





Analysis of Red Blood Cells and their Components in Medical Workers with Occupational Exposure to Low-Dose Ionizing Radiation

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Abstract

Plenty of reports focus on the effects of low-dose radiation (LDR) on peripheral blood lymphocytes in radiation workers. However, studies on red blood cells (RBCs) in radiation workers are rarely reported. Many studies focused on investigate the hemogram of radiation staffs without detecting other components of RBCs. To explore the potential effect of LDR on RBCs, we detected the level of RBC count, hemoglobin, 2,3-disphosphoglycerate (2,3-DPG), and glutathione (GSH), and then analyzed the factors on these indices in 106 medical radiation workers. As a result, RBC count was affected by sex, age, type of work, length of service (only for females), and annual effective dose (only for males). Hemoglobin status was affected by sex, type of work, and annual effective dose (only for males). Sex, age, and type of work had no effects on the concentration of 2,3-DPG and GSH. Length of service affected 2,3-DPG concentration, and annual effective dose affected GSH level. In conclusion, chronic occupational LDR exposure may have an effect on RBC count, hemoglobin status, and the concentration of 2,3-DPG and GSH in radiation workers to some extent. However, it is still unknown how this kind of influence affects the health of radiation workers.

Keywords

red blood cells, hemoglobin, 2,3-disphosphoglycerate, glutathione, low-dose radiation

Introduction

The application of ionizing radiation technology is increasingly widespread in medicine nowadays. A growing number of medical workers could be exposed to ionizing radiation during diagnosis, treatment, or surgery by using X-rays or γ -rays. Occupational exposure of medical radiation staffs is chronic low dose radiation (LDR). Low-dose radiation is defined as less than or equal to 100 mSv, and the LDR rate is defined as less than or equal to 6 mSv per hour.¹ Although radiation protection measures are adopted in occupational exposure, radiation epidemiology studies indicated that LDR has an effect on the health of radiation workers, such as radiation-induced cataracts, cardiovascular disease, and mental health,²⁻⁷ to a certain extent.

Several reports focused on the effects of radiation on peripheral blood lymphocytes genetically⁸⁻¹⁰ and immunologically.¹¹⁻¹⁵ Chromosome aberration and micronucleus analyses in

peripheral blood lymphocytes are often used to examine radiation workers to reflect the extent of radiation damage to the human body.^{8,16} In practice, chromosome aberration frequencies and/or micronucleus frequencies in most radiation workers are in the normal range.⁸ However, it is still unclear whether LDR has no influence on the health of radiation workers. Only focusing on

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changes in some indices from lymphocytes is not sufficient for health monitoring in radiation workers. Although hemogram examination is also performed in the physical examination of radiation workers, the results of red blood cells (RBCs) from hemogram are not as much concerned as the lymphocyte results. RBC count or hemoglobin in some radiation workers becomes abnormal year after year. Therefore, RBCs should be paid further attention in terms of health monitoring of radiation workers.

Red blood cells, as the most numerous cells in peripheral blood, play an important role in many biological processes in humans and animals. Red blood cells may take critical roles in promoting immune activation or maintaining immune quiescence in humans.^{17,18} A mouse experiment revealed that LDR could induce RBC system hormesis.¹⁹ Although animal studies showed that LDR could affect RBC metabolism, the potential effects of LDR on RBC metabolism in radiation workers are still unclear. Previous studies only investigated the change in hemogram of radiation staffs without detecting other components of RBCs. Thus, the alterations of RBC components induced by ionizing radiation are worthy to be explored.

Red blood cells are responsible for carrying oxygen in mammals, and their metabolic level is pivotal for regulating the oxygen affinity of hemoglobin. The capacity of hemoglobin to release O₂ especially depends on 2,3-diphosphoglycerate (2,3-DPG), which is a byproduct of glycolysis unique to RBCs. The increase in 2,3-DPG content reduces the affinity of hemoglobin-oxygen,²⁰ leading to the dissociation of oxygen from hemoglobin. This phenomenon facilitates the release of oxygen to tissue and improves the tolerance of tissue cells to hypoxia.^{21,22} Thus, 2,3-DPG plays a critical role in regulating oxygen-carrying capacity. Patients with anemic and chronic hypoxia and people living in high-altitude areas have a compensatory increase in 2,3-DPG levels. It was found that the 2,3-DPG content in mouse peripheral blood could be markedly elevated after whole-body LDR exposure.¹⁹ However, reports about the effects of LDR on 2,3-DPG levels in the human body are limited.

Apart from affecting the oxygen-carrying capacity of hemoglobin, ionizing radiation may affect the antioxidant status of medical staffs in the radiology department.²³⁻²⁷ Most of the harmful effects are produced indirectly in medical radiation staffs. In general, the harmful effects mainly result from radiolysis of water and generation of reactive oxygen species (ROS).²³ Glutathione (GSH) is one of the most crucial intracellular antioxidants in the metabolism of ROS.²⁸ The content of GSH in RBCs is of great significance to protect the sulfhydryl group of protein on the RBC membrane and prevent hemolysis. Therefore, the effect of LDR on GSH in RBCs is worthy of investigating.

Based on the above information, reports about the effect on RBC and its components, such as hemoglobin, 2,3-DPG, and GSH, induced by occupational exposure to LDR are still limited. Thus, the potential effect of LDR on RBC and its components is worthy of exploring. In the present study, 106 medical workers occupationally exposed to LDR were included. Red blood cell count, hemoglobin, 2,3-DPG

concentration, and GSH in peripheral blood samples were investigated. For the sake of exploring LDR effect, the levels of RBC and its components were also analyzed among groups with different sex, age, type of work, length of service, and annual effective dose.

Methods

Subjects and Samples

This work was conducted at the National Institute for Radiological Protection (NIRP), China CDC. All experiments in this research were approved by the Ethics Committee of NIRP (LLSC2019-004). The scope of the study was explained to each subject, and the written informed consents were obtained.

Human peripheral blood samples were obtained from 106 medical radiation workers through venipuncture and collected using heparinized (3 mL) and ethylene diamine tetraacetic acid (EDTA) (2 mL) vacutainer tubes (Becton Dickson). Basic personnel information was collected (Table 1), including sex, age, smoking status, and drinking status. Type of work, length of service, and annual effective dose in 2018 were also collected. The subject inclusion criteria are as follows: age of the donor is from 18 years to 60 years; subjects should have incurred occupational LDR exposure for at least 1 year; the annual accumulative doses of each subject should include four quarter records; subjects should have no congenital anemia, other RBC disease, history of chronic occupational disease, special medication, and family inheritance; and subjects should not be exposed to ionizing radiation for medical requirements within 6 months before sample collection. The criterion for smokers is those who have a smoking history regardless of smoking amount. The criterion for non-smokers is those who have never had experience smoking. The criterion for drinking/

Table 1. General characteristics of studied subjects.

Parameters	Radiation workers
Number of subjects	106
Mean age (range)	37.48 (24–58)
Sex (%)	
Female	46(56.60%)
Male	60(43.40%)
Mean length of service	11.30 ± 10.36
Range	1–39
Mean dose (mSv/year)	.58 ± 1.18
Range	.09–10.71
Smoking status (%)	
Smoking	10 (9.43%)
Non-smoking	96 (90.57%)
Drinking status (%)	
Drinking	28 (26.42%)
Non-drinking	78 (73.58%)

non-drinking is the same as that criterion for smoking/non-smoking. The effective dose was determined using a thermoluminescence dosimeter. The thermoluminescence dosimeter was worn in front of the left chest or at the clavicle. Interventional radiology operation requires staff to perform it closely with a radioactive source for a long time. Interventional radiation workers wear a lead coat during working to protect themselves from radiation. Thus, they wore the dosimeter inside and outside the lead coat. The effective doses of interventional radiation were collected from their inside dosimeter records.

Hemogram Examination

Two milliliters of peripheral blood was collected into a vacuum tube containing EDTA anticoagulant for examination. The ADVIA2120 hematology system (Beijing, P.R. China) was applied for blood testing following the manufacturer's instruction. Hemogram examinations included the RBC count ($\times 10^{12}/L$) and hemoglobin content (g/L) examination.

Investigation of Human 2,3-DPG

The concentration of 2,3-DPG in each sample was obtained using a human 2,3-DPG ELISA kit. The procedures were summarized as follows: the samples prepared in GSH extraction were used for 2,3-DPG detection. Then, 100 μL standard or sample was added to each well and incubated at 37°C for 2 hours. The liquid was discarded without washing well. Afterward, 100 μL of biotin antibody was added to each well and incubated at 37°C for 1 hour. Then, the liquid was discarded, and each well was washed three times. Subsequently, 100 μL of HRP-avidin was added to each well and incubated at 37°C for 1 hour. The liquid was then discarded, and each well was washed five times. Ninety microliters of TMB substrate was added to each well and incubated at 37°C for 15–30 min while keeping away from light. Finally, 50 μL of stop solution was added to each well, and the results were read at 450 nm with a plate reader within 5 min. The standard curve was fitted by the concentration of the standard and its OD₄₅₀ value. The concentration of the standard was taken as the X-axis, while its OD₄₅₀ value was taken as the Y-axis. The OD₄₅₀ values of unknown samples were substituted into the standard curve to determine the 2,3-DPG content ($\mu mol/mL$).

Investigation of Human GSH

Human GSH testing was performed using a Glutathione Assay kit (Catalog NO. CS0260, Sigma). Glutathione was extracted from the RBCs of each 300 μL sample following the protocol of the assay kit.

The testing procedures were shown as follows: the prepared samples and standards were mixed with the working mixture as instructed. All samples and standards were incubated at room temperature for 5 min, and then the substrate was added into each well to start the enzyme reaction. The

OD₄₁₂ value was measured in each well by using a plate reader with kinetic read at 1 min intervals for 5 min. The values of GSH standard solutions were used to fit the standard curve and calculated the $\Delta A_{412}/min$ equivalent to 1 nmole of reduced GSH per well. The following formula was used to calculate the nmoles of GSH in the unknown sample: GSH concentration (nmoles/mL) = ($\Delta A_{412}/min$ (sample) \times dil)/($\Delta A_{412}/min$ (1 nmole) \times vol), where $\Delta A_{412}/min$ (sample) means slope generated by the sample, $\Delta A_{412}/min$ (1 nmole) refers to the slope calculated from the standard curve for 1 nmole of GSH, dil denotes the original sample dilution factor, and vol refers to the sample volume in the reaction in mL.

Statistical Analysis

All statistical analyses in this study were performed on SPSS 21.0. All values were expressed as the mean \pm standard deviation. Normal distribution tests were performed for all data. Differences among groups were assessed using one-way ANOVA or Kruskal–Wallis *H* test. *P* < .05 was considered statistically significant.

Results

General Characteristics of Studied Subjects

This study included 106 radiation workers (Table 1). Their age ranged from 24 years old to 58 years old with an average of 37.48 years. The ratio of sex was 1.3:1 (female: male). The average length of service was 11.30 ± 10.36 (1–39) years. The 2018 average annual effective dose was $.58 \pm 1.18$ (.09–10.71) mSv. The ratio of smoking to non-smoking was 1:10. The ratio of drinking to non-drinking was 3.6:10.

Overall Levels of RBCs, Hemoglobin, 2,3-DPG, and GSH of Radiation Workers

The levels of RBCs, hemoglobin, 2,3-DPG, and GSH of each sample were detected. The average levels of these indices in 106 radiation workers were $(4.61 \pm .50) \times 10^{12}/L$, 145.60 ± 16.00 g/L, 1.64 ± 1.44 $\mu mol/mL$ and 560.62 ± 561.80 nmol/mL, respectively. ANOVA results indicated that the status of smoking and drinking had no influence on the levels of RBCs, hemoglobin, 2,3-DPG, and GSH.

Influence of Sex and Age of Radiation Workers on RBC Count, Hemoglobin, Concentration of 2,3-DPG, and GSH

The differences in RBC count, hemoglobin, the concentration of 2,3-DPG, and GSH between male and female were compared in this study (Table 2). A statistically significant difference was found in the RBCs and hemoglobin of different sex groups (*P* < .01). The results also showed that there was no difference

Table 2. Comparison of RBC Count, levels of hemoglobin, 2,3-DPG, and GSH between male and female of radiation workers ($\bar{x} \pm s$).

Sex	Number	RBC count ^a ($\times 10^{12}/L$)	Hemoglobin (g/L)	2,3-DPG ^b ($\mu\text{mol}/\text{mL}$)	GSH ^c (nmol/mL)
Male	60	4.93 \pm .41	156.70 \pm 10.46	1.84 \pm 1.52	530.50 \pm 456.42
Female	46	4.20 \pm .26	131.13 \pm 8.69	1.38 \pm 1.30	599.91 \pm 678.62
<i>F</i> ^d		112.873	179.708	2.744	.395
<i>P</i>		.000	.000	.101	.531

^aRBC means the red blood cell.

^b2,3-DPG means 2,3-diphosphoglycerate.

^cGSH means glutathione.

^d*F* means the statistical value of one-way ANOVA.

in the concentration of 2,3-DPG and GSH between male and female subjects, indicating that the sex of radiation workers may not be a factor influencing the levels of 2,3-DPG and GSH.

Due to sex difference, radiation workers of the same sex were grouped by age. In accordance with the age of radiation workers, they were divided into four groups, starting from 20 years at 10-years interval. The results showed a difference in RBCs other than the hemoglobin of male among different age groups ($P < .05$) (Table 3). In the female groups, the RBC count was decreased with age ($P < .05$). The RBC count in the group aged 40 years was lower than that in the group aged 20–29 years ($P < .05$) (Table 3). However, any difference was not observed in the hemoglobin of female among different age groups.

For 2,3-DPG and GSH, the concentration did not vary with age (Table 4). This finding indicated that age of radiation workers may not be a factor affecting the level of 2,3-DPG and GSH.

Effect of Type of Work on RBC Count, Hemoglobin, Concentration of 2,3-DPG, and GSH

Due to different job duties and workloads, radiation workers may be exposed to different degrees of radiation in different types of work. The 106 radiation workers in this study work in radiodiagnosis, nuclear medicine, radiotherapy, and interventional radiology. They were grouped in accordance with their type of work, and differences in RBC count, levels of hemoglobin, the concentration of 2,3-DPG, and GSH were analyzed among groups (Table 5). The results showed that the RBC level of the interventional radiology group was higher than those of other groups ($P < .01$). The hemoglobin of the interventional radiology group was also significantly higher than those of other groups ($P < .05$, $P < .01$). No statistically significant differences were observed on concentrations of 2,3-DPG and GSH in all types of work.

Influence of Length of Service on RBC Count, Hemoglobin, Concentration of 2,3-DPG, and GSH

Radiation dose, especially accumulated dose, is affected not only by type of work but also by length of service of individuals with chronic occupational exposure. Therefore, it is

Table 3. Influence of age on RBC count and hemoglobin level of radiation workers in different sex groups of radiation workers ($\bar{x} \pm s$).

Sex	Age	Number	RBC count ^a ($\times 10^{12}/L$)	Hemoglobin (g/L)
Male	20–29	16	5.02 \pm .33	158.75 \pm 7.77
	30–39	20	5.08 \pm .40	159.70 \pm 11.12
	40–	24	4.75 \pm .41 ^b	152.83 \pm 10.61
<i>F</i> ^c			4.443	2.955
<i>P</i>			.016	.060
Female	20–29	12	4.33 \pm .26	135.75 \pm 6.74
	30–39	18	4.22 \pm .28	129.22 \pm 10.17
	40–	16	4.08 \pm .18 ^d	129.81 \pm 7.28
<i>F</i> ^c			3.814	2.462
<i>P</i>			.030	.097

^aRBC means the red blood cell.

^bSignificantly lower than that of male subjects in other age groups, $P < .05$.

^c*F* means the statistical value of one-way ANOVA.

^dSignificantly lower than that in the group aged 20–29 years, $P < .05$.

Table 4. Influence of age on the concentration of 2,3-DPG and GSH of radiation workers ($\bar{x} \pm s$).

Age	Number	2,3-DPG ^a ($\mu\text{mol}/\text{mL}$)	GSH ^b (nmol/mL)
20–29	28	1.43 \pm 1.18	617.86 \pm 572.03
30–39	38	1.83 \pm 1.64	561.35 \pm 503.38
40–49	24	1.31 \pm 1.12	570.38 \pm 770.71
50–	16	2.05 \pm 1.73	442.69 \pm 251.88
<i>F</i> ^c		1.269	.328
<i>P</i>		.289	.805

^a2,3-DPG means 2,3-diphosphoglycerate.

^bGSH means glutathione.

^c*F* means the statistical value of one-way ANOVA.

necessary to investigate the difference in RBCs, hemoglobin, concentration of 2,3-DPG and GSH in varying lengths of service. For RBC count and the level of hemoglobin, the subjects of the same sex were divided by length of service. The results showed no statistically significant differences in males' RBCs and hemoglobin and females' hemoglobin among groups (Table 6). A difference was found in the RBC count of females among different length-of-service groups. The RBC count of females in the 10 years length-of-service group was

Table 5. Comparison of RBC Count, levels of hemoglobin, 2,3-DPG, and GSH in different types of work of radiation workers ($\bar{x} \pm s$).

Type of work	Number	RBC count ^a ($\times 10^{12}/L$)	Hemoglobin (g/L)	2,3-DPG ^b ($\mu\text{mol}/\text{mL}$)	GSH ^c (nmol/mL)
Radiodiagnosis	39	4.55 \pm .46	144.69 \pm 14.78	1.83 \pm 1.51	457.05 \pm 227.90
Nuclear medicine	20	4.48 \pm .44	139.85 \pm 16.93	1.53 \pm 1.51	685.00 \pm 600.24
Radiotherapy	17	4.41 \pm .50	140.18 \pm 13.73	1.55 \pm 1.38	629.41 \pm 888.13
Interventional radiology	30	4.91 \pm .49 ^d	153.70 \pm 15.48 ^e	1.51 \pm 1.39	573.37 \pm 612.73
<i>F</i> ^f		5.668	4.533	.855	.353
<i>P</i>		.001	.005	.467	.787

^aRBC means the red blood cell.

^b2,3-DPG means 2,3-diphosphoglycerate.

^cGSH means glutathione.

^dSignificantly higher than that of other groups, $P < .01$.

^eSignificantly higher than that of other groups, $P < .05$, $P < .01$.

^f*F* means the statistical value of one-way ANOVA.

Table 6. Comparison of RBC count and hemoglobin level of radiation workers in different length-of-service groups according to sex ($\bar{x} \pm s$).

Gender	Length of service (Years)	Number	RBC count ^a ($\times 10^{12}/L$)	Hemoglobin (g/L)
Male	0–5	15	4.99 \pm .34	156.80 \pm 8.80
	5–10	17	4.99 \pm .37	156.65 \pm 9.56
	10–	28	4.87 \pm .46	156.68 \pm 12.02
	<i>F</i> ^b		.675	.001
<i>P</i>		.513	.999	
Female	0–5	16	4.23 \pm .25	131.12 \pm 9.55
	5–10	14	4.31 \pm .28	133.29 \pm 8.53
	10–	16	4.08 \pm .22 ^c	129.25 \pm 8.69
<i>F</i> ^b		3.337	.797	
<i>P</i>		.045	.457	

^aRBC means the red blood cell.

^b*F* means the statistical value of one-way ANOVA.

^cSignificantly lower than that of 5–10 years length-of-service group, $P < .05$.

lower than that of females in the 5–10 years length-of-service group ($P < .05$).

The 2,3-DPG analysis showed statistically significant difference among groups. The status of 2,3-DPG increased with longer length of service. The 2,3-DPG concentration of 10– years length-of-service group was significantly higher than that of the 0–5 years length-of-service group ($P < .01$) (Table 7). A difference in GSH concentration was not identified in all length-of-service groups (Table 7).

Influence of Annual Effective Dose on RBC Count, Hemoglobin, Concentration of 2,3-DPG, and GSH

The annual effective dose of 85 radiation workers was collected. The results revealed that each accumulative annual dose was below the permissible limit of the International Commission on Radiological Protection. The influences of annual effective dose on RBCs, hemoglobin, the concentration of 2,3-DPG, and GSH were analyzed. For RBCs and

Table 7. Comparison of 2,3-DPG and GSH of radiation workers in different length of service groups ($\bar{x} \pm s$).

Length of service (Years)	Number	2,3-DPG ^a ($\mu\text{mol}/\text{mL}$)	GSH ^b (nmol/mL)
0–5	31	1.06 \pm 1.06	653.77 \pm 743.15
5–10	31	1.75 \pm 1.24	489.71 \pm 221.61
10–	44	1.97 \pm 1.70 ^c	548.45 \pm 595.03
<i>F</i> ^d		3.830	.659
<i>P</i>		.025	.520

^a2,3-DPG means 2,3-diphosphoglycerate.

^bGSH means glutathione.

^cSignificantly higher than that of 0–5 years length-of-service group, $P < .01$.

^d*F* means the statistical value of one-way ANOVA.

Table 8. Influence of annual effective dose in 2018 on RBC count and hemoglobin level of radiation workers in different sex groups of radiation workers ($\bar{x} \pm s$).

Gender	2018 annual effective dose (mSv)	Number	RBC count ^a ($\times 10^{12}/L$)	Hemoglobin (g/L)
Male	.0–0.3	10	4.83 \pm .29	153.30 \pm 4.72
	.3–0.4	12	4.81 \pm .36	153.83 \pm 11.12
	.4–0.5	11	4.69 \pm .37	152.27 \pm 8.63
	.5–	11	5.13 \pm .44 ^{b,c}	163.60 \pm 12.40 ^d
<i>F</i> ^e		2.849	3.198	
<i>P</i>		.049	.033	
Female	.0–0.3	9	4.23 \pm .29	130.89 \pm 7.54
	.3–0.4	11	4.10 \pm .17	131.91 \pm 4.50
	.4–0.5	13	4.23 \pm .34	131.62 \pm 10.52
	.5–	8	4.20 \pm .24	130.50 \pm 13.70
<i>F</i> ^e		.615	.045	
<i>P</i>		.610	.987	

^aRBC means the red blood cell.

^bSignificantly higher than that of male subjects in the .3–4 mSv group, $P < .05$.

^cSignificantly higher than that of male subjects in the .4–5 mSv group, $P < .01$.

^dSignificantly higher than that of male subjects in other dose groups, $P < .05$.

^e*F* means the statistical value of one-way ANOVA.

hemoglobin, same sex subjects were divided into four groups on the basis of annual effective dose (Table 8). In male groups, the RBCs of the .5 mSv group were significantly higher than those of the .3— .4 and .4— .5 mSv groups ($P < .05$, $P < .01$). The hemoglobin of male samples in the .5 mSv group was higher than that of male subjects in other groups ($P < .05$). No difference in RBCs and hemoglobin was observed in the female groups.

For 2,3-DPG and GSH, a statistically significant difference was found in the GSH among dose groups (Table 9). From .0 mSv to .5 mSv, a trend of increasing GSH concentration with the increase in annual effective dose was observed. The level of GSH in the .4—0.5 mSv group was significantly higher than those in the .0— .3 mSv and .3— .4 mSv groups ($P < .01$).

Discussion

In this study, the levels of RBCs, hemoglobin, 2,3-DPG and GSH in 106 medical radiation workers with chronic occupational exposure were detected. To our best knowledge, this study was the first to describe the 2,3-DPG status of medical staff occupationally exposed to long-term LDR.

Red blood cells play a vital role in human metabolism. They interact with and transport a series of substrates, such as oxygen, carbon dioxide, nitric oxide, chloride ions, protons, and organic phosphates.^{29,30} Red blood cells have plenty of hemoglobin. In general, RBC status and the amount of hemoglobin are affected by sex.^{31,32} A similar result was found in the present study. The background level of RBCs in males was higher than that in females. This finding was similar among radiation workers. Thus, different standards for male and female should be established to assess the effect of LDR on RBC level. Age may influence RBC count rather than hemoglobin level. Regardless of sex, the RBC status of groups

aged over 40 years was markedly lower than that of groups aged less than 40 years.

Type of work, length of service, and annual effective dose are main factors that affect the health of radiation workers during occupational exposure. In accordance with varying job duties and workloads, the absorbed dose of persons engaged in radiological diagnosis, nuclear medicine, radiotherapy, and interventional radiology increased. The present study found that the RBCs and hemoglobin of the interventional radiology group were extensively higher than those of groups with other types of work. A report showed that the RBC membrane became more fragile and vulnerable to breakage after γ -ray irradiation in whole-blood samples from healthy volunteers.³³ Another study found a marked elevation in the susceptibility of RBCs to hemolysis among radiographers compared with controls.³⁴ If RBCs become easily hemolysis, it may lead to hemolysis anemia. Erythropoiesis is enhanced as a compensatory in the human body to deal with hemolysis.³⁵ Therefore, the results of RBC count and hemoglobin among groups with different types of work showed that radiation workers may be predisposed by LDR to hemolysis anemia, especially those who work in interventional radiology. However, a thorough investigation is needed in the future. A previous study reported lower values of mean hemoglobin in radiation staff with at least a 10-year length of service than those in the control group.³⁶ Another report revealed that the level of RBCs and hemoglobin markedly decreased with longer duration of employment of radiation workers in Tangshan, China.³⁷ However, in the present study, the males' RBCs and hemoglobin in both sexes did not change with longer length of service. This finding may be due to the different kinds of radiation workers, which included medical staffs and industrial workers in the Tangshan study, whereas the radiation workers in the present study only came from hospital. In addition, the case number in the present study was not sufficient, and the sample size must be increased in further research. A notable detail that the RBCs of females with over 10 years duration of employment was lower than those with 5—10 years group. This finding was consistent with the result of lower RBC count in females aged over 40 years, probably because older people usually get longer length of service. The information of 2018 annual effective dose was collected as the accumulate dose. The results indicated that this dose had more influence on males' RBCs and hemoglobin than on females. The RBC count and hemoglobin status in over .5 mSv group were markedly higher than those of under .5 mSv groups for male. This finding may be due to the difference in radiosensitivity between male and female.³⁸ A previous study showed that the change in RBC count was more significant in male workers occupationally exposed to LDR, while the change in lymphocytes was more extensive in female radiation workers.³⁹ This result indicated that the effects of ionizing radiation on some indicators of peripheral blood differed between male and female. However, the mechanism is still

Table 9. Influence of annual effective dose in 2018 on the concentration of 2,3-DPG and GSH of radiation workers ($\bar{x} \pm s$).

2018 annual effective dose (mSv)	Number	2,3-DPG ^a ($\mu\text{mol/mL}$)	GSH ^b (nmol/mL)
.0—0.3	19	2.04 \pm 1.89	401.32 \pm 218.02
.3—0.4	23	1.13 \pm 1.09	539.13 \pm 771.50
.4—0.5	24	1.61 \pm 1.02	702.08 \pm 546.63 ^c
.5—	19	1.96 \pm 1.61	513.16 \pm 257.04
χ^2		4.687	10.361
<i>P</i>		.196	.016

^a2,3-DPG means 2,3-diphosphoglycerate.

^bGSH means glutathione.

^cSignificantly higher than that in the .0— .3 mSv and .3— .4 mSv groups, $P < .01$.

unclear, and further systematic research needs to be conducted.

One of the most important functions of RBCs is transporting oxygen to the tissues to support oxidative phosphorylation.³⁰ As matured RBCs have no cellular nucleus and mitochondrion, they obtain energy mainly through anaerobic glycolysis. 2,3-DPG is a byproduct of glycolysis unique to RBCs. It is synthesized in the Rapoport—Luebering glycolytic shunt. The anabolic BPGM and catabolic BPGP reactions, two components of the shunt, are responsible for the regulation of the 2,3-DPG status.^{40,41} Furthermore, 2,3-DPG plays a pivotal role in regulating hemoglobin when transporting oxygen.^{19,42-44} During oxygen transportation, the conformation of hemoglobin undergoes a transition from the low affinity state to the highly oxygenated state. 2,3-DPG binds to Val1 of one β chain and to the His2, Lys82, and His143 of two β chains. These positively charged amino acid residues are located at the entrance of the central cavity of deoxydative hemoglobin.³⁰ An increasing in 2,3-DPG content reduces the affinity of hemoglobin-oxygen and improves the tolerance of tissue cells to hypoxia.^{21,22} An animal study showed that low-dose total body ionizing radiation could enhance the 2,3-DPG content.¹⁹

This study showed that 2,3-DPG status was not affected by sex, age, type of work, and annual effective dose, except for length of service. The 2,3-DPG level increased with longer length of service. Patients with anemia and chronic hypoxia and people living in high-altitude areas have a compensatory increase in 2,3-DPG levels. In addition, severe bleeding, polycythemia, and other pathological reasons could result in the elevation of 2,3-DPG level. The radiation workers in this study obviously suffered from neither severe bleeding nor polycythemia or other illnesses that could cause the 2,3-DPG level to increase. Therefore, we speculated that the reason behind the 2,3-DPG increase may be chronic LDR exposure resulting in enhancing the risk of anemia among radiation workers. Thus, the elevation of 2,3-DPG may indicate a potential anemia risk for radiation workers who chronically work in an environment with LDR. However, this speculation needs further systematic study to confirm the effect of LDR on 2,3-DPG. No extensive changes in 2,3-DPG were found in subjects grouped by type of work and annual effective dose. The reasons may be as follows: (1) the number of cases in each type of work or each annual effective dose group was not sufficient; (2) the data of annual effective dose collected in this study was only 1-year records, which may not be sufficient to reflect the effect of cumulative dose on the 2,3-DPG content of those people with long-term exposure to ionizing radiation; (3) dosimeters were not correctly worn by some radiation workers, making the data of annual effective dose inaccurate. Thus, more subjects and annual effective dose data are needed, and the effect of type of work and accumulative dose on the 2,3-DPG level of radiation workers requires further study. The effect of variation in the 2,3-DPG content on the health

of radiation workers exposed to LDR is also worthy of investigating.

Occupational exposure to LDR may affect the antioxidant status of radiation workers. GSH is a key antioxidant and well-reported biomarkers of oxidative stress in human and other mammalian tissues.^{45,46} There are different conclusions about the effect of LDR on GSH status in medical staff. Russo GL et al found that the GSH content in interventional cardiologists had a 1.7-fold increase compared with that in unexposed control. Long-term exposure to LDR was associated with an altered redox balance and with an increased antioxidant defense (increase in GSH, counteracting the enhancement of oxygen radical stress) in interventional cardiologists.⁴⁵ On the contrary, another study found no difference in GSH status between medical radiation workers and medical workers without radiation exposure.⁴⁷ In the present study, the GSH level was not affected by sex and age. Although the GSH content of those working in nuclear medicine, radiotherapy, and interventional radiology are higher than that of staff working in radiodiagnosis, the significant differences of GSH levels were not found. This result may be due to the insufficient subject number. Thus, more subjects are needed to confirm the effect of type of work on GSH content. In addition, the GSH content increased with annual effective dose from 0 mSv to .5 mSv but reduced after .5 mSv. This finding indicated that the GSH level may increase with accumulative dose first and then decrease later. Hormesis can be induced by LDR in human cells and tissues.⁴⁸ Under low-dose range, ionizing radiation may induce the elevation of GSH level. It may be helpful for the health of radiation workers to facilitate scavenging ROS. When the accumulation dose continues to increase, the decline in GSH concentration may be a disadvantage to scavenging ROS. In addition, the GSH status in groups with over 5 years of length of service was lower than that in the group with 0—5 years of length of service despite no statistically significant difference was found among groups. The results of GSH in each length-of-service group may be attributed to the increase in length of service resulting in increased accumulative dose. As only 1 year of annual effective dose was collected, the effect of accumulative doses on GSH levels was not sufficiently clarified. Thus, more annual effective dose data and subjects must be collected, and the effect of accumulative dose on the GSH level of radiation workers need be further explored.

The results suggested that the changed levels in RBC and its components may have a role in prompting the potential effect of LDR. This study has also some limitations. For better clarification of the effect of LDR on RBC and its components, the length of time workers incurred LDR will be collected in the next study. In addition, the present study mainly focused on the effect of ionizing radiation on RBCs in this study. However, other environmental exposures and confounding factors are worthy of investigation. For instance, chemical agents may have an influence on RBCs. Lifestyle may also be a factor that affects RBC metabolism. Therefore, more subjects must be included and more confounding factors must be

considered to further study the effect of LDR on RBC and its components in radiation workers.

Conclusion

Chronic occupational exposure to LDR may have an effect on the level of RBCs, hemoglobin, 2,3-DPG, and GSH of radiation workers to some extent. Red blood cells should be monitored in radiation workers because of the important role of RBCs for human health. However, how these alterations affect the health of radiation workers remains unknown. Whether LDR affects the RBC maturity and the RBC metabolism of radiation workers and whether LDR leads to cell hypoxia need further systematic study.

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Author Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Xue-Lei Tian, Xue Lu, Yu-Min Lyu, and Hua Zhao. The first draft of the manuscript was written by Xue-Lei Tian. Qing-Jie Liu and Mei Tian commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

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Ethics Approval

Approval was obtained from the ethics committee of NIRP. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

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