

Original Research Article

**IONIZING RADIATION AND REDOX BALANCE
IN DIAGNOSTIC RADIOLOGY PERSONNEL**

ABSTRACT

Aim: Determine the influence of ionizing radiation on the redox balance of health personnel.

Introduction: Ionizing radiation has carcinogenic, genetic and immunological effects, among others. Exposure to low radiation doses has been reported to increase the damage induced by high dose exposures. Although it has been used in the therapy of various disorders, its mechanism damages cellular DNA and membrane.

Study design: Transversal, comparative, descriptive.

Place and Duration of Study: It is a study based in the community in Radiology Centers, Public and Private, that did accepted to participate, between January 2015 and November 2015.

Methodology: A total of 60 individuals, divided into 2 groups (n = 30), exposed and not exposed to ionizing radiation, all belonging to diagnostic radiology team, have participated in this research study. Blood was drawn, blood plasma was separated for determine the Malondialdehyde (MDA) concentration and the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG).

Results: No statistically significant differences were found when comparing both groups in any of the two biochemical determinations.

Discussions: Perhaps the influence of environmental conditions, nutrition and other aspects not considered in this study may provide guidelines to clarify the factors underlying the effect of ionizing radiation on the redox balance in diagnostic radiology personnel.

Conclusions: This study, showed that ionizing radiation did not intervene in the redox balance in diagnostic radiology personnel. Enzymatic dynamics studies are required to determine the effect of ionizing radiation on the activity of each of the enzymes involved in the response to oxidative stress.

Keywords: *Ionizing radiation, Diagnostic radiology personnel, Redox Balance, Lipid peroxidation, Glutathione*

17 **1. INTRODUCTION**

18

19 Environmental risk is defined as the probability that a human activity, as the use of a
 20 chemical, may produce adverse effects. Ionizing radiation has important effects on the
 21 exposed individual's health, since it can have carcinogenic, genetic and immunological
 22 effects, among others. It has been reported that exposure to low-dose radiation (less than 50
 23 millisieverts mSv/year) causes high-dose exposure to subsequently have greater effects on
 24 humans¹. Not only is the patient exposed to radiation effects; diagnostic radiology personnel
 25 are also affected by low, prolonged and fractionated doses of ionizing radiation, which
 26 extend throughout their professional activities.²

27 Although ionizing radiation has been used as part of the therapy for the treatment of various
 28 types of cancer, its mechanism, which causes the death of cancer cells, increase the
 29 number of free radicals which can induce DNA and cell membrane lipids damage³. Together,
 30 the direct and indirect effects of radiation initiate a series of biochemical and molecular
 31 signaling events that can repair the damage or culminate in permanent physiological
 32 changes or cell death⁴. These therapies create not only an internal redox environment in
 33 cancer cells, but also affect healthy cells, as the vitality of the cells depends on their
 34 responsiveness through the action of antioxidant mechanisms⁵.

35 In a study carried out by Skiöld et al., they show that when comparing the oxidative stress
 36 response induced by ionizing radiation in patients with breast cancer, patients developed
 37 skin lesions during radiation⁶. When analyzing the peripheral blood, they were able to isolate
 38 a biomarker of oxidative stress, 8-oxo-dG, which depend on the dose of radiation, implying
 39 that the presence of 8-oxo-dG in peripheral blood is an excellent marker of oxidative stress
 40 secondary to cellular damage⁶. Other in vitro studies show a significant increase in
 41 malondialdehyde levels by exposing cell cultures for 30 to 60 minutes⁷, in vivo murine
 42 exposure⁸ and in humans exposed for 30 minutes to electromagnetic radiation⁹.
 43 Nevertheless, the response of the oxidant capacity is altered in the cancer cells compared to
 44 the normal cells, so they propose to address these qualities appropriately, valuing the use of

45 treatment regimens that selectively activate the signals of cell death in the tumor cells with
 46 minimal consequences for normal cells⁴. A cell under normal conditions cope with induced
 47 stress and changes in the redox environment, provoking transient responses at the
 48 molecular, cellular and tissue levels to counter the toxic effects of radiation¹⁰. In vivo
 49 measurement of cellular redox status is an important challenge for future research. The
 50 improvement in the methodologies for the detection of reactive oxygen species (ROS) and
 51 their antioxidants: glutathione and its enzyme, glutathione peroxidase and superoxide
 52 dismutase (SOD), among others, will allow a better understanding of events related to the
 53 redox microenvironment; there is a greater potential for its application in the development of
 54 new therapies for the treatment of diverse diseases like cancer¹¹. The objective of this study
 55 was to determine the influence of ionizing radiation on the redox balance in health
 56 personnel.

57 **2. METHODOLOGY**

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 59 The project was approved by the Division of Health Sciences and Engineering Research
 60 Committee and by its Bioethics Committee with registration number CBDCSI-87141126,
 61 both situated on University of Guanajuato Celaya-Salvatierra campus, in Mexico.

62 **2.1 Study design**

63 Transversal, comparative, descriptive.

64 **2.2 Universe**

65 Workers in X-ray rooms, such as doctors, radiology technicians, nurses, assistants. For the
 66 control group, there were nursing students, doctors, maintenance personnel and nurses who
 67 did not have regular contact with x-rays.

68 **2.3 Sampling**

69 An invitation was made to public and private x-ray centers in the city of Celaya, Mexico, to
 70 participate in the study, and for the control group, students, teachers and administrators of a
 71 public university in the same city were invited to participate.

72

73 **2.4 Selection of participants**

74 1. Inclusion criteria.

75 For the exposed group (men or women), working in x-ray rooms: doctors, radiology
76 technicians, nurses, assistants and maintenance personnel who attended daily the
77 radiodiagnosis center.

78 For the control group, not being in x-ray rooms and not having contact with them.

79 2. Exclusion criteria.

80 For both groups, having been diagnosed with any type of cancer or acute disease at the time
81 of blood sampling.

82 **2.5 Variables**

83 **2.5.1. Sociodemographic:** age, gender, residence, marital status, scholar level.

84 **2.5.2. Exposure**

85 Occupation is a nominal categorical variable, it is the principal occupation of an individual's
86 life; some possible values are doctor, nurse, radiologist, secretary or receptionist; it is
87 summarized by means of frequencies and percentages.

88 Working life is a discrete quantitative variable; it represents the seniority at present work in
89 complete years; it is measured in years and summarized in terms of mean and standard
90 deviation.

91 Working hours per day is a discrete quantitative variable; it is the time devoted to your
92 occupation daily; it is measured in hours divided by 24 hours and summarized in terms of
93 mean and standard deviation.

94 Smoking is a dichotomous categorical variable; it was categorized smoking as either "Yes" (if
95 the individual smok 2 or more cigarettes a day) or "No" (one or no cigarettes a day); is
96 summarized by means of frequencies and percentages.

97 Radiation measurement, is a dichotomous categorical variable; it is the measurement of 1-
98 month ionizing radiation capture (mSv) with two tablets connected to each dosimeter;

99 radiation measurement was categorized as “High” (≥ 4.1 mSv) or “Low” (≤ 4.0 mSv),
100 according to Official Mexican Standards¹².

101 2.5.3. Variable result

102 MDA is a continuous quantitative variable; it represents the amount of MDA expressed in μM
103 per unit of fluorescence contained in blood plasma.

104 GSH / GSSG rate is a continuous quantitative variable; it is the ratio of reduced glutathione
105 to oxidized glutathione expressed in μM per unit of fluorescence contained in blood plasma.

106 2.6 Procedures

107 The subjects went to the Lifestyle Laboratory at the Health Sciences and Engineering
108 Division on the University of Guanajuato Celaya-Salvatierra campus; here, the objectives of
109 the study were explained. An information sheet for participants was provided and the
110 questions they asked were answered. A signed informed consent form was requested and
111 the socio-demographic background questionnaire was applied. All participants were given a
112 dosimeter to be carried during working hours for a month, to measure their exposure to
113 ionizing radiation. After this time, the dosimeters were collected and sent to the Medical
114 Physics and Biomedical Instrumentation Group for reading.

115 2.6.1 Sample size

116 If the group working in radiology has 60% of oxidative stress damage while the unexposed
117 group has 20%, the minimum sample size is 30 exposed and 30 unexposed, with a 95%
118 accuracy and an 80% power (EpiInfo 7, 2013, CDC, Atlanta, GA, USA).

119 2.6.2 Extraction of blood plasma

120 After antisepsis, 5 ml of whole blood were collected of each participant by puncture with
121 vacutainer. This blood was kept in a blood sample tube with EDTA. Subsequently, it was
122 centrifuged at 1500 rpm for a period of 10 minutes, for the separation of the formed elements
123 and the plasma portion. The portion corresponding to the blood plasma was collected and
124 stored in Eppendorf tubes, kept freezing at -70°C until assayed for biomarkers
125 determination.

126 **2.6.3 Lipid Peroxidation (MDA)**

127 For the determination of malondialdehyde, we used the Abcam ab118970 Lipid Peroxidation
 128 (MDA) Assay Kit according to the manufacturer's instructions, which are briefly described
 129 below. After mixing the product, the standard was prepared by maintaining a constant
 130 temperature of 4°C. The MDA 0.1 M standard was prepared by dilution, to a total number of
 131 6, contain a double concentration between each of them, to perform the calibration curve.
 132 Subsequently, the sample was prepared using 10 µl of plasma with 500 µl of 42 mM H₂SO₄
 133 in a microfuge tube. Phosphotungstic acid was added and mixed, then incubated for 5 min in
 134 a short at room temperature. The aqueous portion is separated by centrifugation at 13,000 x
 135 g for 3 min and the pellet is collected, then resuspended in cold water with distilled water
 136 containing 2% BHT, adjusting the final volume to 200 µl. The MDA-TBA adducts are
 137 generated and the spectrophotometer of the Thermo brand is detected by fluorometric test
 138 with an Ex / Em = 532 / 553nm wavelength. The results are expressed in nmol per units of
 139 fluorescence.

140 **2.6.7 GSH / GSSG ratio**

141 To determine the ratio of reduced / to oxidized glutathione, the Abcam ab138881 GSH /
 142 GSSG ratio Detection Assay Kit was used according to the manufacturer's instructions. After
 143 mixing the solutions, 50 µl of GSH and GSSG are added separately and 50 µl of the sample
 144 to give a final volume of 100 µl. The reaction was incubated in a dark room at room
 145 temperature for 20 min. The fluorescence reading is performed on the Thermo brand
 146 Multiscan Go UV / Vis plate reader at a wavelength of Ex / Em = 490/520 nm. The results
 147 are expressed in µM.

148 **2.7 Statistical analysis**

149 Descriptive statistics were used for the distribution of socio-demographic and study
 150 variables. Student t, and p value, were calculated for two independent means to compare
 151 the presence of MDA levels and the GSH/GSSH ratio by groups. In all cases, to

152 demonstrate statistical significance of the results, the p value was set at 0.05. All statistical
153 analysis was performed on STATA 13.0® (Stata Corp., College Station, TX, USA).

154 155 **3. RESULTS AND DISCUSSION**

156
157 **3.1 Sociodemographic:** The analysis of the results for the sociodemographic variables
158 (Table 1) shows that women with urban residence, married with a technical career, working
159 as technicians and being smokers predominated in the group exposed to ionizing radiation
160 (x-rays). Participants who were not exposed, were, in general, single women having urban
161 residence, undergraduate studies, working occasionally as nurses and being smokers.
162 The analysis of participant's marital status and educational attainment showed statistically
163 significant differences between the unmarried individuals of both groups ($P < 0.05$), married
164 ($P < 0.05$), with technical career ($P < 0.05$) and technical occupation ($P < 0.05$).

165 As for age, there were differences in the mean of both groups ($P < 0.05$), working life (P
166 < 0.05) and daily work ($P < 0.05$). This is because the professionals in the control group have
167 been engaged since a few years and some of them are students. (Table 2).

168 The reading of the dosimeters was categorized and, in its analysis, it was determined if there
169 was a statistically significant association between the high exposure and being in the
170 exposure group (Table 3), showing that the exposed group has more exposure to ionizing
171 radiation.

172 **3.2 Lipid Peroxidation (MDA)**

173 The analysis of the results of the biochemical test of lipid peroxidation to obtain the levels of
174 malondialdehyde did not show statistically significant differences when comparing the means
175 of both groups by means of the student t test for independent groups, obtaining values of the
176 mean $\pm$ SE 129 ± 19.1850 , for the control group and 111 ± 17.9150 , for the exposed group,
177 with a value of $t = 6015$, $P = 0.5024$, (see Graph 1).

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180 **3.3 GSH / GSSG ratio**

181 The analysis of the results of the biochemical test for the determination of the relationship
182 between reduced glutathione and oxidized glutathione (GSH/GSSG) did not show significant
183 differences when comparing the means by the Student t test for independent groups,
184 obtaining values of mean $\pm$ SE of 0.613 ± 0.195 , for the control group and 0.695 ± 0.111 , for
185 the exposed group with value of $t = -0.307$, $P = 0.7126$, (see Graph 2).

186 As human beings, we find ourselves constantly under the effects of ionizing radiation, being
187 exposed to environmental, artificial and cosmic elements. Occupational exposure in health
188 care centers is the result of exposure to radiation at work, where radiological safety is the
189 main cause of multiple health problems, mainly its carcinogenic effect associated with
190 chronic exposure to low doses of ionizing radiation. The use of ionizing radiation in medicine
191 is rapidly expanding nowadays due to the introduction of new diagnostic and therapeutic
192 practices working with ionizing radiation. Multiple CT scans and radiographic imaging are
193 extremely valuable as a diagnostic tool in medicine, but ionizing radiation also carries well-
194 known potential risks^{13,14}.

195 The analysis of the biochemical results in the determination of MDA levels did not show
196 significant differences between the exposed group versus the control one, opposite to data
197 shown by Eken et al., who reported finding lower levels of MDA in subjects exposed to a
198 radiation ionizing agent. Perhaps this effect may be due to the technique used for the
199 detection of MDA; in our study, it was performed at the plasma level, whereas the data
200 reported by the group of Eken et al., were obtained in erythrocytes¹⁵. However, in another
201 study by Akköse, Omer and Yiğitbaşı, they show an insignificant increase in plasma MDA
202 levels and a significant decrease in GSH/GSSG ratio levels in radiology technicians¹⁶. In this
203 study, the findings were directly related to the smoking factor. Various contradictory results
204 have been found so far in the literature. However, the effect of ionizing radiation on health
205 personnel on redox homeostasis could be due to intrinsic factors involving health personnel
206 rather than directly from ionizing radiation. Another study, conducted in health personnel

207 exposed to ionizing radiation showed no significant differences in total plasma GSH content,
 208 these data being consistent with our results; however, in their study, there were differences
 209 in the activity of the enzyme superoxide dismutase (SOD)¹⁷, as in other studies¹⁵⁻¹⁸, which
 210 might suggest that maintenance of redox homeostasis in personnel exposed to a radiation
 211 ionizing agent is not due to the concentration of biological antioxidants such as glutathione,
 212 but to the activity of the different enzymes involved in biological redox reactivity.

213 It is important to mention that our study has a small sample n, so it would be important to
 214 increase the number of participants; however, the data obtained are concordant with other
 215 studies. Nevertheless, the results remain controversial up to these days; perhaps the
 216 influence of environmental conditions, nutrition and other aspects not considered in this
 217 study may provide guidelines to clarify the factors underlying the effect of ionizing radiation
 218 on the redox balance in diagnostic radiology personnel. It is important, as well, to carry out
 219 studies to clarify the mechanism maintaining this balance, which will propose new lines of
 220 action in prevention of diseases linked to exposure in the future.

221

222 **Table 1. Sociodemographic characteristics by group, Celaya, 2015**

Variable	Exposed group		Group not exposed		X ²	gl	P value
	f	%	f	%			
Gender					2.58	1	0.11
Male	14	46.67	8	26.67			
Female	16	53.33	22	73.33			
Home					4.10	2	0.13
Urban	26	86.67	21	70.00			
Suburban	1	3.33	6	20.00			
Rural	3	10.00	3	10.00			
Civil Status							
Single	9	30.00	25	93.33	4.17*		0.00001
Married	15	50.00	5	16.67	2.74*		0.006
Separated	1	3.33	0	0.00	1.01*		0.31

Widower	2	6.67	0	0.00	1.44*	0.15	
Divorced	1	3.33	0	0.00	1.01*	0.31	
Free Union	2	6.67	0	0.00	1.44*	0.15	
Scholarship							
Elementary School	2	6.67	0	0.00	1.44*	0.15	
Secondary School	1	3.33	1	3.33	0.00*	1.00	
High School	3	10.00	1	3.33	1.04*	0.30	
Technical degree	15	50.00	0	0.00	4.47*	0.0001	
Bachelor's degree	2	6.67	26	86.67	-6.12*	0.0001	
Postgraduate	7	23.33	2	6.67	1.81*	0.07	
Occupation							
Receptionist	2	6.67	1	3.33	0.59*	0.55	
Assistant	2	6.67	0	0.00	1.44*	0.15	
Technician	15	50.00	2	6.67	3.72*	0.0002	
Nurse	5	16.67	15	50.00	2.74*	0.006	
Doctor	4	13.33	7	23.33	1.00*	0.32	
Maintenance personnel	2	6.67	5	16.67	1.21*	0.23	
Smoking					1.67	1	0.20
Yes	4	13.33	8	26.67			
No	26	86.67	22	73.33			

* Z for two proportions, gl degrees of freedom, **Source:** Study measurements

Table 2. Quantitative sociodemographic characteristics by group, Celaya, 2015

Variable	Range	Mean ± SD	t	gl	P value
Age (years)			5.04	58	0.00001
Exposed	20 a 61	37.80± 11.59			
Not exposed	20 a 49	25.30± 7.09			
Working life (years)			5.77	58	0.00001
Exposed					
Not exposed	0.5 a 29	10.83± 7.86			
	0.17 a 10	2.13± 2.52			
Daily working time (hours)			3.05	58	0.0034

Exposed	4 a 24	11.17± 6.14
Not exposed	5 a 12	7.63± 1.63

228 **SD** Standard deviation, **gl** degrees of freedom, **Source:** Study measurements

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230 **Table 3 Distribution of exposure to ionizing radiation by group, Celaya, 2015**

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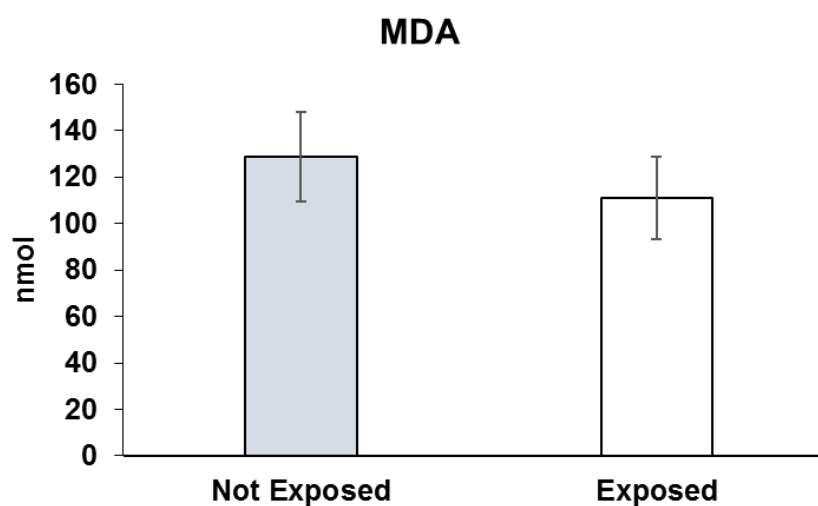
Exposure to radiation (mSv)*	Exposed (n=30)		Not exposed (n=30)		Z	gl	P value
	n	%	n	%			
≥4.1	4	13.33	0	0.00	2.07	1	0.039
<4.1	26	86.67	30	100.00			

232 * Z for two proportions, **gl** degrees of freedom, **Source:** Study measurements

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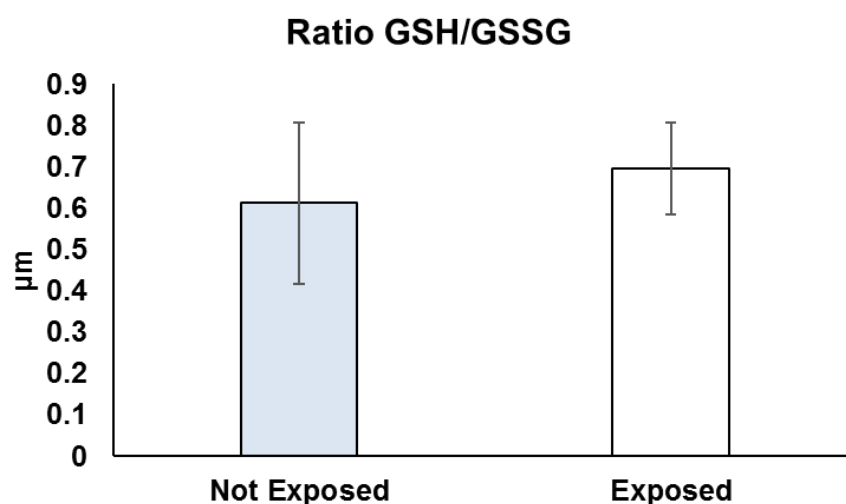
237 **Graph 1.** Shows the mean ± SE, between the not exposed group and the exposed group, P

238 = 0.5024.

239 **SE:** standard error, **MDA:** Malondialdehyde, nmol: nanomoles

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Graph 2. Shows the mean $\pm$ SE, of the relationship between reduced glutathione and

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oxidized glutathione, between the not exposed group and the exposed group, $P = 0.7126$.

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247

4. CONCLUSION

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This study, showed that ionizing radiation did not intervene in the redox balance in diagnostic radiology personnel.

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Enzymatic dynamics studies are required to determine the effect of ionizing radiation on the

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activity of each of the enzymes involved in the response to oxidative stress.

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COMPETING INTERESTS

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The author(s) declared no potential conflicts of interest with respect to the research,

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authorship, and/or publication of this article.

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INFORMED CONSENT

258

All participants signed informed consent

259

BIOETHICS COMMITTEE

260

The Bioethics Committee of the Division of Health Sciences and Engineering of the Campus

261

Celaya-Salvatierra of the University of Guanajuato reviewed and approved the protocol, with

262

registration CBDCSI-87141126.

263 **Ethical and legal considerations**

264 The project was approved by the Division of Health Sciences and Engineering Research
265 Committee and Bioethics Committee, on University of Guanajuato Celaya-Salvatierra
266 campus, with registration number CBDCSI-87141126.

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