Effect of Gamma Radiation on CBC parameters of Male Guinea Pigs

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Abstract

This study examined the impact of gamma radiation on hematological parameters of male guinea pigs. Comparison between exposed and unexposed guinea pigs to acute gamma radiation dose (9 kGy) showed that there was great significant effect of gamma radiation on WBCs count, MCHC, HCT, Hb concentration, MCH, MCV and RDW-CV (%) (p-value $\leq$ 0.05).The most severe effect of gamma radiation was on the WBCs count. The results showed also that the percentage standard deviation of these parameters in the exposed group was higher than that of non-exposed.

Gamma radiation had no significant effect on RBCs count, platelet count or PDW-CV (%) since P -value is greater than 0.05. The results showed also that their percentage standard deviations values were near.

Keywords: *Complete Blood Count (CBC);White Blood Cells (WBC); Platelets (PLT) Count; Ionizing Radiation; Free radical; Male guinea pigs***.**

1. Introduction

The increasing use of nuclear technology either in medicine, industry and other applications increased the risk of exposure to radiation. As a result, exposure to radiation induced harms on human beings. These harms are considered to be due to free radicals that formed by water [1].

The main products of water radiolysis are hydrogen radical ($H^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), e^{-}_{aq}, hydrogen (H_2), and hydrogen peroxide (H_2O_2). Hydrogen peroxide and hydroxyl radical act as oxidizing agents, while hydrogen radical and solvated electrons act as reducing agents. In presence of oxygen, hydrogen radical reacts with oxygen to produce $HO_2^{\bullet}$ as well as with solvated electrons to produce O_2^{-} which are strongly oxidizing species [2].

The mechanism of radiation effect on rats was suggested to be due to interaction of formed free radicals with cells compounds with subsequent induced damage. Also radiation altered cellular metabolism [3].

The blood forming organs are one of the most sensitive to radiation and in some cases bone marrow transplantation is required to produce new blood cells. Reproducing cells also are most sensitive for radiation and the presence of oxygen increases its sensitivity to radiation. Anoxic cells (as interior cells of a tumor) are less affected. When people are exposed to high radiation doses, blood count changes and death may occur due to acute radiation syndrome. At doses more than 50 Gy, the central nervous system (brain and muscles) can no longer control breathing and blood circulation. Radiation causes ionizations of atoms which affect molecules, cells, tissues, organs and the whole body. The risk increases with the exposed radiation dose [4].

Comparison between exposed and unexposed workers to radiation showed significant decrease in MCH, platelet-count, hematocrit and lymphocytes [5].

When adult male rats were exposed to gamma radiation, the apparent viscosity increased due to cell aggregation in doses up to 7Gy and decreased at higher doses. The reason was attributed to the decrease of membrane surface charge after exposure that facilitated aggregation [6].

Exposure of rats to electromagnetic rays of mobile phone showed a decrease in hemoglobin, hematocrit, red blood cells count, and platelets count. The average number of white cells and lymphocytes increased. Also the viscosity increased due to cells adhesion. Variation in the cell sizes was observed [7].

During exposure of human to X-ray, PDW (platelet distribution width) and P-LCR (platelet large cell ratio) did not show any significant difference. The study showed that long-term exposure to low X-ray might have toxic effects on thrombocytes and coagulation function [8].

Cancer patients treated by radiation showed significant difference in WBCs and RBCs count.

Bone-marrow radiation can cause damage of hematopoietic stem cells (HSCs) and produce bone-marrow syndrome [9].

There was significant difference between radiation-exposed people to uranium radiation in mine and unexposed people. There was a decrease in hemoglobin (HB), white blood cells (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets(PLT), hematocrit (HCT) and neutrophils (NEUT). There was an increase in red blood cells (RBC) and lymphocyte count (LYM) [10].

At 25 to 50 Gy dose of radiation, bullfrog erythrocytes were decreased, swollen, have bulging nuclei, and cytological abnormalities. High radiation dose caused aggregation of membrane proteins and destruction of lipoprotein. Aggregation of membrane proteins may be due to disulfide bridges. Destruction of lipoprotein, the basic structural unit of the membrane, increased permeability of red cells to water and ions. Exposure to γ-rays or proton radiation induces decreases in total white blood cells (WBCs) and lymphocyte counts [11].

2. Materials and Methods

2.1 Animals

Twenty healthy male guinea pigs were used in this study. Animals were housed in a temperature of 24 ± 3 °C, with free access to food and water under good supervision. Examinations were carried out following the "Principles of Laboratory Animal Care" in the Faculty of Veterinary Medicine, Cairo University.

The animals were maintained under care conditions. They were divided into 2 equal groups, control (n=10) and irradiated (n=10). The irradiated group was exposed to acute gamma radiation dose (9 kGy). The source of gamma radiation was 60Co source, Research Model machine, Atomic Energy of India Ltd., Commercial Products Division. It has dose rate of 9.5 KGy/h. The available chamber volume equals 5L and the weight of cell was 5600 Kg.

2.2 Collection of Blood Samples

Blood samples were collected from the external jugular vein soon after radiation and immediately placed in tubes containing EDTA and these samples were used in assessment of blood profile.

The collected blood was mixed well and inserted into the following machine: Italian Hematology Analyzer, PDW and PCT for Q.C. and CBC parameters were analyzed. Ten CBC parameters were considered for this study and these were: red blood cells (RBC in 10^6/ mm^3),hemoglobin (HB in g/dl), white blood cells (WBC in 10^3/mm3), platelet count (PLT in 10^3/ mm3), hematocrit (HCT in %), mean corpuscular volume (MCV in fl), mean corpuscular hemoglobin (MCH in g/dl), mean corpuscular hemoglobin concentration (MCHC in %), relative distribution width of red blood cells by volume coefficient of variation (RDW-CV in %) and relative width of the distribution of platelets in volume index of the heterogeneity of platelets (PDW-CV in %) were analyzed in the two groups.

2.3 Functional method

Guinea pigs were anesthetized by Ether (Merck, Germany) in a particular lacuna.

2.4 Statistical analysis

Statistical Package for Social Sciences (SPSS) version 17.0 was employed for statistical calculations and data analysis. All of ten CBC parameters are represented as means ± standard deviation (%). Results were analyzed using independent t-test to analyze those parameters which show significant difference between radiation exposed and radiation unexposed guinea pigs. Confidence level was set at $p \leq 0.05$.

3. Results and Discussion

Table (1) showed that there is no significant effect of gamma radiation on RBCs count ($p > 0.05$). The number of RBCs of group II is higher than that of group I (108.05%) as found by other authors [10, 11].Variation in the number of RBCs are increased (156.26%).

Table (1): Comparison between the control and radiated Guinea pigs in RBCs count $x10^6/mm^3$
(Normal: 4.5-7.0 $x10^6/mm^3$).

SN	Group I Control N=10	Group II Radiated N=10	
1	5.110	6.040	Independent t-test P-value= 0.229
2	4.640	3.610	
3	5.750	6.310	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 108.05%
4	5.600	4.960	
5	4.200	5.230	
6	4.330	5.500	
7	4.180	4.500	$\frac{\sigma\text{ \% Radiated}}{\sigma\text{ \% Control}}$ = 156.26%
8	4.320	4.300	
9	4.200	4.700	
10	4.590	4.800	
Mean±SD (%)	4.623 ±0.480 (10.38 %)	4.995 ±0.8 (16.22 %)	

WBCs are markedly decreased in the irradiated group (4.72%) as shown in table (2). There is great significant effect of gamma radiation on WBC ($p \leq 0.05$). % SDII (56.03 %) is higher than % SDI (25.37 %) by 220.58%. Similar results were obtained [5, 9]. The significant decrease in the number of WBCs is due to bone-marrow radiation injury. It was reported

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that high radiation dose can damage hematopoietic stem cells (HSCs) and produce bone-marrow syndrome [9].

Table (2): Comparison between the control and radiated Guinea pigs in WBCs count x 10^3 / mm^3
(Normal: 4.5-11.0 $x10^3/mm^3$).

SN	Group I Control N=10	Group II Radiated N=10	Independent t-test
1	7.800	0.100	P-value = 0.00
2	8.100	0.700	
3	6.700	0.100	
4	9.800	0.200	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 4.72%
5	7.900	0.275	
6	7.500	0.450	
7	9.200	0.520	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ = 220.58%
8	4.800	0.490	
9	3.700	0.380	
10	8.200	0.260	
Mean ± SD (%)	7.370 ±1.870 (25.37%)	0.348 ±0.195 (56.03%)	

From table (3), the hemoglobin (Hb) level is markedly decreased in the irradiated group (44.50 %). There is great significant effect of gamma radiation on Hb ($p \leq 0.05$). % SDII (26.58 %) is higher than % SDI(7.96 %) by 333.92%.Similar results are shown due to destructive effect of radiation [7].

Table (3): Comparison between the control and radiated Guinea pigs in Hb concentration (Normal:11 - 15 g/dl).

SN	Group I Control (g/dl)	Group II Radiated (g/dl)	Independent t-test
1	13.50	7.30	P-value = 00.00
2	12.90	2.60	
3	13.20	7.90	
4	14.20	5.40	
5	11.50	5.80	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 44.50 %
6	11.80	4.90	
7	11.40	5.10	
8	11.80	6.20	
9	11.40	5.60	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ =333.92 %
10	12.60	4.50	
Mean ± SD (%)	12.43 ±0.99 (7.96%)	5.53 ±1.47 (26.58%)	

The hematocrit (HCT) value in table (4) is markedly decreased in the irradiated group (43.40 %).There is great significant effect of gamma radiation on HCT ($p \leq 0.05$). % SDII (26.21 %) is higher than % SDI(2.70 %) by 970.74%. Similar results are shown due to destructive effect of radiation [10].

Table (4): Comparison between the control and radiated Guinea pigs in hematocrit (HCT) value (Normal: 37 - 48 %).

SN	Group I Control (%)	Group II Radiated (%))	Independent t-test
1	38.30	22.90	P-value = 00.00
2	40.50	8.90	
3	39.10	24.80	
4	41.80	17.90	
5	38.90	17.10	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 43.40 %
6	39.10	14.80	
7	38.70	15.50	
8	39.90	18.80	
9	38.90	17.20	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ =970.74 %
10	40.40	13.80	
Mean ± SD (%)	39.56 ±1.07 (2.70%)	17.17 ±4.50 (26.21%)	

Table (5) showed that there is significant effect of gamma radiation on MCV ($p \leq 0.05$), while there is no significant effect of gamma radiation on RBCs count (Table 1). The MCV value is decreased in the irradiated group (96.69 %). % SDII (3.30 %) is near % SDI (2.83 %) by 116.61%.

Table (5): Comparison between the control and radiated Guinea pigs in MCV (Normal: 37.8 - 56.0 fl)

SN	Group I Control (fl)	Group II Radiated (fl)	Independent t-test
1	38.50	37.80	P-value = 0.025
2	38.20	36.40	
3	39.50	39.10	
4	40.10	37.70	
5	39.40	38.90	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 96.69 %
6	41.20	39.40	
7	37.90	36.60	
8	38.20	37.70	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ =116.61 %
9	37.80	35.60	
10	38.40	37.10	
Mean ± SD (%)	38.92 ±1.102 (2.83%)	37.63 ±1.24 (3.30%)	

The MCH (content of hemoglobin in single erythrocytes in absolute units) is decreased in the irradiated group (58.55 %) with great significant effect of gamma radiation on MCH ($p \leq 0.05$) as shown in table (6). % SDII (14.90 %) is higher than % SDI (6.73 %) by 221.40%.
MCH is proportional to the relative "hemoglobin / red blood cells number". MCH is decreased due to marked decrease of the hemoglobin (Hb) level in the irradiated group (44.50 %)as shown in table (3) and no significant change on the RBCs count (Table 1). Similar results are shown due to destructive effect of radiation [10].

Table (6): Comparison between the control and radiated Guinea pigs in MCH (Normal:14.2 - 20.1 pg)

SN	Group I Control (pg)	Group II Radiated (pg)	Independent t-test
1	16.40	12.10	P-value = 00.00
2	16.70	7.30	
3	16.10	12.50	
4	17.90	8.90	
5	19.30	10.70	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 58.55%
6	19.10	9.80	
7	18.80	10.10	
8	17.40	11.20	
9	16.50	10.60	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ =221.40%
10	17.20	9.50	
Mean± SD (%)	17.54 ±1.180 (6.73%)	10.27 ±1.53 (14.90%)	

The MCHC (mean concentration of hemoglobin in erythrocytes) in table (7) is decreased in the irradiated group (40.13 %) with great significant effect of gamma radiation on MCHC ($p \leq 0.05$). % SDII (13.95 %) is higher than % SDI (1.68 %) by 830.36%. Similar results were shown due to destructive effect of radiation [10].

Table (7): Comparison between the control and radiated Guinea pigs in MCHC (Normal: 31.7- 40.4 g/dl).

SN	Group I Control (g/dl)	Group II Radiated (g/dl)	Independent t-test
1	35.20	16.30	P-value = 00.00
2	36.30	10.20	
3	35.60	17.20	
4	36.27	15.10	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 40.13%
5	37.20	14.20	
6	36.30	12.60	
7	36.90	13.70	
8	35.70	14.80	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ =830.36%
9	36.50	15.30	
10	36.60	16.10	
Mean± SD (%)	36.26 ±0.61 (1.68%)	14.55 ±2.03 (13.95%)	

MCHC is proportional to the relative "hemoglobin/ red blood cell volume". MCHC is decreased due to marked decrease of the hemoglobin (Hb) level in the irradiated group (44.50 %) as shown in table (3) with slight significant decrease of MCV (96.69%) as shown in table (5).

The number of platelets (PLTs)of group II in table (8) is less than that of group I (89.04 %) but there is no significant effect of gamma radiation on PLTs count ($p > 0.05$). % SDII (25.92 %) is higher than % SDI (16.32 %) by 158.82 %.Similar results are shown [10]. This may be due to platelets adhesion. Similar results of cell adhesion were obtained [7].

Table (8): Comparison between the control and radiated Guinea pigs in platelet (PLT) count x 10^3/ mm^3 (Normal: 250 - 850 x10^3/mm^3).

SN	Group I Control	Group II Radiated	Independent t-test
1	441	516	P-value = 0.232
2	440	246	
3	402	307	
4	471	300	
5	394	342	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 89.04 %
6	388	410	
7	322	490	
8	470	385	
9	382	520	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ =158.82 %
10	580	299	
Mean ± SD (%)	4.29 ±0.70 16.32%)	3.82 ±0.99 (25.92%)	

RDW-CV is the relative distribution width of red blood cells by volume, coefficient of variation. It is an indicator of heterogeneity of red blood cells. Table (9) showed that RDW-CV is higher in the irradiated guinea pigs (117.91 %) with great significant effect of gamma radiation on RDW-CV (%) ($p \leq 0.05$). % SDII is higher than % SDI by 184.43%. Similar results are shown due to effect of radiation [10].

Table (9): Comparison between the control and radiated Guinea pigs in RDW-CV (%).

SN	Group I Control (%)	Group II Radiated (%)	Independent t-test
1	12.10	16.10	P-value = 00.00
2	12.30	17.30	
3	13.10	13.50	
4	12.90	13.60	
5	12.30	15.13	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 117.91%
6	13.90	14.20	
7	11.90	15.20	
8	12.70	13.90	
9	13.50	16.40	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ =184.43%
10	12.60	14.80	
Mean± SD (%)	12.73 ±0.63 (4.95%)	15.01 ±1.27 (8.46 %)	

Table (5) showed that the percentage standard deviation of MCV "anisocytosis" is increased by 116.61% and MCV mean is slightly decreased by (96.69%) in the irradiated group. Since RDW-CV is directly proportional to MCV standard deviation and inversely proportional to MCV mean, RDW-CV is increased.

Table (10) showed that there is no effect of gamma radiation on PDW-CV (%) ($p > 0.05$). % SDII (4.54 %) is higher than % SDI (3.19 %) by 142.63%. Similar results are shown due to effect of radiation [10].

Table (10): Comparison between the control and radiated Guinea pigs in PDW-CV (%).

SN	Group I Control (%)	Group II Radiated (%)	Independent t-test
1	28.70	27.70	
2	28.60	31.30	P-value = 0.365
3	28.30	30.30	
4	30.50	28.10	
5	28.40	29.35	
6	28.60	30.60	$\frac{\text{Mean (Radiated)}}{\text{Mean (Control)}}$ = 101.67 %
7	28.10	27.40	
8	28.00	29.60	
9	28.50	28.40	
10	30.50	30.20	$\frac{\sigma \text{ \% Radiated}}{\sigma \text{ \% Control}}$ =142.63 %
Mean ± SD (%)	28.82 ±0.92 (3.19 %)	29.30 ±1.33 (4.54 %)	

PDW-CV "coefficient of variation" is the relative distribution width of platelets by volume (%). PDW is the relative width of the distribution of platelets in volume as an index of the heterogeneity of platelets.

Figure (1) shows that gamma radiation dose 9 KGy for guinea pigs induced significant decrease in WBCs count, MCHC, HCT, Hb concentration, MCH and MCV, significant increase in RDW-CV while it had no significant effect on RBCs count, platelet count and PDW-CV. The most severe effect of radiation was on the WBCs count (p-value ≤ 0.05).

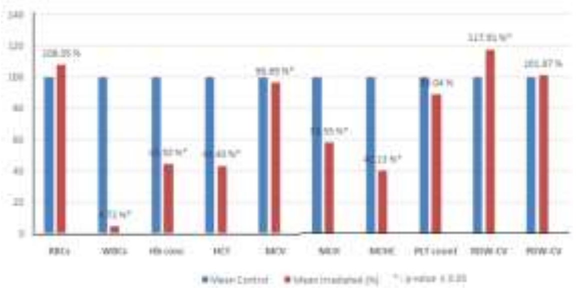

Figure (1): Comparison between the mean controls to the mean (%) irradiated for CBC parameters in male Guinea pigs.

4. Conclusion

Gamma radiation had great significant effect on WBCs count, MCHC, HCT, Hb concentration, MCH, MCV and RDW-CV (%) (p-value ≤ 0.05). The most severe effect of gamma radiation was on the WBCs count. Gamma radiation had no significant effect on RBCs count, platelet count or PDW-CV (%) since (P -value > 0.05).

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