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Cell phone radiation effect on osteocalcin and bone alkaline phosphatase

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ABSTRACT

Background: *Electromagnetic fields are forces associated with moving electric charges and have electrical, magnetic components and contain electromagnetic energy, one of which is radio frequency (RF) energy which is commonly used in telecommunications. Cell phones are one of the RF electromagnetic radiation devices that can emit 90-2450 MHz waves and are often placed near the head. The human body works like an electromagnetic field in that each cell has its own electrical circuit characteristics. As the number of electromagnetic radiation devices in the environment increases, the electromagnetic balance in the human body may be disturbed by the magnetic waves produced by cell phones. Electromagnetic radiation is known to have the ability to induce oxidative stress, which is characterized by the accumulation of reactive oxygen species (ROS) in tissues. The accumulation of ROS in the body leads to osteoblast cell death. Osteoblasts are needed for mineralization of the extracellular matrix during bone growth. Therefore, bone growth is not optimal and can caused malocclusion.* ***Purpose:*** *This study aims to evaluate the relationship between electromagnetic radiation and osteocalcin and bone alkaline phosphatase (BALP) serum levels.* ***Methods:*** *Experimental laboratory research with a pre- and post-control group design approach was carried out on 12 Rattus norvegicus Wistar strain. Osteocalcin and BALP serum levels were calculated before and after treatment. This study used the t-test as a comparative study ($p<0.05$).* ***Results:*** *There are significant differences in osteocalcin and BALP values between the treatment groups before and after treatment.* ***Conclusion:*** *Cell phone radiations (electromagnetic field exposure) reduce osteocalcin and BALP serum levels.*

Keywords: *bone alkaline phosphatase; cell phone radiation; electromagnetic radiation; osteocalcin*
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INTRODUCTION

The prevalence of malocclusion in Indonesia is still very high, around 80% of the population, and is one of the major dental and oral health problems.[1] The World Health Organization (WHO) defines malocclusion as a handicapping dentofacial anomaly, which refers to abnormal occlusion or disturbed craniofacial relationships, affecting appearance, aesthetics, function, facial harmony, and psychosocial well-being.[2,3] Clinical features in individuals can include crowding, protrusive, and crossbite with the most common clinical picture during the mixed dentition stage is crowding. The clinical prevalence of crowding was more common in patients with malocclusion than in others. Crowded teeth are caused by a difference between the size of the mandibular and the size of the teeth, which can eventually lead the teeth to overlap. Malocclusion itself is strongly influenced by genetic and environmental factors such as early primary tooth loss, tooth persistence, the discrepancy between jaw and tooth size, an abnormal number of teeth and eruption problems, and oral habits.[4,5]

With the current state of the environment in the COVID-19 pandemic, the duration of cell phone use by children will undoubtedly increase with restrictions on activities

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outside the home; this causes children to be unable to attend school in person, and, as an alternative, they use cell phones to communicate with teachers and obtain subject matter. In addition, this means children are often in contact with cell phones for an extended period time. Cell phones send signals to (and receive them from) nearby cell towers using RF waves which are a form of non-ionizing radiation. The excessive use of electromagnetic radiation has some adverse impacts on human health. Several papers say that long-term exposure to electromagnetic radiation affects the human body and can cause various types of cancer and disorders in human internal organs, such as the brain, kidneys, and lungs.[6,7] Previous studies on electromagnetic radiation have also shown a decrease in BALP levels.[8] Osteocalcin is a bone biomarker along with BALP. Both (osteocalcin and BALP) have an essential role in bone matrix formation. Children experience an increase in the concentration of bone markers because of the speed of bone growth.[9]

The use of cell phones, when making calls near the facial bones, and placing the cell phone close to the head during sleep leads to malocclusion in children because the electromagnetic radiation generated by cell phones can produce ROS, which can eventually accumulate and activate stress. Oxidative stress may ultimately decrease the expression of serum levels of osteocalcin and BALP.[10,11] Repeated exposure to electromagnetic radiation by cell phones can induce selective tissue damage. Damage rate will increase with exposure time.[12] Current social norms requiring increased use of cell phones may be related to the cause of the malocclusion itself, so mandibular growth is not optimal. Based on the above background, researchers are interested in analyzing the effect of electromagnetic radiation equivalent to cell phones on bone growth markers as measured by osteocalcin and BALP serum levels.

MATERIALS AND METHODS

The study design was in vivo experimental laboratory study, using osteocalcin and BALP serum levels carried out from 14 white rats (*Rattus novergicus*), male Wistar rats at 180-200 grams (2-3 months old), generally in good condition. After a week of acclimation, the 14 rats were divided into two (2) groups, namely the control group (group I) and the group exposed to an electromagnetic field (group II). Blood samples were taken from experimental animals to determine values for BALP and osteocalcin pre-test serum levels. In group 2, the RF Signal Generator was set at 2100 MHz (equivalent to a cell phone) and exposed to the treatment group for six (6) hours a day for 14 days. Subsequently, blood samples from the experimental animals were taken as to determine values for BALP and osteocalcin post-test serum levels.

The blood of the experimental animals was collected in a gel cloth and centrifuged at 3000 rpm for 10 minutes so that the serum was separated from the blood plasma. ELISA was performed using the BALP ELISA Kit, Bioenzy, Germany, and Osteocalcin ELISA Kit, Bioenzy, Germany, then continued with calculations using the Biotek Microreader Elisa Reader to determine BALP and osteocalcin serum levels before and after treatment. Data recapitulation and analysis were performed using SPSS software.

Quantitative data analysis was used in the results of this study. The normality test in each group was carried out using the Shapiro-Wilk tests to see the distribution of the data and continued with the homogeneity test of sample variance using Levene's test. After the normality and homogeneity of the data were met, it was continued with the ANOVA test to determine the differences in the entire sample group, and the paired t-test was carried out to determine whether there were differences between the sample groups ($p<0.05$).

RESULTS

The average results of the pre- and post-treatment from the radiation treatment group indicate differences in the values of the pre- and post-treatment groups (Figure 1, Table 1 and Table 2). The data normality test was carried out in each group using the Shapiro-Wilk test (Table 3 and 4). From the two groups, both pre (sig .281 and sig .186) and post (sig .263 and sig .209), sig > 0.05, which means the data is normally distributed. Homogeneity tests were carried out on both groups, both pre (sig .820) and post (sig .699); the results were sig > 0.05, which means that the data is homogeneous (Table 5 and 6). Paired t-test was conducted to compare the pre- and post-treatment in each group. The paired t-test can describe whether there is a significant difference between the pre- and post-treatment, which is stated to be a difference if a sig value < 0.05 is obtained (Table 7).

As seen in Table 7, in the control group, the paired t-test was 0.316, which means sig > 0.05; this shows no significant difference between the pre- and post-control groups. In group II Radiation, the paired t-test results were 0.001, which means sig < 0.05; this shows a significant difference between the pre- and post-radiation groups. This shows that there is an effect on the sample by giving radiation.

The results of data analysis were obtained from the research, which was seen from the mean, which was divided into two (2) treatment groups, namely control and radiation. The data normality test was carried out in each group using the Shapiro-Wilk test. From the two groups, both pre (sig .992 and sig .856) and post (sig .107 and sig .196), the sig value was > 0.05, which means the data is normally distributed. Homogeneity tests were carried out on both groups, both pre (sig .107) and post (sig .740); the results were sig > 0.05, which means that the data is homogeneous. The average results of the pre- and post-treatment from the radiation treatment group show differences in the values of the pre- and post-treatment groups (Figure 2).

Paired t-test was conducted to compare the pre- and post-treatment in each group. The paired t-test can describe whether there is a significant difference between the pre-

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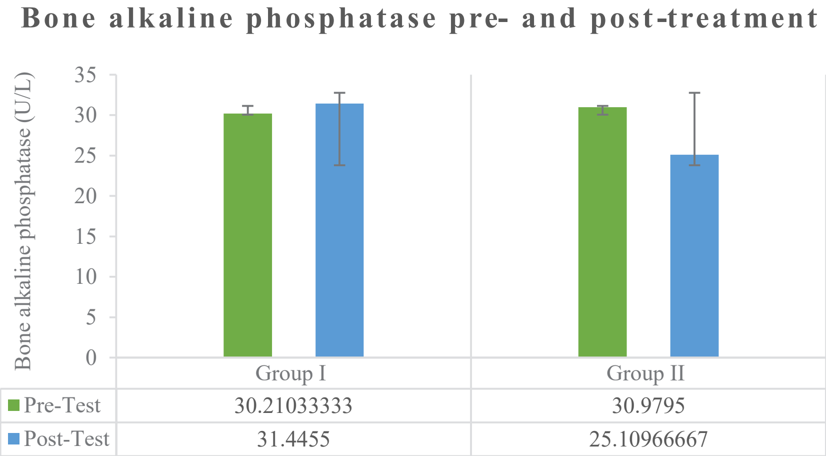

Figure 1. Diagram of mean BALP serum levels.

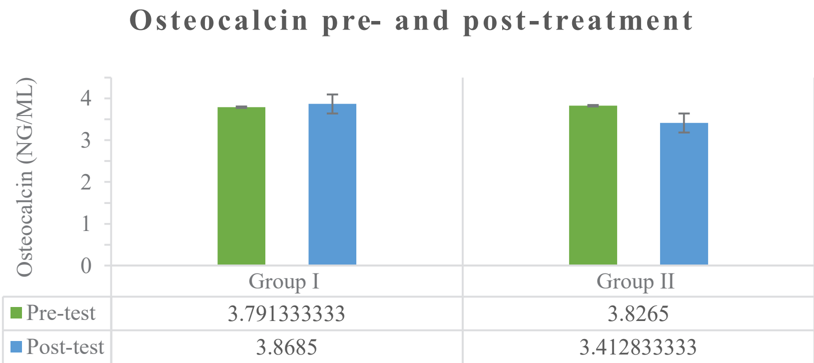

Figure 2. Diagram of mean Osteocalcin serum levels.

Table 1. Mean and standard deviation of pre-test

Group	N	Pre-test			
		Osteocalcin		BALP	
		Mean	SD	Mean	SD
I	6	3.791	.095	30.210	2.684
II	6	3.826	.129	30.979	2.388

Table 2. Mean and standard deviation of post-test

Group	N	Post-test			
		Osteocalcin		BALP	
		Mean	SD	Mean	SD
I	6	3.868	.065	31.445	1.753
II	6	3.412	.070	25.109	2.014

Table 3. Test of normality pre-test (Shapiro Wilk)

Group	N	Pre-test	
		Osteocalcin	BALP
		Sig.	Sig.
I	6	.992	.281
II	6	.856	.186

Table 4. Test of normality pre-test (Shapiro Wilk)

Group	N	Post-test	
		Osteocalcin	BALP
		Sig.	Sig.
I	6	.107	.263
II	6	.196	.209

Table 5. Test of homogeneity (pre-test)

Pre-test	
Osteocalcin	BALP
Sig.	Sig.
.820	.252

Table 6. Test of homogeneity (post-test)

Post-test	
Osteocalcin	BALP
Sig.	Sig.
.740	.699

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Table 7. Test of paired t-test

Pairs	Sig.
Pair 1 (Group 1 Pre-BALP - Group 1 Post-BALP)	.316
Pair 2 (Group 2 Pre-BALP - Group 2 Post-BALP)	.001
Pair 3 (Group 1 Pre-Osteocalcin - Group 1 Post-Osteocalcin)	.123
Pair 4 (Group 2 Pre-Osteocalcin - Group 2 Post-Osteocalcin)	.001

and post-treatment, which is stated to be a difference if the sig value < 0.05 is obtained. In control group I, the paired t-test result was 0.123, which means sig > 0.05. This shows no significant difference between the pre- and post-treatment in the control group. In group II Radiation, the paired t-test results were 0.001, which means sig < 0.05. This shows that there is an effect on the sample by giving radiation. This shows a significant difference between the pre- and post-treatment in the radiation group.

DISCUSSION

Until now, the use of cell phones is one of the communication technologies that is considered practical in daily use. Even though it has various benefits, electromagnetic wave radiation can have a bad impact on the body.[13] Lately, with the conditions of the COVID-19 pandemic, the use of cell phones in children has increased because many activities, such as learning, that should be done in school have been shifted to using digital media, one of which is using cell phones. Current social norms dictate increased cell phone use among children. Previous studies on electromagnetic radiation have identified a decrease in alkaline phosphatase.[12] However, there has not been much research on the electromagnetic radiation produced by cell phones on bone formation markers, which in this study are BALP and osteocalcin serum levels. Therefore, further research is needed.

In this study, exposure to electromagnetic radiation equivalent to a cell phone was carried out using an electromagnetic radiation device that had been set with a frequency of 2100 MHz with exposure for six (6) hours.[14] The drawback of this study is that the exposure dose given to rats, even though it is representative of cell phone radiation, is an average dose calculated from several points in the experimental animal cages during the treatment. In addition, the exposure was not carried out directly around the head, due to our concern that if radiation exposure was carried out around the head, the rats would have to be placed in a narrow space which could cause stress that could lead to death in experimental rats during treatment. The results of this study showed that the serum levels of BALP and osteocalcin decreased significantly when compared to the control group.

The effects of electromagnetic radiation arise in connection with the environmental impact on human health. There is enough research to suggest that electromagnetic radiation can generate ROS or free radicals in biological systems.[12] In other studies, the effects of electromagnetic radiation can activate oxidative stress pathways that play an important role in tissue damage.[15,16]

The decrease in osteocalcin and BALP serum levels in this study may be caused by the generation of ROS caused by exposure to electromagnetic radiation. Kunt et al., in 2016, conducted a study using Guinea pigs which showed that exposure to electromagnetic radiation caused a significant increase in oxidant products and decreased activity of antioxidant enzymes.[16] With a low antioxidant capacity in overcoming the emergence of ROS, it can eventually trigger oxidative stress which is a negative effect of ROS.[17] If this happens continuously, it can stimulate a decrease in the production of osteoprotegerin (OPG) and an increase in the production of Receptor activator of nuclear factor-κB ligand (RANKL).[18]

OPG is a strong bone-protective agent, whereas RANKL is a resorptive factor.[16] OPG is a membrane that surrounds and secretes a protein attached to RANKL to inhibit RANKL from binding to the Receptor activator of nuclear factor-κB (RANK) receptor.[19] RANKL will bind to RANK to stimulate differentiation and activation of osteoclasts. RANKL, together with the RANK receptor, plays a role in the formation and function of osteoclasts.[19] A study conducted by Kunt et al. on workers who work around high-voltage electric transmission lines (HVETL) supports the idea that the levels of RANK, RANKL, and OPG in these workers have a strong tendency for bone loss to increase as a result of exposure to electromagnetic radiation and not due to age.[16]

Repeated exposure to electromagnetic radiation by cell phones can induce selective tissue damage. Damage rate will increase with exposure time.[12] If this happens continuously, it can cause repeated apoptosis of osteoblasts so that osteoblast levels decrease. Osteocalcin and BALP are two bone markers used as an indicator of bone formation formed by osteoblasts.[9,20] Osteoblasts are responsible for the formation of new bone; under physiological conditions, bone resorption and formation will remain stable. However, if the balance is disturbed, bone architecture or bone function will become abnormal.[21] Repeated osteoblast apoptosis, may allow the mandibular bone to not develop which can eventually lead to crowding malocclusion in which the growing teeth do not get enough space. Previously, it was also mentioned that the damaging effects of electromagnetic radiation can induce selective tissue damage, which is usually where cell phones are placed near the facial bones.[12] The results of research on experimental animals show a correlation that exposure to electromagnetic radiation can affect the serum levels of osteocalcin and BALP which are two of the biomarkers of bone growth. In conclusion, cell phone radiation (electromagnetic field exposure) reduces osteocalcin and BALP serum levels.

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