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Effect of Low Dose (Diagnostic X-Rays) on Peripheral White Blood Cells Count in Guinea Pigs (*Cavia porcellus*)

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Abstract

Exposure to ionizing radiation is known to affect some hematological parameters of biological sample. This study was aimed at evaluating the effect of ionizing radiation within the diagnostic range on some hematological parameters in guinea pigs. Thirty six (36) apparently healthy adult guinea pigs of both sexes weighing between 700 and 1200 g were used. The guinea pigs were categorized into three groups, 12 per group; group A (control), group B, and C were exposed to X-rays within the diagnostic range, using 70 kV and 12.5mAs; using X-ray machine MS-185, serial no. 0904 GE at a source to skin distance (SSD) of 90 cm. Blood samples were collected from all the guinea pigs at intervals of 1, 24, 72, 168 and 336 hours post-irradiation, and subjected to standard hematological analysis. A continuous decline in the mean total white blood cell count and mean lymphocyte, monocyte, neutrophil and eosinophil count after 1 hour in both groups was observed, and more pronounced after 24 hours post-irradiation. However, stability was observed 72 hours post-irradiation in both groups. In conclusion, a depleting effect of low dose ionizing radiation on white blood cell count was found, with appreciable recovery occurring after 72 hours onward.

Keywords: white blood cell, irradiation, guinea pigs, ionizing radiation, hematological parameters

1. Introduction

Radiation is a wave or particle traveling through space which can transmit all or parts of its energy on contact with matter [1]. It could be ionizing and non-ionizing in nature [2].

Ionizing radiation is a very high-energy form of electro-magnetic radiation which has the energetic potential to break apart electrically neutral atoms resulting in the production of negative and/or positive ions [3]. Non ionizing radiation is relatively a low-energy radiation that does not have sufficient energy to ionize atoms or molecules [4]. Although considered less dangerous than ionizing radiation, over exposure to non-ionizing radiation can also be hazardous [4].

Exposure to radiation results in a deposition of energy in tissues that can damage cellular structures including DNA [5]. The degree of the damage due to the radiation depends on the type of radiation, energy of the radiation, intensity of the radiation, and exposure time [6]. Depending on the duration of exposure, the area exposed and the dose received, radiation exposure in the immediate aftermath could lead to a myriad of deleterious effects including acute radiation syndrome [7]. Acute radiation syndrome (ARS) includes hematopoietic syndrome, gastro-intestinal syndrome and cardiovascular/central nervous system syndrome among others. Hematopoietic syndrome may occur after exposure to significant radiation dose and all blood components may be affected adversely [8]. Blood being a vital special circulating tissue composed of cells suspended in a fluid (plasma) with a major function of maintaining homeostasis [9] may experience decline in cell count on exposure to ionizing radiation leading to drop in circulating blood cells which is detrimental to the health of the individual [10]. The white blood cells also called leukocytes are the mobile units of the body's protective system [11]. Decrease in the WBC count leaves the individual at risk of infection. Low WBC count is known as leucopenia.

Guinea pigs are rodents of the family Caviidae and the genus *Cavia* which are mostly kept as pets and also used as laboratory animals for biomedical experiments. *Cavia porcellus* are small stout-bodied short-eared tailless domesticated rodent of South American origin [12]. They are not related to swine neither are they from Guinea Republic [13]. They are used for meat, local medicine and play important roles in religious and cultural ceremonies especially in South America [13]. Guinea pigs are used for biomedical research because they are biologically similar to humans as they share more than 90% DNA with humans, diseases that affect humans are also likely to affect them and they have shorter life span making it possible for them to be studied throughout their life time and they also are easy to handle [14].

Ionizing radiation is widely used in the medical field for both diagnostic and therapeutic purposes in form of X-rays, gamma rays, and particles (α -particle, β -particle, protons and neutrons) radiations [15].

The biological effect of ionizing radiation arises from the deposition energy in the tissues which can cause changes in the chemical composition of the cell. The energy of the ionizing radiation is significantly greater than the bond energies of many molecules and can cause homolytic bond scission and generation of secondary electrons [6]. Ionizing radiation is thus seen to affect biological tissues by directly dissociating molecules following their excitation and ionization, or indirectly by the production of free radicals and hydrogen peroxide in the water of the body fluids [15], and the severity of the effect increases with dose and dose rate [16].

Although the use of ionizing radiation involves a certain level of risk, its use in medicine results in such numerous benefits that if judiciously employed, the benefits greatly exceed the risk to the individual [17]. The hematopoietic system is highly sensitive to radiation, and peripheral blood examination may serve as a biological indicator of such damage that may occur even at very low doses of ionizing radiations like X-rays or gamma rays [15, 18]. Peripheral blood examination may serve as a screening test for various hematological as well as non-hematological disease states [18]. Radiographic imaging is extremely valuable as a diagnostic and therapeutic tool in medicine, but ionizing radiation also carries well-known potential risk [19]. It is generally known that exposure to high energy ionizing radiation like X-ray is known to have effect on rapidly dividing cells of the bone marrow, blood, and mucosal linings. Often, patients are required to undergo repeated exposure which usually increases the risk of damage by ionizing radiation damage on the hematopoietic system [17]. There is no known safe period for the patient to undergo repeated exposure with less or minimal risk to ionizing radiation. There is dearth of information on the studies bordering on the Effect of Ionizing Radiation on White blood cells within the locality of study.

The white blood cells fights off infections and defends the body against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response [20]. Decrease in the white blood cell count leaves the individual at risk of infection. Low white blood cell count is known as leucopenia [11]. It has been observed that there is always a slight decrease in the total white cells count after the first few days of exposure to ionizing radiation; hence, white blood cells count may be a reliable indicator of degree of exposure [17]. Irradiating animal models to a single whole-body dose of ionizing radiation result in complex sets of symptoms whose onset, nature, and severity are functions of both total radiation dose and radiation quality which are classified into three syndromes: the hematopoietic syndrome, the gastrointestinal syndrome, and the central nervous syndrome. The hematopoietic syndrome occurs at very low radiation doses and is manifested by depletion of hematopoietic stem cells and ultimately by depletion of matured hematopoietic and immune cells [21]. This study was aimed at observing the changes that may occur on the white blood cells counts after exposure to low dose ionizing radiation (X-rays) within the diagnostic range, using guinea pigs (*Cavia porcellus*) as animal sample.

1.1. White blood cells (WBC)

The white blood cells also known as leukocytes make up approximately 1% of the total volume of the cells in the blood [22]. The WBCs are primarily involved in the immune response and defense of the body. The WBCs differs from the red blood cells (RBC) as they do not have nuclei and do not contain hemoglobin [11, 23]. The WBCs are formed in the bone marrow and lymph tissue which are then transported to different locations of the body where it is needed. The number of WBCs in the blood is often an indicator of disease, significant increase in the number is known as leukocytosis and significant decrease is called leucopenia [22].

1.1.1. Classification

There five types of WBCs which are classified into two major groups: granular and agranular WBC [24]. The granular WBCs are: neutrophils, eosinophils, and basophils. The granulocytes are characterized by a lobed nucleus and granular inclusions in the cytoplasm. Granulocytes are typically first-responders during injury or infection [11, 23]. The agranular WBCs are; lymphocytes and monocytes. The lymphocytes include B and T cells and are responsible for adaptive immune response. The monocytes differentiate into macrophages and dendritic cells, which in turn respond to infection or injury [11, 23].

1.2. Biologic effect of radiation

Soon after the discovery X-rays and radioactivity it became evident that ionizing radiation could cause damage to cells and tissues [16]. For risk estimation, scientists presently rely on molecular, cellular and animal experiments. The immediate effect of ionizing radiation is directly cellular damage through ionization, excitations and indirect damage by formation of radicals that initiate chemical reactions occur within a very short period following exposure. Subsequently these effects induce changes at the level of molecules (e.g., DNA) [25]. The interaction of radiation and the tissue is governed by the energy and mass of the incident radiation (alpha, beta particle, gamma ray or X-ray) and the properties of the tissue [26]. If the damage is not or not correctly repaired, cell, tissues and finally the whole organism may be affected. Above small doses (few grays), cell death is the dominant effect, which may cause severe damage to organs and tissues [25]. Other effects occur long after the exposure and involve the risk of developing radiation-induced cancer and hereditary disease in the offspring of following generations of the exposed persons.

1.2.1. Deterministic effect

Radiation kills cells at high exposures. Low numbers of dead cells will usually be replaced through cell division in a tissue or organ, but if the numbers of killed cells is too large, harm occurs to the tissue or organ [25]. The deterministic effects occur at high dose level, in which below the dose the effect will not be observed. The severity of the effect increases with dose and dose rate [25]. Fortunately deterministic effects are perceived at relatively high doses are there hardly observed in diagnostic radiology because of the low doses used. Exceptions are incident with deterministic radiation induced skin injury after prolong fluoroscopy-guided procedures. Most deterministic effects come early to expression even though some can occur later [25].

1.2.2. Stochastic effects

Radiation-induced malignancies and heritable effect are referred to as stochastic effects. These effects do not have threshold, this implies that there is finite probability they can occur after exposure to very low doses of radiation [25]. For stochastic effects not the severity but the likelihood of occurrence of the effect depend on the dose, therefore the probability of occurrence depends on with increasing dose [2]. Theoretically a single ionization track has the potential to result in a detrimental stochastic effect.

2. Materials and methods

Institutional approval to conduct the study was obtained from the committee on ethics of the Veterinary teaching hospital, University of Maiduguri (VTH). Thirty six (36) guinea pigs were obtained and kept at the large animal clinic of the VTH, Faculty of Veterinary Medicine University of Maiduguri, under good ventilation and adequate light. The guinea pigs were fed with standard commercial prepared diet (pelletized feed) and vegetables (such as cabbage and carrots) and given free access to clean drinking water. The guinea pigs were kept in this condition for 14 days in order to acclimatize before starting the experiment [27]. The guinea pigs were routinely screened for ectoparasites, endoparasites, and hemoparasites using standard methods by a veterinary doctor, and randomly divided into three groups, 12 guinea pigs per group. Group A served as the control group, group B and group C were exposed to low dose X-rays at a dose that is within the diagnostic range, using factors for chest X-ray of an adult patient in the study center (70 kV and 12.5mAs) using X-ray machine MS-185, serial no. 0904 GE, on which quality assurance check was routinely performed by a medical physicist with over 8 years experience, at a source to skin distance (SSD) of 90 cm. The guinea pigs in each group were irradiated together using a vertical central ray on a horizontal table top (couch) within the same cage, with the radiation properly collimated to include all the guinea pigs. Group C was irradiated twice with the same exposure factors 5 minutes after the first exposure.

2.1. Recruitment of subjects

A total of 36 adult guinea pigs of both sexes, weighing between 700 and 1200 g, were used for the study although 50 was obtained in case of accidental death, straying away, and some may be sickly.

2.1.1. Inclusion criteria

Thirty six apparently healthy adult guinea pigs of both sexes were used for the study.

2.1.2. Exclusion criteria

Apparently (physically) unhealthy and diseased guinea pigs were not selected for this study.

2.2. Irradiation procedures

Group A served as the control group. Group B and group C were irradiated with X-ray dose of about 70 kV and 12.5mAs which is within the diagnostic range from X-ray machine MS-185, serial no. 0904 GE at focus to film distance (FFD) of 90 cm. The guinea pigs in each group were irradiated together with a vertical central ray on a horizontal table top. Group C were irradiated again with the same exposure factors 5 minutes after the first exposure.

2.3. Blood sample collection

Blood sample from each guinea pig was collected into EDTA bottle from direct cardiac puncture with a 2 ml syringe and appropriately labeled. The blood samples were collected at the

intervals of 1, 24, 72, 163 and 336 hours post irradiation of the experimental groups. Blood samples were also collected during same time interval from the control group. No same syringe was used to collect blood sample more than once. The blood cell count was done by a veterinary doctor with over 10 years experience in veterinary parasitology at the veterinary teaching hospital University of Maiduguri, who performed the procedure alone to avoid inter-observer error. Hemocytometric method was used to count the white blood cells using Neubauer counting chamber. This method was used due to availability and convenience, as the automatic analyzer was not readily available at the time of analysis.

2.4. Hematological examination

All blood samples collected were subjected to standard hematological procedures to determine PCV, Hb, WBC and differential WBC count.

White blood cell count: Bulk dilution of the white blood cell count was employed. 0.02 ml of well mixed EDTA anticoagulant blood was pipetted into 0.38 ml of Turks solution contained in Khan tube and mixed. A clean cover slip was put in place on the improved Neubauer counter. Using a capillary tube held at an angle of 45° to the counting chamber, the diluted blood sample was carefully discharged into the counting chamber. The chamber was then placed in a petri dish and left undisturbed for 2 minutes, allowing the cells to settle. The underside of the chamber was dried and placed on a microscope and examined with 10× objective. The cells in the four large corners of each chamber were counted, including cells on the lines of two sides of the large squares. The number of white cells (per liter of blood) was recorded using a correction factor of 10×.

Differential white blood count: Longitudinal method of differential white blood cell count was adopted. A drop of blood was placed on a clean dry glass slide and a thin film was made. The film was dried in the air, fixed and stained by flooding with Leishman stain and allowed to stand for 30 minutes. Then the excess stain was washed off and allowed to dry in the air. A drop of immersion oil was placed on the film and covered with a clean dry cover slip. The film was viewed under 100× objective of the microscope. The differential white cells seen in each field was counted using the automated differential cell counter and recorded appropriately. Thus, the observed number of WBC indices in response to irradiation is used as an indicator of exposure [28].

2.5. Statistical analysis

The mean values of hematological parameters of control, single and double exposure groups were determined using one way analysis variance (ANOVA). P-values <0.05 was considered significant and the mean ± SE for hematological parameters were presented using descriptive statistics.

3. Results

The mean ± SE of white blood cell count values of guinea pigs for the control group is shown in **Table 1**, while **Tables 2** and **3** shows the mean ± SE of white blood cell count values of guinea pigs following single and double exposures to X-rays within diagnostic range respectively.



Figure 1. Mean total white blood cell counts of guinea pigs (*Cavia porcellus*) following single and double exposure to radiation.

There was an observed decline in the mean total white blood cell count of guinea pigs after 1hour post exposure to single and double exposures **Figure 1**. This was more pronounced after 24 and 72 post exposures. Marked recovery of WBC was noticed after 168 and 336 hours post exposure in both single and double exposure groups.

A decline in the mean total white blood cell count of guinea pigs was observed at 1 hour after single exposure, and was more pronounced 24 hours post irradiation. However, recovery of WBC commenced at 72 hours post exposure after single exposure. This was found to be significant ($p < 0.05$) as shown in **Table 2**.

Parameters	Control group					
	Mean \pm SE					
	0	1 hr	24 hr	72 hr	168 hr	336 hr
WBC	10.4 \pm 0.8	10.7 ^a \pm 0.8	10.2 ^a \pm 0.6	10.5 ^a \pm 0.5	11.0 \pm 1.0	10.7 \pm 0.4
PCV	40 \pm 1.8	40.3 \pm 1.8	40.5 \pm 1.5	38.5 \pm 1.9	38.8 \pm 0.8	39.3 \pm 1.4
HB	13.0 \pm 0.4	12.8 \pm 0.9	12.8 \pm 0.9	12.5 \pm 0.3	12.3 \pm 0.3	12.3 \pm 0.3
Monocyte	0.4 \pm 0.2	0.3 ^a \pm 0.1	0.3 ^a \pm 0.1	0.4 ^a \pm 0.1	0.4 ^a \pm 0.1	0.3 ^a \pm 0.1
Lymphocyte	4.7 \pm 0.7	5.2 \pm 0.6	4.9 ^a \pm 0.5	4.7 ^a \pm 0.6	4.9 \pm 0.7	4.7 \pm 0.4
Neutrophil	5.5 \pm 0.7	5.7 \pm 0.8	5.2 ^a \pm 0.6	5.2 ^a \pm 0.5	5.7 \pm 0.8	5.5 \pm 0.3
Eosinophil	0.4 \pm 0.07	0.4 ^a \pm 0.1	0.4 ^a \pm 0.1	0.4 ^a \pm 0.1	0.4 ^a \pm 0.1	0.4 ^a \pm 0.06
Basophil	00	00	00	00	00	00

Key: a—not significant and b—significant ($P > 0.05$).

Table 1. Effects of low radiation dose exposures on hematological parameters of guinea pigs (*Cavia porcellus*).

Parameters	Single exposure					
	Mean \pm SE					
	0	1 hr	24 hr	72 hr	168 hr	336 hr
WBC	10.5 \pm 0.5	8.7 ^a \pm 0.9	5.8 ^b \pm 0.8	6.2 ^b \pm 0.9	8.0 \pm 1.1	8.3 \pm 1.2
PCV	40 \pm 1.1	35.3 \pm 3.3	28.9 \pm 4.0	27.4 \pm 3.8	30.3 \pm 4.2	31.6 \pm 4.4
HB	12.6 \pm 0.3	11.2 \pm 1.1	9.0 \pm 1.2	8.8 \pm 1.2	9.3 \pm 1.3	10.0 \pm 1.4
Monocyte	0.3 \pm 0.0	0.2 \pm 0.0	0.2 ^b \pm 0.0	0.1 ^b \pm 0.0	0.2 ^b \pm 0.0	0.2 ^b \pm 0.0
Lymphocyte	3.7 \pm 0.5	4.0 \pm 0.5	2.5 ^b \pm 1.3	2.6 ^b \pm 0.4	3.2 \pm 0.5	3.7 \pm 0.6
Neutrophil	5.5 \pm 0.4	4.3 \pm 0.6	2.7 ^b \pm 0.0	2.8 ^b \pm 0.5	3.8 \pm 0.6	4.2 \pm 0.6
Eosinophil	0.4 \pm 0.03	0.2 ^b \pm 0.0	0.1 ^b \pm 0.4	0.1 ^b \pm 0.0	0.1 ^b \pm 0.0	0.1 ^b \pm 0.0
Basophil	00	00	00	00	00	00

Key: a—not significant and b—significant ($P > 0.05$).

Table 2. Effects of low radiation dose single exposures on hematological parameters of guinea pigs (*Cavia porcellus*).

Parameters	Double exposure					
	Mean \pm SE					
	0	1 hr	24 hr	72 hr	168 hr	336 hr
WBC	11.0 \pm 0.4	8.7 ^a \pm 0.4	6.1 ^b \pm 0.2	6.1 ^b \pm 0.2	8.9 \pm 0.3	9.3 \pm 0.3
PCV	41 \pm 1.1	38.5 \pm 1.0	31.8 \pm 1.0	34.3 \pm 1.3	38.0 \pm 1.2	39.5 \pm 0.9
HB	13.1 \pm 0.3	11.9 \pm 0.5	9.8 \pm 0.4	10.8 \pm 0.3	11.7 \pm 0.3	12.3 \pm 0.2
Monocyte	0.3 \pm 0.1	0.1 ^b \pm 0.0	0.1 ^b \pm 0.0	0.1 ^b \pm 0.0	0.2 ^b \pm 0.0	0.2 \pm 0.0
Lymphocyte	6.0 \pm 0.4	4.4 \pm 0.3	2.9 ^b \pm 0.2	2.7 ^a \pm 0.1	3.8 \pm 0.2	4.7 \pm 0.3
Neutrophil	5.8 \pm 0.3	4.3 \pm 0.3	2.8 ^b \pm 0.1	2.7 ^b \pm 0.1	4.1 \pm 0.2	4.5 \pm 0.2
Eosinophil	0.5 \pm 0.04	0.2 ^b \pm 0.0	0.1 ^b \pm 0.0	1.1 ^b \pm 0.0	0.2 ^b \pm 0.0	0.2 ^b \pm 0.0
Basophil	00	00	00	00	00	00

Key: a—not significant and b—significant ($P > 0.05$).

Table 3. Effects of low radiation dose double exposures on hematological parameters of guinea pigs (*Cavia porcellus*).

There was also a decline in the mean total white blood cell count of guinea pigs at 1 hour after double exposure. This was more pronounced after 24 hours post irradiation. The recovery of WBC was observed 72 hours after double exposure group. This was found to be significant ($p < 0.05$) as seen in **Table 3**.

A decline in the mean absolute monocyte count at 1 hour post irradiation was also noted. This decrease was more pronounced at 24–72 hours post irradiation. However, recovery of monocytes was evident at 168–336 hours post irradiation in both single and double exposure groups, with no significant difference ($p < 0.05$) as shown in **Tables 2** and **3**.

There was also a slight decline in the mean absolute lymphocyte count of guinea pigs at 1 hour in the exposure groups, which was more pronounced 24–72 hours post irradiation in both exposure groups. However, recovery of the mean absolute lymphocyte count was evident at 168 and 336 hours post irradiation as seen in **Tables 2** and **3**.

There was an observed decline in the mean absolute eosinophil count of guinea pigs in both single and double exposure group at 1 hour following single and double exposure to irradiation. This decrease was sustained and was more pronounced 24–72 hours post irradiation. However, there was slight recovery of the mean absolute eosinophil count at 168–336 hours post irradiation, as shown in **Tables 2** and **3**.

An observed decline in the mean absolute neutrophil count of guinea pigs at 1 hour in both single and double exposure groups, and became more pronounced 24 hours after irradiation. However, slight recovery of mean absolute neutrophil count in guinea pigs was observed at 168–336 hours post irradiation as seen in **Tables 2** and **3**.

4. Discussion

A decrease in total white blood cell count; lymphocytes, monocytes, neutrophils, eosinophil was observed; however, basophils were not seen. This probably could be because basophils naturally are rarely encountered granulocytes in the peripheral blood, therefore, it is not unusual for basophils to be absent [29]. Previous studies have reported similar findings [6, 17, 30]. The observed decline in the white blood cell counts could be attributed to high radio-sensitivity of hematopoietic tissues [6, 31]. The results are consistent with the previous findings that irradiation induces leucopenia and reduces lymphocytes, neutrophils and monocytes count [32, 33]. However, the recovery was evident 72 hours post irradiation and onward, even though the recovery and repair took longer time than the damage [17, 33]. This could be due to the fact that the recovery might be as a result of the repair at the cellular level where sub-lethally damaged cells recover their viability and proliferation of undamaged cell elements [17, 30]. The effect on the double exposure group was severe, which proves the fact that severity of damage increases with increase in dose or exposure [16, 30, 33].

5. Conclusion

This study found a depleting effect of low dose ionizing radiation on the white blood cell counts of guinea pigs (*Cavia porcellus*). This was found to be more pronounced with repeated exposures. However, recovery occurred from 3 days (72 hours) post irradiation onwards. Thus, a proposed interval of 3–14 days (72–336 hours) before repeating an exposure is recommended for subjects that may require a series of follow up and repeat radiographic examinations.

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Conflict of interest

Nil.

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