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Analysis of CD4 Cell Production Using PBMC Culture after Exposure to Radiofrequency Electromagnetic Waves

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Abstract**—** *This research was analyze of CD4 cell production in lymphocytes after exposure radiofrequency electromagnetic waves. The sample use lymphocytes from normal human donor blood that have been cultured pheripheral blood mononuclear cell (PBMC). The exposure process is carried out in a box exposure using VSG25A device. The frequencies used were 900 MHz and 1800 MHz. The measurement variable use variation in distance and time. Calculation of CD4 cell production using flowcitometry. The result show that the longer of exposure time, CD4 cell production was increases. The highest CD4 cell changes occured at distance of 2.5 cm (900 MHz frequency). The highest CD4 cell changes occured at distance of 6.25 cm (1800 MHz frequency).*

Keywords**—** *Radiofrequency, PBMC, lymphocytes, CD4.*

I. INTRODUCTION

The health effects of exposure radiofrequency electromagnetic waves have been widely tested in the last decade, due to increasing intensity of cellphone use as a communication and information media. The frequency of cellphone that are widely used is the GSM frequency (900 MHz and 1800 MHz) [1].

The effect of exposure radiofrequency electromagnetic waves can interfere the body's cell balance, especially blood cells. Radiofrequency exposure can affect the number of white blood cell, red blood cell and platelet. The changed number of blood cells that significantly can give an indication damage to the body cells [2].

The exposure of GSM radiofrequency, the frequency of 850 MHz can change the number of white blood cells, blood monocyte cell production and lymphocytes are increases, but different results at platelets that are decrease [3].

Radiation exposure of GSM 900 MHz for 2 hours duration caused the production of ROS (Reactive Oxygen Species) in monocytes and lymphocytes are increase significantly. The number of ROS monocytes and lymphocytes that are exposed RF radiation is very different compared to those that are not exposed [4].

Lymphocytes are a large percentage of white blood cell components. Lymphocytes have an important role to provide information about the condition of cells that are still healthy or that have been damaged or dead. Components of lymphocytes consist of T cells, B cells and NK (Natural Killer). Lymphocyte cells provide information about dysregulation of the immune system in the body. T cells can produce cytokines. If the production of cytokines decreases, will affect at activity of cell production in the body [5].

The lymphocyte component in T cells is CD4. CD4 cell counts can be an indication of the stability immune response in the body. Amino acids are CD4 components obtained from the body's mechanism. CD4 cells function as senders of signals to antibodies when there are foreign objects that enter the body [6].

Peripheral Blood Mononuclear Cell (PBMC) is white blood cell that has a rounded single core, found in lymphocyte cells and monocyte cells. These cells are important components of the immune system that are involved in humoral and cellular immunity. Mononuclear cells are widely used in clinical research and applications such as in the fields of microbiology, virology, oncology, vaccine development, transplantation biology, regenerative biology, and toxicology [7].

CD4 is a part of lymphocytes which indicates the stability of cell conditions, so it must be further investigated how RF radiation affects at CD4 cell production in lymphocytes.

II. RESEARCH METHOD

This research was used lymphocytes from adult human who does not have abnormalities in blood cells as the sample. Donor blood obtained from ± 24 people with male and female gender. The frequencies used were 900 MHz and 1800 MHz. The time variations was used 15 minutes, 30 minutes, 45 minutes and 60 minutes. The distance variation that used at 900 MHz was 0 cm, 2.5 cm, and 5 cm. The distance variation that used at 1800 MHz was 5 cm, 6.25 cm and 25 cm. The exposure box was used during the measurement process. The exposure process is carried out in exposure box using VSG25A device.

Before the isolation process, blood cell was added by EDTA (Ethylenediaminetetraacetic acid) nutrition. Nutrition EDTA to maintain stability in the blood. Blood cells that was given EDTA nutrition was not easily damaged [8].

The mixture of blood and EDTA will be placed in a centrifuge tube that has been filled with Ficoll-Hypaque liquid (Fig. 1). Parts of the blood that have a higher density than the Ficoll-Hipaque will pass through the Ficoll layer and parts of the blood (lymphocytes and monocytes). Parts of blood that have a lower density than Ficoll-Hypaque will accumulate in the plasma. The sample in Fig. 1 was centrifuged at room temparature which had a speed 1000 rpm for 30 minutes that resulting 4 layers (Fig. 2).

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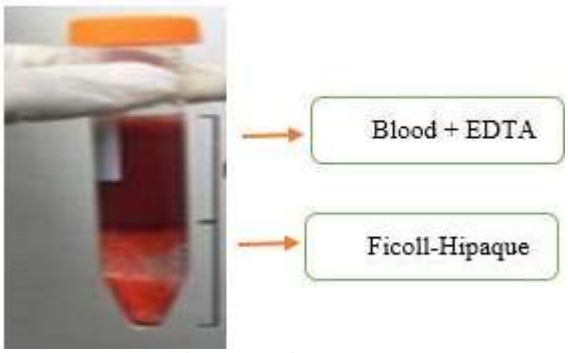

Fig. 1. Centrifuge tube before separating cell part.

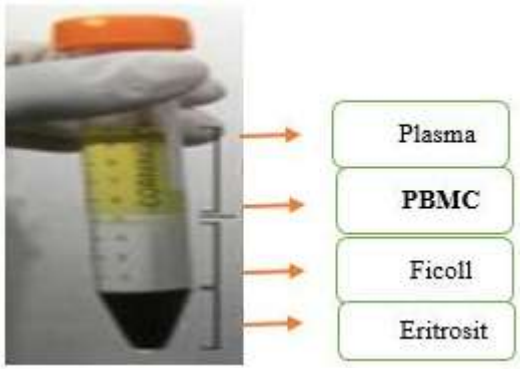

Fig. 2. Centrifuge tube after separation of cell part.

After the isolation process, the blood cells will separate into 4 layers (Plasma, PBMC, Ficoll and Erythrocytes). PBMC consists of lymphocytes and monocytes. The radiofrequency exposure process was carried out at lymphocytes.

The frequency was used 900 MHz and 1800 MHz. In this experiment, 900 MHz and 1800 MHz radiation are similar to GSM cellphone frequencies. The sample was protected with exposure boxes that made by aluminum and lead. The temperature control thermostat was used to adjust temperature at 37^{o}C during radiofrequency exposure process. The exposure distance was adjusted from radiofrequency (RF) generator antenna to the well plate. Laptop to set the time and frequency of exposure (Fig. 3).

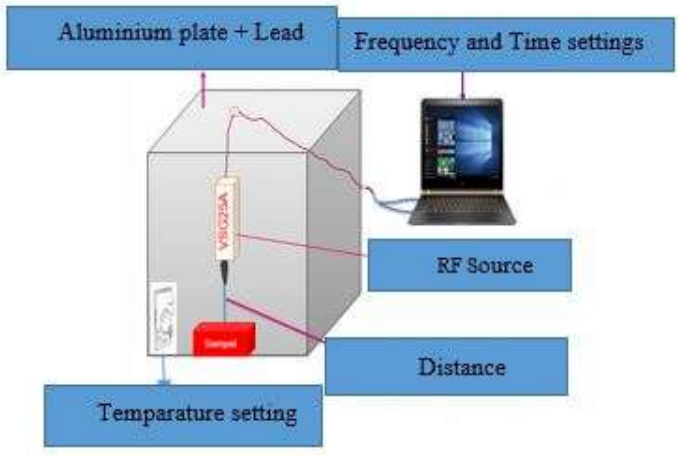

Fig. 3. RF exposure process in the sample.

After the radiofrequency exposure process, the sample culture process was carried out by incubating for 48 hours in incubator. Brefeldin A (Golgiplug) was gave to the sample before harvesting cells to maintain the condition of cytokines.

The process of cell surface coloring with CD4 markers.. The sample was added with cell staining buffer according to the number of treatments.

Calculation the number of CD4 cells with flowitometry. The calculation of percentage CD4 cell changes is shown by "(1)".

$$x = \frac{B}{A} * 100 \quad (1)$$

Where x is the percentage of CD4 cell changes, B is the number of CD4 cell after being exposed to radiofrequency, and A is the number of CD4 cell before being exposed to radiofrequency.

III. Result and Discussion

Based on the research, it shows a graph of the relationship between the percentage value of CD4 cell changes and radiofrequency exposure time with distance variations. At 900 MHz and 1800 MHz frequencies was identified that CD4 cell production was increase. The percentage of CD4 cell changes and Radiofrequency exposure time with distance variations are shown in Fig. 4 and Fig. 5.

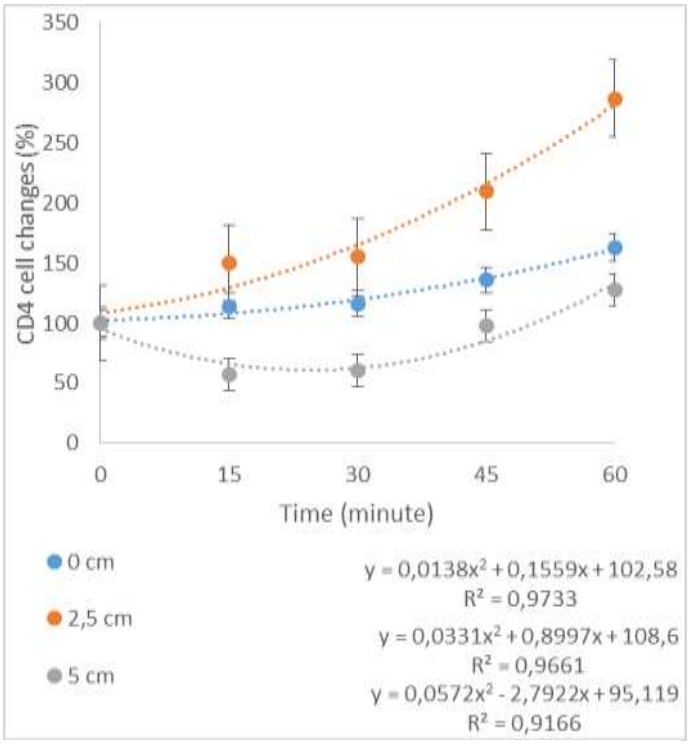

Fig. 4. The relationship between percentage of CD4 cell changes and radiofrequency exposure time with distance variations at 900 MHz.

Fig 4 and Fig 5 shows the percentage value of CD4 cell change and radio frequency exposure time is associated with the second order polynomial chart. the percentage value of CD4 cell changes decreases at the farthest distance. Distance affects the radiation intensity of the sample.

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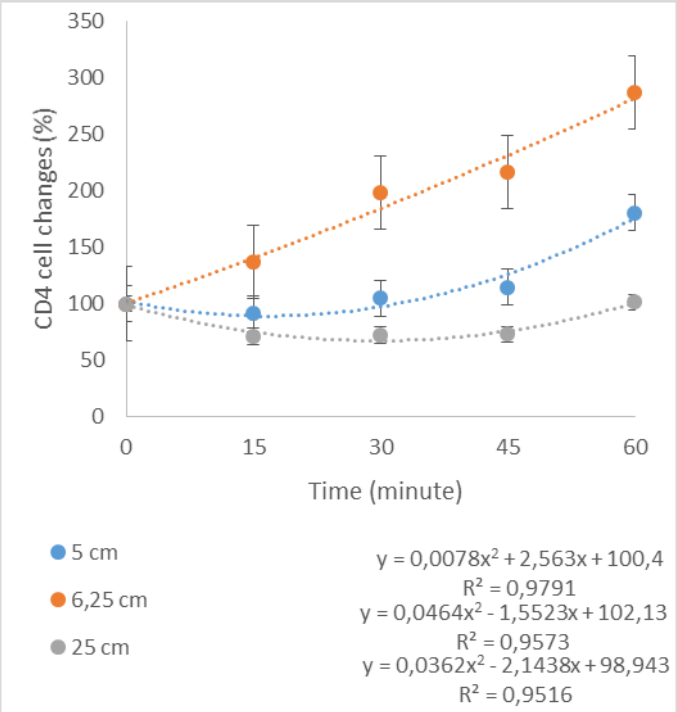

Fig. 5. The relationship between percentage of CD4 cell changes and radiofrequency exposure time with distance variations at 1800 MHz.

Percentage CD4 cell changes increase when radiofrequency exposure time is longer. Increased of percentage CD4 cell changes is related to its function as an introduction to signals antibodies when there are foreign objects or material that enter the body. Exposure of radiofrequency radiation is a type of exposure non ionizing electromagnetic radiation. Non-ionizing radiation can accelerate the movement of leukocyte constituent cells [1].

Radiofrequency interactions with cells are not absorbed and are not dispersed. Radiofrequency radiation can penetrate deep tissue and skin layers. Radiofrequency will transfer the energy to molecules in cells. When cells have more energy, cell mobility increases, causing a heat effect. Radiofrequency energy causes heat effects and stimulates the movement of cells. Electric currents that oscillate in RF waves cause thermal effects [9].

Lymphocyte cells increase due to several factors, such as large amounts of radiation energy induced in the body, length of radiation exposure, and temperature [10]. There is a correlation between CD4 behavior when were exposed radiofrequency radiation with the length of time exposure. The longer radiofrequency exposure was given, the CD4 percentage increases. Increased CD4 cell production shows the function of CD4 as a marker of antibodies will respond to RF exposure in the body. CD4 can produce cytokines. Cytokines help antibodies to repair damaged cells.

Radiofrequency passes microwave radiation to water, protein, mineral, and salt molecules in blood cells. Molecules will absorb induced electromagnetic energy. Cells in the blood are electrically polarized, meaning the molecule has a negative charge on one side and a positive charge on the other side. Electric field that change can cause the movement of molecules. The movement of molecule will produce heat energy due to friction between molecules [11].

CD4 cells as the main regulator of the immune response. When CD4 cells are activated by contact with antigens, they will respond through cell division and produce lymphokines such as interferon, interleukin and tumor necrosis factor (TNF). This lymphokine functions as a mediator in the immune response that controls the growth and maturation of other types of lymphocytes such as cytotoxic T cells (CD8) and antibody-producing B lymphocytes. Lymphokine 38 also triggers the maturation and function of tissue monocytes and macrophages [12].

IV. Conclusion

The percentage value of the comparison CD4 cell counts increases during the duration of radiofrequency exposure. Radiofrequency exposure to white blood cells (lymphocytes) affects the value of CD4 cell production. The highest CD4 cell changes occur at distance of 2.5 cm (900 MHz frequency). The highest CD4 cell changes occur at distance of 6.25 cm (1800 MHz frequency).

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