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Effects of High Doses of X-Ray on Hematological Parameters and Morphology of Red Blood Cells in Human Blood

Ali H. Taqi[1*], Kharman A. Faraj[2], Sarah A. Zaynal[1], Jalil J. Said[3], Ahmed M. Hameed[4]

1. Department of Physics, College of Science, Kirkuk University, Kirkuk, Iraq.
2. Department of Physics, College of Science, University of Sulaimani, Kurdistan Region-Iraq.
3. CT Scan and MRI Department, Kirkuk General Hospital, Kirkuk, Iraq.
4. Hematology Laboratory, Kirkuk General Hospital, Kirkuk, Iraq.

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ABSTRACT

Introduction: The aim of the current study was to investigate the effects of X-ray radiation on some hematological parameters, morphology of red blood cells (RBC) and erythrocyte sedimentation rate (ESR) analysis of human blood using computed tomography (CT) scanner.

Material and Methods: For the purpose of the study, 5 ml of whole blood was drawn from vein puncture of 28 healthy people and divided into two equal parts in ethylendiamintetracetic acid (EDTA) tubes. The first 28 tubes were assigned as the controls. The second 28 tubes were divided into 4 groups of 7 cases, irradiated to (3, 6, 9, and 12) Gy, X-ray from a computed tomography CT-scan machine.

Results: The results showed that no significant difference was observed for the hematological parameters and ESR analysis. However, there was a significant decrease in the radius of RBCs.In this regard, the mean RBC counts were obtained as 6.267 ± 0.528, 6.867 ± 0.476, 7.167 ± 0.535, and 6.55 ± 0.295 µm after exposure to the radiation doses of 3, 6, 9, and 12 Gy, respectively. The crenation of the cells was also observed, and the percentage of crenation were <5%, 15%, 40%, and 60% after irradiation to 3, 6, 9, and 12 Gy, respectively.

Conclusion: The in vitro irradiation of human blood to different X-ray doses (i.e.,3, 6, 9, and 12 Gy), resulted in the enhancement of RBCs crenation with increasing the dose, and reduction of the cell radius compared to those in the control groups. However, the hematological parameters and ESR analysis were not statistically affected.

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Introduction

The effects of ionizing radiations on people can arise from both low and high doses. The interaction of radiation with the DNA of the cells may cause the radiolysis of water inside the cell producing free radicals [1]. The penetration of radiation into living cells can resulting in the transfer of radiation energy to the biological material. The absorbed energy can induce the formation of reactive oxygen species, chemical bond-breaking and ionization of different biologically essential macromolecules, e.g., DNA, membrane lipids, and proteins. The DNA damage induced by radiation, such as base alteration, cross-linking, strands break or chromosomal aberration can lead to mutation [2]. Radiation doses to radiosensitive organs such as the ovaries, testicles, and thyroid may induce harmful effects, e.g., cancer and genetic effects [3].

Both non-stochastic (deterministic) and stochastic effects may result from exposure to ionizing radiation. Non-stochastic effects refer to the acute exposure and immediate health effects that are induced at a certain dose, below which they cannot occur. On the other hand, stochastic effects refer to chronic exposure, where there is no threshold and the probability of having the effects is not proportional to the dose absorbed [4]. However, the effects of low doses of ionizing radiations are still controversial. The effects depend on several factors such as age, gender, and time of exposure. Taqi et al. [5] concluded that the chronic exposure can alter significantly some hematological parameter in human blood. On the contrary, Talab et al. [6] showed that long-term exposure cannot alter the level of blood factor. However, they reported that with ageing and continuous work, the number of white blood cell decreases.

The life span of red blood cells (RBCs) is 110-120 days in human blood [7]. Macrophages remove senescent RBCs in the spleen, liver, and bone marrow [8]. Mechanical properties of healthy RBCs depend on some factors such as their shapes, spherical surface area, flexibility of the membrane and viscosity of the cytoplasm. Any changes in these factors can affect the membrane and cytoskeletal

*Corresponding Author: Tel: + 964-07710128455; Email: alitaqi@uokirkuk.edu.iq

structure [9], increase the hemoglobin released from the RBCs with a concomitant decrease in the hemoglobin concentration as the dose increases. In addition, there is evidence regarding the increased damage in the cell membrane after irradiation. Phospholipid bilayer membrane has a main role in producing damage in erythrocytes. The inner surface of the membrane is supported by a complex arrangement of the molecular interaction of proteins structures [10].

With this background in mind, the present study aimed to investigate the effects of relatively high doses of X-ray (i.e., 3, 6, 9, and 12 Gy) on some hematological parameters, morphology (radius and the shape) of RBCs and Erythrocyte Sedimentation Rate (ESR) analysis of human blood. The significance of this study lies in our selected dose range since most of the previous studies investigated the effects of doses greater than 20 Gy.

Materials and Methods

For the purpose of the study, 5 ml of whole blood was drawn from vein puncture of 28 healthy people. The blood from each donor was divided into two equal parts in EDTA tubes. The first 28 tubes were considered as the controls group, and the second 28 tubes were divided into 4 experimental groups of 7 cases, irradiated to 3, 6, 9 and 12 Gy X-ray from computed tomography (CT) scan machine (Siemens, Germany) with a dose rate 0.2Gy/sec. The specimens were analyzed directly.

The measurements of hematological parameters included White blood cells, Neutrophils, Lymphocyte, Monocyte, Eosinophil, Basophil, Reactive Lymphocyte, RBC, hemoglobin (Hb), hematocrit, mean cell volume, mean Corpuscular hemoglobin, mean cell hemoglobin concentration, Red cell Distribution Width and platelet. The parameters were measured by hematological analyzer (Swelab-alpha, Sweden).

The analysis of ESR was accomplished using Westergren rack [11, 12]. After the preparation of the slides, morphology (i.e., radius and percentage of crenation) of RBCs were measured using an optical microscope (Labomed, USA). A CT-scan machine was employed as a tool for obtaining these ranges of X-ray doses. The statistical analysis was performed by using the t-test to compare the means of the groups. The data were examined using the GraphPad Software Inc. vesion 8.0.0 (California, USA). P-value less than 0.05 was considered statistically significant.

Results

Effects on hematological parameters

Tables 1, 2, 3 and 4 illustrate the results related to the measurements of the hematological parameters in the control group and the four experimental groups irradiated with 3, 6, 9 and 12 Gy. There was no significant difference between the control and experimental groups in terms of all parameters.

Table 1. Comparison of complete blood count between the control group and experimental group at 3 Gy

Parameters	Control (Mean±SD)	Irradiation (Mean±SD)	P-value
WBC×10^9/l	6.25±0.52	6.35±0.404	0.7715
Neutrophils×10^9/l	4.25±0.404	4.3±0.346	0.8572
Lymphocyte×10^9/l	1.65±0.058	1.7±0.00	0.1340
Monocyte×10^9/l	0.25±0.058	0.25±0.058	1.0000
Eosinophil×10^9/l	0.05±0.058	0.05±0.058	1.000
Basophil×10^9/l	0.5±0.58	0.5±0.58	1.0000
RBC×10^{12}/l	4.595±0.0981	4.67±0.1155	0.3605
Hb (g/dl)	13.75±0.635	13.95±0.52	0.6432
HCT%	39.95±0.404	40.65±0.52	0.0776
MCV (fl)	86.95±0.981	87.15±0.981	0.7829
MCH (pg)	29.95±0.751	29.95±0.404	1.000
MCHC (g/dl)	34.5±1.27	34.35±0.866	0.8517
RDW%	11.4±0.693	11.35±0.52	0.9118
PLT×10^9/l	220.50±5.20	224.00±6.93	0.4498

WBC: white blood cell
HCT: hematocrit
MCH: mean corpuscular hemoglobin
RDW: red cell distribution width
RBC: red blood cell
MCV: mean cell volume
MCHC: mean cell hemoglobin concentration
PLT: platelet

Table 2. Comparison of complete blood count between the control group and experimental group at 6 Gy

Parameters	Control (Mean±SD)	Irradiation (Mean±SD)	P-value
WBC×10^9/l	7.650±1.799	7.350±1.112	0.7862
Neutrophils×10^9/l	2.85±0.173	3.000±0.115	0.1996
Lymphocyte×10^9/l	3.075±0.613	3.000±0.432	0.8481
Monocyte×10^9/l	0.15±0.058	0.15±0.058	1.000
Eosinophil×10^9/l	0.05±0.058	0.05±0.058	1.0000
Basophil×10^9/l	0.5±0.58	0.5±0.58	1.0000
RBC×10^{12}/l	5.3975±0.291	5.38±0.2741	0.9331
Hb(g/dl)	15.5±1.283	15.825±0.954	0.6984
HCT%	46.525±3.323	46.1±2.947	0.8546
MCV (fl)	85.125 ± 1.7	85.325 ± 1.475	0.8648
MCH (pg)	28.675 ± 0.727	29.2 ± 0.476	0.2726
MCHC (g/dl)	31.925 ± 1.097	31.400 ± 1.602	0.6081
RDW%	11.275 ± 0.32	14.55 ± 3.811	0.1376
PLT×10^9/l	205.5 ± 19.64	204.25 ± 14.8	0.9223

Table 3. Comparison of complete blood count between the control group and experimental group at 9 Gy

Parameters	Control (Mean±SD)	Irradiation (Mean±SD)	P-value
WBC×10^9/l	8±0.523	8.1±0.082	0.7185
Neutrophils×10^9/l	4.8±0.577	4.95±0.3	0.6610
Lymphocyte×10^9/l	2.5±0.231	2.375±0.35	0.5728
Monocyte×10^9/l	0.5±0.231	0.525±0.15	0.8619
Eosinophil×10^9/l	0.15±0.058	0.175±0.05	0.537
Basophil×10^9/l	0.05±0.058	0.025±0.05	0.5370
RBC×10^{12}/l	5.48±0.2945	5.5275±0.226	0.8066
Hb(g/dl)	15.8±0.408	15.95±0.238	0.5490
HCT%	48.27±2.09	49.025±1.578	0.5876
MCV (fl)	88.55±1.502	88.65±0.926	0.9135
MCH (pg)	28.95±0.81	28.9±0.744	0.9305
MCHC (g/dl)	32.4±0.432	32.625±0.532	0.5356
RDW%	13.300±2.149	12.700±2.434	0.7244
PLT×10^9/l	228±89.01	239.5±72.12	0.8475

Table 4. Comparison **of** complete blood count between the control group and experimental group at12 Gy

Parameters	Control (Mean±SD)	Irradiation (Mean±SD)	P-value
WBC×10^9/l	7.7±1.329	7.15±1.038	0.5384
Neutrophils×10^9/l	4.9±0.808	4.9±0.693	1.000
Lymphocyte×10^9/l	2.05±0.289	1.9± 0.2	0.4258
Monocyte×10^9/l	0.45±0.058	0.5±0.00	0.134
Eosinophil×10^9/l	0.25±0.173	0.175±0.15	0.5370
Basophil×10^9/l	0.05±0.058	0.025±0.05	0.5370
RBC×10^{12}/l	5.695±0.3637	5.53±0.4377	0.5831
Hb(g/dl)	16.6±0.346	16.5±0.346	0.6973
HCT%	49.6±0.231	50.25±0.975	0.2420
MCV (fl)	87.35±5.947	91.1±5.335	0.3841
MCH(pg)	29.2±1.27	29.95±1.652	0.4987
MCHC(g/dl)	33.5±0.808	32.85±0.37	0.1939
RDW%	13.500±1.915	13.550±1.570	0.9691
PLT×10^9/l	185.5 ± 42.15	180.75±40.01	0.8755

Effects on Morphology

After irradiation of blood to 3, 6, 9 and 12 Gy X-ray, the percentages of RBCs crenation were <5%, 15%, 40% and 60%, respectively. Table 5.

Figures 1, 2, 3 and 4 represent the crenation study of the control and four experimental groups. The results of the crenation analysis revealed explicit morphological changes.

Table 5. Percentage of red blood cell crenation after irradiation to X-ray doses

X-ray Dose (Gy)	Percentage of crenation of RBC
3	<5%
6	15%
9	40%
12	60%

RBC: red blood cell

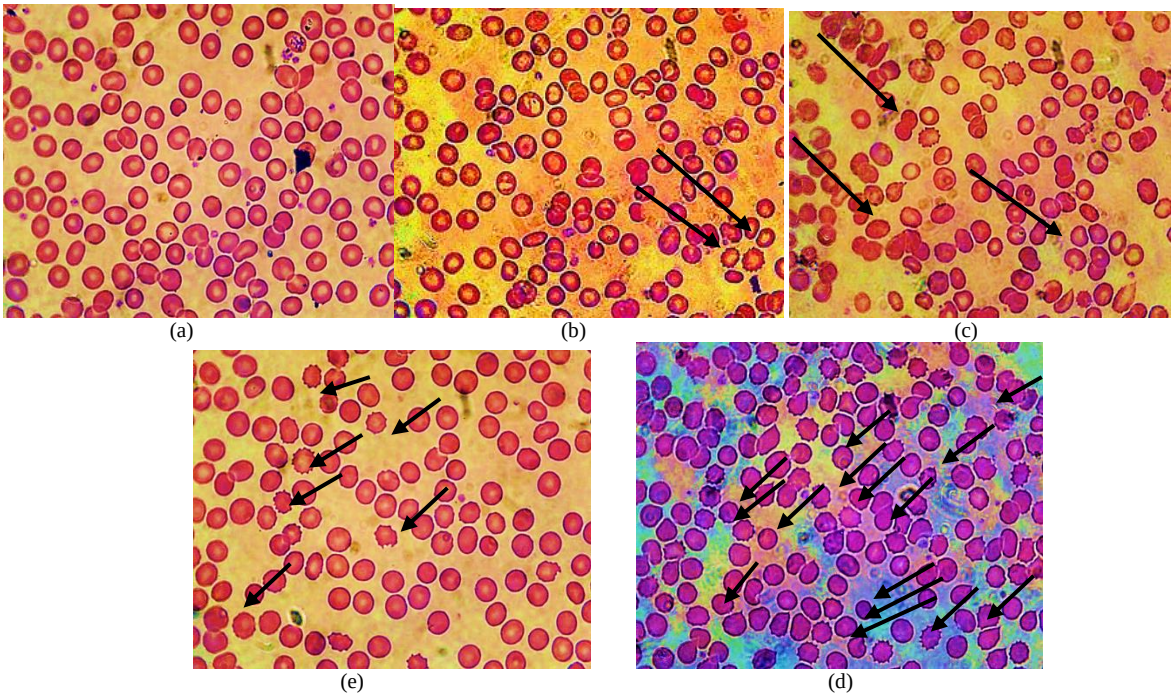

Figure1. Morphology of sample1: a) control, b) 3 Gy, c) 6 Gy, d) 9 Gy, e) 12 Gy

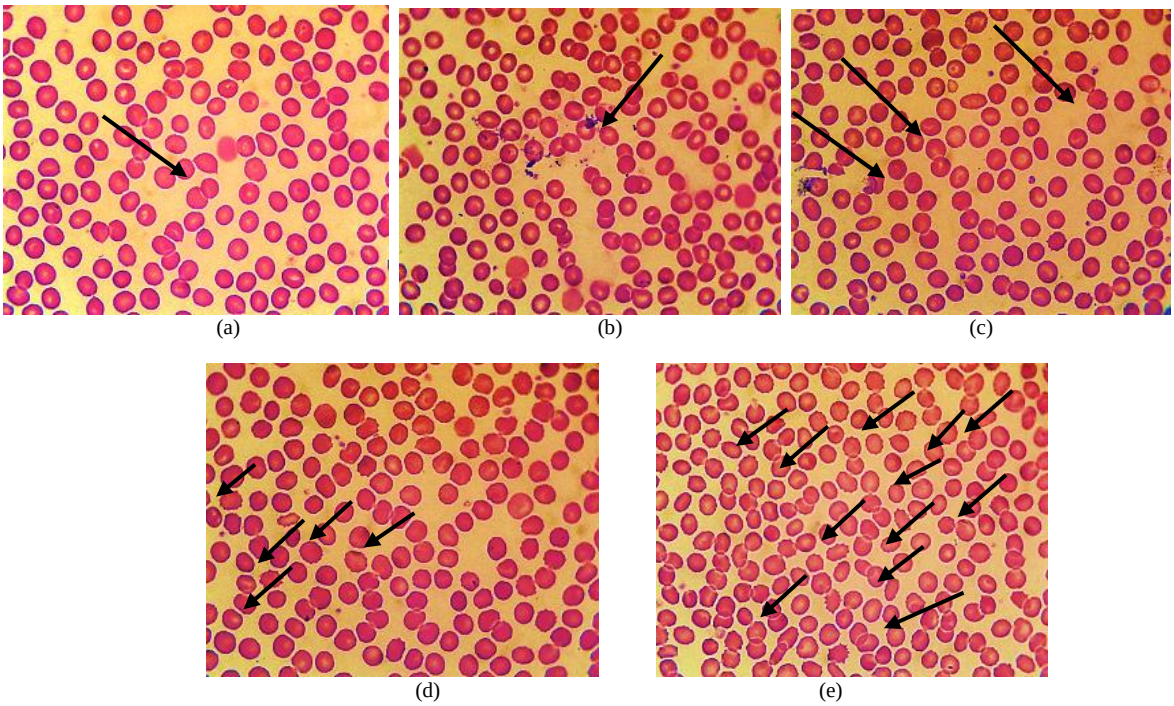

Figure 2. Morphology of sample 2: a) control, b) 3 Gy, c) 6 Gy, d) 9 Gy, e) 12 Gy

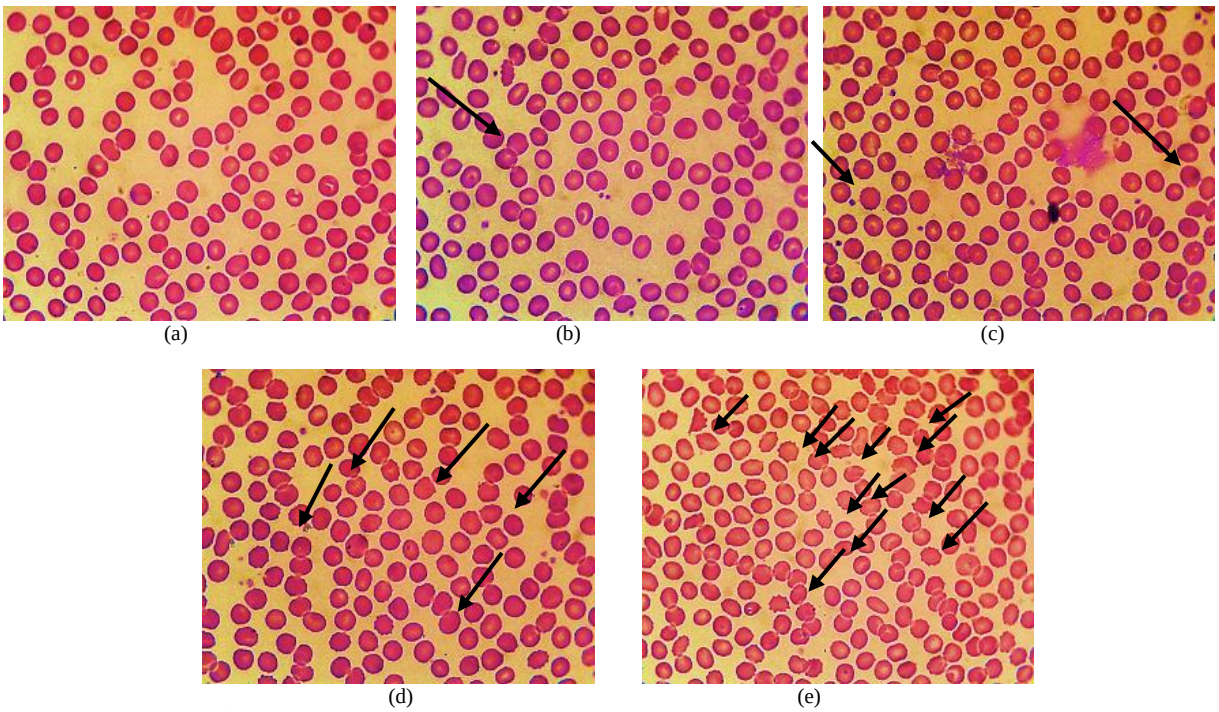

Figure 3. Morphology of sample 3: a) control, b) 3 Gy, c) 6 Gy, d) 9 Gy , e) 12 Gy

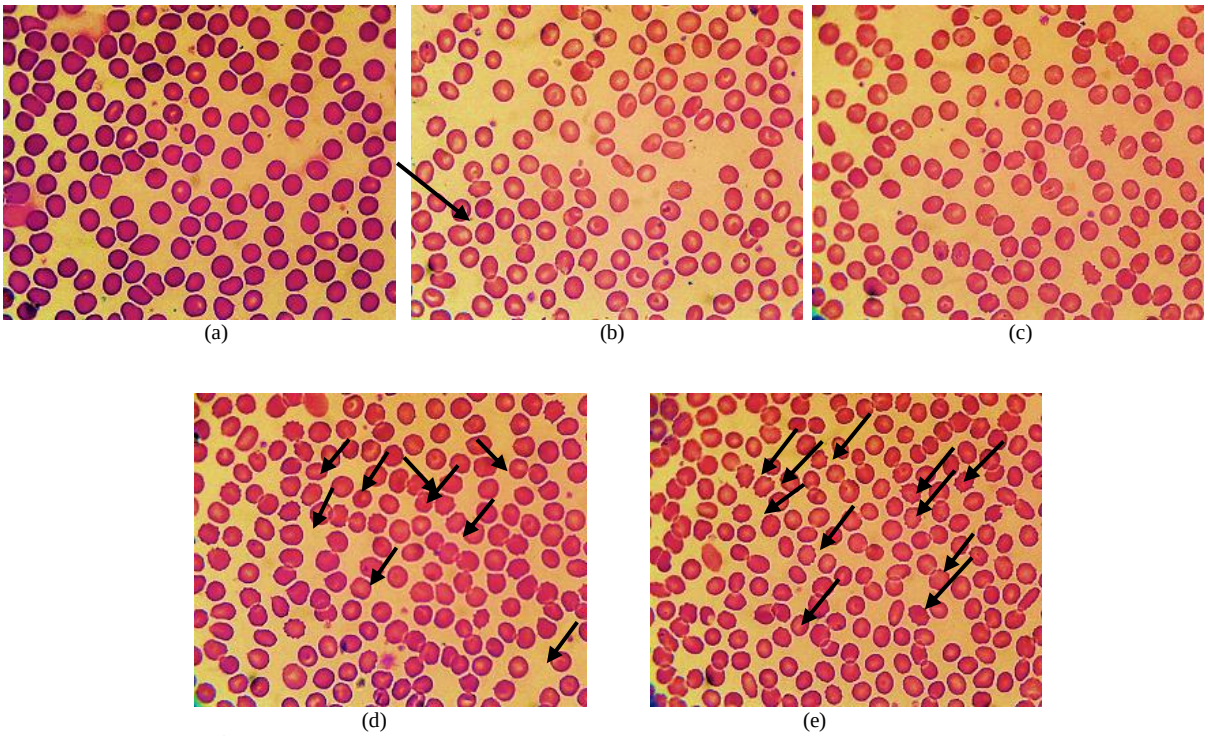

Figure 4. Morphology of sample 4: a) control, b) 3 Gy, c) 6 Gy, d) 9 Gy, e) 12 Gy

The RBCs radii showed a significant decreas after irradiation, compared to their controls, as presented in table 6. The mean RBCs radii were 6.267±0.528, 6.867±0.476, 7.167±0.535, 6.55±0.295 μm for 3, 6, 9, and12 Gy, respectively.

Effects of X-ray doses on erythrocyte sedimentation analysis

Table 7 tabulates the effects of X-ray doses of 3, 6, 9 and 12 Gy on ESR analysis. The results indicated no significant difference between the control and experimental groups in terms of ESR.

Table 6. Comparison of mean radii of red blood cells between control and four experimental groups

Dose (Gy)	Control (mean±SD)	Irradiation (mean±SD)	P-value
3	7.933±0.333	6.267±0.528	<0.0001
6	7.667±0.197	6.867±0.476	0.0035
9	7.867±0.513	7.167±0.535	0.0433
12	8.033±0.242	6.55±0.295	<0.0001

Table 7. Comparison of erythrocyte sedimentation rate between control and four experimental groups

Dose (Gy)	Control (Mean±SD)	Irradiation (Mean±SD)	P-value
3	4.50±1.29	4.25±0.96	0.7663
6	2.75±0.96	3.50±0.58	0.2283
9	4.25±2.87	2.75±1.50	0.3903
12	3.00±1.15	3.25±0.96	0.7502

Discussion

There is an increasing interest in the effects of exposure to ionizing radiation on individuals health status [13]. Different cells respond to ionizing radiations differently due to the amount of absorbed dose and the type of the cell. Generally, this reflects the damage caused to the cell wall, cellular components, and molecular structures [14]. In the current study, no significant changes were observed between the control and four experimental groups in terms of hematological parameters.

In a study investigating the effects of X-ray on leukocytes Sokolov [15] demonstrated that the radiation dose of 90 Gy can cause the leukocytes count to drop by 30% when measured one day following irradiation. However in the mentioned study, no reduction was observed in the number of RBCs following an X-Ray dose of up to 97 Gy, and hemolysis was evident at doses greater than 60 Gy.

In another study conducted by Snyder et al. [16], the results of irradiating platelets with 100 Gy followed by 24 hour storage at 20-24˚C did not induce an increased release of β-thromboglobulin nor enhanced discharge of lactate dehydrogenase. Any variation between the results of current study and the abovementioned ones may be attributed to the difference in the amount of the doses. In general, the in vitro irradiation of human blood cells needs high doses of ionizing radiations in order to alter the cells [17].

It was shown that some characteristics of RBCs, such as the biconcave shape, phospholipid bilayer and cytoskeleton were related to some erythrocyte diseases. Any changes in these characteristics may induce difference between membrane morphology and mechanical properties of the RBC, thereby altering the cells [18], Therefore, these characteristics not only are responsible for the deformation and damage of RBCs, but also alteration in biological function [19, 20]. Some examples of RBC diseases related to erythrocyte dysfunction include malaria, sickle cell anemia, hemolytic anemia, cancer, spherocytosis, elliptocytosis and diabetes mellitus [21-29]. Therefore, there is a need to study the pathophysiology of these diseases is [25, 30, 31]. The formation of the highly active species in the hydrocarbon chains in the bilayer membrane of the RBCs can disturb the packing properties of the phospholipid bilayer macromolecules forming the cell membrane. Variation in the packing properties of these molecules may result in the changes of the intermolecular Van der Waals forces forming the cellular membrane, which in turn alters the membrane permeability and morphology [13].

In our study, the increase of crenation of RBCs with increasing doses and the radius of the cells decreased significantly compared with the controls. Crenation and decreasing of the radius indicate changes in morphology after the irradiation. Schuurhuis et al. [32] investigated that irradiation of RBCs to 450 Gy X-ray their incubation for 4hrs at 37°C produce crenation of RBCs by 26% while in the un-irradiated control cells the produced crenation was by 6%. Irradiation the cells above 450Gy caused aggregation of membrane proteins. Fadel et al. [33] examined how radiation exposure can damage living cells, cause death in some of them and modify others. Green stock CL. and Trivedi A. [34] reviewed the existing techniques to evaluate the radiation exposure by making a correlation between membrane damage and the observed doses of radiation. Shishkina et al. [35] concluded that exposures to low doses of gamma and X-Rays showed a high sensitivity of the characteristics of the lipid metabolism in erythrocyte. Allehayanim et al. [36] studied erythrocytes of rats to investigate the effects of γ-rays in the dose rate range of 0-5.6 Gy on RBCs membrane solubilization by using sodium dodecyl sulfate (SDS). The results showed a shift in the detergent critical concentration of the irradiated dose.In vivo investigation blood films showed irregularly shaped red blood cells, while those photos graphed 10 days post-irradiation revealed some sort of repair. Regarding the fact that red blood cells are not very radiosensitive they cannot considered as biomarker to detect cellular radiation damage in vivo [37]. The RBCs membrane contains polyunsaturated lipid and hemoglobin is considered a source of free radical reaction which may lead to lipid peroxidation. The lipid peroxidation can affect the characteristics of the cell membrane such as fluidity, transport of an ion through the membrane and enzyme activates of the cell.

Membrane oxidation may affect properties of RBCs cell, especially Hb state [38].

The findings of the current study revealed that the radiation doses of 3, 6, 9 and 12 Gy X-ray had no effect on ESR analysis, while the study conducted by Ibrahim [39] showed that ESR could increase with increasing the dose up to 59.94 Gy of gamma ray. The contradictory results of these two studies may be due to the difference in the size of absorbed doses and the radiation types.

Conclusion

Ionizing exposure can result in stem cell injuries of hematopoietic system,one of the radiosensitive systems in the human body. This type damage may lead to the alteration in the production of bone marrow stromal cells. Moreover, it is known that exposure to ionizing radiations has direct determintal effects on blood cells. Blood cells respond to the radiations in a variey of ways which may result in different amounts of the effects. It was observed that the in vitro irradiation of human blood to different X-ray doses (i.e.,3, 6, 9, and 12Gy) resulted in the enhancement of RBC crenation with increasing the dose, and reduction of the cell radius, compared to those in the control group. However, the hematological parameters and ESR analysis were not statistically affected.

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