*
Breaks%	r	0.276
	p	0.033*
Fragments%	r	0.264
	p	0.042*
Dicentric%	r	0.077
	p	0.558
8-OHdG concentration (ng/ml)	r	0.187
	p	0.153

r: Pearson's coefficient.
* Statistically significant at $p \leq 0.05$.

Moreover, industrial radiographers occupationally receive the highest individual radiation doses.

In agreement with our results, (Zakeri, Assaei, & Varergar, 2003) reported that the incidence of all types of chromosomal aberrations were significantly higher in all exposed groups than in controls with the highest rate of chromosomal aberrations was found in the industrial radiographers and the lowest one was obtained in the personnel of medical X-ray diagnostic centers. Contradictory to our results, (Cigarran et al., 2001) reported no significant difference in the frequencies of chromosomal abnormalities among hospital workers and the matched control group.

With respect to serum 8-OHdG concentration, the current study showed that the mean values of serum 8-OHdG in all radiation workers were significantly higher than in control group ($P = 0.001$) with significant higher values observed among industrial radiographers compared to diagnostic radiologist or radiotherapists ($P_2 = 0.003$ and $P_2 = 0.009$ respectively). This finding reflects a higher degree of oxidative stress among radiographers making them more vulnerable to the oxidant stress on different organs. 8-OHdG is one of the predominant forms of free radical-induced oxidative lesions. 8-OHdG has been used to estimate the DNA damage in humans after exposure to cancer-causing agents, such as ionizing radiation. The majority of the studies revealed an increase of the concentration of 8-OHdG in urine in exposed subjects to ionizing radiation compared with controls (Sajous, Botta, & Sari-Minodier, 2008). (Silva et al., 2013) found that, 8-OHdG levels were significantly higher in pilots occupationally exposed to cosmic radiation than the unexposed group which agrees with our results.

Moreover, there was a significant positive correlation between serum 8-OHdG and gaps%, breaks%, fragments% and dicentric% ($P = 0.045$, 0.001, 0.043 and 0.042 respectively). To the best of our knowledge, this is the first study found a positive correlation between different types of chromosomal aberrations and oxidative DNA marker 8-OHdG in radiation workers. Ionizing radiation leads to the production of free radicals (reactive oxygen species) (Azzam, Jay-Gerin, & Pain, 2012) also in the air (Dizdaroglu, Jaruga, Birincioglu, & Rodriguez, 2002). Since free radicals are heavier than other

Table 10 – Correlation between 8-OHdG concentration with hematological parameters and chromosomal aberrations in all radiation workers.

All radiation workers (n = 60)		
		8-OHdG-concentration (ng/ml)
M.C.V (fl)	r	−0.356
	p	0.005*
H.B (g/dl)	r	−0.014
	p	0.916
RDW (%)	r	0.298
	p	0.041*
M.C.H (pg)	r	−0.090
	p	0.494
WBCs (10^3/uL)	r	0.098
	p	0.445
Gaps%	r	0.260
	p	0.045*
Breaks%	r	0.498
	p	0.001*
Fragments%	r	0.261
	p	0.043*
Dicentric%	r	0.263
	p	0.042*

r: Pearson's coefficient.
* Statistically significant at $p \leq 0.05$.

molecules, they may even despite their shorter half-life precipitate to the ground. Therefore, air conditioning in nuclear medicine departments is very important and it must come from the ground.

Regarding the impact of ionizing radiation on hematological parameters, a significantly lower mean corpuscular volume (M.C.V) was found among radiation workers versus the controls reflecting erythrocyte microcytosis. But we did not find any change in the other parameters namely total leucocytic count, hemoglobin or other red cell indices. This agrees in part with (Ghadhia et al., 2004) who observed no significant change in blood pictures of radiation workers when compared to controls. They attributed this to that the parameters considered in their study might not have been influenced much by low level irradiation.

In the present study, a significantly lower neutrophil/lymphocyte (N/L) ratio was observed in industrial radiographers compared to diagnostic radiologists, radiotherapists and controls ($P_2 = 0.036$, $P_2 = 0.046$ and $P_1 = 0.036$ respectively). This makes them more vulnerable to acquire bacterial infection.

In the present study, there was a significant positive correlation between age with gaps%, breaks%, dicentric% and serum 8-OHdG concentration (P = 0.010, 0.019, 0.026, 0.017 respectively). While this correlation failed to reach statistical significance in case of fragments% (P = 0.096). In multiple regression analysis, age had significant effect on the frequency of chromosomal aberrations. These findings disagree with (Maffei et al., 2004) who reported no influence of age on the frequencies of chromosomal damage. However, the effect of age as confounding variable on the yield of chromosomal aberration cannot be entirely discounted. In agreement with our results, (Kasuba et al., 2008) stated that age significantly influenced the incidence of dicentrics in the exposed groups. Some biomonitoring studies found no relationship between age and chromosomal aberrations whereas others reported on age effect (Bolognesi et al., 1997; Santovito, Cervella, & Delpero, 2015).

In relation to smoking, the present study revealed that, the mean gaps% in smokers radiation workers was significantly higher than in non-smokers radiation workers (P = 0.007). While there was insignificant difference between smokers and non-smokers workers in mean breaks%, fragments%, dicentric% and serum 8-OHdG concentration (P = 0.098, 0.151, 0.118, 0.374 respectively). In multiple regression analysis, smoking status significantly affect the frequency of chromosomal aberrations. These findings obviate the additive effect of smoking on inducing chromosomal aberration in workers exposed to ionizing radiation. Yet, smoking in the control group did not induce any chromosomal aberration.

As regards the effect of smoking on chromosomal damage, the data reported in biomonitoring studies are contradictory. It has been reported that only heavy smokers (Those consuming > 30 cigarettes/day) exhibited a significant increase in genotoxic damage in lymphocytes as measured by chromosomal aberration analysis (Au, Cajas, & Salama, 1998) or micronucleus assay among nuclear medicine workers (Bonassi et al., 2003, Sahin et al., 2009). While many authors did not find any influence of smoking on the aberration level (Ballardi et al., 2007, Lazutka et al., 1999), others have indicated greater aberration frequency in smokers than in non-smokers (Alsatari, Azab, Khabour, Alzoubi, & Sadiq, 2012; Balakrishan & Rao, 1999).

(Roland and Hardeny., 1999) reported that the cells of cigarette smokers might have DNA repairs problems. The major problem is a delay in repairing damaged DNA with respect to the cells of non-smokers. So far, (Maffei et al., 2002) pointed out that smoking significantly increased micronucleus frequency in exposed workers, but not in controls. It seems that cigarette smoking is a potential confounding variable for the frequency of chromosome aberrations.

As regards to gender, we found that, there was insignificance difference between males and females in mean gaps%, breaks%, fragments%, dicentric% and serum 8-OHdG concentration in all radiation workers (P = 0.265, 0.151, 0.620, 0.167, 0.868 respectively). The same is true for control group. Moreover, in multiple regression analysis, gender had no effect on the frequency of chromosomal aberrations. Although, there is no evidence that gender influences the frequency of chromosomal aberrations in the general population (Maffei et al., 2002; Mozdarani, Hejazi, & Hejazi, 2002), (Maffei et al., 2004) reported that female gender was associated with increased frequencies of both aberrant cells and chromosome breaks.

Regarding the accumulated dose per year, it was significantly correlated with all types of chromosomal aberrations and with serum 8-OHdG levels. In multiple regression analysis, the annual accumulated dose (mSv) highly affects the frequency of chromosomal aberrations. (Mozdarani et al., 2002) reported that, the total chromosomal deletions and gaps increased with increasing average annual exposure dose which in line with the current study. In contrast to our result (Gricienė, Slapšytė, & Mierauskienė, 2014) found no

correlation was found between chromosomal aberrations frequency and occupational exposure dose.

The current study showed that, there was a significant positive correlation between working period with gaps%, breaks% and fragments% (P = 0.026, 0.033 and 0.042 respectively), while the correlation not reach the statistical significance in case of dicentric% and 8-OHdG concentration (P = 0.558 and P = 0.153 respectively). Furthermore, working period (years) highly affect the frequency of chromosomal aberrations in multiple regression analysis. Contradictory to our results (Zakeri and Hirobe, 2010) reported that, no obvious trend of increased chromosomal aberrations as a function of duration of employment was observed.

Our findings agree with (Tucker J.D., 2008) who reported that the concepts of induction, accumulation and persistence of low dose ionizing radiation are important for understanding the effects of exposure time to ionizing radiation. Each dose or dose fraction, no matter how small or large, has the potential to induce double strand breaks that ultimately lead to translocations. Thus, the concept of accumulation assumes multiple exposures. Persistence refers to the amount of time that translocations exist following their formation. What distinguishes translocation from other aberrations is that their persistence is substantially greater. The fact that no type of aberrations, even translocations, shows complete persistence emphasizes the importance of one month vacation in allowing for sufficient damage elimination, either by removal of damaged cells from peripheral blood through apoptosis or perhaps by DNA repair (Sahin et al., 2009).

5. Conclusions

Our results are particularly interesting for developing countries where biological safety controls are not so strict and extended work days are common.

From this study may conclude the following:-

1. Scoring of chromosome aberrations such as breaks, fragments and dicentrics is a reliable method to detect exposure to ionizing radiation. This type of monitoring may be used as a biological dosimeter which gives informations on the effects of ionizing radiation, on previous exposures or on differences in individual radiosensitivity. Biological dosimetry is needed when physical dosimetry cannot be used or does not provide sufficient information.
2. 8-OHdG is a useful oxidative DNA adduct as a marker among radiation workers and those exposed to environmental carcinogens.

6. Recommendations

From the above findings, it is to be recommended that:

1. Periodic cytogenetic study is of utmost importance in individuals occupationally exposed to low dose ionizing radiation.
2. Biomarkers of oxidative stress namely 8-OHdG should be measured and antioxidant supplements be instituted for workers exposed to ionizing radiation.
3. The new ratio N/L should be included in the medical checkups of hospital staff and workers exposed to ionizing radiation.

REFERENCES

Agrawala, P. K., Adhikari, J. S., & Chaudhury, N. K. (2010). Lymphocyte chromosomal aberration assay in radiation biodosimetry. *Journal of Pharmacy and Bioallied Sciences, 2*, 197–201.

Alsatari, E. S., Azab, M., Khabour, O. F., Alzoubi, K. H., & Sadiq, M. F. (2012). Assessment of DNA damage using chromosomal aberrations assay in lymphocytes of waterpipe smokers. *International Journal of Occupational Medicine and Environmental Health, 25*, 218–224.

Au, W. W., Cajas, N., & Salama, S. (1998). Factors contributing to discrepancies in population monitoring studies. *Mutation Research, 400*, 467–478.

Azzam, E. I., Jay-Gerin, J. P., & Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Letters, 327*, 48–60.

Balakrishan, S., & Rao, S. B. (1999). Cytogenetic analysis of peripheral blood lymphocytes of occupational workers exposed to low levels of ionizing radiation. *Mutation Research, 442*, 37–42.

Ballardi, M., Antonelli, A., Cipollini, M., Fallahi, P., Scarpato, R., & Tomei, A. (2007). Induction of chromatid-type aberrations in peripheral lymphocytes of hospital workers exposed to very low doses of radiation. *Mutation Research, 626*, 61–68.

Bolognesi, C., Abbondandolo, A., Barale, R., Casalone, R., Dalprà, L., De Ferrari, M., et al. (1997). Age-related increase of baseline frequencies of sister chromatid exchanges, chromosome aberrations, and micronuclei in human lymphocytes. *Cancer Epidemiology Biomarkers & Prevention, 6*, 249–256.

Bonassi, S., El-Zein, R., Bolognesi, C., Fenech, M., Tinnerberg, H., Forni, A., et al. (2011). Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis, 26*, 93–100.

Bonassi, S., Neri, M., Lando, M., Ceppia, M., Linb, Y., Chang, W. P., et al. (2003). Effect of smoking habit on the frequency of micronuclei inhuman lymphocytes: results from the human micronucleus project. *Mutation Research, 543*, 155–166.

Cigarran, S., Barquinero, J. F., Barrios, L., Ribas, M., Egozcue, J., & Caballín, M. R. (2001). Cytogenetic analysis by fluorescence in situ hybridization (FISH) in hospital workers occupationally exposed to low level of ionizing radiations. *Radiation Research, 155*, 417–433.

Dizdaroglu, M., Jaruga, P., Birincioglu, M., & Rodriguez, H. (2002). Free radical-induced damage to DNA: mechanisms and measurement. *Free Radical Biology and Medicine, 32*, 1102–1115.

Eken, A., Aydin, A., Erdem, O., Akay, C., Sana, H. T., Soykut, B., et al. (2010). Cytogenetic analysis of peripheral blood lymphocytes of hospital staff occupationally exposed to low doses of ionizing radiation. *Toxicology and Industrial Health, 26*, 273–280.

Ghadhia, P. K., Shah, N., Nahata, S., Patel, S., Patel, K., Pithawala, M., et al. (2004). Cytogenetic analysis of radiotherapuetic and diagnostic workers occupationally exposed to radiations. *International Journal of Human Genetics, 4*, 65–69.

Gricienė, B., Slapšytė, G., & Mierauskienė, J. (2014). Cytogenetic monitoring of nuclear workers occupationally exposed to ionising radiation. *Radiation Protection Dosimetry, 159*, 10–19.

Karbownik, M., & Reiter, R. J. (2000). Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. *PSEBM, 225*, 9–22.

Kasuba, V., Rozgaj, R., & Jazbec, A. (2008). Chromosome aberrations in peripheral blood lymphocytes of Croatian hospital staff occupationally exposed to levels of ionising radiation. *Archives of Industrial Hygiene and Toxicology, 59*, 251–259.

Lazutka, J. R., Lekevicius, R., Dedonyte, V., Maciuleviciute-Gervers, L., Mierauskiene, J., Rudaitiene, S., et al. (1999). Chromosomal aberrations and sister-chromatid exchanges in Lithuanian populations: effects of occupational and environmental exposures. *Mutation Research, 445*, 225–239.

Lewis, S. M., Bain, B. J., & Bates, I. (2006). *Dacie and Lewis practical hematology* (10th ed.). Philadelphia: Churchil Livingstone Elsevier,pp.40-57.

Maffei, F., Angelini, S., Forti, G. C., Lodi, V., Violante, F. S., Mattioli, S., et al. (2002). Micronuclei frequencies in hospital workers occupationally exposed to low levels of ionizing radiation: influence of smoking status and other factors. *Mutagenesis, 17*, 405–409.

Maffei, F., Angelini, S., Forti, G. C., Violante, F. S., Lodi, V., Mattioli, S., et al. (2004). Spectrum of chromosomal aberrations in peripheral lymphocytes of hospital workers occupationally exposed to low doses of ionizing radiation. *Mutation Research, 547*, 91–99.

Martínez, A., Coleman, M., Romero-Talamás, A. C., & Frías, S. (2010). An assessment of immediate DNA damage to occupationally exposed workers to low dose ionizing radiation by using the comet assay. *Rev Invest Clin, 62*, 23–30.

Mozdarani, H., Hejazi, A., & Hejazi, P. (2002). Chromosomal aberrations in lymphocytes in individuals with chronic exposure to gamma radiation. *Archives of Iranian Medicine, 5*, 32–36.

Roland, R. E., & Hardeny, K. M. (1999). Increased sister chromatid exchange in the peripheral blood lymphocytes of young women who smoke cigarettes. *Herediates, 131*, 143–146.

Sahin, A., Tatar, A., Oztas, S., Seven, B., Varoglu, E., Yesilyurt, A., et al. (2009). Evaluation of genotoxic effects of chronic low-dose ionizing radiation exposure on nuclear medicine workers. *Nuclear Medicine and Biology, 36*, 575–578.

Sajous, L., Botta, A., & Sari-Minodier, I. (2008). Urinary 8-hydroxy-2'-deoxyguanosine: a biomarker of environmental oxidative stress. *Ann Biol Clin, 66*, 19–29.

Santovito, A., Cervella, P., & Delpero, M. (2015). Baseline frequency of chromosomal aberrations and sister chromatid exchanges in peripheral blood lymphocytes of healthy individuals living in Turin (North-Western Italy): assessment of the effects of age, sex and GSTs gene polymorphisms on the levels of genomic damage. *Annals of Human Biology, 24*, 1–10.

Sharma, A. K., & Sharma, A. (1980). Chromosomal analysis following short and long-term culture in animals including man. In A. K. Sharma, & A. Sharma (Eds.), *Chromosome techniques* (pp. 492–499). London: Butter Worth.

Shirazi, A., Ghobadi, G., & Ghazi-Khansari, M. (2007). A radiobiological review on melatonin: a novel radioprotector. *Journal of Radiation Research, 48*, 263–272.

Silva, R., Folgosa, F., Soares, P., Pereira, A. S., Garcia, R., Gestal-Otero, J. J., et al. (2013). Occupational cosmic radiation exposure in Portuguese airline pilots: study of a possible correlation with oxidative biological markers. *Radiation and Environmental Biophysics, 52*, 211–220.

Sperati, A., Abeni, D. D., Tagesson, C., Forastiere, F., Micell, M., & Axelson, O. (1999). Exposure to indoor background radiation and urinary concentrations of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage. *Environmental Health Perspectives, 107*, 213–215.

Terzic, S., Milovanovic, A., Dotlic, J., Rakic, B., & Terzic, M. (2015). New models for prediction of micronuclei formation in nuclear medicine department workers. *Journal of Occupational Medicine and Toxicology, 10*, 25.

Tucker, J. D. (2008). Low-dose ionizing radiation and chromosome translocations: a review of the major considerations for human biological dosimetry. *Mutation Research, 659*, 211–220.

Zakeri, F., Assaei, R., & Varergar, R. (2003). Chromosomal aberration in workers occupationally exposed to chronic low-level ionizing radiation. *Biomonitoring by Cytogenetic Analysis of Radiation Exposed Workers, 9*, 33–38.

Zakeri, F., & Hirobe, T. (2010). A cytogenetic approach to the effects of low levels of ionizing radiations on occupationally exposed individuals. *European Journal of Radiology, 73*, 191–195.