Original Article

Expression of DNA repair genes in association with ionizing radiation

Amaury Machi Jr[1], Matheus Moreira Perez[1], Glaucia Luciano da Veiga[1], Edimar Cristiano Pereira[2], Fernando Adami[3], Beatriz da Costa Aguiar Alves[1], Fernando Luiz Affonso Fonseca[1,2]

[1]Laboratório de Análises Clínicas do Centro Universitário Faculdade de Medicina do ABC, Santo André, SP, Brazil; [2]Departamento de Ciências Farmacêuticas da Universidade Federal de São Paulo, Diadema, SP, Brazil; [3]Laboratório de Epidemiologia e Análise de Dados do Centro Universitário Faculdade de Medicina do ABC, Santo André, SP, Brazil.

Abstract. *Background and aim:* DNA repair systems are functionally essential for the maintenance of life and among these, we can highlight the MutS system, subdivided into MutSα (hMSH2 and hMSH6) and MutSβ (hMSH2 and hMSH3). The objective of this study was to analyze the expression of hMSH2 and hMSH6 repair genes in radiology technicians exposed to low radiation doses. *Methods:* Thirty workers occupationally exposed to ionizing radiation and twenty-five non-exposed were included in this study. Gene expression was analyzed by qPCR. Peripheral blood samples were collected from both groups for total RNA isolation. *Results:* It was observed a five-fold increase (p=0.006) in the hMSH2 repair gene expression in those exposed to radiation and a weak but significant correlation (p=0.041) with the hMSH6 genes when we associated the number of hours of exposure with gene expression. *Conclusions:* The longer the exposure time, the greater the activation of this component of the repair system. Application to Practice: Blood count parameters could did not alter with radiation exposure. X-rays used by radiology technicians in imaging tests can damage the DNA to the point of activating the MutS repair system and that there is a greater tendency of expression of this system in professionals that had undergone longer exposure. (www.actabiomedica.it)

Key words: radiology, DNA damage, repair system, MutSα

Introduction

Ionizing radiation is an inseparable part of human life and can be categorized as that of natural background radiation or as produced by man (1). It was estimated that natural background radiation account for 82% of the annual exposure of the population while the man-made sources accounts with the remaining 18% (approximately 10.44% being exposure to medical X-ray) (2). The latter can be much greater when there is an occupational exposure. We currently have over 2 million workers worldwide who are occupationally exposed to prolonged low-dose ionizing radiation from medical sources (3). It is estimated that in the early 1980s the effective radiation dose per capita for the North American population was 3.6 mSv per year. In 2006 the dose had nearly doubled to 6.2 mSv per year due to a revolution in medical imaging (4). Increasing the number of medical imaging procedures may result in increased occupational exposure. Among the professionals involved, the radiology technician is one who ends up being exposed directly and indirectly to the ionizing radiation beams.

Ionizing radiation such as X-rays can directly reach the cell nucleus and due to its energy can cause damage to the DNA. A critical conclusion on the mechanisms of radiation tumorigenesis is that the revised data reinforce the view that there are intimate connections between induction of DNA damage in cells, the onset of mutations in genes or in chromosomes through incorrect DNA repair, and the development of cancer (5).

Recognition of carcinogenicity and consequently the long-term effects of radiation exposure were reported in 1902 by Frieben based on his observations of a carcinoma in the hands of a worker at an X-ray tube factory (6). Approximately 1% of cancer cases is considered as a result of a simple exposure to 0.1 Sv of a low energy LET (linear energy transfer) X-ray radiation for example (2,7).

Sensing molecules can detect DNA damage and activate signaling factors. In turn these factors can induce cell cycle arrest and facilitate the repair of DNA and other defense mechanisms (8,9). Evolutionarily, the cell system has developed various forms of DNA repair, such as: base excision repair, nucleotide excision repair, recombination repair, DNA mismatch repair, among others. Damaged DNA, without repair, has a high potential to cause the development of neoplasms in germ and/or somatic cells. Each type of injury requires a specific repair mechanism, although pathway overlaps have been frequently reported (10).

In eukaryotes, MutS proteins are composed of MSH proteins (MutS Homolog). The MSH2-MSH6 complex forms the MutSα complex which recognizes small incompatibilities and small insertion/deletion loops (called indel). The MutSβ complex is formed by the MSH2 and MSH3 proteins, responsible for the recognition of larger indel loops (11).

Currently, the complementary examination for monitoring the occupational exposure of radiology technicians to ionizing radiation is the complete blood and platelet count test. Overall analysis of gene and protein expression is promising for a better understanding of the dose-response to radiation (12).

The objective of this work was to analyze *ℏMSH2* and *ℏMSH6* gene expressions in professionals exposed to ionizing radiation, X-rays, in comparison to non-exposed professionals. In addition, to associate the expressions of these genes with the working conditions of said professionals and with the hours worked per week.

Methods

Ethics

The characteristics and objectives of the study were explained to all subjects through the Free and Informed Consent Form (FICF), previously approved by the Ethics and Research Committee from Faculdade de Medicina do ABC and in accordance with Helsinki Declaration of 1975. After signing the FICF, approximately 15.0 mL of blood sample was collected via peripheral venous puncture, with times ranging from a few minutes (shortly after the procedure) to up to two hours after X-ray exposure.

Selection and description of participants

The research was carried out in health units managed by the Social Health Organization - ABC Foundation. Thirty professionals working with radiology from the age of 21 to 52 in any of the ABC Foundation units were included. As control group, 25 people from the general public (not exposed to ionizing radiation) were included. We excluded volunteers (workers or control) who have already had received a diagnosis of cancer and consequent treatment, those who had a first-degree relative who had cancer, those who had type I and type II diabetes, those who had used corticosteroids in the last 6 months, those who had been hospitalized in the last 6 months due to any inflammatory disease, and those who tested positive for HIV I and II, Hepatitis B and Hepatitis C. In order to qualify the sample, a questionnaire with the general clinical condition of each individual and family history was also applied.

Regarding worker protection, dosimeters were used and monitored, as well as the use of lead aprons and barite-concrete walls. In the case of at least one of these protective measures not existing we considered the worker unprotected.

RNA Isolation

Total RNA was isolated from 15 mL of peripheral blood obtained from workers and controls, using TRIzol reagent (TRIzol LS Reagent Thermo Fisher Scientific cat. no. 10296-010, Waltham, MA, USA), according to the manufacturer's recommendations. A260/A280 ratio and concentration of total RNA were measured by spectrophotometry with NanoDrop

Lite (Thermo Fisher Scientific, Waltham, MA, USA). RNAs samples with a concentration greater than 200 ng/μL and a ratio between 1.5 and 2.0 were used for reverse transcription.

Synthesis of complementary DNA (cDNA)

One microgram of total RNA was used in reverse transcription for complementary DNA synthesis (cDNA) using the QuantiNova Reverse Transcription Kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations.

RT-PCR for *hMSH2* and *hMSH6*

The real-time quantitative technique RT-qPCR was performed in an Applied Biosystems® 7500 Real-Time PCR Systems thermal cycler (Applied Biosystems, Foster City, CA, USA) with initial hot-start step at 95°C for 10 min, followed by 40 repetitions at 95°C for 15 sec and at 60°C for 25 sec, in a final volume of 15 μL containing: 1X SYBR Green mix (Quantitec SYBR Green PCR kit, QIAGEN catalog number 204054), 25 pmol of each specific primer and 2 μL cDNA. Gene expressions were normalized by the reference gene RPL13a. To estimate the gene expression levels, the formula $2^{-(\Delta Cq)}$ was used (13).

Specific primers for the target genes (*hMSH2* and *hMSH6*) were designed using Primer3 Input 0.4.0 software available at http://frodo.wi.mit.edu/primer3/. Table 1 shows the nucleotide sequences and amplicons length generated by these primers and the reference gene *RPL13α*.

Statistics

Absolute and relative values were used to describe the qualitative variables. For the quantitative (Shapiro-Wilk, $p>0.05$) mean and standard deviation were used. Student's t-test was performed to analyze the association between the variables *hMSH2* and *hMSH6* with white blood cells. The correlation between the variables *hMSH2* and *hMSH6* with white blood cells was studied using the Pearson test. The Spearman test was used to study the correlation between ambulatory variables and hours worked. For all analyzes, a confidence level of 95% was used. The program utilized was Stata version 11.0.

Results

The characteristics of included workers can be found in Table 2. As can be seen, workers were 29 radiology technicians and one radiology supervisor, 19 males (63.3%) and 11 (36.7%) females, aged from 21 to 52 years. Professional practice time is also a very discrepant variable that varies from months of work to professionals with 29 years of experience, with a mean of 9.8 ± 7.3 years. For comparison, 25 healthy subjects (non-radiology workers) were included as well, with 8 (32%) males and 17 (68%) females, with a mean age of 30.2 ± 10.3 years.

Of the workers interviewed, 14 (46.7%) worked more than one job. With regard to the weekly workload, 18 (60%) workers have a workload of 24 hours per week, 1 (3.3%) has a workload of 36 hours a week, 8 (26.7%) have a workload of 48 hours a week and 3 (10%) have a workload of 72 hours per week.

Table 1. Primers nucleotide sequences

Gene	Primer Sequence (5´- 3`)	Amplicon (bp)
RPL13a	F- TTGAGGACCTCTGTGTATTTGTCAA R- CCTGGAGGAGAAGAGGAAAGAGA	126
hMSH2	F- CCTTGTAAAACCTTCATTTGATCC R- ATCCAAACTGTGCACTGGAA	157
hHMSH6	F- GAACATTCATCCGCGAGAAA R- TGAGGGCTCATCACAAACTG	250

Table 2. Characteristics of the group exposed to radiation

Variables	n	%
Sex		
Male	19	63.33
Female	11	36.67
Smoker		
No	27	90.00
Yes	3	10.00
Protection		
No	6	20.00
Yes	24	80.00
Profession		
Radiology technician	29	96.67
Radiology supervisor	1	3.33
	Mean (sd)	**Minimum - Maximum**
Age	35.23 (7.41)	21.0 – 52.0
Amount of jobs	1.53 (0.62)	1.0 – 3.0
Hours worked	35.6 (16.21)	24.0 – 72.0

* Gene expression accessed by $2^{-(\Delta Cq)}$

Associations of the *hMSH2* and *hMSH6* gene expressions were performed in the different groups (exposed/non-exposed or workers/ controls). We found that there is a significant difference in the expression of the hMSH2 gene between these two groups, with expression being approximately 5 (five) times higher in the group exposed to radiation than in the non-exposed group (p=0.006). When we evaluated the association of the hMSH6 gene between the groups, there was a little and non-significant difference in gene expression. Table 3 shows the mean expression of *hMSH2* and *hMSH6* in the groups.

The correlation between hMSH2 and hMSH6 genes and the white blood cells (or globules) (WG) count (blood count parameter) was also performed. These correlations were negative and non-significant for both genes.

Verifying if the accumulation of hours worked interfere with complete blood count parameters and expression of the *hMSH2* and *hMSH6* genes, a significant positive correlation was found, however weak (p=0.041), with the hours worked and hMSH6 gene expression data (Table 4).

Table 3. Mean expression of *hMSH2* and *hMSH6*

Gene	Exposed	Non-exposed	
	Mean (sd)*		***p***
hMSH2	0.10 (0.12)	0.02 (0.05)	0.006
hMSH6	0.05 (0.09)	0.03 (0.14)	0.561

Red blood cells or globules (RG), hemoglobin (HB) and hematocrit (HT) parameters were lower in females; however, we did not obtain any difference in complete blood count parameters with respect to the gene expression (Table 5).

Discussion

The analyzed group of workers exposed to radiation were in the majority male and the mean age of the group was 35.2 years. Epidemiological and experimental data show that age is an essential parameter and that young people are associated with a greater susceptibility to radiocarcinogenesis (14).

When comparing the groups exposed (workers) and not exposed (controls) to ionizing radiation X, we obtained a 5-fold increase in the expression of the hMSH2 repair gene. The human hMSH2 protein is responsible for the initial recognition of nucleotide incompatibility during post-replication of the repair process (15), and together with the hMSH6 protein forms the MutSα and MutSβ complex when it binds to the hMSH3 protein. hMSH2 protein action in relation to the deleterious effects caused by the ionizing radiation is greater than that of the hMSH6 protein. It acts both via the Mutα complex and the Mutβ and MutL complexes, all with the same goal: to repair the damage caused to the DNA.

The MutSα heterodimer (hMSH2 and hMSH6) binds to incompatibilities in DNA and small insertion and deletion loops whereas the MutSβ heterodimer (hMSH2 and hMSH3) binds to large insertion and deletion loops. In addition, there is also MutL (MLH1 heterodimer and either PMS2 or PMS1) which is subsequently recruited by the MSH2 protein to form a tertiary complex with one of the MutS complexes and

Table 4. Correlation between hours worked with blood parameters and gene expression

Variables		Hours worked	
		rho	*p*
Complete blood count parameters	White blood cells (per mm3)	0.047	0.853
	Red blood cells (per mm3)	0.113	0.654
	Hemoglobin (g/ dL)	0.0435	0.864
	Hematocrit (%)	.03	0.905
	RDW (%)	0.114	0.652
	Lymphocytes (per mm3)	- 0.233	0.352
	Monocytes (per mm3)	- 0.100	0.692
	Neutrophils (per mm3)	0.146	0.562
	Eosinophils (per mm3)	- 0.006	0.979
	Basophils (per mm3)	- 0.003	0.991
Gene expression[a]	*hMSH2*	0.231	0.218
	hMSH6	0.375	0.041

RDW stands for Red Cell Distribution Width; [a]Gene expression accessed by $2^{-(\Delta Cq)}$ formule.

promote intracellular signaling to initiate the excision and repair of incompatibilities (16).

Even with low-dose radiation (less than 100mSv), the DNA undergoes damage that is then repaired by the defense system (17). Budworth et al. (18) analyzed 40 repair genes exposed to 2 Gy (high dose), ex vivo. Of these, 12 genes were significantly expressed 24 hours post-exposure, ranging from 2.3-fold for the LIG1 gene to 17-fold for the FDXR gene, characterizing repair genes as robust biomarkers of radiation exposure in peripheral blood cells (18). This research included cell cycle regulators (CDKN1A, GADD45a, PCNA and CCNG1), apoptosis regulators (BAX, BBC3 and FDXR) and genes involved in specific repair functions (XPC, DDB2, LIGI, POLH and RAD51). Depending on the dose and dose rate, these genes are not always transcribed (14).

Understanding that exposure time is a determining factor for the characterization of occupational hazards, a Brazilian law (7.394/85) that regulates the practice of the profession of Radiology Technician among other provisions states that the maximum workload of these professionals should be 24 (twenty-four) hours per week.

Our results showed a positive and weak but significant correlation (p=0.041) with the hours worked and *hMSH6* gene expression data. It means that the longer the exposure time, the greater the activation of this component of the repair system. The study reveals that the average hours worked per week was 35.6 hours ± 16.21, with workers performing their activities for up to 72 hours per week. Among the main causes, low wages lead professionals to work two or more jobs resulting in greater exposure to radiation.

In a study involving about 90,268 radiology technicians in the United States, the researchers associated certain types of neoplasias such as lung cancer with the action of workers maintaining the patient in the correct place for X-ray application, consequently with longer exposure to ionizing radiation (19). In that study they also concluded that technicians who started their activities before 1950 and worked for many years thereafter increased the risk of death from neoplasias such as breast cancer and leukemias, as well as diseases of the cardiovascular system, resulting from the non-use of protective equipment such as a lead apron and non-monitoring using dosimeters.

Due to the fact that radiation is cumulative, the longer the exposure time, the greater the radiation dose absorbed and associated with lower the levels of protective control, the greater the probability of the

Table 5. Association between the variables Smoker, Sex and Protection with Complete Blood Count

Variables	WG	*p*	RG	*p*	HB	*p*	HT_relative	*p*	RDW_relative	*p*	HT	*p*
Smoker	**Mean (sd)**		**Mean (sd)**		**Mean (sd)**		**Mean (sd)**		**Mean (sd)**		**Mean (sd)**	
No	7,78 (1,45)	-	5,13 (0,74)	-	13,98 (1,82)	-	43,41 (5,35)	-	14,57 (1,82)	-	43,41 (5,35)	-
Yes	-		-		-		-		-		-	
Sex												
Male	7,72 (1,37)	0.844	5.50 (0.62)	0.003	15.12 (1.15)	<0.001	46.68 (3.89)	<0.001	14.57 (2.09)	0.988	46.68 (3.89)	<0.001
Female	7.78 (1.67)		4.53 (0.49)		12.2 (1.05)		38.28 (2.37)		14.58 (1.45)		38.28 (2.37)	
Protection												
No	7.76 (1.49)	-	5.13 (0.76)	-	13.95 (1.87)	-	43.45 (5.51)	-	14.71 (1.78)	-	43.45 (5.51)	-
Yes	8.1 (0.0)		5.0 (0.0)		14.5 (0.0)		42.8 (0.0)		12.3 (0.0)		42.8 (0.0)	

RDW	*p*	LYM	*p*	MON	*p*	NEU	*p*	EOS	*p*	BAS	*p*	*hMSH2*	*p*	*hMSH6*	*p*
Mean (sd)		**Mean (sd)**		**Mean (sd)**		**Mean (sd)**		**Mean (sd)**		**Mean (sd)**		**Mean (sd)**		**Mean (sd)**	
14.57 (1.82)	-	2.60 (0.73)	-	0.63 (0.19)	-	4.23 (0.98)	-	0.23 (0.13)	-	0.04 (0.03)	-	0.09 (0.12)	0.307	0.05 (0.09)	0.722
-		-		-		-		-		-		0.17 (0.07)		0.07 (0.03)	
14.57 (2.09)	0.988	2.49 (0.70)	0.454	0.65 (0.18)	0.76	4.26 (0.91)	0.904	0.24 (0.15)	0.713	0.05 (0.02)	0.363	0.12 (0.14)	0.099	0.07 (0.11)	0.132
14.58 (1.45)		2.77 (0.79)		0.62 (0.20)		4.20 (1.16)		0.22 (0.11)		0.03 (0.03)		0.05 (0.05)		0.02 (0.02)	
14.71 (1.78)	-	2.65 (0.71)	-	0.63 (0.19)	-	4.19 (0.99)	-	0.22 (0.12)	-	0.04 (0.03)	-	0.19 (0.22)	0.116	0.08 (0.05)	0.511
12.3 (0.0)		1.74 (0.0)		0.8 (0.0)		4.96 (0.0)		0.48 (0.0)		0.06 (0.0)		0.08 (0.10)		0.05 (0.09)	

WG – White Globules, RG – Red Globules, HB – Hemoglobin, HT – Hematocrit, RDW – Red Cell Distribution Width, LYM – Lymphocytes, MON – Monocytes, NEU – Neutrophils, EOS – Eosinophils, BAS – Basophils.

workers developing deleterious effects in their body, and in addition, damage to the DNA can be seen even with the worker showing no evident disease. Preston et al. (20) point to a higher incidence of breast cancer in women born after 1930 who began their activities from 1950 when the average annual radiation was of 37mGy, being considerably higher than in the last years (1.3mGy).

Of the sample studied, 20% of the interviewed workers did not have the protection of lead apron and/or barite-concrete walls and/or monitoring of radiation with the use of dosimeters. These protective devices are essential for decreasing the dose of radiation over time as well as quantifying the levels of absorbed radiation.

In conclusion, analyzes of repair gene expressions are more promising in the initial detection of the dose level received since the dosimeter can be neglected by the employer/worker and also because it can present errors in detecting the radiation the professional received. The complete blood and platelet count, in the medical surveillance of the exposed workers does not offer any benefit in relation to the doses of radiation received (21). One of the major limitations in this study was not knowing the value of the individual doses of radiation as well as the limited sample numbers. There were some obstacles in obtaining information from some units which did not allow us to conduct the interviews or collect the blood samples from the workers due to the fear of a future labor suit.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Correspondence:
Received: 10 August 2021
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Fernando Luiz Affonso Fonseca, PhD
Laboratório de Análises Clínicas,
Centro Universitário Faculdade de Medicina do ABC
Av. Príncipe de Gales, 821
Santo André, 09060-650 Brazil
Phone: 55 11 49935488
E-mail: profferfonseca@gmail.com