academicJournals

Vol. 10(29), pp. 604-613, 8 August, 2016
DOI: 10.5897/AJPP2016.4584
Article Number: B062DAC59760
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Toxicogenetic biomonitoring of workers to the ionizing radiation

Ronald Gerard Silva[1], Md. Torequl Islam[2,3]*, Ricardo Melo de Carvalho[4], Marcus Vinícius Oliveira Barros Alencar[2,4], Antonio Luiz Gomes Júnior[2,4], Rai Pablo Sousa de Aguiar[4], Ana Maria Oliveira Ferreira da Mata[4], João Marcelo de Castro e Sousa[4,5], Márcia Fernanda Correia Jardim Paz[4], Paulo Michel Pinheiro Ferreira[6], Sandra Maria Mendes de Moura Dantas[7], Ana Amélia de Carvalho Melo-Cavalcante[2,4] and Jaqueline Nascimento Picada[1]

[1]Postgraduate Program in Cellular Biology Applied to Health, Lutheran University of Brazil, Canoas (Rio Grande do Sul) - 92.425-900, Brazil.
[2]Postgraduate Program in Biotechnology (RENORBIO), Federal University of Piauí, Teresina (Piauí) - 64.049-550, Brazil.
[3]Department of Pharmacy, Southern University Bangladesh, Mehedibag (Chittagong) - 4000, Bangladesh.
[4]Postgraduate Program in Pharmaceutics Sciences - Laboratory of Genetic Toxicology, Federal University of Piauí, Teresina (Piauí) - 64.049-550, Brazil.
[5]Department of Biological Sciences, Federal University of Piauí, Picos (Piauí) - 64.607-670, Brazil.
[6]Department of Biophysics and Physiology, Federal University of Piauí, Teresina (Piauí) -64.049-550, Brazil.
[7]Department of Biological Sciences, Federal University of Piauí, Teresina (Piauí) - 64.049-550, Brazil.

Received 28 April, 2016; Accepted 14 June, 2016

Occupational exposure to ionizing radiation (IR) can cause systemic acute and chronic effects on human health, including genetic instability that may be etiology of various diseases, including cancer. The aim of this study was to evaluate the possible toxicogenetic changes in haematological and biochemical parameters, and cytogenetic biomarkers (micronuclei and nuclear abnormalities) indicators of mutagenicity and apoptosis, as well as seek their correlation with lifestyle, age and gender. In accordance with the ethical aspects, 45 professionals (technicians and technologists in radiology) occupationally exposed to low doses of IR participated in this study. For control, 45 healthy individuals were not exposed to IR and/or genotoxic chemicals were included. Peripheral blood and oral epithelium samples were used in the toxic evaluations. The results suggested unchanged hematological biomarkers but a significant ($P < 0.05$) increases in the frequency of micronuclei, sprouts, binucleate cells and bridges, as well as karyolysis and karyorrhexis in professional radiology sector. Hepatic and nephritic toxicity were not observed. Without protection, a significant ($P < 0.01$) correlation ($P < 0.05$) was observed between toxicogenetic biomarkers with age, smoking, alcohol consumption, time and place of work. In conclusion, IR may be associated with genetic instability in health diseases, like cancer.

Key words: Ionizing radiation, micronuclei, occupational risk; hematological profile.

INTRODUCTION

Cancer is the second leading cause of death worldwide and is an important public health problem. According to the World Health Organization (WHO), in the coming decades, the impact of cancer in the population will be 80% of the more than 20 million new cases estimated for 2025. In Brazil, in the biennium 2016-2017, about 600,000 new cases of cancer were estimated (INCA, 2015). Among the risk factors, nowadays, radiation and its impacts on human health is a major concern to the etiology of cancer (Samet, 2011). On this occasion, extensive researches are needed in order to diagnose the problems to avoid or at least minimize the deleterious

effects of ionizing radiation (IR) (Pernot et al., 2012).

Notably, ionizing radiation (IR), even at low dose may be harmful and can cause dangerous events in biological systems (Kadhim et al., 2013). An increased dose is always harmful. Sometimes, a particular radiation may act by different mechanistic pathways (Yang et al., 2012). Low doses (0.05 to 0.5 Gy) of IR can cause genomic instability, such as chromosomal alterations and cell death (Kadhim et al., 2013), and can lead to a double failure of DNA and epigenetic alterations in histones (Sasakil et al., 2014). Studies with mass spectrometry based on proteome analysis in human skin model, indicated that after 48 h exposure to 3, 10, and 200 cGy x-rays alter and disrupt 135 proteins, where carboxipeptidases and ubiquitin carboxyl terminus isoenzyme hydrolase were the most sensitive, indicating that radiations at any dose can alter enzymatic proteins (Zhang et al., 2014).

IR have a potential for induced genetic instability in germ and somatic cells, characterized by the production of chromosomal groupings, aneuploidy, micronuclei (MN), gene amplification, mutations in derived and other cells having effects of radiation (Camats et al., 2008). Such changes can be highlighted and monitored by the use of any tests, among them the micronucleus (MN) test is one. It is an easy, economical and reproducible test procedure (Maluf and Riegel, 2011). The MN are formed from the chromosomal fragments or entire chromosomes that are not incorporated into the nucleus of the daughter cell during cell division, they are corpuscles containing DNA without structural connection to the core. The presence of MN and nuclear abnormalities can be considered as a preliminary indication for assessing the mutagenic and/or carcinogenic agents, such as ionizing radiation (Sari-Minodier et al., 2002).

To contribute to the health and prevention of cancer in health professionals occupationally exposed to IR, the present study evaluated the toxicogenetic risks of exposure to the IR using hematological and biochemical parameters in peripheral blood; and the application of cytogenetic MN test in the evaluation of the frequency of MN, nuclear-type abnormalities: binucleate cells, buds, bridges nucleo-plasmatics and apoptosis stages (karyorrhexis and karyolysis) in oral epithelial cells. Secondly, hematological parameters and toxicogenetic biomarkers were correlated to the lifestyle (vegetable consumption, smoking, alcohol consumption, protection of use, age, place of work and working time), age and gender of the participants.

MATERIALS AND METHODS

Ethics and legal aspects

All studies were performed in accordance with Brazilian research guidelines (Law 466/2012, National Council of Health, Brazil) and with the Declaration of Helsinki and all procedures were approved by the Ethics Committee on Human Research, based at the Lutheran University of Brazil (ULBRA, Rio Grande do Sul - CAAE: 38570914.8.0000.5349).

Research subjects

This study was conducted with 45 professionals occupationally exposed to IR including radiology technicians, technologists in radiology and radiologists from two clinics: Diagnostic Unit Imaging (UDI 24 hours, Teresina, Brazil) Lucidio Portela Hospital (Teresina, Brazil) and 45 professionals who do not work in sectors related to diagnostic radiology. All participants answered the health questionnaire recommended by the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) (Carrano and Natarajan, 1998) in order to select the unexposed group as well as for obtaining data on health and lifestyle of the participants to correlate with toxicogenetic parameters.

Haematological and biochemical parameters analysis

Blood samples were collected for hematological and biochemical tests to quantify liver and kidney enzymes such as AST (glutamic-oxalacetic transaminase) and ALT (glutamic pyruvic transaminase), creatinine, urea and alkaline phosphatase (ALP). The tests were performed according to the protocol established by LabtestTM.

Micronucleus (MN) test in oral epithelial cells

Buccal cell samples were obtained by gently rubbing the inside of the cheeks (right and left side) with a cytobrush, which was immersed in 5 ml cold saline (0.9% (w/v) aqueous NaCl) in a conical tube and transported under refrigeration to the Laboratory of Genetic Toxicology, Federal University Piauí (Teresina, Brazil). The samples in saline were centrifuged at 1500 rpm for 10 min and the sedimented cells were then washed with saline (twice) and fixative solution (methanol and glacial acetic acid 3:1) (once) under the same centrifugation conditions. The cell suspension was spread onto a slide with fixative solution and dried at room temperature. The slides were stained with 2% Giemsa solution for 10 min, rinsed in distilled water, and air-dried. Biomarkers of DNA damage (MN), cytokinetic defects (binucleated cells) and cell death (karyorrhexis, pyknotic and karyolytic cells) were scored according to the criteria set by Thomas et al. (2009). For each volunteer, 2000 buccal cells (1000 from each of the duplicate slides) were scored using bright-field optical microscopy at a magnification of 1000. MN were identified taking into consideration in the following conditions: cells with intact main nuclei and cytoplasm; diameter of one-third of the main nucleus; same staining and texture as the main nucleus, and MN was in the same focal plane as the main nucleus. Other anomalies in cells were measured as binucleated, karyolysis, karyorrhexis,and pyknotic cells.

Statistical analysis

Personal characteristics and lifestyle of the groups obtained from the personal health questionnaire were statistically compared by *t*-student's test using the statistical program SPSS 16.0. The relationship between lifestyle characteristics and cytogenetic data were performed by correlation of Spearman's rho. Cellular data

*Corresponding author. Email: mti031124@gmail.com.

Table 1. General characteristics of control group and workers exposed to ionizing radiation.

Subject characteristics		Control group (n = 45)	Exposed group (n = 45)
Age[1]		31.73 ± 7.44 (18 - 45)	31.47 ± 7.34 (19- 47)
Gender[2]	Male	68.9 (n=31)	66.7 (n=30)
	Female	31.1 (n=14)	33.3 (n=15)
Alcoholism[2]	Yes	66.7 (n=30)	44.4 (n=20)
	No	33.3 (n=15)	55.5 (n= 25)
Smoking[2]	Yes	22.2 (n= 10)	-
	No	77.8 (n = 35)	100 (n=45)
Prescribed medication use[2]	Yes	22.2 (n=10)	33.3 (n=15)
	No	44.4 (n=20)	66.7 (n=30)
	Not reported	33.4 (n=15)	00.0
Vegetable intake[2]	Yes	20 (n = 9)	13.3 (n=6)
	No	80 (n= 36)	86.7 (n=39)
PPE use[3]	Yes	-	100.0 (n=45)

[1]Mean ± standard deviation; [2]Data in percent form (%); [3]Personal Protective Equipment: coat, mask, apron and boots. Alcoholism was considered as mean of 5 bottles/week. $P < 0.05$; ANOVA, *t*-Student.

on the frequency of micronuclei and nuclear abnormalities between the groups were assessed by the multiple *t*-test with significance in Holm-Sidak method, using the statistical program Graphpad Prism 5.0 (Graphpad Inc., San Diego, CA). Values of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Characteristics of population in the study

The characteristics of the population obtained from data collected from the health questionnaire are summarized in Table 1. The mean age of people in the exposed group was 31.47 ± 7.34 years and about 68.9% were male, mostly white (68.9%) and average household 1,097.7 reais (Brazilian currency). With regard to alcohol consumption, 13.3 and and 20,0% of the exposed and unexposed groups answered yes for it, respectively. For smoking, 22.2% of the people of exposed group answered yes and 100% of the members of the unexposed group said that they did not consume cigarettes. Regarding the use of drugs, 66.7% of respondents belonging to the group not exposed and 44.4% of the exposed group reported not using any type of medicine in the last two weeks before the application of the questionnaire. No statistical difference in relation to these characteristics was observed ($P < 0.05$).

Evaluation of haematological an biochemical parameters in health professionals occupational exposure to IR

Hematological (red and,white series and platelets) and biochemical (AST, ALT, creatinine, urea and ALP) were seen within the normal reference standards. Then, no signs of hematological changes and liver and kidney toxicity were detected (Table 2).

Genetic instabilities induced by occupational exposure to IR in oral epithelium of health professionals

The risk assessment for occupational exposure to hazards has social importance and for worker health (Montano, 2014). Epidemiological studies show an association between IR and cancer, like brain cancers (Smoll et al., 2016). High and low doses of IR induced circulatory diseases are indicative of risks following occupational exposure to mortality risk, as well as with the cancer-inducing ability (Little et al., 2012). The MN test is well founded to establish the risk of DNA damage and studies show a strong association between IR, increased frequency of MN and susceptibility to cancer (Bolognesi et al., 2014).

Workers exposed occupationally at IR in Radiological Diagnostics Clinics in Teresina- -Piauí, showed genetic instability in oral epithelium cells ($P < 0.001$) since it occurred increase in MN frequencies, nuclear bud (NB), binucleate cells (BN) and nucleoplasmatic bridges (NP), when compared individuals not exposed to IR (Figure 1). Previously cytogenetic studies reported impacts in mammalian cells exposed to 0.5-3 Gy with presence of DNA damage confirmed by chromosomal aberrations and MN, which are coming from breaks and loss of chromosomes, suggesting a genetic instability (Plamadeala et al., 2015).

Oral epithelium is important tool for toxicological

Table 2. Hematologic and biochemistry parameters of workers occupationally exposed to ionizing radiation.

Parameter	Reference range		Control group		Exposed group	
Red line	**Man**	**Woman**	**Man**	**Woman**	**Man**	**Woman**
Red cell Count/mm^3	4.3 – 5.7	3.9 – 5.0	4.9 ± 0.2	4.6 ± 0.2	4.6 ± 0.2	4.5 ± 0.3
Haemoglobin g/dL	13 – 17.5	12 – 15	15.5 ± 1.0	13.7 ± 1.2	14.1 ± 1.1	13.2 ± 1.0
Haematocrit (%)	38 – 50	36 – 44.5	45.6 ± 2.4	42.2 ± 2.1	43.0 ± 2.9	40.3 ± 3.0
White line						
White cell Count/mm^3	4.000		5588 ± 1000	5148 ± 1065	5221 ± 990	5473 ± 1100
Neutrophils/mm^3	45 – 69		58 ± 5.8	56.4 ± 5.3	5221 ± 990	5473 ± 1100
Lymphocytes/mm^3	20 – 47		37 ± 4.7	39.5 ± 4.4	37.7 ± 5.4	36.3 ± 3.5
Monocytes/mm^3	1 – 10		3 ± 1.2	2.4 ± 1.3	3.0 ± 1.4	2.8 ±1.0
Eosinophils/mm^3	0 – 5		1.6 ± 1.0	2.2 ± 1.2	1.9 ± 1.1	1.2 ± 0.8
Basophils/mm^3	0 – 1		0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Platelet Count	150 – 450 K		281 ± 60	281 ± 55	270 ± 42	299 ± 84
Biochemistry parameters						
AST (U/L)	37	31	31.4 ± 7.1	23.9 ± 8.4	21.4 ± 5.0	19.2 ± 3.9
ALT (U/L)	42	32	33.5 ± 10.1	26.2 ± 15.3	20.8 ± 12.0	14.9 ± 5.3
Creatinine (mg/dl)	0.4 – 1.4		0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.1
Urea (mg/dl)	15 – 40		29.8 ± 4.8	34.8 ± 11.0	36.4 ± 8.8	34.0 ± 5.3
Alkaline phosphatese (U/L)	27 – 100		42.3 ± 11.3	69.7 ± 22.9	76.5 ± 18.6	73.4 ± 18.9

studies, especially due to its rapid division. MN and other nuclear abnormalities such as sprouts and bridges are considered biomarkers for genotoxic damage and chromosomal instability. MN are fragments of chromosomes or loss of chromosomes during anaphase of cell division due to the poor chromosome segregation as a result of hipometilations in repeated sequences of the centromeric regions (Fenech et al., 2011).

MN, extra nuclear bodies, may be observed in oral exfoliated mucosa cells, as a biomarker for genotoxicity and carcinogenicity. The formation mechanism of NBs may be related to the elimination and amplification or DNA repair. The BNs may be indicative of cytokinesis failure at the end of cell division (Sabharwal et al., 2015). The MN formation may be due to the breakage, indicating clastogenic and chromosomes aneugenic agents (Suzuki et al., 2003; Fenech et al., 2011).

Nuclear changes induced by occupational exposure to IR in oral epithelium of health professionals

Exposure to occupational and environmental radiation sources can cause damage and genetic instability due to its effects as carcinogens. Epidemiological studies show an association between IR at low doses (1 to 5 mGy) and development of leukemia. Leukemia is a complex disease that may have etiologies related to the lifestyle and occupational and environmental exposure, but there are also associations between leukemia (Polychronakis et al., 2013) due to genetic instability related to increased single and double strand DNA breaks that can lead to the chromosomal alterations and formation of MN (Saha et al., 2014), as noted in mutagenicity analysis of professionals occupationally exposed to IR in this study. There are also reports that IR can lead to apoptosis, as observed in embryonic neuronscells (Leuraud et al., 2015).

Nuclear abnormalities such as BNs and apoptosis, karyorrhexis (nuclear fragmentation), and karyolysis (nuclear dissolution) are indicative of cytotoxicity were observed in health professionals exposed to IR. Data were statistically significant ($P < 0.05$) when compared with the control group (Figure 2). BNs may be indicative of cytokinesis failure at the end of cell division and cells with condensed chromatin are indicative of apoptosis (karyorrhexis and karyolysis), as well as picnoses (Sabharwal et al., 2015). Exposure to IR can lead to oxidative events, one of the mechanisms of cytotoxicity and apoptosis, due to the fact that these have direct action on the macromolecules *via* radiolysis product and intracellular modulation of the communication mechanisms of redox system, which induce stress in cells, tissues and mitochondria, led to the toxic effects of radiation depending on the doses (Einor et al., 2016).

IR, even at low doses, induce gene amplification and reactive oxygen species (ROS), for transducing signals for the enzyme superoxide dismutase (SOD), as observed in studies of brain cells from rats submitted to 2, 10 and 50 cGy, with the modulation about 88 signaling molecules which can induce apoptosis (Veeraraghavan et al., 2011). The accidental exposure to IR in doses 0 to 8 Gy in rats cause damage to brain cells that can generate neurodegenerative and cognitive complications with an induction of p53 and a decreased expression of proliferating cell nuclear

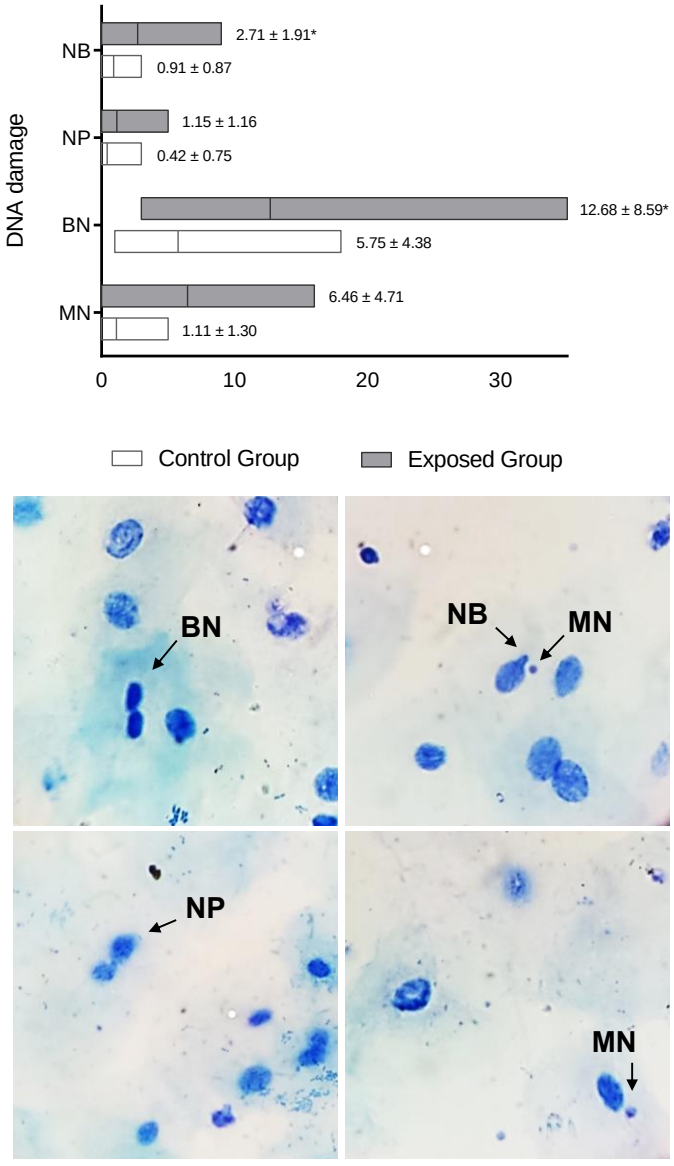

Figure 1. DNA damage assessed by cytogenetic biomarkers front of occupational exposure to ionizing radiation. NB: Nuclear buds; NP: nucleoplasmic bridges; BN: binucleated cells; MN: micronuclei. Means ± standard deviation, multiple t test and Holm- Sidak method, *P <0.05 compared to control group.

antigen (PCNA) and cell proliferation. The reduction of PCNA may be associated with the induction checks during the cell cycle (Chen et al., 2015).

Ionizing radiations have adverse responses in human cells, including as discussed previously in apoptosis, necrosis, premature senescence stress, autophagy and endoploidy. The p21 and p53 genes are important for cell responses front to the DNA damage processes and apoptosis induction (Mirzayans et al., 2013). Apoptosis has an important role in the homeostasis as well regulation of apoptosis levels related to the prevention of diseases and may be an indicative of genotoxic stress. Several signaling pathways regulate apoptosis among them involving the BCL-2 family protein, and inhibitors of apoptosis (IAP) (Hassan et al. 2014). The effects of IR involve endogenous signaling events that culminate in oxidative damage to DNA, lipids, proteins and many metabolites, in addition to changes in gene expression, metabolism and epigenetic factors (Reisz et al., 2014). It is well established that IR induce instability in chromosomes and have effects on activation and inactivation of DNA repair mechanisms, induce oxidative damage, but is not yet well established role of IR on telomeric proteins, which are considered to be protective of genome and related to carcinogenesis (Shim et al., 2014).

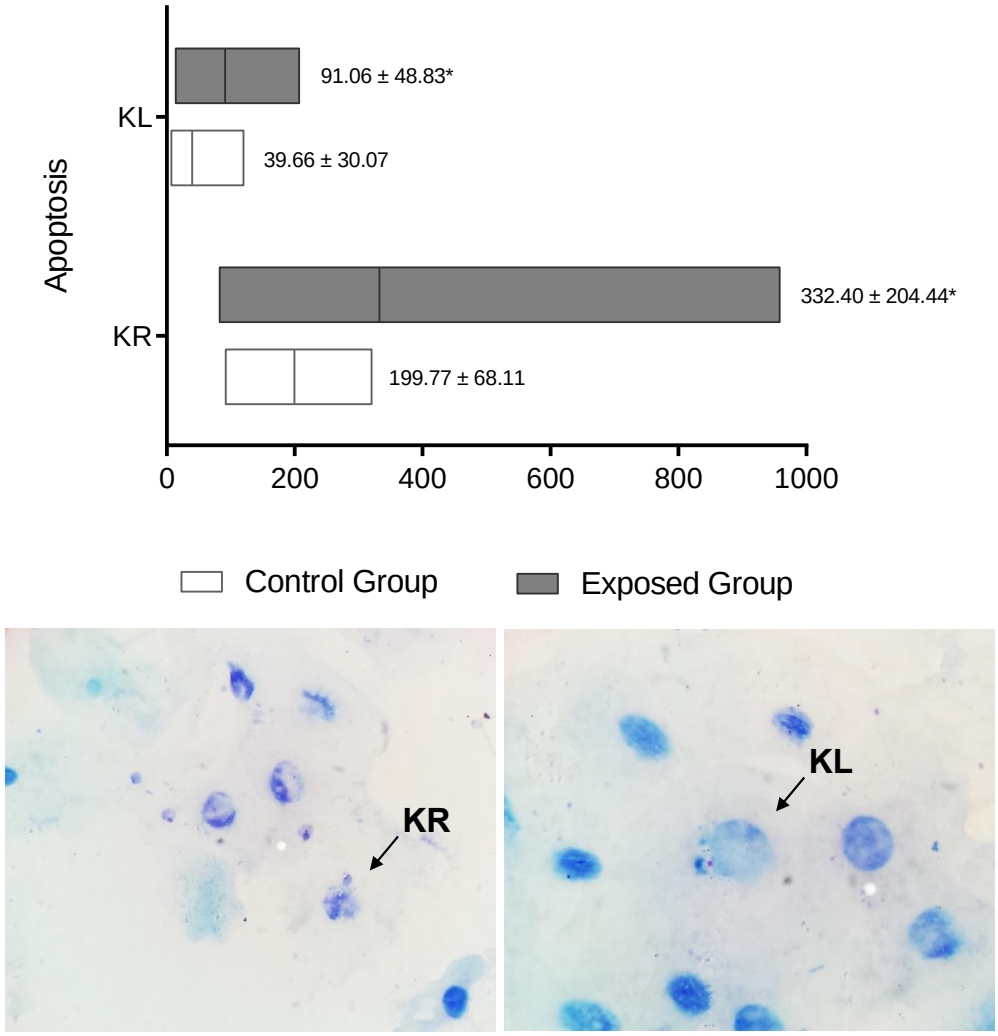

Figure 2. Apoptotic DNA fragmentation in buccal mucosa cells from workers exposed to ionizing radiation and control group through Micronucleus test. ANOVA. Non-parametric Mann-Whitney U test. *P <0.05 compared to the control group.

Numerous effects of radiation have been identified in association with mutations and genomic instability (breaks and actions in repair genes), angiogenesis (vascularisation and hypoxia), apoptosis (changes in p53), proliferative signaling (EGCR and TGF-α), suppression of cell cycle (ATM, p53 lock), energy dysregulation (HIF, c-MyC, glycolytic pathways inhibitors), tumor promotion and inflammation (p53 and ROS) and activation and inactivation of metastases (hypoxia, lactate) (Boss et al., 2014). Given the risks of cancer, studies suggest biological dosimeters guided by genetic instability mechanisms, is noted in summary in Table 3, especially for chromo-somal aberrations that may be markers for evaluation of dose and biological effects induced by IR (Higueras et al., 2015). Numerous effects of radiation (Table 3) have been identified in association with mutations and genomic instability (breaks and actions in repair genes), angiogenesis (vascularization and hypoxia), apoptosis (changes in p53), proliferative signaling (EGCR and TGF-α), suppression of cell cycle (ATM, locking p53), energy deregulation (HIF, c-MyC, glycolytic pathways inhibitors), tumor promotion and inflammation (p53 and ROS) and activation and inactivation of metastases (hypoxia, lactate) (Boss et al., 2014; Lee et al., 2014). Relation among age, gender, DNA damage and cytotoxicity.

Populations occupationally exposed to chemical and physical genotoxic agents are being carried out with the application of toxicogenetic tests. However, the understanding of the influence of factors related is necessary to age, gender and lifestyle and disease in relation to MN rates (Bonassi et al., 2011), as well as other nuclear abnormalities (Holland et al., 2008).

To evaluate the influence of age for MN induction, cells with karyorrhexis (indicative of apoptosis) and the mean ages were compared with those toxicogenetic biomarkers. The mean of MN of different age intervals

Table 3. Possible action mechanisms of ionizing radiations in workers occupationally exposed.

Mechanisms	Dose/Biological sample	References
Chromosome damage, dicentric chromosomes, nucleoplasmic bridges, chromosomal rings, fragments	Low dose (0-100 mGy)/ Blood	Manning and Rothkamm (2013)
Chromosomal aberrations and micronuclei	0.5-5.0 Gy/Blood	Liu et al. (2009)
Chromosomal aberrations, micronuclei and apoptosis	0.325 mGy/Buccal mucosa cells	Arora et al. (2014)
Changes in DNA, lipids and expression of proteins and epigenetic factors	15-30 mGy and 0.01-0.15 mGy/Blood	Reisz et al. (2014)
Dicentric chromosomes	0-5 Gy/Peripheral blood	Al-Hadyan et al. (2014)
Metabolic and immune dysfunction	0.5-5 Gy/Peripheral blood	Lee et al. (2014)
Activation and inactivation of telomeric proteins	High and low doses	Shim et al. (2014)
Mutations and genomic instability. Angiogenesis, apoptosis, proliferation, energy imbalance, inflammation and metastases	High and low doses	Boss et al. (2014)

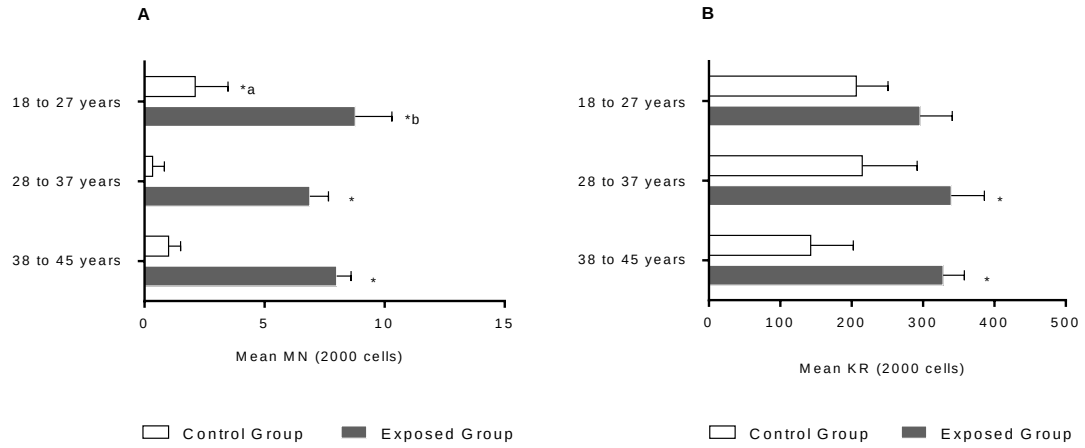

Figure 3. Mutagenicity (A) and apoptotic DNA fragmentation (B) according to tertis of age in buccal mucosa cells from workes exposed to ionizing radiation and control group. ANOVA, Bonferroni's multiple comparison test. *P <0.05 compared to control group. a,b compared to age intervals of 28 to 37 and 38 to 45 years. MN: Micronuclei; KR: Karyorrhexis.

of exposed workers was significantly higher compared to the unexposed group. Workers exposed that age range of 18 to 27 years had more production of the MN when compared to other age ranges (Figure 3A). For karyorrhexis, the data were significant only for the group unexposed to X-rays (Figure 3B).

MN test in relation to the age of the exposed workers showed that in all ages, the exposed workers are more susceptible to IR than the unexposed

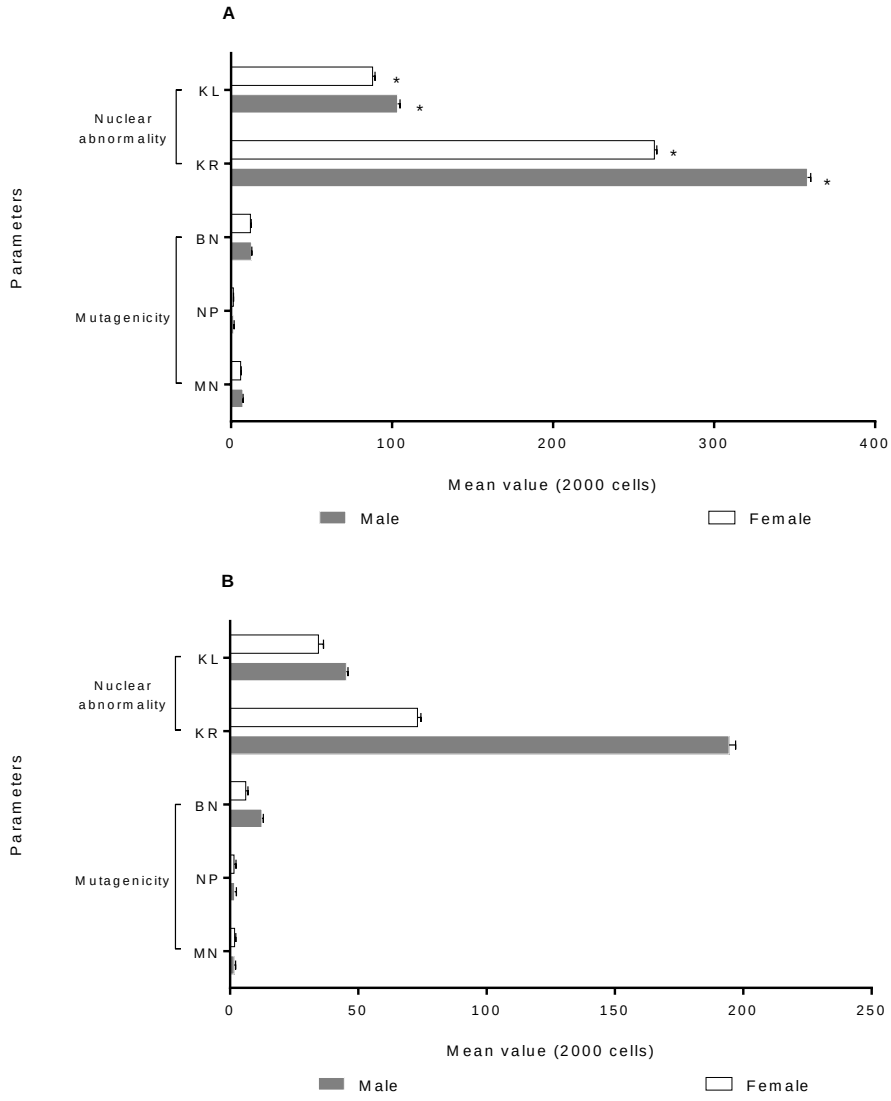

Figure 4. Comparative study of cytogenetic biomarkers in relation to gender. ANOVA, MannWhitney U test. A: Exposed group; B: control group; *P <0.05 compared group B; NP: Nucleoplasmic bridges; NB: nuclear buds; BN: binucleated cells; MN: micronuclei; KR: Karyorrhexis; KL: Karyolysis.

group. These data are in agreement with Maluf and Erdtmann (2001), who evaluated the presence of cellular with Maluf and Erdtmann (2001), who evaluated abnormalities in workers exposed to radiation than the consumption of oxide ethylene and cytostatic agents. In comparison to the age range, the age of professionals between 18 and 27 years showed more significant levels than the other groups with respect to the mutagenicity, and the range of 38 to 45 showed more significant indices with respect to apoptotic changes. These data differ from the data presented by Ladeira et al. (2011), where the evaluation of abnormalities found in the MN test showed no statistical difference in age and sex. However, Jha and Sharma (1991) revelaed an apoptosis and nuclear abnormalities in workers age between 35 to 50 years exposured to IR.

Regarding gender, it was observed that only karyorrhexis level is statistically significant with a greater predisposition to nuclear fragmentation in males. On the other hand, Fenech and Bonassi (2011) showed that the changes in MN test are always higher in women than the men in all age groups. This occurs due to the hormonal cycle in women and especially the low intake of some micronutrients such as vitamins, folic acid and ions. Although, there were no statistically significant differences (P >0.05) for cytogenetic biomarkers tested in relation to gender differences in groups exposed to IR in the unexposed group. In this study, we observed differences only karyorrhexis when assessed alone (P <0.05, Figure 4).

Correlation between lifestyle factors with cytogenetic biomarkers

Genomic damage has great important in the etiological

Table 4. Correlations between lifestyle and cytogenetic damages.

Parameter	Sperman's rho	P value
Life style *vs.* biomarkers	-	-
Smoking *vs.* Micronuclei	0.426	0.002*
Alcoholism *vs.* Micronuclei	0.521	0.000*
Age *vs.* Nucleoplasmic bridges	0.313	0.036*
Age *vs.* Karyolysis	0.382	0.010*
Time of work *vs.* Karyolysis	0.319	0.330
Time of work *vs.* Binucleated cells	0.416	0.005*
Work place *vs.* Karyorrhexis	0.424	0.004*

Sperman's correlation. * P < 0.05.

analysis for the development of degenerative diseases. Radiation and the use of chemicals are needed in different therapies, but also micronutrient deficiencies, lifestyle and other genetic factors are needed in biomonitoring, diagnosis and treatment of diseases in the evaluation of genetic damage (Tolbert et al., 1992).

Herein, after statistical Spearman's Herein, after statistical, positive correlations were observed between smoking and alcohol consumption with MN, age bridges and karyolysis and working time with karyolysis and binucleate cells in oral epithelium (Table 4).

Cytogenetic damage shown in this study did not show positive correlations with age, smoking, alcohol consumption, and genetic diseases, with exposure to IR. Hartmann et al. (1998) also demonstrated an insignificant relationship between smokers/nonsmokers and DNA damage. However, epidemiological studies show that smoking induces DNA damage, and may cause lung cancer (Sram, 1998). To be noted that age-related increased in the risk of aneuploidy and non-disjunction mitotic, changes in chromosomes with a reduced DNA repair are suggested by Migliore et al. (1991), despite of a controversial talk by Betti et al. (1994). These different responses can be related to the size of the samples, as well as varied and individual susceptibility of the population under study.

CONCLUSION

Occupational exposure of ionizing radiation to the workers caused genetic instability in a independent way of age and gender, induce micronuclei, bridges, buds and binucleate cells. However, an unchanged haematological parameters and hepatic as well as nephritic function were observed. A correlation with risk factors related to lifestyle (smoking and drinking) and IR was established. Our study demonstrates biomonitoring of genetic risks, applying mutagenicity biomarkers and nuclear abnormalities can be alternative tools for the diagnosis and prevention radiation-induced health hazards.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Alexandrou AT, Li JJ (2014). Cell Cycle Regulators Guide Mitochondrial Activity in Radiation-Induced Adaptive Response. Antioxidants Redox Signaling 20:1463-1480.

Al-Hadyan K, Elewisy S, Moftah B, Shoukri M, Alzahrany A, Alsbeih G (2014). Establishing cytogenetic biodosimetry laboratory in Saudi Arabia and producing preliminary calibration curve of dicentric chromosomes as biomarker for medical dose estimation in response to radiation emergencies. 3 Biotech. 4:635-645.

Arora P, Devi P, Wazir SS (2014). Evaluation of Genotoxicity in Patients Subjected to Panoramic Radiography by Micronucleus Assay on Epithelial Cells of the Oral Mucosa. J. Dentistry Tehran Univ. Med. Sci. 11:47-55.

Betti C, Davini T, Giannessi L, Loprieno N, Barale R (1994). Microgel electrophoresis assay (comet test) and SCE analysis in human lymphocytes from 100 normal subjects. Mutat. Res./Fundamental Molecul. Mech. Mutagen. 307:323-333.

Bolognesi C, Bruzzi P, Gismondi V, Volpi S, Viassolo V, Pedemonte S, Varesco L (2014). Clinical Application of Micronucleus Test: a case-control study on the prediction of breast cancer risk/susceptibility. Plos One 9:1-18.

Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S (2011). The HUman MicroNucleus project on eXfoLiated buccal cells (HUMNXL): The role of life-style, host factors, occupational exposures, health status, and assay protocol. Mutat. Res. 728:88-97.

Boss MK, Bristow R, Dewhirst MW (2014). Linking the History of Radiation Biology to the Hallmarks of Cancer. Radiat. Res. 181:561-557.

Camats N, García F, Parrilla JJ, Calaf J, Martín M, Caldés MG (2008). Trans-generation al radiation-induced chromosomal instability in the female enhances the action of chemical mutagens. Mutat. Res. 640:16-26.

Carrano AV, Natarajan AT (1998). International Commission for Protection against Environmental Mutagens and Carcinogens. ICPEMC publication no. 14. Considerations for population monitoring using cytogenetic techniques. Mutat. Res. 204:379-406.

Chen H, Goodus MT, Toledo SM, Azzam EI, Levison SW, Souayah N (2015). Ionizing Radiation Perturbs Cell Cycle Progression of Neural Precursors in the Subventricular Zone Without Affecting Their Long-Term Self-Renewal. Am. Soc. Neurochem. 7:1-16.

Einor D, Bonisoli-Alquati A, Costantini D, Mousseau TA, Møller AP (2016). Ionizing radiation, antioxidant response and oxidative damage: A meta-analysis. Sci. Total Environ. 548-549:463-471.

Fenech M, Bonassi S (2011). The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. Mutagen. 26:43-49.

Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P (2011). Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagen. 26:125-132.

Hartmann A, Fender H, Speit G (1998). Comparative biomonitoring study of workers at a waste disposital site using cytogenetic tests and comet (Single-Cell Gel) assay. Environ. Mol. Mutagen. 32:17-24.

Hassan M, Watari H, Abualmaaty A, Ohba Y, Sakuragi N (2014).

Apoptosis and Molecular Targeting Therapy in Cancer. BioMed Res. Int. p. 1-23.
Higueras M, Puig P, Ainsbury EA, Rothkamm K (2015). A new inverse regression model applied to radiation biodosimetry. Proceedings of the Royal Society a Mathematical, Physical Eng. Sci. 471:1-18.
Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M (2008). The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. Mutat. Res. 659:93-108.
INCA (2015). Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA). Incidência de Câncer no Brasil - Estimativa 2016. Disponível em: <http://www.inca.gov.br/estimativa/2016/>. Data de acesso: 13/01/2016. Rio de Janeiro: INCA; 2015.
Jha AN, Sharma T (1991). Enhanced frequency of chromosome aberrations in workers occupationally exposed to diagnostic X-rays. Mutat. Res. 260:343-348.
Kadhim M, Salomaa S, Wright E, Hildebrandt G, Belyakov OV, Prise KM, Little MP (2013). Non-targeted effects of ionizing radiation–implications for low dose risk. Mutat. Res. 752:84-98.
Ladeira C, Viegas S, Carolino E, Prista J, Gomes MC, Brito M (2011). Genotoxic biomarkers in occupational exposure to formaldehyde. The case of histopathology laboratories. Mutat. Res./Fundamental Molecul. Mech. Mutagen. 721:15-20.
Lee KF, Weng JTY, Hsu PWC, Chi YH, Chen CK, Liu IY, Chen YC, Wu LS (2014). Gene Expression Profiling of Biological Pathway Alterations by Radiation Exposure. BioMed Res. Int. pp. 1-9.
Leuraud K, Richardson DB, Cardis E, Daniels RD, Gillies M, O'hagan JA, Hamra GB, Haylock R, Laurier D, Moissonnier M, Schubauer-Berigan MK, Thierry-Chef I, Kesminiene A (2015). Ionising radiation and risk of death from leukaemia and lymphoma in radiation-monitored workers (INWORKS): an international cohort study. Lancet Haematol. 2:276-281.
Little MP, Azizova TV, Bazyka D, Bouffler SD, Cardis E, Chekin S, Chekin S, Chumak VV, Cucinotta FA, de Vathaire F, Hall P, Harrison JD, Hildebrandt G, Ivanov V, Kashcheev VV, Klymenko SV, Kreuzer M, Laurent O, Ozasa K, Schneider T, Tapio S, Taylor AM, Tzoulaki I, Vandoolaeghe WL, Wakeford R, Zablotska LB, Zhang W, Lipshultz SE (2012). Systematic Review and Meta-analysis of Circulatory Disease from Exposure to Low-Level Ionizing Radiation and Estimates of Potential Population Mortality Risks. Environ. Health Perspectives 120:1503-1511.
Liu L, Lee S, Zhang J, Peters SB, Hannah J, Zhang Y, Yin Y, Koff A, Ma L, Zhou P (2009a). CUL4A abrogation augments DNA damage response and protection against skin carcinogenesis. Molecul. Cell 34:451-460.
Liu Q, Cao J, Wang ZQ, Bai YS, Lü YM, Huang QL, Zhao WZ, Li J, Jiang LP, Tang WS, Fu BH, Fan FY (2009b). Dose estimation by chromosome aberration analysis and micronucleus assays in victims accidentally exposed to 60Co radiation. Br. J. Radiol. 82:1027-1032.
Maluf SW, Erdtmann B (2001). Genomic instability in Down syndrome and Fanconi anemia assessed by micronucleus analysis and single-cell gel electrophoresis. Cancer Genet. Cytogenet. 124:71-75.
Maluf SW, Riegel M (2011). Citogenética Humana. Porto Alegre: Artmed. 2011.
Manning G, Rothkamm K (2013). Deoxyribonucleic acid damage-associated biomarkers of ionising radiation: current status and future relevance for radiology and radiotherapy. Br. J. Radiol. 83:1-11.
Migliore L, Guidotti P, Favre C, Nardi M, Sessa MR, Brunori E (1991). Micronuclei in lymphocytes of young patients under antileukemic therapy. Mutat. Res. 263:243-248.
Mirzayans R, Andrais B, Scott A, Wang YW, Murray D (2013). Ionizing Radiation-Induced Responses in Human Cells with Differing *TP53* Status. Int. J. Molecul. Sci. 14:22409-22435.
Montano D (2014). Chemical and biological work-related risks across occupations in Europe: a review. J. Occupat. Med. Toxicol. 9:1-13.
Pernot E, Hall J, Baatout S, Benotmane MA, Blanchardon E, Bouffler S, El Saghire H, Gomolka M, Guertler A, Harms-Ringdahl M, Jeggo P, Kreuzer M, Laurier D, Lindholm C, Mkacher R, Quintens R, Rothkamm K, Sabatier L, Tapio S, de Vathaire F, Cardis E (2012). Ionizing radiation biomarkers for potential use in epidemiological studies. Mutat. Res. 751:258-286.
Plamadeala C, Wojcik A, Creanga D (2015). Micronuclei versus Chromosomal Aberrations Induced by X-Ray in Radiosensitive Mammalian Cells. Iran. J. Pub. Health 44:325-331.
Polychronakis I, Dounias G, Makropoulos V, Riza E, Linos A (2013). Work-related leukemia: a systematic review. J. Occupat. Med. Toxicol. 8:1-16.
Reisz JA, Bansal N, Qian J, Zhao W, Furdui CM (2014). Effects of Ionizing Radiation on Biological Molecules—Mechanisms of Damage and Emerging Methods of Detection. Antioxidants Redox Signaling 21:260-292.
Sabharwal R, Verma P, Syed MA, Sharma T, Subudhi SK, Mohanty S, Gupta S (2015). Emergence of micronuclei as a genomic biomarker. Indian J. Med. Paeditr. Oncol. 36: 212-218.
Saha S, Woodbine L, Haines J, Coster M, Ricket N, Barazzuol L, Ainsbury E, Sienkiewicz Z, Jeggo P (2014). Increased apoptosis and DNA double-strand breaks in the embryonic mouse brain in response to very low-dose X-rays but not 50 Hz magnetic fields. J. Royal Soc. Interface Royal Soc. 6:1-10.
Samet JM (2011). Radiation and cancer risk: a continuing challenge for epidemiologists. Environ. Health 10:2011.
Sari-Minodier I, Orsière T, Bellon L, Pompili J, Sapin C, Botta A (2002). Cytogenetic monitoring of industrial radiographers using the micronucleus assay. Mutat. Res. 521:37-46.
Sasaki MS, Tachibana A, Takeda S (2014). Cancer risk at low doses of ionizing radiation: artificial neural networks inference from atomic bomb survivors. J. Radiat. Res. 55:391-406.
Shim G, Ricoul M, Hempel WM, Azzam EI, Sabatier L (2014). Crosstalk between telomere maintenance and radiation effects: A key player in the process of radiation-induced carcinogenesis. Mutat. Res./Rev. Mutat. Res. 760:1-17.
Smoll NR, Brady Z, Scurrah K, Mathews JD (2016). Exposure to ionizing radiation and brain cancer incidence: The Life Span Study cohort. Cancer Epidemiol. 42:60-65.
Sram RJ (1998). Effect of glutathione S-transferase M1 polymorphisms on biomarkers of exposure and effects. Environ. Health Perspect. 106:231-239.
Suzuki K, Ojima M, Kodama S, Watanabe M (2003). Radiation-induced DNA damage and delayed induced genomic instability. Oncogen. 22:6988-6993.
Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M (2009). Buccal micronucleus cytome assay. Nat. Protocols 4:825-837.
Tolbert PE, Shy CM, Allen JW (1992). Micronuclei and other nuclear anomalies in buccal smears: Methods development. Mutat. Res. 271:69-77.
Valverde NJ (2005). Radiações Ionizantes. In: Patologia do Trabalho. São Paulo: Atheneu, 2005.
Veeraraghavan J, Natarajan M, Herman TS, Aravindan N (2011). Low-dose Î³-radiation-induced oxidative stress response in mouse brain and gut: Regulation by NFB–MnSOD cross-signaling. Mutat. Res.718:44-55.
Yang F, Waters KM, Webb-Robertson BJ, Sowa MB, Von Neubeck C, Aldrich JT, Markillie LM, Wirgau RM, Gritsenko MA, Zhao R, Camp DG 2nd, Smith RD, Stenoien DL (2012). Quantitative phosphoproteomics identifies filaggrin and other targets of ionizing radiation in a human skin model. Exp. Dermatol. 21:352-357.
Zhang Q, Matzke M, Schepmoes AA, Moore RJ, Webb-Robertson B-J, Hu Z, Monroe ME, Qian WJ, Smith RD, Morgan WF (2014). High and Low Doses of Ionizing Radiation Induce Different Secretome Profiles in a Human Skin Model. Plos One 9:1-8.