Journal of Pharmacopuncture

ISSN 2093-6966 [Print], ISSN 2234-6856 [Online]
Journal of Pharmacopuncture 2017;20[2]:107-111
DOI: https://doi.org/10.3831/KPI.2017.20.014

Original article

Increase in the Th1-Cell-Based Immune Response in Healthy Workers Exposed to Low-Dose Radiation - Immune System Status of Radiology Staff

Gholamreza Karimi[1], Mahdi Balali-Mood[2], Seyed-Ali Alamdaran[3], Hassan Badie-Bostan[1], Elaheh Mohammadi[1], Adel Ghorani-Azam[2], Mahmood Sadeghi[2], Bamdad Riahi-Zanjani[2]*

[1] Pharmaceutical Research Center, Pharmacy School, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Medical Toxicology Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Radiology Department, Faculty of Medicine, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran

Key Words

cellular immunity, hematological indices, immune system, lymphocyte, radiology staff

Abstract

Objectives: Radiation is one of the most important sources of free radical (such as reactive oxygen species) production, which plays an essential role in the etiology of over hundred diseases. The aim of the study was to investigate some immune parameters and hematological indices in healthy workers of the Radiology Department, University Hospital of Mashhad, Iran.

Methods: The study was performed on 50 healthy workers: 30 radiology staff as the case group and 20 laboratory workers as the control group. The radiation dose received by the radiology staff participating in the study was less than the annual maximum permissible level, 50 millisievert. Hematological parameters, lymphocyte proliferation and cytokine production were studied in both groups.

Results: Among healthy radiology workers, the hematological indices did not differ statistically; however, their proliferation indices and IFN-γ levels showed significant increases in parallel with decreases in the IL-4 levels as compared to controls. The immune system of workers exposed to low-dose ionizing radiation was found to be shifted from a Type 2 to a Type 1 response to promote cellular immunity.

Conclusion: Based on our data, exposure to low-dose ionizing radiation may decrease the prevalence, frequency, and recurrence of various cancers and infectious diseases because of an increase in Th1-cell-based response, thus leading to more protection of the human body against tumor cells and foreign agents and possibly increased longevity. However, due to high rate of fluoroscopy use for interventional radiology, we suggest continuing research projects on radiation protection and hazards to prevent irreversible damage. As a recommendation, in future studies, radiology staff with a weakened immunity due to high radiation exposure should be considered as good choices to be treated using acupuncture techniques because acupuncture has been demonstrated to enhance the function and the number of immune cells.

Received: May 04, 2017 **Reviewed:** May 29, 2017 **Accepted:** Jun 13, 2017

1. Introduction

Electromagnetic radiation includes a range of emissions from γ-rays to radio waves. Most X-rays have a wavelength ranging from 0.01 to 10 nanometers. High-

*Corresponding Author
Bamdad Riahi-Zanjani. Medical Toxicology Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
Tel: +98-513-800-2458 Fax: +98-513-800-2467
E-mail: riahib@mums.ac.ir

energy rays, such as X-rays, transfer their energy to atoms and molecules, resulting in excitation and ionization. These rays can also produce reactive oxygen species (ROS) in living organisms. Moreover, free radicals have been shown to be able to change the structures of macromolecules such as DNA organic molecules, lipids (especially unsaturated lipids) and proteins [1-3]. The noxious impact of ROS on cell constituents such as proteins deteriorates their properties and alters their functions. An investigation showed that oxidative stress induced by ionizing radiation could alter the immune system's function [4]. However, by means of scavenging activity, the immune system can handle oxidative stress induced by free radicals through neutralizing them.

Also of note is the observation that the effect of natural ionizing radiation on living cells depends on the level of radiation exposure [5]. Over the past decades, scientific findings have shown that exposure to ionizing radiation can stimulate the immune function and consequently decrease the rate of cancer mortality [6-9]. On the other hand, some studies have produced the opposite result; i.e., exposure to ionizing radiation disrupts the immune function, thereby elevating the incidence of cancer and causing higher mortality rates [10-12].

Furthermore, the immune indices of workers operating X-ray instruments, who are exposed to long-term low doses of ionizing radiation, may be affected by that exposure. For instance, in a study, the lymphocyte function of workers who had been chronically exposed to low doses of ionization radiation was found to be affected. On the other hand, the chemotaxis of neutrophils and the intensity of respiratory burst were not affected [13]. Also, the long-term effect of exposure to low doses of ionizing radiation has been shown to be connected with increased serum IL-2 and decreased serum IL-4 levels [14]. Because of the high importance of the harmful effects of ionizing radiation on the radiology staff and the existence of conflicting information about its impacts on immune parameters, in this study, we aimed to evaluate the effects of low-dose ionizing radiation both on the function of lymphocytes as the main effector cells and on the hematopoiesis indices in a group of healthy workers operating X-ray machinery.

2. Materials and Methods

Fifty healthy workers of Imam Reza Hospital located in Mashhad, Iran, including 30 radiology staff as the case group and 20 laboratory workers as the control group, with ages ranging from 23 to 60 years and work experiences higher than 8 years, were recruited into the study. Radiology staff participating in the study had received radiation doses less than the maximum permissible annual level, 50 millisievert. None of the 20 healthy laboratory workers selected for this study had any past history of exposure to ionizing radiation. Individuals who had been recently exposed to medical or diagnostic radiation were excluded from consideration for inclusion in the study. Subjects who had clinically abnormal complete blood count (CBC) reports due to having had some infectious disease recently were also excluded from consideration. After having obtained approval from the University's Medical Research Ethics Committee and having given the 50 study subjects a thorough explanation of the objectives of the study and the methods to be used, individual written informed consent was obtained.

After an overnight fasting period, about 10 mL of whole blood were obtained from the brachial veins of the subjects in both the case and the control groups and were transferred into sterile tubes with anticoagulant ethylenediaminetetraacetic acid (EDTA) in order to analyze hematological indices, lymphocyte proliferation responses, and cytokine profile status. CBC analyses were undertaken using a hematology cell counter apparatus (Sysmex KX-21, Japan). Ordinary hematological indices, including hemoglobin content (Hb), hematocrit (Hct%), red blood cell (RBC) count, white blood cell (WBC) count, and platelet (Plt) count, were determined. A blood smear was prepared and then stained with Giemsa for each sample. Then, 100 leukocytes per each slide were observed under a light microscope for a differential analysis.

Peripheral blood mononuclear cells (PBMCs) were isolated by using of the Hypaque density method. Then, the mononuclear cells were collected from the interface and transferred to glass tubes. These cells were washed three times with an excess of Roswell Park Memorial Institute (RPMI) culture medium, and the pellets were re-suspended in one milliliter of complete RPMI-1640 culture medium supplemented with 10% fetal bovine serum (FBS), 100 U penicillin/mL, 100 μg streptomycin/ml, and 2-mM L-glutamine (all Gibco, Paisley, UK) prior to running each assay.

MTT [3-(4,5-diamethyl-2-thiazolyl) 2,5-diphenyl-2H-tet razolium] colorimetric assays were performed to measure the proliferation of PBMCs. Briefly, in presence of phytohemagglutinin-A (PHA, final concentration of 5 μg/mL/well) or complete media (100 μL), the aliquots of PBMCs (4×10^6 cells/mL) were dispensed into 96-well microplates. Thereafter, the plates were incubated for 48 h (at 37°C and 5% CO_2), after which MTT assays were performed to determine cell proliferation. For this purpose, 15 μL of a 5-mg/mL solution of MTT was added to each well, and the plates were incubated at 37°C for 4 h. After the addition of 100 μL of dimethyl sulfoxide (DMSO), the blue formazan precipitate produced in each cell was dissolved, and the optical density of each well was measured at 570 nm be using an enzyme-linked immuno assay (ELISA) reader (Stat-Fax, Palm City, FL). Finally, for each sample, the stimulation index (SI) was determined by using the following formula: SI = Absorbance for stimulated cells/Absorbance for unstimulated cells.

For the purpose of cytokine production, after two days of PBMC cultivation in the presence of PHA mitogen (as described above), supernatants were harvested and kept at -70°C until testing. IFN-γ and IL-4 levels were determined using commercially available ELISA kits according to the manufacturer's protocol.

Data were statistically analyzed using the Student's *t*-test to determine significant differences in the data between the two groups. *P*-values less than 0.05 were considered significant. The values are expressed as means ± standard deviations (SDs)

3. Results

Table 1 shows the means ± SDs of the ages, work experiences, and genders of the test and the control subjects, and no significant differences among these parameters were observed between the two groups. Comparisons of the hematological indices between the two groups are shown in Table 2; the hematological parameters for the case group were not significantly different from those for the control group ($P > 0.05$). On the other hand, as shown in Table 3, the exposure of the subjects in the case group to low doses of X-rays caused a significant increase in the stimulation index of PBMCs exposed to PHA compared to the subjects in the control group who had not received such exposure ($P < 0.05$). In addition, as can be seen in Table 3, in comparison to the subjects in the control group, those in the case group showed significant increases in the levels of IFN-γ produced by isolated human PBMCs, but the concentrations of IL-4 were statistically reduced ($P < 0.05$).

4. Discussion

When the human body is exposed to ionizing radiation, the rates of formation of free radicals, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, are increased [15]. Because of their high chemical reactivities, these products show great tendencies to react with mac-

Table 1 Demographic characteristics of the patients in the case and the control groups

Parameter	Case group	Control group	*P*-value
Age (years)	37.48 ± 2.1	35.55 ± 1.9	0.527
Work experience (years)	13.68 ± 1.7	12.05 ± 1.7	0.512
Gender			
Male	16	10	0.583
Female	14	10	0.615

Data shown are in terms of mean ± standard deviations.

Table 2 Comparisons of the patients hematological indices between the case and the control groups

Parameter	Case group*	Control group	*P*-value
WBC (count × 10^3/μL)	6.52 ± 1.58 (10.88)	5.88 ± 1.37	0.153
RBC (count × 10^6/μL)	4.99 ± 0.60 (3.31)	4.83 ± 0.36	0.298
Hct (%)	41.69 ± 4.05 (2.76)	40.57 ± 2.68	0.280
Hb (g/dL)	14.32 ± 1.58 (1.99)	14.04 ± 1.07	0.497
Plt (count × 10^3/μL)	231 ± 54.77 (-2.11)	236 ± 40.24	0.718
Neutrophils (count × 10^3/μL)	3.70 ± 1.20 (10.11)	3.36 ± 0.76	0.276
Lymphocytes (count × 10^3/μL)	2.22 ± 0.66 (16.84)	1.90 ± 0.54	0.070
Mix (count × 10^3/μL)	0.60 ± 0.19 (-3.22)	0.62 ± 0.54	0.827

Data shown are in terms of mean ± SD.
SD, standard deviation; WBC, white blood cell; RBC, red blood cell; Hct, hematocrit; Hb, hemoglobin; Plt, platelet; Mix, the sum of the monocytes and the eosinophil counts. *Mean percentage change relative to the control group is shown in parentheses.

Table 3 Comparisons of the patients immune functional responses between the case and the control groups

Parameter	Case group*	Control group	*P*-value
Stimulation index	1.82 ± 0.80 (25.51)	1.45 ± 0.57	0.040
IFN-γ (pg/mL)	98.8 ± 11.08 (59.87)	61.8 ± 9.20	0.035
IL-4 (pg/mL)	12.5 ± 1.22 (-25.14)	16.7 ± 1.42	0.047

Data shown are in terms of mean ± standard deviations. *Mean percentage change relative to the vehicle is shown in parentheses.

romolecule components, including DNA, lipids, and proteins, in cells. Low levels of these substances are essential for the physiological activities of the cells [16, 17]. However, if ROS are produced in high amounts or the antioxidant defense systems fail, oxidative stress will occur, resulting in tissue damage. Normal cell functions depend on the intracellular redox (reduction-oxidation) state [17]. Fortunately, in normal circumstances, a balance exists between free-radical production and anti-oxidant capacity. Studies have demonstrated that the exposure of humans to high doses of ionizing radiation results in oxidative stress [18-20]. Thus, the balance between pro-oxidant production and antioxidant defense is very important for maintaining cellular homeostasis [21].

In the current study, significant increases in the IFN-γ levels and corresponding decreases in the IL-4 levels produced by cultured PBMCs from the radiology staff were observed, suggesting a shift to a more Th_1-cell-based response. In general, Th_1 cells can initiate processes leading to the activation of many cells, including $CD8^+$ and phagocytes. While the increases in the levels of IFN-γ, which produces Th_1 $CD4^+$ cells, enhance cell-mediated immunity, the increases in the levels of IL-4, which produces Th2 $CD4^+$ cells, cause a increase in the number of regulatory T-cells, thus weakening the immune system [22].

As mentioned earlier, the equilibrium between oxidizing and reducing agents within these cells controls their redox state and consequently their functions. Thus, knowing that transient controlled changes in the redox state, such as elevated production of reactive oxygen species, are vital for signaling and inducing various biological processes is important. Low levels of ROS have been reported to be vital for T-cell activity and other immune system components [23, 24]. For example, small amounts of ROS are important for inducing transcription of NF-κB and for gene expressiosn of the cytokines and the receptors required for T-cell proliferation, highlighting the important role of the cellular redox environment in the function of T-cells [25, 26]. Therefore, the significant increases in the proliferation responses of, and the IFN-γ productions by, isolated PBMCs might be due to the ionizing radiation causing enhanced productions of the ROS needed for T-cell function.

A few controversial studies in this field can be found in the literature. For instance, contrary to the results of our study, Attar and colleagues showed that exposure to high doses of ionizing radiation can induce a shift from Type 1 to Type 2 responses with an unaffected lymphocyte proliferation assay. In addition, they found the neutrophil nitroblue tetrazolium (NBT), phagocytosis, and locomotion to be higher in the exposed group [15]. Similarly, in another study performed on the immune systems of radiology staff in a hospital in Tehran, radiation workers were observed to have decreased levels of IL-2 when compared to controls [19]. That difference may have been due to the greater use of fluoroscopy for interventional radiological procedures in the two aforementioned studies. Fluoroscopy emits a large fraction of the radiation dose delivered in diagnostic medical imaging because of continuous X-ray production and real-time image output. Therefore, that large fraction of radiation might have produced the high level of ROS that consequently disrupted the equilibrium between the oxidizing and the reducing agents within the immune cells, leading to oxidative stress.

5. Conclusion

Our findings showed that hematological indices were not significantly different between healthy radiologists and unexposed controls. On the other hand, exposure to low-dose radiation induced an immunomodulatory effects in radiology workers so that a shift to a more Th1-cell-based response was observed. Therefore, exposure to low-dose radiation may decrease the prevalence, frequency, and recurrence of various kinds of cancer because of the increase in the Th1-cell-based response, leading to increased protection of the human body against tumor cells. As a result, longevity may be increased. However, due to the development of multislice serial CT scanners and digital fluoroscopy applications in Iran, which will lead to high radiation exposure to workers in the Radiology Departments of our medical facilities, research projects involving radiation protection and hazards should be encouraged so as to find ways to prevent irreversible damage.

As a recommendation, in future studies, radiology staff with weakened immunity due to high radiation exposure should be considered as good choices to be treated with acupuncture techniques because acupuncture has been demonstrated to enhance the function and number of immune cells [27, 28]. In other words, acupuncture may be considered as a good strategy for modulating and regulating the immune systems of these workers. However, further research is required if this hypothesis is to be proven.

Acknowledgment

The authors are thankful to the Vice Chancellor of Research, Mashhad University of Medical Sciences, for financial support.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. Int J Radiat Biol. 1994;65(1):27-33.
2. Okamoto H, Tatara A. Effect of low-gamma irradiation on the cell cycle duration of barley roots. Environ Exp Bot. 1994;35(3):379-88.
3. Taguchi Y, Tsutsumi N, Tatara A, Eguchi H, Taano S. Effect of low-dose gamma-irradiation on the root apical meristem of barely. Environ Mut Res Commun. 1994;16:205-9.
4. Pecaut M, Nelson G, Gridley D. Dose and dose rate effects of whole-body gamma-irradiation: I. lymphocytes

and lymphoid organs. In vivo. 2001;15(3):195-208.
5. Cameron JR. Moderate dose rate ionizing radiation increases longevity. Br J Radiol. 2005;78(925):11-3.
6. Luckey T. Hormesis revisited. RSO Magazine. 1998;3:20-1.
7. Mayr A, Pawlas S. [Unexpected effects of a whole body irradiation on the mortality rate of baby mice after an experimental infection with the vesicular stomatitis virus (VSV)]. Zentralbl Veterinarmed B. 1989;36(8):577-83. German.
8. Stone RS. Health protection activities of the plutonium project. Proce Am Philos Soc. 1946;90(1):11-9.
9. Luckey T. Low-dose irradiation reduces cancer deaths. Radiat Protect Manag. 1997;14:58-64.
10. Moss W, Eckhardt R. The human plutonium injection experiments. Los Alamos Sci. 1995;23:177-233.
11. Spiers FW, Lucas HF, Rundo J, Anast GA. Leukaemia incidence in the US dial workers. Health Phys. 1983;44:65-72.
12. Schull WJ, Otake M, Neel JV. Genetic effects of the atomic bombs: a reappraisal. Science. 1981;213(4513):1220-7.
13. Kalamzadeh A, Keihani A, Hajati J, Nooraei M, Latifinia A, Zaker F, *et al*. Total plasma level of antioxidant and immune system function in radiology and nuclear medicine staff. Tehran Uni Med J. 2007;65(9):13-9.
14. Hrycek A, Czernecka-Micinska A, Kłucinski P, Badowski R. Peripheral blood lymphocytes and selected serum interleukins in workers operating X-ray equipment. Toxicol lett. 2002;132(2):101-7.
15. Attar M, Molaie Kondolousy Y, Khansari N. Effect of high dose natural ionizing radiation on the immune system of the exposed residents of Ramsar Town, Iran. Iran J Allergy Asthma Immunol. 2007;6(2):73-8.
16. Boxer LA, Harris RE, Baehner RL. Regulation of membrane peroxidation in health and disease. Pediatrics. 1979;64:713-8.
17. Victor VM, Rocha M, De la Fuente M. Immune cells: free radicals and antioxidants in sepsis. Int Immunopharmacol. 2004;4(3):327-47.
18. Chakraborty S, Singh OP, Dasgupta A, Mandal N, Nath Das H. Correlation between lipid peroxidation-induced TBARS level and disease severity in obsessive-compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2009;33(2):363-6.
19. Kalamzadeh A, Keihani A, Hajati J, Nooraei M, Latifinia A, Zaker F, *et al*. Total plasma level of antioxidant and immune system function in radiology and nuclear medicine staff. Tehran Uni Med J. 2007;65(9):13-9.
20. de Zwart LL, Meerman JH, Commandeur JN, Vermeulen NP. Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med. 1999;26(1-2):202-26.
21. Hughes DA. Effects of dietary antioxidants on the immune function of middle-aged adults. Proc Nutr Soc. 1999;58(1):79-84.
22. Farsam V, Hassan ZM, Zavaran-Hosseini A, Noori S, Mahdavi M, Ranjbar M. Antitumor and immunomodulatory properties of artemether and its ability to reduce $CD4^+$ $CD25^+$ $FoxP3^+$ T reg cells *in vivo*. Int Immunopharmacol. 2011;11(11):1802-8.
23. Griffiths HR. ROS as signalling molecules in T cells-evidence for abnormal redox signalling in the autoimmune disease, rheumatoid arthritis. Redox Rep. 2005;10(6):273-80.
24. Riahi B, Rafatpanah H, Mahmoudi M, Memar B, Fakhr A, Tabasi N, *et al*. Evaluation of suppressive effects of paraquat on innate immunity in Balb/c mice. J Immunotoxicol. 2011;8(1):39-45.
25. Keramati MR, Balali-Mood M, Mousavi SR, Sadeghi M, Riahi-Zanjani B. Biochemical and hematological findings of Khorasan veterans 23 years after sulfur mustard exposure. J Res Med Sci. 2013;18(10):855-9.
26. Los M, Droge W, Stricker K, Baeuerle PA, Schulze-Osthoff K. Hydrogen peroxide as a potent activator of T lymphocyte functions. Eur J Immunol. 1995;25(1):159-65.
27. Liang F, Cooper EL, Wang H, Jing X, Quispe-Cabanillas JG, Kondo T. Acupuncture and immunity. Evid Based Complement Alternat Med. 2015;2015:260620.
28. Arranz L, Guayerbas N, Siboni L, De la Fuente M. Effect of acupuncture treatment on the immune function impairment found in anxious women. Am J Chin Med. 2007;35(1):35-51.