Arthamin MZ, et al. Iran J Immunol. 2020; 17(2):154-166.
https://doi.org/10.22034/iji.2020.84760.1671.

ORIGINAL ARTICLE

Exposure of 1800 MHz Radiofrequency with SAR 1,6 W/kg Caused a Significant Reduction in CD4+ T cells and Release of Cytokines *In-Vitro*

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ABSTRACT

Background: Although there have been many studies investigating the effects of electromagnetic fields on humans cells and tissues, the effects of radiofrequency electromagnetic fields exposure on the cells of the immune system are still controversial. **Objective:** To investigate the effects of 1800 MHz RF-EMF exposure on peripheral blood mononuclear cells by measuring T helper cells count and the cytokine profile under different conditions of durations and distances. **Methods:** The peripheral blood mononuclear cells (PBMCs) from healthy human subjects were exposed to 1800 MHz RF-EMF, with durations of 15, 30, 45, and 60 minutes and distances of 5 and 25 cm. The effects of RF-EMF exposure on the number of CD4+ T cells, and the expression of IL-2, IL-10, and IL-17a after 48 hours of culture were evaluated using flow cytometry. **Results:** Our findings indicated that closer distance and longer exposure induced lower number of CD4+ T cells. Similarly the percentages of IL-2, IL-10 and IL-17a expressing CD4+ T cells were decreased significantly. The number of IL-2 expressing CD4+T cells was increased significantly as the duration of exposure was increased, but the number was decreased after 60 minutes exposure when compared with control group with no exposure. **Conclusion:** Exposure to RF-EMF for 60 minutes at 5 cm distance causes a significant reduction in the number of CD4+ T cells, IL-2, IL-10 and IL-17a expressing T cells.

Received: 2019-12-30, Revised: 2020-05-05, Accepted: 2020-06-13.
Citation: Arthamin MZ, Sulalah A, Resvina R, Widodo C, Endharti AT, Widjajanto E, Juliandhy T. Exposure of 1800 MHz Radiofrequency with SAR 1,6 W/kg Caused a Significant Reduction in CD4+ T Cells and Release of Cytokines In-Vitro. *Iran J Immunol*. 202; 17(2):154-166. doi: 10.22034/iji.2020.84760.1671.

Keywords: 1800 MHz RF-EMF, CD4+ T Cells, IL-2, IL-10, IL-17a

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INTRODUCTION

The rapid development of mobile technology in modern life raises public concerns and fears about the possible effects of EMF exposure on health for users and the general population exposed for 24 hours from base transceiver stations (1,2). However, there is no evidence of the adverse effects of long-term EMF exposure (2). Radiofrequency ranges from 3 kHz to 300 GHz (3-5). Exposure to RF-EMF is associated with non-wired communication devices, a technology widely used. Environmental exposures are also associated radiofrequency emitted by cellular phone base transceiver stations, televisions and radio towers. While operating, cellular telephone antennas emit RF-EMF which can penetrate 4–6 cm into the human brain (6). Various studies have shown that EMF disrupts the body's energy system, thus causing various health problems (7,8). Those studies mentioned that EMF may disrupt immune function through stimulating various allergic and inflammatory responses, autoantibodies, and tissue repair. These disorders increase the risk of various diseases, including malignancy, and can trigger or cause flares in some autoimmune diseases (2,7). In recent years, there has been evidence of the role of immunology and oxidative stress on long-standing EMF exposure. The investigated markers including lymphocytes, activated macrophages, and secretion of several inflammatory factors such as interleukin-1 (IL-1), tumor necrosis factor (TNF), prostaglandin (PG), reactive oxygen species (ROS), lipid peroxides (LP). Reactions occur due to these metabolites play a role in the occurrence of malignancies after prolonged EMF exposure (8). In addition to the unclear mechanism, several studies on the adverse effects of radiofrequency EMF on the immune system showed different results. Therefore, we conducted an 1800 MHz RF-EMF exposure experiment on peripheral blood, CD4+ cells, Th1, Th2, and Th17 cells in PBMC cultures with different distances and durations.

MATERIALS AND METHODS

Study Design. Our study was *in vitro* experimental research with a randomized pre and post-test controlled group for whole blood, and post-test-only controlled group for PBMC. Fresh whole blood with ethylenediaminetetraacetic acid (EDTA) anticoagulant from healthy volunteers, put into chamber where exposure to RF-EMF. Whole blood pre and post-test samples were performed complete blood count with a Sysmex hematology analyzer. Peripheral blood mononuclear cells cultures were mixed *in vitro* with RPMI 1:10 culture media and divided into 13 treatment groups. PBMCs were exposed to 1800 MHz RF-EMF with various durations and distances, then cultured for 48 hours 37°C. Next, PBMCs were examined using flow cytometer for CD4+ T cells expressing IL-2, IL-10, and IL-17a.

Subjects and Samples. Venous blood was collected from 4 healthy volunteers of students and employees in the Medicine Faculty of Universitas Brawijaya, with ages 20 to 35 years. PBMCs sample size was calculated using the following formula: $p(n-1) \geq 15$: $9(n-1) \geq 15$, $n \geq 2.667$, n: Number of samples for each treatment and p: Number of treatments. From the calculation, 2.667 was the minimum number of samples for each group. In this study, the number of samples in each group as 4. The total sample was 36. The samples (12 mL) were taken from the antecubital vein, using a 21G needle, into vacuum tubes containing EDTA for exposure to whole blood or containing heparin

(Becton Dickinson Biosciences) for PBMC isolation. All subjects had previously given their informed consent. Ethics approval was obtained from Medical Faculty of Universitas Brawijaya Research Ethics Committee (letter number: No. 146/EC/KEPK/05/2018).

PBMCs Isolation and Culture. Routine Haematological examination was carried out using an automatic blood cell analyzer (Sysmex XN 2000 hematology analyzer). PBMCs were purified from heparinized peripheral venous blood samples using Ficoll-Hypaque gradients, d=1.077 g/mL (Sigma-Aldrich Co. LLC.). PBMCs were washed with phosphate-buffered saline (PBS) and resuspended in 300 µL RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) and 1% glutamine/penicillin /streptomycin at a concentration of 1 x 10^6 cells/ml in multiwell plates and a tissue-culture incubator at 37°C, 5% CO_2. Before cultured, PBMCs were exposed to 1800 MHz RF-EMF, with four different durations (15, 30, 45, and 60 minutes) and two different distances (5 cm and 25 cm) at 37°C. After 48 hours of culture, PBMCs were harvested, in which 10 µg/ml Brefeldin A (Golgiplug) (BD Pharmingen, San Diego, CA, USA) was given 5-6 hours prior. Cells were taken with a micropipette and put into a 1.5 ml eppendorf tube and centrifuged 2500 rpm for 3 minutes. The formed pellet was washed 2-3 times with 1 ml PBS.

Sample Preparation and Flow cytometric Analysis of CD4, Th17, IL-2, and IL-10. Cell surface staining was performed using mouse anti-human monoclonal antibodies (mAbs) anti-CD4 fluorescein isothiocyanate (FITC) (BD Pharmingen). The staining was performed by adding 20 µL of each mAb to 100 µL of separated PBMCs in the same tube, followed by 30 minutes incubation in the dark at room temperature. The tubes were washed twice with FACs buffer. Next, fixative and permeabilizing solutions were added, followed by intracellular staining using 20 µL of Fastimmune Anti-Human IL-2 phycoerythrin conjugate (PE) (BD Pharmingen), Mouse Anti-Human IL-10 PE (BD Pharmingen), Mouse Anti-Human IL-17a PE (BD Pharmingen). The cells were incubated for 30 minutes in the dark at room temperature and washed twice with FACs buffer. Finally, 0.5 mL of PBS was added to the washed cells prior to the measurement. Sample analysis was performed with a FACS Calibur flow cytometer (Becton Dickinson, San Jose, California, USA). FACs-acquisition and analysis were performed with FACs Cell Quest Pro software (BD Biosciences).

EMF RF Signal Generator. VSG25A Vector Signal Generator is a 100 MHz to 2.5 GHz vector signal generator, which displays a wave generator that can be set at several frequencies from 54 kHz to 180 MHz. The RF-EMF generators are obtained from The Signal Hound® company. In this study, 1800 MHz frequency and specific absorption rate (1.6 w/kg) (SAR) were used to recreate mobile phone radiofrequency radiation at four different duration and two different distances. The Generator was placed in a chamber made of aluminium layered with lead (32 cm × 32 cm) in a temperature of 37°C, with procedures similar to the study by Sulalah *et al.*, 2019, and Resvina *et al.*, 2019 (9,10). The characteristics of the antenna used in this study was an omnidirectional type external antenna. Antennas used in this study can be directed as needed, with a 360-degree radiation pattern that can spread in all directions. By using a detector (mustool MT525), at a frequency of 1800 MHz, VSG25A Vector signal generator was detected to have the same level of electric and magnetic field radiation as a mobile phone. the emitted waveform was also same, the sinus wave.

Radio Frequency Radiation (exposure system). Electromagnetic wave exposure in the radio frequency spectrum of 1800 MHz using the VSG25A signal hound vector signal

generator. The technical specifications and signal generator dosimeters were presented in the following Table 1.

Table 1. Technical specifications and signal generator dosimeters.

Parameter	Value
Type Signal	Vector signal generator VSG25A signal hound
Wave type	Radio frequency
Frequency	1800 MHz
Modulation:	
• **Modulation depth**	0
• **Modulation rate**	0
• **Modulation shape**	Sinusoidal
Amplitude (Power)	
Mechanical/ environmental:	0 dBm (1mWatt)
• RF output connector	SMA (f)
• Power Requirements	USB 2.0 port
• Operating temperature (calibrated)	36°C to 37°C (body temperature)
• Size	5.5" x 2.25" x 1"
• Weight	5 oz

SAR Measurement. SAR measurements are carried out by measuring the large electric field in the chamber after being given a 1800 MHz frequency through the transmitter. The measurement of the electric field is carried out using the Mustool MT525 Electromagnetic Radiation Detector, which was placed just below the antenna with a distance of 5 cm in accordance with the position of the sample. The transmitter was turned on with a frequency of 1800 MHz for ± 3 minutes then the detector was put into the chamber and turned on. After the numbers that appear on the detector were stable, data that often appear as often as 5 times were recorded and averaged. We calculated SAR with the equation:

$$SAR = \frac{\sigma E^2}{\rho}\left(\frac{w}{kg}\right) \quad (1)$$

Where σ was the effective conductivity of a tissue/organ (S/m), ρ was the mass of the tissue/organ type (kg/m^3), E was the magnitude of the electromagnetic field (V/m). The tissue/organ conductivity and density could be seen in Table 2 and 3.

Table 2. Blood conductivity (11).

Frequency	σ **(S/m)**
900 MHz	1,53
1800 MHz	2,0453
2450 MHz	2,54

Table 3. Mass density of organs.

Tissue/organ	Mass density (kg/m^3)
Blood	1850
Muscle	3490
Bone	1042

Where in determining the amount of electrical energy using an electromagnetic wave detector mounted on a chamber. Where σ is the effective conductivity of a tissue/organ (S/m), ρ is the mass of the tissue/organ type (kg/m^3), E is the magnitude of the EMF (V/m). Blood conductivity 1800 MHz 2,0453 S/m, mass density (kg/m^3) of blood is 1850 (kg/m^3), SAR 1.6 W/kg.

Treatment Groups. Whole blood in this study was divided into two treatment groups: 1. Whole blood without exposure, 2. Whole blood with exposure to 1800 MHz RF-EMF, a distance of 5 cm for 60 minutes of exposure. PBMCs in this study was divided into several treatment groups: 1. PBMC without exposure, 2. PBMC with exposure to 1800 MHz RF-EMF, a distance of 5 cm for 15 minutes of exposure, 3. PBMC with exposure to 1800 MHz RF-EMF, a distance of 5 cm for 30 minutes, 4. PBMC with 1800 MHz RF-EMF exposure, a distance of 5 cm for 45 minutes, 5. PBMC with 1800 MHz RF-EMF exposure, a distance of 5 cm for 60 minutes, 6. PBMC with 1800 MHz RF-EMF exposure, a distance of 25 cm for 15 minutes of exposure, 7. PBMC with 1800 MHz RF-EMF exposure, a distance of 25 cm for 30 minutes of exposure, 8. PBMC with 1800 MHz RF-EMF exposure, a distance of 25 cm for 45 minutes of exposure, and 9. PBMC with 1800 MHz RF-EMF exposure, a distance of 25 cm for 60 minutes of exposure.

Table 4. Description of the PBMCs treatment groups.

Group No.	RF-EMF	Distance (in cm)	Duration (in minutes)
1	None		
2	1800 MHz	5	15
3	1800 MHz	5	30
4	1800 MHz	5	45
5	1800 MHz	5	60
6	1800 MHz	25	15
7	1800 MHz	25	30
8	1800 MHz	25	45
9	1800 MHz	25	60

Figure 1 showed an illustration of the chamber and instruments for 1800 MHz EMF RF exposure. Controlling temperature changes was done by using a digital thermometer that had been installed automatically in accordance with body temperature (around 37^{o}C). Digital thermometer connected with a lamp that functions as a heater. The lights were placed inside the chamber, while the digital thermometer was placed outside the chamber so that it was easy to control the temperature. The way the thermometer works was when the temperature has not reached 37°C, the lamp will continue to burn until the temperature

of the chamber reaches 37°C. If temperature in the chamber has reached 37°C, the lamp will automatically turn off and will come back on when the temperature of the box had fallen to 36°C.

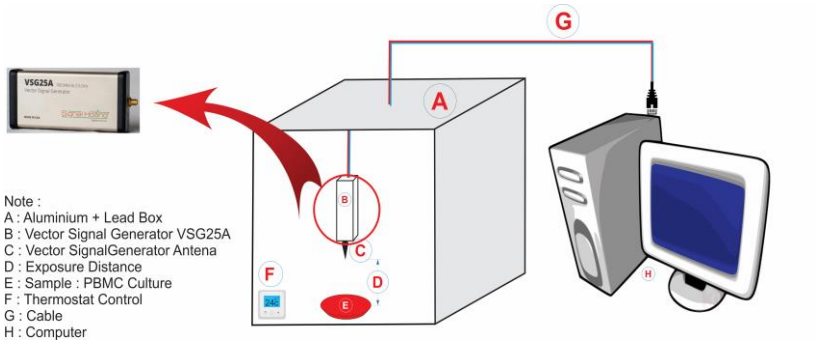

Figure 1. Schematic of the instrument for 1800 MHz EMF RF exposure.

Statistical Analysis. Statistical differences between experimental groups were determined by paired student t-test. Quantitative data were expressed as the mean and standard deviation. Comparisons between two groups were tested for statistical significance using One-Way Anova and Tukey test or the nonparametric Kruskal-Wallis and Mann-Whitney U-test as appropriate. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS statistical software (version 20, SPSS Inc., Chicago, IL, USA).

RESULTS

The results of the 1800 MHz RF-EMF exposure on fresh whole blood from healthy volunteers showed significant differences between pre- and post-exposured groups: hemoglobin level, RBC, MCV, MCHC, RDW-SD, WBC, neutrophil, monocytes, platelets and PDW (Table 5).

Table 5. Comparison of the results of a complete blood count from healthy volunteers pre- and post-exposure to RF-EMF

Parameters (unit)	Pre-exposure (Mean ± SD)	Post-exposure (Mean ± SD)	p-value
Hemoglobin (g/dL)	15.83 ± 2.57	16.48 ± 2.42	0.003*
Hematocrite (%)	45.08 ± 5.71	45.35 ± 4.92	0.673
MCV (fL)	82.35 ± 3.99	79.22 ± 4.38	0.000*
MCH (pg)	28.80 ± 1.64	28.65 ± 1.80	0.452
MCHC (g/dL)	34.97 ± 1.55	36.25 ± 1.89	0.001*
RDW-SD (fL)	37.90 ± 3.58	36.52 ± 3.30	0.000*
RDW-CV (%)	12.75 ± 1.41	12.93 ± 1.39	0.072

RBC (x 10^6/µL)	5.49 ± 0.78	5.74 ± 0.67	0.005*
WBC (x 10^3/µL)	6.30 ± 0.76	5.59 ± 0.62	0.018*
Lymph (x 10^3/µL)	1.96 ± 0.49	1.93 ± 0.49	0.477
Mono (x 10^3/µL)	0.45 ± 0.11	0.31 ± 0.10	0.018*
Neut (x 10^3/µL)	3.71 ± 0.74	3.20 ± 0.60	0.012*
PLT (x 10^3/µL)	200.67 ± 22.24	49.33 ± 9.31	0.000*
PDW (fL)	9.95 ± 2.02	12.45 ± 3.45	0.075
PCT (%)	0.19 ± 0.02	0.05 ± 0.01	0.000*

*Differ significantly. RBC: red blood cell, WBC: white blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: red cell distribution width, PDW: platelet distribution width, PLT: platelet, PCT: plateletcrit, Lymph: lymphocyte, Mono: monocyte, Neut: neutrophil

Table 5 showed the effect of RF-EMF on the number of CD4+ T cells. The data showed the distance of the source of exposure to the PBMCs has a direct relationship to the number of CD4+ T cells. However, the duration of exposure was inversely correlated with the number of CD4+ T cells. On 25 cm exposure, there was an increase in the number of CD4+ up to 45 minutes, but at 60 minutes of exposure, there was a significant decrease.

Table 5. The comparison of the number of CD4+ T cells between the two groups based on variable exposure durations.

1800 MHz	Duration (in minutes)	CD4+ T Cell (mean ± SD)	p-value
5 cm	60[a]	21.34±1.95	0.000*
	45[b]	16.57±0.42	
	30[ac]	21.20±0.53	
	15[d]	26.74±0.61	
	Control[de]	26.86±3.14	
25 cm	60[a]	26.99±2.11	0.000‡
	45[b]	34.03±3.01	
	30[b]	35.58±1.62	
	15[b]	33.72±1.44	
	Control[c]	26.86±3.14	

*Kruskal-Wallis test followed by Mann-Whitney test, ‡ANOVA test followed by Tukey test, [a-e]Mean values followed by different superscript letters differ significantly ($p<0.05$).

Table 6 showed the effect of RF-EMF on several parameters. The CD4+ data showed the distance of the source of exposure to the PBMCs has a direct relationship to the number of CD4+ T cells. However, the duration of exposure was inversely correlated with the number of CD4+ T cells. On 25 cm exposure distance, there was an increase in the number of CD4+ up to 45 minutes, but at 60 minutes of exposure, there was a significant decrease.

The number of IL-2 has a positive correlation with the duration of 1800 MHz RF-EMF exposure (15, 30, and 45 min). But the number was decreasing at the 60-minute when compared to PBMCs without exposure (Table 6).

The longer the exposure the number of IL-10 significantly decreases. On 5 cm exposure distance, there was an increase until 45 minutes, but at 60 minutes there was a significant decrease when compared to the control. On 25 cm, there was a significant increase, except for the 45 minutes (Table 6).
The longer the exposure the number of IL-17a significantly decreases, and increases only at 15 minutes with 5 cm exposure distance (Table 6).

Table 6. The comparison of the number of CD4+ T cells, and IL-2, IL-10, IL-17a-expressing CD4+ T cells between the two groups based on variable exposure

Distance	Duration	CD4+ (mean±SD)	IL-2 (mean±SD)	IL-10 (mean±SD)	IL-17a (mean±SD)
5 cm	60 minutes	21.34±1.95^{a}	15.26±0.38^{a}	9.95±0.81^{a}	22.57±0.98^{a}
	45 minutes	16.57±0.42^{b}	21.26±0.94^{b}	22.71±0.79^{b}	28.28±0.60^{b}
	30 minutes	21.20±0.53^{ac}	28.43±0.54^{c}	28.19±1.30^{c}	28.19±1.30^{c}
	15 minutes	26.74±0.61^{d}	37.14±0.67^{d}	37.51±0.91^{d}	41.95±1.40^{d}
	Control	26.86±3.14^{d}	17.73±8.87^{e}	19.64±12.59^{ae}	31.58±12.48^{e}
	p-value	0.000*	0.001*	0.002*	0.000‡
25 cm	60 minutes	26.99±2.11^{a}	20.93±0.21^{a}	26.43±0.19^{a}	25.44±0.84^{a}
	45 minutes	34.03±3.01^{b}	26.72±1.04^{b}	13.56±0.17^{b}	24.02±0.34^{b}
	30 minutes	35.58±1.62^{b}	19.13±0.60^{c}	21.33±0.68^{c}	29.86±0.56^{c}
	15 minutes	33.72±1.44^{b}	25.90±0.57^{bd}	29.36±0.80^{d}	15.67±0.50^{d}
	Control	26.86±3.14^{c}	17.73±8.87^{e}	19.64±12.59^{e}	31.58±12.48^{e}
	p-value	0.000‡	0.000‡	0.000‡	0.000‡

Notes: *Kruskal-Wallis test followed by Mann-Whitney test, ‡ANOVA test followed by Tukey test, $^{a\text{-}e}$Mean values followed by different superscript letters differ significantly ($p<0.05$).

DISCUSSION

Studies on the biological effects of EMF such as power-frequency, radiofrequency, and microwaves at the cellular level are increasing in number. Many types of EMF are widely used in various places daily (12,13). Exposure to EMF as non-infection or external stress factors on the immune system is not less important than infection factors and internal stress (13,14). Low-frequency EMF has shown to be able to affect various cell functions at the cellular level. The effects were observed in the synthesis of DNA, the transcription of RNA, the expression and phosphorylatioin of protein, proliferation and differentiation of cell, apoptosis, and the reaction of oxidation-reduction (re-dox) (15). Internal stress, infection or non-infection factors such as alcohol, toxins, EMF exposure trigger an

effective and highly sensitive immune cells response (13). The result of studies on the effects of EMF on the immune system is important for evaluating their adverse effects. Laboratory studies on cells aim to explain the basic mechanism of the relationship between RF-EMF exposure and its effects on the cells of the immune system. In our previous study, we used 900 and 1800 MHz frequencies, but the results of exposure with 900 MHz were not significant. Another reason why we use 1800 MHz electromagnetic wave frequency is because many electronic devices in Indonesia utilize that particular radio frequency, i.e. mobile phones operating with Global System for Mobile Communications (GSM). The hemoglobin level post-exposure increased significantly compared to pre-one. The increase in Hb was accompanied by an increase in MCHC. This increase in hemoglobin may be caused by the leakage of hemoglobin from RBC to the outside due to the disruption of erythrocyte membrane integrity but not to cause lysis. This is evident from the number of RBC that significantly increased post-exposure. The increase in RBC may be due to erythrocyte fragments still large enough to be counted as intact erythrocytes. This was proven by the decrease in MCV. Previous study reported that there was a clear imbalance in the forms and function of RBC and their contents when exposed to EMF. Given that, this exposure may be attributed due to a clear change in the form of the membrane that surrounds the red blood and finaly it leads to an imbalance in the function of the red cells (16). The total number of WBC, neutrophils, and monocytes, and platelet decreased significantly after exposure. This was likely due to the disruption of membrane integrity or cell lysis was caused by the effects of non-thermal radiation. No previous studies on the effects of RF-EMF exposure on blood cells have been carried out *in vitro* laboratory experiments. But, in vivo experiments in animals or observational study in human existed. The study by Al-Mayyahi *et al.* (17), regarding the adverse effects of EMF on complete blood parameters of female mice, that showed a significant reduction in granulocytes, erythrocytes, hemoglobin, hematocrit (HCT), mean platelet volume (MPV) and PCT levels in the exposed group compared to control group. Abdolmaleki *et al.* (18), observed furnace workers, welders and computer operators who had at least five years work experience and were 48 hours exposed to waves per week. The study showed that the number of RBC, MCV, and platelets in the exposure group was significantly increased. In contrast, Hb level, lymphocytes, and WBC were decreased. In this study, PBMCs exposed to RF-EMF for 30, 45, and 60 minutes could significantly decrease the number of CD4+ T cells when compared with PBMCs control without exposure. The mechanism of the decrease might be through apoptosis. On exposures at a distance of 25 cm for 15, 30, and 45 minutes there was an increase in CD4+ cell number compared to the control group without exposure. But, there was a tendency to decrease at a longer duration of exposure. This temporary increase was likely due to the normal cellular immune reaction to the external stimuli. RF-EMF more often causes a decrease in cell number rather than stimulates proliferation. The reduction in the number of CD4+ T cells was greater at 5 cm exposure compared to 25 cm, which showed that distance affecting the magnitude of the exposure effect. The main criteria for evaluating cellular reactions to external factors are cell growth and survival, which depends on the extent of the effect. Severe damage inhibits or suppresses cell growth and causes cell death. Very rarely, cell growth is accelerated by external factors, except by specific growth factors. Tian *et al.* reported that at SAR >20 W/kg, cell survival rates decreased (19). Takashima *et al.* compared the effect of 2,450 MHz EMF on high SAR on growth, survival, and cell cycle (20). The results showed that they were not significantly affected (21). Several studies have shown an increase in apoptosis in human myelogenous leukemic cell lines, HL-60

and ML-1, in thymocytes and macrophages of mice exposed to long-term extremely low frequency (ELF)-EMF. A study has shown a decrease in apoptosis in lymphocytes exposed to 100 μT for 16 hours without mitogen (22). But a study by Hirose et al. showed no significant difference in the percentage of cell apoptosis observed in groups exposed to RF signals (2,142.5 MHz, SAR up to 800 mW/kg) and negative controls (20,23,24). In our study, a decrease in CD4+ T cells after exposure, was followed by a decreaseed IL-2 production significantly on the distance of 5 cm and the duration of 60 minutes, when compared with control. Conversely, decreased IL-2 can result in a decrease in CD4+ T cell proliferation. IL-2 plays roles in promoting tolerance and preventing autoimmunity (25). Decreased IL-2 production due to exposure to RF-EMF may lead to a susceptibility to viral infections, a tendency to suffer from Inflammatory bowel disease (IBD), low immune tolerance, and autoimmune diseases. The increased IL-2 on 15, 30, and 45 minutes of exposure appears to be temporary. But, there was a tendency for IL-2 to decrease with the increasing duration of exposure. On a distance of 25 cm, the results were inconsistent. The decrease in IL-2 production at a distance of 5 cm exposure was greater than the 25 cm. The distance from the EMF source to cells influences the magnitude of the effect. The results of our study differ from a study by Tuschl et al. regarding the adverse effects of GSM modulated RF fields on the functional competence of human immune cells. But, the exposure was done with 1950 MHz basic GSM, SAR of 1 mW/g with intermittent mode (5 min "on", 10 min "off") for 8 hours. Emissions from cellular telephones do not adversely affect IL-1, -2, and -4; INF-g; and INF-a (26). In our study, the longer the exposure, the number of CD4+ T cells expressing IL-10 decreased significantly. At an exposure distance of 5 cm, IL-10 increased significantly compared to controls, but there was a tendency to decrease. At a distance of 5 cm with an exposure duration of 60 minutes, IL-10 decreased significantly compared to control, whereas at a distance of 25 cm the results were inconsistent. Interleukin-10 major function is suppressing inflammation and immune system because it heavily inhibits proinflammatory cytokines production. The findings in mice with IL-10 deficiency showed that IL-10 is an important endogenous inhibitor of cell-mediated immunity. Because, mice with IL-10 deficiency acquire autoimmune disease and generate excessive inflammatory responses (25). From our study on IL-10, it could be implied that a decrease in IL-10 due to long exposure to RF-EMF would be potentially harmful to the body which may increase the risk of autoimmune disease and exaggerate inflammatory responses. Interleukin-17a is a pro-inflammatory interleukin produced mainly by Th17 cells, associated with many inflammatory diseases of contact delayed-type hypersensitivity and airway (27). In our study, the expression of IL17a showed a significant decrease in PBMCs exposed to 1800 MHz RF-EMF, except for 15 minutes exposure at the closest distance (5 cm). The longer the exposure, the number of IL-17a decreases significantly. It could be implied that CD4+ T cells exposed to 1800 MHz RF-EMF might suffer a decrease in IL-17a production, which could result in an increased risk of extracellular bacterial and fungal infections. Interestingly, decreasing IL-17a is actually beneficial for autoimmune diseases, but how about the effects of chronic/long-term exposure, still needs further study. Level of exposure decreases with increasing distance from the transmitter (5). The highest local exposure is when using cellular or cordless telephones. In addition to these environmental exposures, RF-EMF exposure from radars and microwaves can occur and safety rules must be applied (26,28–30). The intensity of radiation received will be inversely proportional to the square of the distance between the object receiving radiation and the source of radiation (28). The proposition was consistent with the result

of our study. The decrease in CD4+ cell number and the cytokines produced was greater at exposure from 5 cm than the 25 cm. However, at an exposure distance of 25 cm, the results were inconsistent. Interaction can occur through the thermal or non-thermal mechanism. The thermal mechanism occurs as a result of temperature changes in the tissue caused by the RF field (4,31,32). Non-thermal mechanism is not directly related to changes in temperature but more related to other changes in the tissue caused by electric or magnetic fields (4,32). Federal Communications Commission (FCC) and other regulatory agencies, claimed that only factors with thermal mechanism affecting health. Therefore, exposure limits are set based only on thermal effects. Nevertheless, many studies, *in vivo* and *in vitro*, proved that significant harmful biological effects occur due to the effects of non-thermal RF exposure (32,33). In our study, the temperature was maintained within the physiological range, 36.5-37°C, to eliminate thermal effects disrupting the cellular immune system. Thus, changes occurred in our study must be due to non-thermal mechanisms. The possibility of harmful effects on a subset of CD4+ T cells cannot be underestimated if the results of the study are inconsistent and/or from *in vitro* cell experiments. Further research is needed to explore the mechanism of the effect of exposure to helper T cells. Furthermore, comprehensive and well-coordinated study is needed to overcome all the limitations of previous studies, especially replication studies to concretize previous findings. For now, the community must follow the precautionary principle and limit EMF exposure as much as possible. Our further research is exploring the mechanism of RF-EMF exposure in causing cellular immune system disorders, in PBMCs of normal subjects and autoimmune disease patients. We conclude that exposure to RF-EMF 60 minutes 5 cm causes changes in blood cells with their indexes. The 60 minutes of PBMC exposure to RF-EMF with a distance of 5 cm causes a significant reduction in the number of CD4+ T cells, and the expression of IL-2, IL-10 and IL-17a. Immune cells recognize electromagnetic fields at low exposure levels and produce biochemical stress responses. Exposure to electromagnetic fields at low levels (non-thermal) can impair immune function if it occurs continuously for a certain period of time. Scientific evidence shows that the current safety standards for low-frequency EMF are inadequate because most of the standards were made by only considering thermal effects. Therefore, the authorities need to re-evaluate safety standards.

ACKNOWLEDGEMENTS

The authors thank Mrs. Heni Endrawati for her excellent technical work in PBMC culture, and Mr. Wahyudha Ngatiril Lady for his excellent technical work in flow cytometry.

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