

## ORIGINAL ARTICLE

# The Electromagnetic Radiation Effect of Mobile Phone to the Inflammation Cells in Gingival (*Rattus Norvegicus*)

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## ABSTRACT

**Introduction:** Gingiva is one of the periodontal tissue that protects the tooth. Mobile phone's electromagnetic radiation triggers Reactive Oxygen Species (ROS) production in the body hence causing gingival inflammation. Inflammatory mediators will initiate the migration of neutrophils, macrophages, mast cells, and lymphocytes. The purpose of this study was to investigate the effect of mobile phone electromagnetic radiation on neutrophil, macrophage, mast cell, and lymphocyte count in the gingival tissue of *Rattus norvegicus*. **Methods:** Fifteen rats were divided into three groups randomly. Group A was given electromagnetic radiation (2500-2700 MHz) exposure 24 hours per day for 14 days, Group B six hours per day for 14 days and group C was control group. Radiation was given at 12 cm distance from cages of group A and B. On the 14th day, labial gingiva of rat's mandible was cut. Histological preparations were stained using *Hematoxylin Eosin* and measured the number of neutrophil, mast cell, macrophage, and lymphocyte. The data were analyzed by *Kruskal Wallis* and *U-Mann Whitney*. **Results:** There was significant difference between three groups of lymphocyte but no significant differences for neutrophil, mast cell, and macrophage. **Conclusion:** The conclusion is 2500-2700 MHz electromagnetic radiation of mobile phone for 14 days increase the number of lymphocyte on rat's gingiva, but doesn't increase the number of neutrophil, macrophage, and mast cell.

**Keywords:** Radiation, Lymphocyte, Neutrophil, Mast cell, Macrophage

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## INTRODUCTION

One of the dental and oral health problems that are commonly found in Indonesia is the periodontal tissue problem, which is 25.9% (27). Outer part of periodontal tissue that surrounds the tooth and attaches to alveolar bone is called gingiva. Other part of periodontal tissues are periodontal ligament, cementum, and alveolar bone. The four components have a function to support teeth (4). Problem that often occurs in periodontal tissue is periodontal disease. Periodontal disease is a condition in which periodontal tissue components have inflammatory process (15).

Inflammation is the basis of various diseases (13). Inflammation is a reaction due to a microcirculation injury to the tissue that aims to restore the function

and structure of the tissue back to normal (18). The cells involved in the inflammatory process are neutrophils, macrophages, mast cells, lymphocytes, and eosinophils (4). Neutrophils are inflammatory cells in the gum connective tissue that normally migrate through the junctional epithelium to the gingival pocket or sulcus. Damaged gingival tissue will show infiltration of neutrophil cells microscopically (4).

Functions of macrophages are to phagocytize bacteria, viruses, necrotic tissue and foreign particles in tissue (22). Macrophages also play a role in tissue regeneration or remodeling. In tissue remodeling, macrophages secrete mediators such as Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), proteolytic enzymes, Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) and pro inflammatory cytokines that will activate M1 macrophages (19).

Mast cells spread throughout the body, abundant in oral and gingival mucosa connective tissue (4). Mast cells participate in innate and adaptive immunity

that will secrete pro-inflammatory, vasoactive, and mitogenic mediators in response to pathological conditions (7).

Lymphocytes work as host's defense against pathogenic and foreign body infections (30). Increase of lymphocytes were caused by stimulation from infectious hepatitis viruses, toxoplasmosis, measles, parotitis, allergic reactions, drug sensitivity, malignant lymphoma, and antigens (6). Lymphocytes persist in inflamed tissue for 7 days and gradually decrease until the inflammatory triggering factors disappear (18). Uncontrolled inflammation may cause loss of integrity of the periodontal tissue and alveolar bone (29).

Inflammation is caused by several trigger factors, such as: physical, environmental, emotional, chemical, and nutritional factors (13). One of the environmental factor that can cause inflammation in periodontal tissue is radiation (7).

Mobile phones are one of the wireless communication devices that utilize radio wave radiation as a medium (10). Waves that emitted by mobile phones are electromagnetic wave (26). Mobile phones usage is not limited by space and time (10). Utilization of mobile phones is rapidly increasing in many aspects of life (33). Research shows that the average mobile phone usage by students approximately six hours per day (9). It can even reach 24 hours per day, and people often use mobile phone as bedside alarm (1).

Mobile phones are very beneficial for human life, but electromagnetic radiation from mobile phones have a negative impact to human health (26). Electromagnetic waves can increase Reactive Oxygen Species (ROS), causing oxidative stress, and cell or tissue damage (34). Free radicals that induce oxidative stress are known to trigger inflammation in periodontal tissues (35). Mobile phone with 300 – 3000 MHz may emit destructive effect on human tissue (23).

The purpose of this research was to find out the effects of electromagnetic radiation into the number of neutrophil, macrophage, mast cell, and lymphocyte in the gingival tissue of *Rattus norvegicus* rat.

## MATERIALS AND METHODS

The ethical clearance (No.00225/KKEP/FGK-UGM/EC/2019) and research application permit (No.13415/UN1/FGK1/KM/2019) from the Faculty of Dentistry Universitas Gadjah Mada was attained upon commencement of the study. Research was conducted in Laboratorium Penelitian dan Pengujian Terpadu Universitas Gadjah Mada. Xiaomi Mi 4i with 2500 – 2700 MHz frequency (based on manufacturer information) was chosen for this research. The research subjects in this study were *Rattus norvegicus*

rats obtained from the Laboratorium Penelitian dan Pengujian Terpadu Unit IV, Universitas Gadjah Mada, Yogyakarta. *Rattus norvegicus* rats were chosen as research subjects because they are easily manipulated in the laboratory, and their gingival tissue is similar to humans (27). Another reason is because they have high reproductive abilities and genetic traits can be made uniform in a short time (14).

Number of research samples were determined by the "Resource" method:

$$E = \text{Total number of experimental animals} - \text{Total number of groups}$$

The value of E is the degree of freedom of ANOVA (between 10-20) (5). There are 3 groups in this study, which are group A as treatment group exposed to electromagnetic radiation 24 hours/day for 14 days, group B exposed for 6 hours/day for 14 days, and group C as a negative control (not exposed to electromagnetic radiation). Based on the calculation of the formula, the total number of experimental animals in each group is 5 rats. This number is determined to get the value of E between 10 to 20 and minimize the number of experimental animals used. This study used 5 research subjects for each cage for each group, so 15 rats were used.

Mobile phone was placed 12 cm from the cage of group A and B, while group C was placed in different room with same conditions. Mobile phone was kept on for 24 hours while charged and mobile data was on, and then author came every day at 06.30 AM to put group B into the treatment room, and at 00.30 PM took out group B to separate room from group A and C. Fifteen *Rattus norvegicus* rats were divided into three groups randomly by one stage random sampling method, five rats for each group. Inclusion criteria for animal subject were 2 – 3 months old male rats with 250-350 grams of bodyweight and fed with pellet and drinking water. The food in the form of pellets contains antioxidants, that contain vitamins and sodium bicarbonate. The temperature was set to 28° C and humidity 65 g/m<sup>3</sup>.

On the 14th day, euthanasia was performed by intraperitoneal overdose injection 0,1 ml each for ketamine and xylazine. Histological preparations were obtained from the labial gingival tissue of the mandibular incisors and then stained by *Hematoxylin Eosin*. Radiation effect was observed by the increase of inflammatory cells in gingiva because it is the outermost part of teeth supporting tissues, so it is more sensitive to mobile phone radiation.

The number of neutrophil, macrophage, mast cell, and lymphocyte were counted using a binocular microscope with 400x magnification (objective lens

40x and ocular lens 10x). Three visual fields (one third upper third, middle third and lower third of the gingiva) were observed from each sample by two calibrated observers and analyzed with IBM SPSS Statistics Ver. 25.

## RESULTS

Research was conducted on 15 rats divided into three groups equally for 14 days. Group A was given radiation for 24 hours per day, group B was given radiation for six hours per day, and group C was control group.

Histological preparations of *Rattus norvegicus* gingiva were dyed with *Hematoxylin eosin* and observed using a binocular microscope (Optilab) with a 400x magnifications. The calculation of neutrophil, macrophage, mast cell, and lymphocyte can be seen in table I.

From table I, we can see the differences in the number of neutrophil, macrophage, mast cell, and lymphocyte between groups A (24 hours), B (six hours), and C (control). The data is ratio scale data, so the normality test is performed which is a prerequisite for parametric data analysis. A large standard

deviation indicates that there is a lot of variance in the observed data around mean.

The normality test result was insignificant ( $p < 0.05$ ) in all treatment groups. This mean that the data wasn't normally distributed, so data transformation was performed. Normality test of the transformed data was carried out.

The transformed data also wasn't normally distributed, so *Kruskal Wallis* test was performed to determine whether there was a difference in the influence of mobile phone electromagnetic radiation on lymphocytes in groups A, B, and C. The results of the *Kruskal Wallis* test can be seen in table II.

*Kruskal Wallis* test results shows there were no significant value for neutrophil, macrophage and mast cell group, while there was a significant value for lymphocyte group. Only lymphocyte group had statistically significant differences. *U-Mann Whitney* test was carried out to determine differences in the number of lymphocytes between groups that were significant. The test results are significant if the value obtained is less than 0.05. *U-Mann Whitney* test results are presented in table III.

**Table I : The inflammatory cells calculation results based on the treatment group**

Exposure Group	n (number of sample)	Number of lymphocytes (mean $\pm$ std deviation)	Number of mast cells (mean $\pm$ std deviation)	Number of neutrophils (mean $\pm$ std deviation)	Number of macrophages (mean $\pm$ std deviation)
Group A	5	2,40 $\pm$ 1,67	0.80 $\pm$ 1.30	1,60 $\pm$ 1,14	0,80 $\pm$ 1,09
Group B	5	0,60 $\pm$ 0,54	0.20 $\pm$ 0.44	0,20 $\pm$ 0,45	0,20 $\pm$ 0,44
Group C	5	0,10 $\pm$ 0,22	0.30 $\pm$ 0.44	0,50 $\pm$ 0,71	0,20 $\pm$ 0,44

Information :

Group A = group given 24 hours per day for 14 days

Group B = group given six hours per day for 14 days

Group C = group that was not given exposure for 14 days

**Table II : Kruskal Wallis test results between the four groups**

	Neutrophil	Macrophage	Mast cell	Lymphocyte
Kruskal-Wallis	5,190	1,218	0,652	9,470
df	2	2	2	2
p	0,075	0,544	0,722	0,009*

Information :

df = degree of freedom

p \* = Significance ( $p < 0.05$ )

**Table III : U-Mann Whitney test results**

	Group A	Group B	Group C
Group A	-	0,034*	0,007*
Group B	-	-	0,120
Group C	-	-	-

Information :

Group A = group given 24 hours per day for 14 days

Group B = group given six hours per day for 14 days

Group C = group that was not given exposure for 14 days

\* = significance (p <0.05)

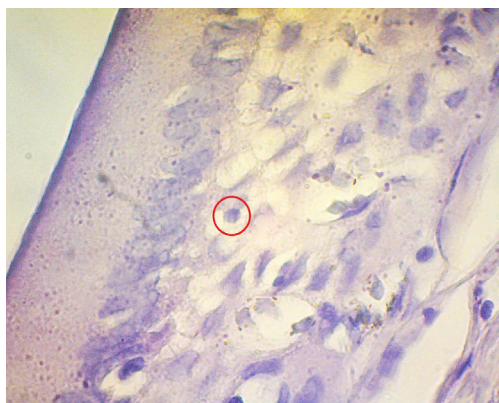
*U-Mann Whitney* test results show:

1. Group A (24 hours) ≠ Group B (six hours): Group A and B had significant differences.
2. Group A (24 hours) ≠ Group C (control): Group A and C had significant differences.
3. Group B (six hours) = Group C (control): Group B and C had no significant differences.

**DISCUSSION**

The results showed only lymphocyte in Group A, B, and C had significant differences. This indicate that mobile phone electromagnetic radiation (2500-2700 MHz) for 14 days didn't affect neutrophils, mast cells, and macrophages, but had effect on lymphocytes. The histological of neutrophils and lymphocytes can be seen on figure 1 and 4.

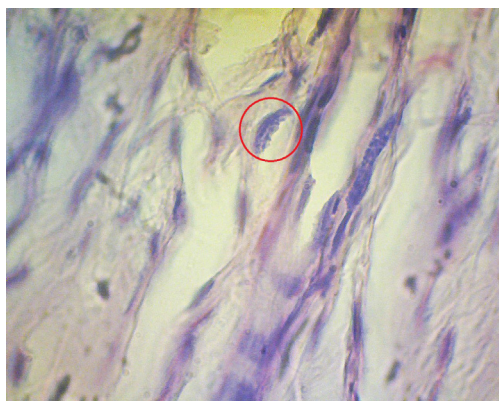
Radiation exposure causes gingival cell damage, leading to initial inflammation. This inflammatory response activates pro-inflammatory cytokines, chemokines, and growth hormone in microvasculature thereby initiating innate immune system comes to the damaged tissue. Continued inflammation will trigger the adaptive immune system, namely B and T lymphocytes (7). Lymphocytes will remain until the 7th day and will decrease if the trigger factors were removed (18). Increased number of lymphocytes in tissue exposed to electromagnetic radiation is because the nature of lymphocytes which are very sensitive to radiation compared to other cells, even at very small doses (31). Electromagnetic radiation has a harmful effect that will stimulates the hematopoietic system to continue to produce more lymphocytes a lot (3). These theories explain how electromagnetic



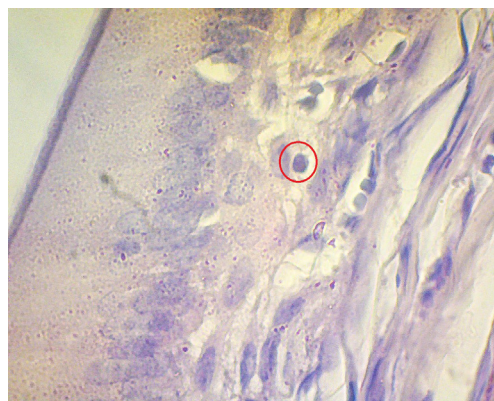
**Figure 1 : Neutrophil**



**Figure 2 : Macrophage**



**Figure 3 : Mast Cell**



**Figure 4 : Lymphocyte**



radiation affects lymphocytes production. Lymphocytes can be seen on Figure 4.

Another study examined the effect of chemotaxis and phagocytosis of neutrophil cells exposed by electromagnetic radiation 2450 MHz for four hours and 24 hours. There are no neutrophil cells undergoing apoptosis, this is supported by the research of Koyama et al. (2015) that chemotaxis and phagocytosis of neutrophil cells are not affected by 2450 MHz electromagnetic radiation. Neutrophils were chosen because they are part of innate immunity that defense against pathogen invasion by chemotaxis and phagocytosis. The outcome was no difference in the chemotactic and phagocytic activity of neutrophil cells after being exposed to 2450 MHz electromagnetic radiation for four and 24 hours. Researchers explain there was no effect because the exposure duration and frequency were minimal. Another study investigate ROS production after being exposed to 1800 MHz electromagnetic radiation, but the outcome wasn't significant. Superoxide anion production was also not seen even though the group was given exposure to electromagnetic radiation added to lipopolysaccharides, so that oxidative stress and tissue damage did not occur (20). Other factors that are likely to influence the results of this study are the distance of exposure, environmental factors, and internal factors of the mobile phone itself. Neutrophils can be seen on figure 1.

Macrophage's result wasn't significant because of some point. Previous studies examined 2450 MHz electromagnetic radiation exposure to macrophage and there was no effect (8). Low duration and exposure distance play a major role in decreasing ROS production. Decreased ROS reduces oxidative stress in tissue (21). Macrophages are activated by chemokines produced by resident cells at the site of lesions (macrophages, mast cells, fibroblasts, and adipose cells) and apoptosis of neutrophil cells (18). Chemokines that activate macrophages include Tumor Necrosis Factor- $\alpha$ , Interleukin-1 $\beta$ , Interleukin-6, Interleukin-13, Interleukin-4, Interleukin-5, and Interferon- $\gamma$  (11)(32). Decreased ROS production also decrease chemokines production by the resident cells. There are no neutrophil cells undergoing apoptosis, this is supported by the research of Koyama et al. (2015) that chemotaxis and phagocytosis of neutrophil cells aren't affected by 2450 MHz electromagnetic radiation (17). Macrophage can be seen on figure 2.

Mast cell's result also wasn't significant because of some point. In the histological observations, there was a change in the number of mast cells but it wasn't significant because there was no possible inflammation due to several things. The non-thermal biological

effects of electromagnetic radiation were mediated by the ROS and decreased activity of catalase, Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH). Oxidative stress happens when number of ROS is more than antioxidants, which makes cell damage and secretes chemokine (TNF- $\alpha$ , IL-1, IL-4, IL-6, IL-8, IL-13, platelet-activating factors (PAF), and leukotriene C4) which will induce inflammation (16). In a study conducted in vitro of mast cells irradiated with 2450 MHz microwave waves did not have a significant effect on mast cell viability, mast cell percentage, and histamine secretion in mast cells in peritoneal cells. This is because the energy per photon of electromagnetic radiation from the microwave is not enough to affect mast cells histamine secretion (24). In our study we use 2500-2700 MHz microwave with 12 cm distance, our research has higher microwave but still the result showed no significant results in mast cell percentage. Mast cell can be seen on figure 3.

Neutrophil, macrophage, and mast cell's result weren't significant because of three reasons. The first possibility is related to the distance from the radiation source to the cage. Previous studies had examined Specific Absorption Rate (SAR). SAR was affected by the mobile phone network, the characteristics of the mobile phone, the energy emitted by the mobile phone, and the position of the mobile phone. The outcome, there are differences in SAR between the position of mobile phones held parallel to the cheeks, 15° away from the cheeks, and 30° away from the cheeks, so distance plays a major role for SAR (12). A built up of Reactive Oxygen Species in cells is caused by the effects of electromagnetic waves from mobile phone (16). Based on that, the farther distance of the mobile phone towards rats will cause the smaller amount of energy absorbed, so that the damage to the rat's gingival tissue does not significantly visible and the amount of neutrophil, mast cells, and macrophage are stable.

Second possibility is about food content. The food contains antioxidants, vitamins and sodium bicarbonate. Previous studies have investigated the antioxidant effects caused by vitamin C combined with sodium bicarbonate. Vitamin C has function as an effective antioxidant by eliminating free radicals in the body in the form of Reactive Oxygen Species. Sodium bicarbonate plays a big role on acid base stability, electrolyte stability, and relieving respiratory alkalosis. The results of previous study was the use of vitamin C and sodium bicarbonate (C-Na) effectively increase endogenous antioxidants in the body (35). Vitamin E (A-Tocopherol) and vitamin A also function as antioxidants (35). Rat's food containing vitamins and sodium bicarbonate increases antioxidant in the rat's body which decreases ROS, so that tissue

damage is reduced and didn't increase the amount of neutrophil, macrophage, and mast cells in rat's gingival tissue.

There are also variable factors that are not controlled by mobile phones that can affect the number of inflammatory cells in the gingival tissue are radiation strength, frequency level, nature of modulation, duration of exposure related to radiation strength (23). Other studies have shown that mice that received 10 days of electromagnetic radiation experienced less histopathological damage compared to mice that had 40 days of exposure. From these statements it can be seen that the nature of mobile phones that are difficult to control and the lack of duration of exposure causes electromagnetic radiation does not have a significant impact on neutrophil, macrophage, and mast cell increase in rats (2).

Group B (exposed to mobile phone radiation six hours per day) and group C (control) showed no different results, while group A (exposed to mobile phone radiation 24 hours per day) was different. The results occurs due to differences in the duration of exposure to electromagnetic radiation, which means that electromagnetic mobile phone exposure at six hours per day for 14 days to the gingival tissue of rats was not enough to increase the amount of lymphocytes. The difference in results occurs due to variation of exposure time. It is also affects the magnitude of radiation effect on biological tissue(19).

Mobile phone electromagnetic radiation affects human health by increasing ROS and reduces antioxidants such as catalase, Superoxide Dismutase (SOD), and Glutathione Peroxidase (GSH). The number of ROS that has exceeded the amount of protective antioxidants will cause oxidative stress and tissue damage (34). ROS induce damage to lipids, proteins, and nucleic acids. It will trigger membrane peroxidation and cause cell damage, such as disruption of membrane transport, changes in structure, changes in protein receptors, and changes in cell membrane enzyme activity(16).

Radiation exposure causes gingival cell damage, leading to initial inflammation. This inflammatory response activates pro-inflammatory cytokines, chemokines, and growth hormone in microvasculature thereby initiating innate immune system comes to the damaged tissue. Continued inflammation will triggers the adaptive immune system, namely B and T lymphocytes (7). Lymphocytes will remain until the 7th day and will decrease if the trigger factors were removed (18). Increased number of lymphocytes in tissue exposed to electromagnetic radiation is because the nature of lymphocytes which are very sensitive to radiation compared to other cells, even at very small doses (31). Electromagnetic radiation has a

harmful effect that will stimulates the hematopoietic system to continue to produce more lymphocytes a lot (3). These theories explain how electromagnetic radiation affects lymphocytes production. Lymphocytes can be seen on Figure 4.

## CONCLUSION

The conclusion of this study was the electromagnetic radiation of mobile phones for 14 days didn't increase the number of neutrophils, macrophages, and mast cells, but the electromagnetic radiation of mobile phone for 14 days with 24-hour exposure time did increase the amount of lymphocytes in the gingival tissue of *Rattus norvegicus* rats.

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