

The effects of gamma-radiation on red blood cell corpuscles and dimensional properties in rats

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Abstract: In an attempt to understand the toxicity and the potential role of gamma-radiation as a therapeutic tool, the effects of different Gamma-radiation doses on haematological and dimensional properties of rats' blood were investigated *in vivo*. 60 healthy male Wistar-Kyoto rats were used, which were randomly divided into five groups, 4 Gamma-radiated rat groups (1st group was radiated with five Gamma-radiation dose, 2nd group 25Gy; 3rd group with 50Gy, 4th group with 100Gy, and 5th group was control). Different haematological and dimensional parameters were measured using the standard haematological technique. A significant decrease in red blood cells (RBCs) count, haemoglobin (HGB), and haematocrit (HCT) was observed compared with the control. While a significant increase in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), red distribution width (RDW) were observed compared with the control. This study suggested that low RBCs, HGB, and HCT might produce anemia and cessation of erythrocytes production in the bone marrow. Moreover, the RBCs size increase might be attributed to changes in the morphology and deformability of RBCs, which was confirmed by a slightly increase in RDW.

Keywords: Gamma-radiation, In-vivo, RBCs, HGB, HCT, MCV, MCH, MCHC, RDW.

INTRODUCTION

The blood is circulating in the human body, transferring oxygen to all the important organs of the body. The RBCs are suspended in the blood plasma. The blood is crucial for removing the waste products (e.g. carbon dioxide, lactic acid, urea, etc.) from the same cells and tissues, it is responsible for the immune system, clotting, and the regulation of body temperature and many other functions (Karsheva, *et al.*, 2009).

The setting up of large number of nuclear reactors for power generation has resulted in additional sources of radiation exposure in the form of nuclear wastes. The discovery of radioactive rays has proved a milestone in the history of medical research. Numerous studies on the radio-exposure have yielded plenty of information on its biological effects on the living systems at the molecular, cellular, neuro-endocrine and haematological levels (Daga *et al.*, 1995).

Earlier it has not been understood, nor appreciated that how a radiation could alter the life of an organism. It became apparent that, the radiation can penetrate the living cells and deposit within them in a random fashion, leading to radiation damage (Heda and Bhatia, 1986; Jacob and Jagetia, 1992).

The haematocrit gives valuable information about the

changes in the number and dimension of RBCs as well as about the volume of the blood plasma. Its value decreases not only with the decrease of the total RBCs number, but also when more liquid enters into the intravasale space (hydration). In contrary, the increase in its value is observed when the organism loses liquids, so decreasing the plasma volume (dehydration due to exposure to Gamma-radiation). The blood viscosity increases rapidly with the haematocrit increase (Karsheva *et al.*, 2009).

The changes in RBCs count still considered the most sensitive biological evidences for excessive acute exposure to both internal and external radiation. A complete CBC gives important information about kinds and numbers of cells in the blood, especially RBCs. CBC helps in diagnosing conditions, such as anemia, infection, and many other disorders (Jacobson, 1954; Jacob and Jagetia, 1992; Kumar *et al.*, 1984). In medicine, there is a limited knowledge on the haematological indices, the alterations induced by the different gamma-radiation doses. This study not only aimed to involve routine haematological indices measurement but also to identify the dimensional parameters of rat blood *in vivo*.

MATERIALS AND METHODS

Experimental protocol

A total of 60 healthy male Wistar-Kyoto rats were used in this study. Animals were randomly divided into five groups. Four rat group were n = 10 and were exposed to Gamma-radiated as follow: (1st group was irradiated with

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5Gy, 2nd group was radiated with 25Gy, 3rd group was radiated with 50Gy, 4th group was radiated with 100Gy, and 5th control group (NG: n = 20). The four rat group under Gamma-radiated were maintained on standard laboratory rodent diet pellets and housed in humidity and temperature-controlled ventilated cages for a period of 24hr day/night cycle. The different haematological parameters were measured using the standard haematological techniques. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. Blood sample of nearly 2ml collected into EDTA-polypropylene tubes for whole blood. All experiments conducted in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

Haematological and dimensional measurements

A haematological auto-analyzer (Orphee Mythic 22 Haematological Analyzer; Diamond Diagnostic; USA) were used to determine different haematological and dimensional parameters. These parameters were RBCs, HB, HCT, MCV, MCH, MCHC, RDW, PDW%, and PCT%.

STATISTICAL ANALYSIS

The results of this study were expressed as mean \pm standard Error (Mean \pm SE). To assess the significance of the differences between the control group and the four Gamma-radiated rat groups (5, 25, 50 and 100Gy), a statistical analysis were performed using one-way analysis of variance (ANOVA) for repeated measurements, with significance assessed at the 5% confidence level.

RESULTS

RBCs count, HGB, and HCT showed a significant decrease with the different Gamma-radiation doses 5, 25, 50 and 100Gy compared with the control (table 1).

The dimensional blood indices MCH, MCHC, MCV and RDW showed a significant increase with the different Gamma-radiation doses compared with the control (table 1).

DISCUSSION

This study not only was aimed to assess the Gamma-radiation effects on haematological properties, but also to identify the dimensional properties of rat blood *in vivo* induced by Gamma-radiation. Table 1 showed significant decreases in RBCs count with 5, 25, 50 and 100Gy Gamma-radiation doses compared with the control. A low RBCs count or low HGB or HCT may suggest anemia, which can be attributed to many causes. The damage effect of Gamma-radiation on RBCs count may be

attributed to the cessation of erythrocytic production in bone marrow, the loss of cells from the circulation by haemorrhage or leakage through capillary walls and/or direct destruction of mature circulation cells (Chlebovsky *et al.*, 1983, Abdel-Gawad *et al.*, 2003, Manisha *et al.*, 2011, sharma *et al.*, 2012 and El-Deeb *et al.*, 2006. Selim, 2010 and Nikishkin *et al.*, 1992), reported that increased permeability in the haemolytic process and the erythrocytic membrane stability was major cause of the expressed drop in RBCs count following Gamma-radiation. However, haemolysis was the major cause of the developed anemia following whole body Gamma-radiation and no decrease in erythropoiesis was evident. After whole-body exposure to Gamma-radiation, injury to animal tissues well reflected in the peripheral blood (Down *et al.*, 1990; Kumar *et al.*, 1984; Shaheen and Hassan, 1991).

It is generally agreed that Haematopoietic organs, i.e., spleen, thymus and bone marrow are markedly sensitive to the ionizing radiation (Ellinger, 1957). The clinical symptoms, which are largely due to damage in the radiosensitive haematopoietic organs (Heineke, 1904), a very small dose of radiations to a blood-forming organ causes an arrest of the haematopoiesis with changes in the peripheral blood (Sharma *et al.*, 2012).

The increase in the haemoglobin release from the RBCs with a concomitant decrease in the haemoglobin concentration as the dose increases. In addition, they have illustrated the increasing damage in the cell membrane with the dose (Selim, 2010). The reduction in haemoglobin due to Gamma-radiation may be attributed to the incorporation of iron into haemoglobin due to disturbance in the biogeneration structure of the haemoglobin molecule as evidenced by pronounced hyperferraemia post-irradiation (Saad El-Din *et al.*, 1988) and oxidation of haemoglobin molecule (Szweda-Lewandowska *et al.*, 1976).

Stohlman *et al.* (1957), reported that the decrease in RBCs count may be due to defective haemopoiesis as well as intravascular RBCs damage. According to the kinetics of the RBCs, the fall in RBCs count after irradiation might be due to changes in plasma volume, leakage of cells through capillaries secondary to thrombopenia and in some instances, by severe haemorrhage (Bond *et al.*, 1965). However, at higher doses the bone marrow also suffers from hypoplasia (Saini, 1977). Our results indicated a significant decrease in HCT level. The decreased HCT values are observed when the globular level decreases, a case similar to the anemia disease (Karsheva, *et al.*, 2009).

The dimensional blood indices include MCH, MCHC, MCV, RDW and PDW. An increase in MCH, MCHC, MCV and RDW with 5, 25, 50 and 100Gy Gamma-radiation doses were observed compared with the control

Table 1: Red blood cells (RBCs) count and Blood indices Hb, MCH, MCHC, MCV, and RDW compared with different gamma radiation doses

Blood Indices	Normal	5Gy	25Gy	50Gy	100Gy
Red blood cells count (RBCs) x 10 ³ /μl	8.45 ± 0.02	6.95 ± 0.03	7.30 ± 0.07	6.92 ± 0.03	6.40 ± 0.03
Haemoglobin (Hb)	14.21 ± 0.16	12.48 ± 0.34*	13.17 ± 0.33*	12.61 ± 0.30*	12.98 ± 0.21*
Haematocrit (HCT %)	43.76 ± 0.51	37.71 ± 0.88*	39.41 ± 0.99*	38.58 ± 0.98*	38.64 ± 0.57*
Mean Corpuscular Hemoglobin (MCH)	16.93 ± 0.24	18.22 ± 0.66*	17.69 ± 0.49*	18.43 ± 0.34*	19.22 ± 0.31*
Mean Corpuscular Haemoglobin Concentration (MCHC)	32.55 ± 0.17	33.1 ± 0.18*	33.42 ± 0.17*	32.7 ± 0.21*	33.58 ± 0.16*
Mean Corpuscular Volume (MCV) (μm) ³	51.98 ± 0.82	55.19 ± 1.94*	52.94 ± 1.53*	56.43 ± 1.24*	57.16 ± 0.83*
Red Blood Cell Distribution Width (RDW%)	15.65 ± 0.47	17.63 ± 0.58*	16.59 ± 1.07*	18.8 ± 0.97*	16.15 ± 0.79*

All results are represented as M ± SE [* means that the means are significantly different (p<0.05)]

while a decrease in PDW was observed. The increase in size of RBCs indicates changes in morphology and deformability of RBCs, which confirmed by slightly increase in RDW%. The PLTs count decreased with the 5, 25, 50 and 100Gy Gamma-radiation doses compared with the control. This study suggests that additional experiments will be performed taking in consideration the morphology and deformability changes in RBCs, inflammatory infection, toxic reactions, bone marrow problem and disturbance in the function of immune system efficiency after irradiating the rats with different gamma radiation doses *in vivo*.

CONCLUSIONS

RBCs count, HGB, and HCT showed a significant decrease with the different Gamma-radiation doses compared with the control. A low RBCs count or low HGB or HCT may suggest anemia. The damages effect of Gamma-radiation on RBCs count may be attributed to the cessation of erythrocytes' production in the bone marrow, the loss of cells from the circulation by haemorrhage or leakage through the capillary walls, and the direct destruction of mature circulation cells. The dimensional blood indices MCH, MCHC, MCV and RDW showed a significant increase with the different Gamma-radiation doses compared with the control while a decrease in PDW was observed. The increase in size of RBCs indicates changes in morphology and deformability of RBCs, which confirmed by slightly increase in the red cell distribution width RDW%.

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