Journal of Biology and Today's World

Journal home page: http://journals.lexispublisher.com/jbtw

Received: 03 April 2016 • Accepted: 28 July 2016

Research

doi:10.15412/J.JBTW.01050702

Assessment of some Immunological and Hematological Factors among Radiation Workers

Samira Heidari[1], Mohammad Taheri[2], Alireza Pouyandeh Ravan[3], Abbas Moghimbeigi[4], Maryam Mojiri[1], Yousef Naderi-Khojastehfar[1], Sahel Paydari-Banyarani[1], Zahra Hassanpour[1], Mohammad Mahdi Eftekharian[3,5,6*]

[1] Department of Radiology, Faculty of Paramedicine, Hamadan University of Medical Sciences, Hamadan, Iran

[2] Department of medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Faculty of Paramedicine, Hamadan University of Medical Sciences, Hamadan, Iran

[4] Modeling of Noncommunicable Diseases Research Center, Department of Biostatistics, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran

[5] Research center for molecular medicine, Hamadan University of Medical Sciences, Hamadan, Iran

[6] Molecular Immunology research group, Hamadan University of Medical Sciences, Hamadan, Iran

*Correspondence should be addressed to Mohammad Mahdi Eftekharian, Faculty of Paramedicine, Hamadan University of Medical Sciences, Hamadan, Iran; Research center for molecular medicine, Hamadan University of Medical Sciences, Hamadan, Iran; Molecular Immunology research group, Hamadan University of Medical Sciences, Hamadan, Iran ; Tel: +988138381017; Fax: +988138381017; Email: eftekharian@umsha.ac.ir.

ABSTRACT

Although the ionizing radiations cause various disorders in different parts of the human body, the importance and extensive application of radiations in medicine are undeniable. In this study, we have tried to evaluate the effect of radiation on immunological and hematological parameters in the radiation workers compared with the control group. Two groups including radiation workers (test group) and a control group were determined. Test and control groups were selected from imaging staffs in teaching hospitals of Hamadan and healthy employees working in other parts of the hospital, respectively. They were matched according to age and sex. The serum levels of cytokines IFN-γ, IL-4, IL-10 and IL-17 were determined and the results were analyzed by using SPSS 18. The obtained results indicated that the level of the IFN-γ and IL-10 in the radiation workers were significantly lower than the control group ($P < 0.05$). There was a statistically significant difference in the white blood cell count and percentage of peripheral blood lymphocytes between the radiation workers and the control group ($P < 0.05$). Based on the results of this study and comparison with the results obtained from other studies, the effects of ionizing radiation (in the radiation workers) appear certain on blood and immune systems. However, type, the exact location and severity of the effect, require more extensive researches on the number of people under study.

Key words: Hematological factors, Immunological factors, Radiation workers, Ionizing radiation, Cytokines.

Journal of Biology and Today's World is published by *Lexis Publisher*; Journal p-ISSN 2476-5376; Journal e-ISSN 2322-3308.

1. INTRODUCTION

The increasing use of radiation in diagnosis and treatment is causing an increasing concern about the importance of the protection of radiation related workers and also the patients (1-3). It should be considered that the radiographers of the diagnostic and therapeutic sections inevitably exposed to long-term and low-dose radiation ions even if the personal protective equipment were used appropriately and the rules and regulations were performed (3). Based on the results of research in molecular biology, cancer and malignant risk for ionizing radiations were simple function of the dose of radiation without threshold. Combined this fact with epidemiologic data led to this hypothesis that they are low-level radiation in every place and no place is protected from ionizing radiation (2, 3). Cell sensitivity to radiation is different. Among different cells, hematopoietic cells are the most sensitive cells against radiation (4). The range of blood cells in healthy people was relatively stable. This could alter by many factors such as occupational hazards. Several studies have been emphasized on the importance of the correlation CBC with the effects of partial and total radiation (4-6). Accordingly, blood cell count can be used as a biological indicator in the investigation of the damage caused by radiation (7-9). The immunological studies indicated that the ionizing radiation influenced the

immunological factors of individuals exposed to radiation (10). Therefore, in this study, some blood and immunological factors of radiation workers in diagnostic sections of Hamadan hospitals were investigated and then compared with the results obtained from the control group. In hematological studies, the most accessible and common tests were complete blood count (CBC). The assessment indicators in this section were a complete blood count and measurement of blood indexes including MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), Hb (Hemoglobin), HCT (Hematocrit), RDW (Red Cell Distribution Width), MPV (Mean platelet volume) and PDW (platelet distribution width)

2. MATERIALS AND METHODS

This investigation was performed as a test-control study. The test group was selected from staffs of imaging sections of teaching hospitals in Hamadan. The control group was healthy employees working in other parts of the hospital who did not have contact with radiation. These two groups were matched for age, cases with a mean age of 35.0±1.2 years and controls with a mean age of 34.5±0.53 years; also the sex ratio in the patients group was 20/20 (Female/Male) versus adjusted number in controls. The samples were collected from the diagnostic imaging staffs including radiology, CT scan and angiography of Beasat, Fatemiyyeh, Beheshti and Farshchian hospitals. An informed consent form was also obtained from the participants in the project. Personal information of participants was recorded. They included their age, gender, work experience and service location, as well as records and genetic risk of various diseases including acquired and inheritance disorders. Samples were collected in the form of a blood clot as well as in the tubes containing anticoagulant EDTA (Ethylene Diamine Tetra Acetic Acid). They were used for ELISA and hematology tests, respectively. The counting of red and white blood cells as well as platelets along with the level of hemoglobin and hematocrit and other indexes such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were performed by cell counting device(Sysmex cell counter, model kx21) in the Shariati laboratory in Hamadan. Differential leukocyte counts were manually done by the blood slides stained with wright. These slides were observed under light microscope. The percentage of neutrophils, lymphocytes, monocytes, eosinophils, and basophils in the blood sample were determined by this method. The measurement of cytokines in the serum was carried by sandwich ELISA in the laboratory of Besat hospital. Plasma was obtained from fasting venous blood of study participants by centrifugation and was then stored at −80 °C until the assay was performed. Plasma IFN-γ, IL-4, IL-10 and IL-17 level were measured using a high sensitivity human enzyme-linked immunosorbent assay kit (Abcam, Cambridge, MA, USA) according to the manufacturer's instructions. Briefly, 100 µL of standards, controls and plasma samples were added into 96-well plates, respectively, coated with purified anti-human cytokine antibody in duplicate and 50 µL of 1× Biotinylated anti-cytokine were then added into the appropriate wells. The wells were covered and incubated at room temperature for 2 h respectively. The contents of the test wells were then aspirated and rinsed with wash buffer repeatedly three times. 100 µL of 1× Streptavidin–Horseradish peroxidase solution 1 were added into all wells and incubated at room temperature for 20 min. The plates were washed three times as previously described. About 100 µL of 1× Amplifier were added and incubated for 15 min at room temperature. The plates were washed three times as previous 100 µL of 1× Streptavidin–Horseradish peroxidase solution 2 were added into all wells, including the blank wells and incubated at room temperature for 20 min. The plates were washed three times as previous 100 µL of chromogen TMB substrate solution were added into each well and incubated in the dark for 10–20 min at room temperature. Finally, the reaction was terminated by adding 100 µL of stop reagent and the absorbance was read immediately at 450 nm, or within 1 h, if the microplate is stored at 2–8 °C in the dark, the absorbance was used an Infinite M200 (Tecan, Männedorf, Switzerland) microplate reader. The lower detection limit of cytokine assay was 1.30 pg/mL.

The results obtained from laboratory tests were analyzed using SPSS 18 and independent T-test, ANOVA and Pearson correlation coefficient.

3. RESULTS AND DISCUSSION

In this study, 19 blood sample and immune factors were investigated in cases and controls. At first, the normal distribution of variables was studied by Kolmogorov-Smirnov test. The obtained results were shown in

Table 1.

Table 1. Normal distribution of variables by Kolmogorov-Smirnov test

Normality	Kolmogorov-Smirnov Z	Asymp.Sig.(2-tailed)
Basophil	4.753	0
Eosinophil	2.528	0
Lymphocyte	0.724	0.672
Neutrophil	0.505	0.96
Monocyte	1.565	0.015
MPV[a]	0.805	0.537
PDW[b]	1.691	0.007
PLT[c]	0.703	0.706
MCHC[d]	0.464	0.982
MCH[e]	0.785	0.568
MCV[f]	0.976	0.296
HCT[g]	0.58	0.889
Hb[h]	2.095	0
RBC[i]	0.596	0.87
WBC[j]	1.181	0.123

a: Mean platelet volume, b: platelet distribution width, c: Platelets, d: Mean corpuscular hemoglobin concentration, e: Mean corpuscular hemoglobin, f: Mean corpuscular volume, g: Hematocrit, h: hemoglobin, i:Red blood cell, j: White blood cell.

According to the results, the variables except basophil, eosinophil, monocyte, Platelet distribution width (PDW) and Hb had the normal distribution. A T-test was used to compare the mean of variables with normal distribution. As represented in Table 2, the amount of IFN-gamma in the radiation worker was significantly lower than the control group (P-Value =0.029). The similar data was observed for the serum IL-10 level (P-Value=0.032).

Table 2. The results obtained from tests on blood and serum of the studied groups regarding with variables with normal distribution. These data were analyzed by t-test.(Control group=2, case group=1)

	group	N	Mean	Std.Deviation	Std. Error	t	P-Value
Lymphocyte	1	40	40.65%	7.90	1.24	2.155	0.034
	2	40	36.1%	10.76	1.70	2.155	
Neutrophil	1	40	56.82%	8.27	1.30	-2.157	0.034
	2	40	61.6%	11.29	1.78	-2.157	
MPV[a]	1	40	10.42 FL	1.183	0.18	-0.118	**0.907**
	2	40	10.45 FL	1.094	0.17	-0.118	
PLT[b]	1	40	220000/µl	49.83	7.87	-2.075	0.041
	2	40	243000/µl	51.67	8.17	-2.075	
MCHC[c]	1	40	33.96%	1.455	0.23	-1.53	**0.13**
	2	40	34.39%	1.05	0.16	-1.53	
MCH[d]	1	40	29.54 pg	1.88	0.29	-0.18	**0.858**
	2	40	29.61 pg	1.44	0.22	-0.18	
MCV[e]	1	40	87.00 FL	4.11	0.65	1.113	**0.269**
	2	40	86.08 FL	3.24	0.51	1.113	
HCT[f]	1	40	43.84%	4.21	0.66	0.773	**0.442**
	2	40	43.14%	3.87	0.61	0.773	
RBC[g]	1	40	5.04/µl	0.45	0.07	0.18	**0.857**
	2	40	5.02/µl	0.54	0.08	0.18	
WBC[h]	1	40	6.02/µl	1.01	0.16	-2.53	0.013
	2	40	6.75/µl	1.50	0.23	-2.53	
IFN-γ[i]	1	40	1.88 ng/ml	50.31	7.95	-2.22	0.029
	2	40	2.16 ng/ml	60.70	9.59	-2.22	
IL-17[j]	1	40	2.47 ng/ml	78.62	12.43	0.33	**0.740**
	2	40	2.42 ng/ml	50.50	7.98	0.33	
IL-10[k]	1	40	2.39 ng/ml	83.86	13.26	-2.18	**0.032**
	2	40	2.92 ng/ml	130.34	20.60	-2.18	
IL-4[l]	1	40	94.90 ng/ml	45.30	7.16	1.32	**0.189**
	2	**40**	**81.70 ng/ml**	**43.74**	**6.91**	**1.32**	

a: Mean platelet volume, b: Platelets, c: Mean corpuscular hemoglobin concentration, d: Mean corpuscular hemoglobin, e: Mean corpuscular volume, f: Hematocrit, g: red blood cell, h: white blood cell, i: Interferon gamma, j: Interleukin-17, k: Interleukin-10, l: Interleukin-4.

Although the results showed that there was no significant difference between the amount of Interleukins 4 and 17 in the radiation workers and control groups (p=0.189 and p=0.740, respectively), the serum levels of IL-4 in the test group was higher than the control group (94.9 VS. 81.7). the results obtained from the blood factors indicated that the percentage of blood neutrophils and lymphocytes showed a statistically significant difference between the radiation workers and control group (P-Value =0.034). Based on these results, the number of neutrophils in the radiation workers was lower than the control group. In contrast, the number of lymphocytes was higher in the radiation workers. On the other hand, it was found that the total number of white blood cells and platelets was significantly lower in the peripheral blood of radiation workers than the control group (P-Value =0.013 and P-Value =0.041, respectively). To determine the mean of variables that did not have normal distribution, the non-parametric Mann-Whitney test was used. The r esults were presented in Table 3.

Table 3. The results obtained from tests on blood and serum of the studied groups regarding the variables with non-normal distribution. These data were analyzed by Mann-Whitney test. (Control group=2, case group=1)

	group	N	Mean	Std.Deviation	Std. Error Mean	Z	P-Value
Basophil	1	40	0.1%	0.49	0.07	-1.423	0.155
	2	40	0%	0			
Eosinophil	1	40	0.85%	1.02	0.16	-0.073	0.942
	2	40	0.92%	1.24	0.19		
Monocyte	1	40	1.57%	1.17	0.18	-1.05	0.294
	2	40	1.37%	1.39	0.21		
PDW[a]	1	40	13.53	3.05	0.48	-0.149	0.881
	2	40	13.21	2.22	0.35		
Hb[b]	1	40	14.89	1.63	0.25	-0.236	0.814
	2	40	15.6	5.10	0.80		

a: platelet distribution width, b: hemoglobin.

As can be considered in this section, there was no significant difference between these two groups. As mentioned, exposure to X-ray radiation in radiation workers may be harmful for living their cells or may adversely effects on the function of human immune cells. It may lead to life-threatening diseases associated with the hematopoietic system (11-13). Therefore, a continuing monitoring of staff associated with ionizing radiation was considered as an essential care in many countries. One of these important tests was to check blood count including total and differential counts of white blood cells, platelets, red blood cells, the average of red blood cells volume, mean of hemoglobin concentration, the mean of cell hemoglobin, hematocrit and hemoglobin (7). The results in the present study showed that there was no significant difference in the factors related to red blood cells between these two groups. In other words, exposure to ionizing radiation did not have any side effects on the quantity and quality parameters of red blood cells such as total count of red blood cells, hemoglobin, hematocrit, MCH and MCV. On the other hand, the number of platelets showed a significant decrease in the radiation workers compared with the control group. In contrast, their average volume (MPV) is the same in both groups. According to the results, the total number of white blood cells significantly decreased in the radiation worker than the control group. The differential counts indicated that the number of monocytes, eosinophil and basophil cells did showed no significant changes in these two groups. However, the number of neutrophils and lymphocytes had a significant decrease and increase in the radiation workers compared to the control group, respectively. Therefore, in summary, the radiation workers group showed a significant decrease of the total number of platelets and leukocytes than the control group. This decrease in the total number of leukocytes also accompanied with the changes in the balance between lymphocytes and neutrophils. The obtained results from previous studies were very varied and controversial**.** Mesa et al (2006) studied the individuals who were exposed to low levels of X-ray. They found that the leukocytes number of these individuals show no significant difference compared to the control group. However, their phagocytic activity (phagocytosis) as one of the most important defense mechanisms against infectious agents was greatly reduced. These findings suggested that exposure to X-rays can seriously influence on the function of neutrophils (11). Davoodi et al (2012) were also studied the effects of ionizing radiation on staff depending on their experience. The obtained results indicated that the total number of leukocytes and platelets showed a significant decrease in the radiation workers with more experience than the control group (12). As observed, our results were consistent with the results of the study mentioned. However, the correlation of experience of radiation workers with the effects of radiation on them was not evaluated in our study. In a similar study done by Taher et al, no difference in hematological parameters including total count of white blood cells, red blood cells and platelets were observed between these two groups**.** However, the number of atypical lymphocytes significantly

increased in the radiation workers (13). It appears that extensive exposure to ionizing radiation may also affect blood cell morphology**.** Riahi-Zanjani et al were studied the staff with more than three years of work experience in sections related to ionizing radiation. They were not found any difference in hematological parameters of these individuals compared to the control group. Exceptionally, the increase of platelet distribution width (PDW) as an indicator of the heterogeneity and homogeneity of platelets volume in circulation were reported in the radiation workers (14). As a result, the continuous encounter with the X-rays can affect the maturation, survival, activation or death processes of platelet precursors. This can lead to changes in the PDW. However, our results indicated that the reduction in platelet count did not accompany the changes in PDW. Therefore, it is possible that continuous exposure to X-rays can cause adverse effects on platelet precursors and their production in bone marrow (14). Tavakoli et al (2011) found similar results in radiation workers in Birjand, East Iran. They found that the radiation group had a leukocyte count less than the control group (15). In some studies, the effectiveness of radiation on the lymphocytes has been also investigated. These studies showed different and sometimes contradictory results. Our results showed that the percentage of peripheral blood lymphocytes significantly increased in the radiation workers group while Dainik et al found that ionizing radiation can significantly reduce the percentage of peripheral blood lymphocytes. The mechanism of this reduction has been evaluated. It is believed that the rearrangement and redistribution of lymphocytes or exit of circulating lymphocytes to the lymph nodes or induction of apoptosis by radiation may be reasons of this observation (16). By summarizing the research results, it is possible that the total number of leukocytes, neutrophils and platelets reduced while the structure and number of red blood cells did not change in the radiation workers. However, the assumptions about the number of lymphocytes were controversial and required further studies**.** As previously mentioned, some immunological parameters were also evaluated in this study. The cytokines levels of IFN-γ representing Th1, IL-4 and IL-10 responses were measured as well as levels of Th2, IL-17 representing Th17 response. The ratio between lymphocytes Type 1 helper (Th1) to type 2 helper (Th2) [Th1 / Th2] was an important indicator in determination of shifting the immune responses to the cellular or humoral responses. The differentiation of T cells into Th1 and Th2 will cause that the responses directed to cellular and humoral immunity, respectively. This ratio was subject to change in many common diseases including multiple sclerosis and cancer. The Th17 cells were the most important source of IL-17 production and secretion. They also play a key role in the inflammatory and destructive responses especially in autoimmune diseases (17-19). Based on the results obtained from this study, IFN-gamma and IL-10 levels in the radiation workers group was significantly lower than the control group ($p<0.05$). As considered, some inconsistency was observed in results. The reduction of gamma interferon level means to overcome humoral immunity (as opposed to cellular immunity). On the other hand, the reduction of IL-10 means to increase cell-mediated immunity (as opposed to humoral immunity). Increasing the number of subjects along with reduction of other confounding factors may eliminate this paradox. Although the obtained results showed that there was no significant difference ($p>0.05$), serum levels of IL-4 in the test group was higher than the control group (94.9 VS 81.7). The increased levels of IL-4 as one of the main indices of the humoral arm of the immune responses indicated that the immune system had the greater tendency to produce antibodies and, in contrast, it showed the lower tendency to function through the cellular immunity pathway. However, these two groups did not show any difference in terms of IL-17 (2.42 VS 2.47, $p>0.05$). This means that the risk of shifting the immunity responses to the generation of lymphocytes Th17 represented no significant difference in these two groups. The lymphocytes Th17 produced pro-inflammatory and inflammatory cytokines. As noted, normally, there should be a balance between two arms of the cellular and humoral immune responses. Any deviation leading to imbalance can cause various diseases including autoimmune disorders. Accordingly, the shifting the immune response towards the humoral arm was one of the factors affecting the development of autoimmune diseases including systemic lupus erythematous (SLE), Graves and non-insulin dependent diabetes. For this reason, researchers tried to search the treatment of these diseases by improvement of Th1 / Th2 ratio and bring it back to normal level. The IL-4 plays an important role in switching antibody to IgE class through the effect on B lymphocytes. It is the most important factor in induction of type 1 hypersensitivity diseases. Ghiassi-Nejad et al were studied the effects of radioactive waves on immune system in Ramsar, in the north of Iran. Residents of this city naturally were subjected to radioactive waves five times more than other individuals. These researchers found that production level of IgE antibody in the serum of individuals exposed to radiation was significantly higher than other individuals. The results obtained from this study were consistent with our results (20). Athar et al were studied the residents of two villages in Ramsar. The results obtained from their study represented that although people living in these two villages seem healthy, but they had the serum levels of IL-4, IL-10, IL-2 and INF-γ less than the control group (10). The results obtained from this study indicated inhibition of cellular immune responses after the effects of radioactive radiation. As noted, IL-4 altered the class of antibody towards IL-4. Acleev et al (2008) in Russia showed that the immune system of individuals exposed chronically to radioactive radiation had more inhibitory and regulatory characteristics than the normal individuals. Plasma cells of the individuals exposed to radioactive radiation had more

tendencies to produce IgA antibody (21). Godekmerdan et al (2004) found that the number of CD4+, some components of the complement (C3 and C4), and antibodies of class IgG, IgM and IgA decreased in the radiation workers compared to the control group (22).

4. CONCLUSION

According to the results of the present study and other studies, the effects of ionizing radiation were assured on the immune system of radiation workers. However, the type, the exact location and severity of the effect required the extensive research on more individuals exposed to radiation. In addition, multiple confounding factors must be excluded from the study. It can cause that we achieved accurate and more uniform results.

ACKNOWLEDGMENT

Not mentioned any acknowledgment by authors.

FUNDING/SUPPORT

This study was supported financially (grant number 9308063773) by vice chancellor of research and technology in Hamadan University of medical sciences.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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