Characteristic of Peripheral Blood Mononuclear Cells after Diagnostic Irradiation in Term of Morphology and Differentiation Potency

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Abstract

Radiation is the emission or transmission of energy in the form of waves or particles through space or a material medium. There are many benefits for patients from medical imaging, but radiation is absorbed within our bodies, it can damage molecular structures and potentially cause harm. The aims are to characterize the peripheral blood mononuclear cells (PBMCs) after diagnostic x-ray irradiation and analyze the potency of morphology and differentiation potency. The PBMCs were isolated by ficoll-centrifugation technique and analyzed morphology and differentiation potency by inverted microscope. The cells were observed in 1hr, day 1, day 5, day 10, day 15 and day 20 after diagnostic irradiation base on diagnostic references level in the range of 70-110 kVp, 5-40 mAs, and 0.47-2.30 mGy. The freshly PBMCs isolated were found spherical shape and measured by flow cytometer that can be divided into two groups were composed of lymphocyte and monocyte. After x-ray irradiation in 20 days, it revealed that the morphology was changed in all groups and the adherent cells in the control group were found more than x-ray irradiation group. The overall results indicated that the PBMCs might be considered as biomarkers were found to have a significant radiation effect on the morphology and differentiation of PMBCs and raises awareness on the use of radiation in medicine including being careful when using diagnostic radiation

Keywords: *Peripheral blood mononuclear cells, Diagnostic irradiation, Cell morphology, Cell differentiation, Diagnostic references level, Biomarkers*

1. Introduction

Radiation in medicine is considered useful for the treated or diagnostic disease. However, the effect of radiation on one side, which found that the use of radiation for medical diagnosis may affect the operation of the cell, including the effects on organisms. Ionizing radiation has a direct effect to the cells in the body including peripheral blood mononuclear cells (PBMCs) (Ghardi, Moreels, Chatelain, Chatelain & Baatout, 2012), stem cells (Greenberger & Epperly, 2009) and maybe the cause of cancer (a carcinogen) (Little, 2000) in humans. However, in previous studies, the effect of radiation in medical diagnostics on living organisms and their effects on cell levels including peripheral blood mononuclear cells are still arguments and ongoing research on the effects of radiation during the medical examination.

In the human body, there is a variety of radiation responses, which are determined by various factors. As mentioned humans may be exposed to radiation from the diagnostic radiation commonly used in medical equipment such as x-rays and serious may affect the cells or cause mutation of a gene which may develop a disease such as cancer (Ron, 2003), In general, the medical use x-rays to diagnostic the disease with a general x-ray machine, the dose received is below 100 mGy (Nguyen & Wu, 2011). Recent research has conducted studies the effect of x-rays in medical diagnostics has a direct effect to the characteristic and behavior on PBMCs, that are isolated from the blood and consists of cells with round nuclei such as lymphocytes, monocytes, natural killer cells (NK cells) and dendritic cells (Fuss, Kanof, Smith & Zola, 2009).

PBMCs can grow into various cells. (differentiation) such as blood cells, endothelial cells, hepatocytes, cardiomyogenic cells, smooth muscle cells, osteoblasts, osteoclasts, epithelial cells, neural cells, myofibroblasts (Zhang & Huang, 2012). However, various behaviors and abilities of cells depend on the environment and the factors that are at that time (Moonkum et al., 2018). In which various environments, including the amount of natural and medical radiation affect all living organisms and the

cellular systems within the body. The recent studies have shown that x-rays affect both direct and indirect PBMCs and found that 0.3-0.7 Gy of x-rays caused the PBMCs were apoptosis to 80% (KERN, 1999) and found that the PBMCs obtained from healthy volunteers and then irradiated x-rays of 0.1, 0.25, 0.5, 1, 2, and 4 Gy were shown the fracture of DNA after irradiation at 0, 24, 48, 72, and 96 hours (Ghardi et al., 2012).

The objective of this research to study the effect of medical diagnostic radiation by using diagnostic reference levels in radiography (Compagnone, Pagan & Bergamini, 2005), which is used in plan film imaging, such as chest, abdomen, pelvis, lumbar on PBMCs from healthy volunteers by studying in term of morphology and differentiation potency. In this research, we will know the effect of medical diagnostic radiation on the morphology and differentiation potency of mononuclear white blood cells.

2. Objectives

In this research aims to study the effects of the x-ray on the characteristics of PBMCs in terms of morphology and differentiation potency

3. Materials and Methods

3.1 Isolation of PBMC from human peripheral blood

Criteria of donor selection: The project was approved from the ethical review committee for research in human subjects, faculty of Radiologic technology, Rangsit University. (RSU-ERB2019/026)

Blood samples were collected from 4 healthy donors (female and male, age 18-35 years). The donors have a healthy lifestyle. They have not had diseases, including diabetes, hypertension, heart disease, cerebrovascular diseases, cancer, or HIV+. They are not overweight and not smoking or consume alcohol. The PBSCs samples were collected after an agreement and signed on consent form by donors.

3.2 Collection of human peripheral blood mononucleated cells (PBMC) from donors.

The blood samples (10ml) were collected and were centrifuged using a swing-out rotor with 4000 rpm for 20 minutes. The buffy coat portions were aspirated and transferred into a sterile conical tube and completed the final volume of 5 mL with isotonic solutions following gently mixed. A ficoll-paque solution of 5 mL was injected into the bottom of the tube and then centrifuged at 4000 rpm for 20 minutes. The PBMCs fraction was isolated and washed once using 5 mL of PBS and resuspended in 5 ml of fresh RPMI 1640 with 10% FBS. The culture flasks were placed in an incubator that controls the temperature to 37°C, 5% CO_2 (g) atmosphere and 95% humidity.

3.3 Expansion of adult PBMC in conventional culture

Cells (10^6 cell/mL) were cultured in 24-well plates with RPMI-1640 supplemented with 10 % fetal bovine serum and 1 % penicillin /streptomycin at 37 °C in 5 % CO2(g) atmosphere in a humidified incubator at 95% humidity. The cell morphology was examined under an inverted light microscope at 1hr., 24hr., day 5, day 10, day 15, and day 20 after x-ray irradiation. The experiment was conducted to add medium cell culture after 14 days to study the cells for longer and observe the cells by random 5 points; 4 points are divided by the edges and the middle one point in the well plate (Figure 1).

3.4 Irradiation system

Cells in culture system divided into two groups for the control (non-irradiation) and x-ray irradiation. Irradiations were done at 1hr. after the initiation of PBMCs. At irradiation, the tissue culture flasks were placed in the center of an x-ray beam at 100 cm from the x-ray tube. A medical diagnostic x-ray machine (Shimadzu, Collimator type R-20J, Japan)

Table 1 The parameters obtained by the medical x-ray machine operated at 70-110 kV, 5-40 mAs and absorbed radiation dose

Organ	kV	mAs	mA	sec	mGy
Chest	110	5	100	0.05	0.47
Pelvis	70	20	200	0.1	0.71
Abdomen	90	20	200	0.1	1.16
Lumbar spine	90	40	200	0.2	2.30

kV = kilovoltage, mAs = milliampere-seconds, mA = milliampere, sec = seconds, mGy = milligray

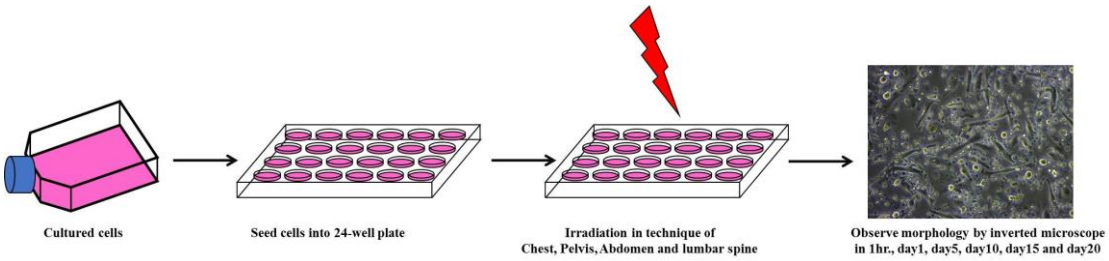

Figure 1 Schematic diagram of the experimental study design

4. Results and Discussion

Analysis of freshly isolated PBMCs from whole blood

PBMCs freshly isolated were spherical shape (Figure 2a, b) and characteristics of PBMCs as measured by a flow cytometer that can be divided into two groups, light scattering and was composed of lymphocytes and monocytes (Figure 2c). As clearly seen that the lymphocyte small than the monocyte.

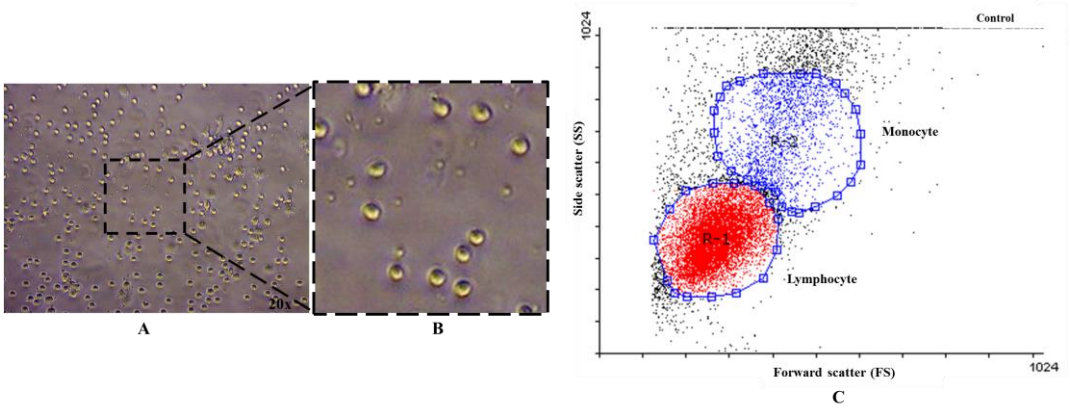

Figure 2 Characterization of Peripheral blood mononuclear cells by inverted microscope (a,b), and cell counts using flow cytometer (c)

Effect of x-ray irradiation on PBMCs culture

We have verified that the colony-forming determined in our system was dependent on the density of PBMCs; only the density of cells lower degree than $5x10^6$ cell/ml can observe cell proliferation and colony-forming units of the stem cells. The behaviors of PBMCs were suspended, and morphology after x-ray irradiations in 1hr was shown not change in all groups and morphology of PBMCs were spherical shape throughout the well plate in Figure 3.

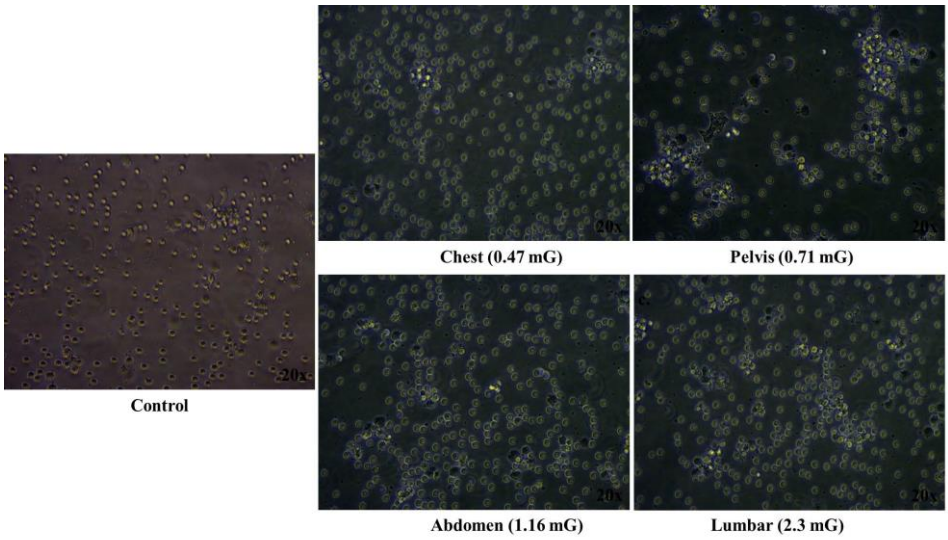

Figure 3 Micrograph of PBMCs at 1hr after x-ray irradiation (20x)

Furthermore, on days 1, 10, and 20, it was found that the cells had more integration characteristics, which were the characteristics of stem cells (a colony of stem cells) in day 1. The cells were changed shape (differentiation) to spindle shape or specific characteristics such as stromal cells, mesenchymal cells and were found to attach on the bottom of the well plate in day 10 and only a few colonies and cell differentiation in day 20 (Figure 4). Additional from 4 donors were found the characteristics of cell changes such as spindle cell and colony-forming. The results were found that the changes in appearance between irradiated group were similar to the control group and were suggested that the effect of radiation might not contribute to changing the morphology of the cell in Figure 5.

However on day 20, when removing condition medium, RPMI 1640 from the well plate were found the cell culture is still cells attached to the well plate called “adherent cells” and were found the large size of adherent cells in the control group (Figure 6).

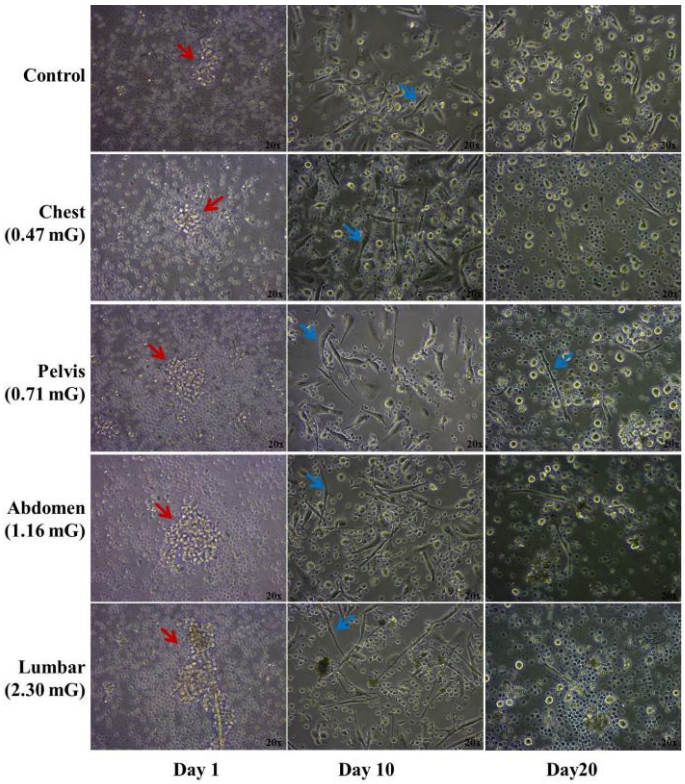

Figure 4 Micrograph of PBMCs on day 1, day 10, and day 20, after x-ray irradiation. The red arrow shows the colony of stem cells and blue arrow show cell differentiation such as stromal cells, mesenchymal cells

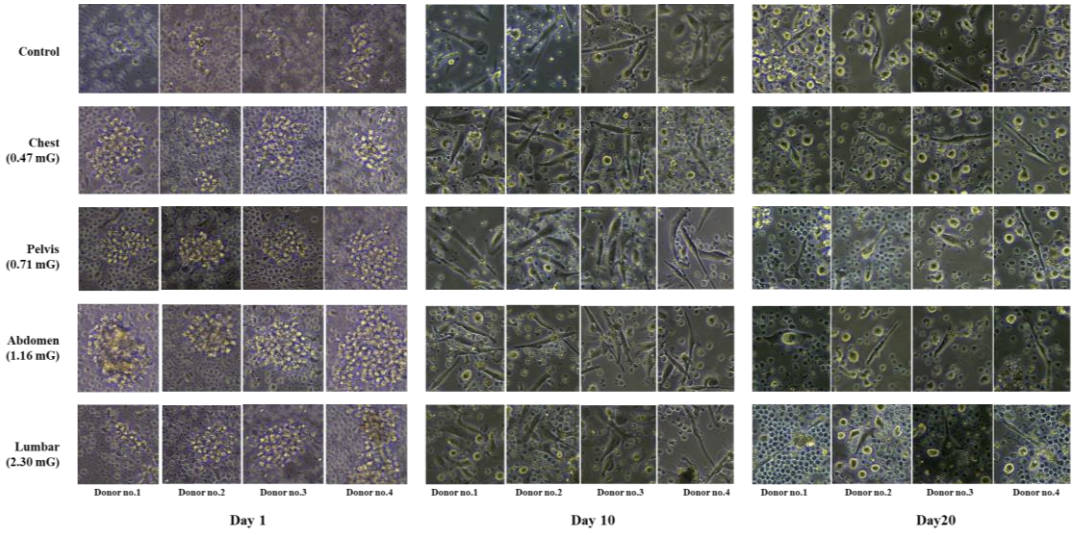

Figure 5 Micrograph of PBMCs on day 1, day 10, and day 20 after x-ray irradiation in 4 donors (20x)

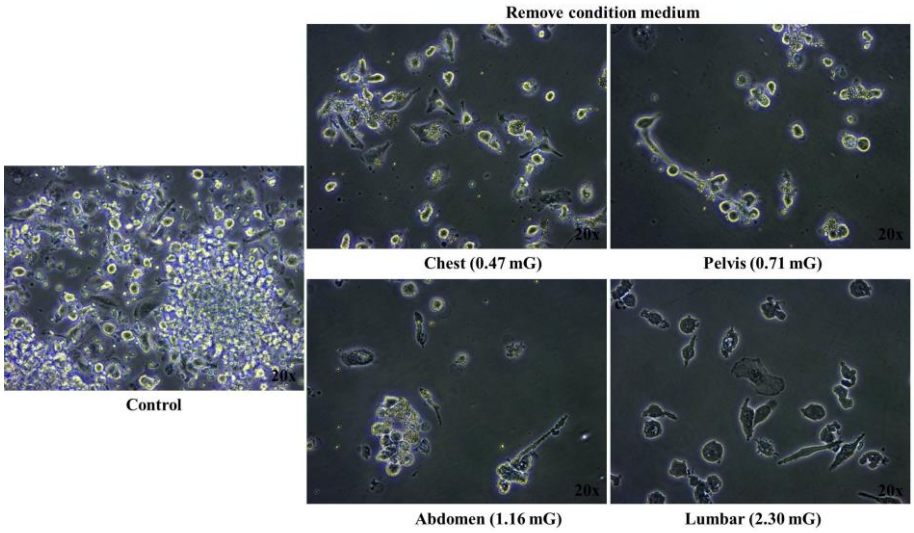

Figure 6 Micrograph of PBMCs at 20 days after x-ray irradiation and remove condition medium (20x)

5. Conclusion

The experimental results can be seen that the radiation levels below 100 mGy may not affect the cell shape change. However, on day 20, when removing condition medium RPMI 1640 from the well plate were found the control group to find more adherent cells than the irradiated group. In previous studies, adherent cells were clearly a group of mesenchymal stem cells at the *in vitro* method (Ab Kadir et al., 2012). But the experiments only were collected quality data. This experiment is therefore based on physical characteristics.

The overall results of the study showed that the effects of radiation from x-ray techniques based on diagnostic references revels (Compagnone et al., 2005) such as chest, pelvis, abdomen and lumbar spine were found to have a significant effect on the PBMCs differentiation, especially when doing experiments until the 20 day, which is a long period enough to observe the changing morphology of PBMCs. Found that diagnostic radiation effects to the morphology and differentiation of the PMBCs when cultured for a long period. Also, the PBMCs in conventional cell culture conditions can differentiate to varieties of specific cells, including, adipocytes, osteocytes, chondrocytes, neurons, leukocytes, and stromal cells. From the low-energy x-ray studies used in the diagnosis. It can be exploited as clinically significant. However, the studies showed the one side effects of low dose radiation (0.4-2.3 mGy) that related to the differentiation of suspension and adherent cells. The result is only an experiment in the laboratory because such experiments are difficult in humans, and it is harmful to humans.

This study makes evidence that PBMCs are one of the important sources of normal cells in the body which were very easy to achieve and expand in culture, as well as being able to a biomarker to detect damage in the molecular level.

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