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The Effect of Mobile Phone Radiation on Sperm DNA Fragmentation

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Abstract : Background: Mobile phones is ubiquitous in everyday life. The need for mobile phones is very important, considering the rapid development in the era of communication and internet. Radiofrequency electromagnetic wave (RF-EMW) emitted from mobile phones can disrupt human body through direct contact. The impact of this exposure on male reproduction system include decreased sperm quality. This study aimed to determine the effect of mobile phone radiation on human sperm quality, particularly in sperm DNA fragmentation.
Methods: Twenty-four healthy normozoospermia men donated their sperm for this research. After being prepared using the swim up method (SpermRinse), the sperm sample is divided into 2 groups: Group A received mobile phone radiation exposure for 180 minutes and Group B serves as control. After 180 minutes, DNA fragmentation was examined through flowcytometry using Spermfunc™ DNAf medium. WHO guidelines were used in the identification and calculation of DNA fragmentation.
Result: After 3 hours, DNA fragmentation was calculated and the result was analyzed using Wilcoxon Signed-Rank Test. In group A, 7.2% of the sperm had DNA fragmentation after 180 minutes of exposure; while in the group B, only 4.8% of the sperm had DNA fragmentation ($p<0.001$). However, further research needs to be performed to find the correlation between this result and its clinical implications on daily life.
Conclusion: In this study, exposure to mobile phone radiation is associated with more DNA fragmentation on human sperm compared to control group. This may have an effect on male infertility.
Keywords : phone radiation, sperm, DNA fragmentation, clinical trial.

1. Introduction

Mobile phone has become an integral part of the human life; this demands a study on the effect of mobile phone use on human life, especially in terms of radiations emitted by mobile phone signals. Mobile phone radiation is categorized as an electromagnetic wave(EMW), a radiofrequency(RF) signal with low frequency. Exposure to this radiofrequency signal depends on the frequency used by each mobile phone. Analog mobile phones operate at 450MHz and 900 MHz frequency, digital (Global System for Mobile

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Communications[GSM]) mobile phones operate at 900MHz or 1800 MHz frequency, and third generation mobile phones (3G phones) operate at approximately 2000 MHz frequency.[1]

The impact of these radiation frequencies on the human body has been studied extensively. Although mobile phone manufacturers and service providers ensured the safety of their mobile phones, the accuracy of their claim remains to be argued. The World Health Organization (WHO) has classified mobile phone electromagnetic field as a class 2B carcinogens (possibly carcinogenic to humans).[2]

Nowadays, men often use their active mobile phones when making a call through a wireless (handsfree/Bluetooth) device, while the phones remain in their pants pocket or hanging on their belts. This habit exposed the testicles directly to RF-EMW. Ashok Agarwal in his article questioned mobile phone's safety on the testicles and other organs.[3] RF-EMW is thought to affect the testicles in two ways: through changes in the hormones produced by the pituitary due to exposure to electromagnetic radiation or by damaging the DNA in male genitalia cells.[4] Damages in sperm DNA may cause structural disruptions, and often negatively correlates with sperm motility.[5] A study by Agarwal, et al. strengthened the evidence that the longer testicles are exposed to electromagnetic radiation, the larger its impact on sperm quality; this will in turn reduce the progressiveness of sperm movements.[6] Semen exposed to electromagnetic radiations will be stimulated to form oxidized NADH in the plasma membrane, which causes oxidative stress and negatively impacts spermatozoa and impacts male fertility.[7] Evidences on the correlation between the duration of radiation exposure and its impact on sperm count, motility, viability and morphology are also available.[6,8] These processes may increase the risk of male infertility.

There were also studies with contrasting results regarding the effect of mobile phone radiation on the quality of sperm. Those studies found no significant changes in the quality of sperm after being exposed to radiation from a certain distance for a specific period of time.[3,9]

Due to these varying results, we aimed to assess the effect of mobile phone radiation on the quality of human sperm, focusing on sperm DNA fragmentation.

2. Experimental

Sperm samples were exposed to mobile phone radiation for 3 hours, with the assumption that maximum mobile phone user talk timeis 3 hours. The effect of mobile phone radiation on mobile phone user is observed through DNA fragmentation analysis.

Subjects

Twenty-four healthy sperm donors who came to the Aster Fertility Clinic in Dr. Hasan Sadikin General Hospital Bandung on October 2017 were included as volunteer participants in this study. Semen samples were selected based inclusion criteria. These criteria include all normozoospermia samples based on WHO sperm analysis criteria. Samples were obtained through masturbation, and analyses were conducted in the same day. Donors have been instructed to reduce the exposure to mobile phone radiation on their pelvic area and were forbidden to ejaculate sperm for 3–5 days.[10] Exclusion criteria include: azoospermia and oligozoospermia semen samples, donors not being healthy, and donors who have just engaged in sexual activity.

Ethical Considerations

This study is conducted after obtaining approval from the Ethical Committee of Dr. Hasan Sadikin General Hospital Bandung. All donors have received information and signed a written consent to participate in this study.

Sperm Analysis

Twenty-four sperm samples were divided into 2 parts, one part assigned as treatment group (n=24) and the other as control group (n=24). DNA fragmentation was assessed in both groups.

Sperm was prepared using the swim up method. This was done by collecting 1 mL of *Spermrinse* medium and sperm sample respectively with a 5 mL syringe, which in turn creates two layers (the medium on

top and sperm on the bottom). The sample was then incubated in room temperature for 1 hour, tilted at 45^0. After an hour, the needle was removed from the syringe, and the bottom part of the sperm sample was discarded, leaving only 0.5 mL of top layer sample. Motility, viability, and DNA fragmentation on minute 0 was assessed on this direct sample.

Group A constitutes treatment sperm samples (n=24), which were prepared and exposed to active mobile phone in speaking mode from a $\pm$ 10 cm distance. Group B constitutes non-treatment sperm samples (n=24), which were prepared with no exposure to active mobile phonefrom their surroundings. Samples from both groups were kept in the same room temperature to avoid the formation of reactive oxygen species(ROS) due to changes in temperature, which may affect the result of the study. DNA fragmentation analysis was conducted on both groups after 3 hours of exposure.

DNA fragmentation analysis on the semen was conducted through flowcytometry using *SpermFunc*™ DNAf medium DNA fragmentation analysis kit: Kit for determination of the DNA fragmentation level in spermatozoa. After reagents and room temperature were prepared, 60 µl of sperm sample with a concentration of 5-10x10^6/ml was poured into a tube containing dissolved gel (while ensuring that this step was conducted at 37^oC temperature) and mixed evenly. Samples were then incubated at 37^oC. Before coating, pre-coated slide is placed in a refrigerator at 2-8^{o}C temperature for 5 minutes. Subsequently, 30 µl of sperm suspension made in the previous step was placed onto a pre-coated *slide* at 37^oC. Slide cover was then quickly and gently placed on top of the sperm suspension (without pressing the slide cover and ensuring no air bubbles were present). The resulting gel was solidified by placing the slide in a refrigerator at 28^oC for 5 minutes. Then, the slide cover was carefully removed by gently pushing the slide cover to one side until one end of the slide cover slightly passes the pre-coated slide. The slide cover was then carefully removed horizontally using a pincer. The pre-coated slidewas dipped vertically into a tube containing solution A, and then incubated at room temperature (20-28^{o}C) for 7 minutes. The pre-coated slide was then removed, and the remaining solution on the sides and back of the slide was cleaned using a filter paper, being careful not to touch the main part of the slide. The slide was then re-dipped vertically in another tube containing solution B and incubated at room temperature (20-28^{o}C) for 25 minutes. The pre-coated slide was then removed and the remaining solution on the sides and back of the slide was cleaned using a filter paper, being careful not to touch the main part of the slide. The slide was placed horizontally in a container with distilled water for 5 minutes. The water was replaced once or twice during this step. The slide was then removed, and the remaining solution on the sides and back of the slide was cleaned using a filter paper, without touching the main part of the slide. The slide was subsequently placed vertically on another container with 70% ethanol for 2 minutes. And then the slide was removed, and the solution on the sides and back of the slide was cleaned using a filter paper without touching the main part of the slide. The slide was then re-dipped vertically in a container with 90% ethanol for 2 minutes, removed, and the remaining solution on the sides and back of the slide was cleaned using a filter paper (without touching the main part of the slide). The slide was then dipped vertically on another container with 100% ethanol for 2 minutes. The pre-coated slidewas left to dry naturally on room temperature. Around 15 - 20 drops of Wright's stainwere poured onto the pre-coated slide, followed by 30-40 drops of Wright's butterfor staining. It must be noted that if 15 drops of Wright's stainwere used, it must be followed by 30 drops of Wright's butter. The mixture was gently mashed with a pipette pump on the slide, being careful not to destroy the surface tension created by the stain. After fifteen minutes, the slide was rinsed gently using cooked water. The slide was then left to dry naturally. The slide was subsequently examined under a normal optical microscope with 40-times magnification and the number of sperms with DNA fragmentation was calculated. DNA fragmentation was identified through the presence of a halo on the head of the sperm, or through the thickness of the halo (less than 1/3 of the diameter of sperm head).

Statistical Analysis

The percentage of sperm DNA fragmentation was calculated using the following formula.

$$\text{Percentage of Sperm with DNA Fragmentation} = \frac{\text{The number of sperm with DNA fragmentation}}{\text{The number of all observed sperm}} x\ 100\%$$

$$\text{Percentage of sperm with intact DNA} = 1 - \text{Percentage of sperm with DNA fragmentation}$$

Notes: The total count of all observed sperm must be more than 500.

We used Wilcoxon Signed-Rank Test. P value of <0.05 is considered statistically significant.

3. Results

In this study, 24 sperm samples from normal donor was divided into 2 parts, one part was used as treatment group (n=24) and the other as control group (n=24).

Characteristics such as general profile and daily habit were obtained from sperm donors, including: age, body mass index, occupation, marital status, and smoking habit. These characteristics were presented in Table 1.

Table 1. Demographic Characteristics of Treatment Sperm Donor

Characteristics		**Results**
Age		25.8 (22– 34)
Body Mass Index		22.2 (18.6– 27.1)
Marital Status		13 (54.2%)
Occupation:	Security Officers	3 (12.5%)
	Housekeeping Staff	4 (16.7%)
	Private Employee	7 (29.2%)
	Aides	3 (12.5%)
	Others	7 (29.2%)
History of Smoking		13 (54.2%)

After 3 hours, the number of sperms with DNA fragmentation was calculated, and the obtained data were analyzed using Wilcoxon Signed-Rank Test, as shown in the following Table.

Table 2.The Difference in Sperm DNA Fragmentation Between Treatment Group and Control Group, as analyzed using Wilcoxon Signed Rank Test

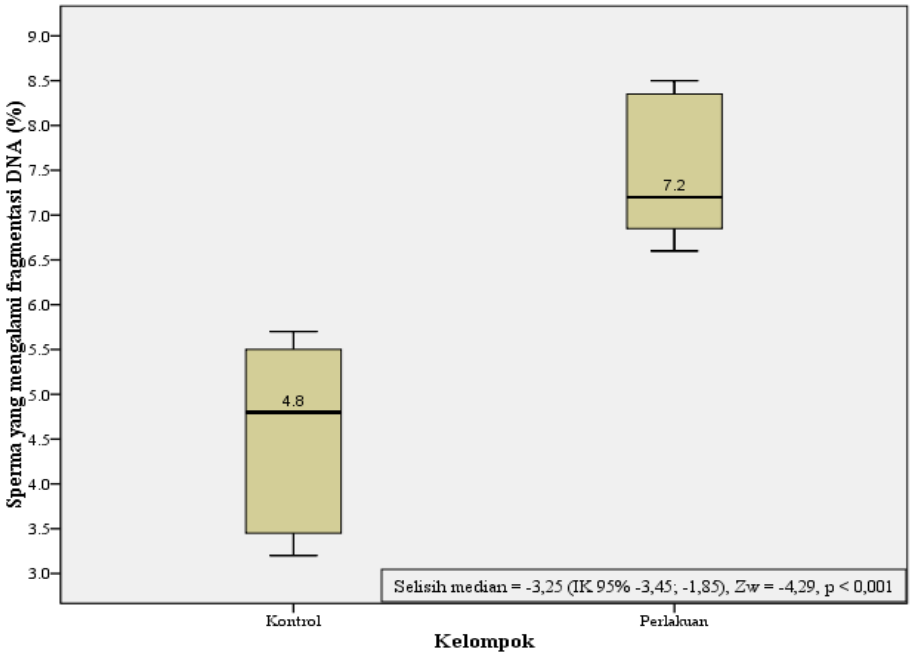

Table 2 showed the difference in the effect of mobile phone radiation on DNA fragmentation in both treatment and control sperm groups. In the treatment group, 7.2% of the sperm had DNA fragmentation on the 180th minute after exposure, while in the control group, only 4.8% of the sperm had DNA fragmentation ($p<0.001$). This showed that DNA fragmentation occurred in higher rate in sperm exposed to mobile phone radiation for 180 minutes compared to control sperm.

4. Discussion

Based on the results of this study, exposure to mobile phone radiation on semen preparation significantly increased DNA fragmentation in the treatment group compared to the control group.

Assessing the exposure to radiation in daily life is important, especially for the reproductive system, as this system is sensitive to physical and chemical changes. In a study on the effect of exposure to radiation on operators of radioactive instruments who have worked in a hospital for more than a year, the exposure was found to have negative impacts on sperm functions, resulting in changes in motility, increased abnormal morphology, DNA fragmentation, and global hypermethylation.[11]An increase of DNA fragmentation process in human have also been observed in several studies, especially an increase in sperm DNA fragmentation after exposure to mobile phone radiation for 2 hours in Paranezhad study, 4 hours in Rago, et al. study, and 5 hours in Gorpichenko, et al. study[12-14] In addition, Zalata et al. also found that mobile phone emission also negatively impacts sperm motility, sperm acrosin activity, DNA fragmentation, and seminal CLU gene expression.[15]

Several literatures have stated that repeated exposure to RF-EMW radiation, and not an increase in temperature, results in single- or double-chain DNA fragmentation.[16] Electromagnetic radiation causes oxidative stress and makes spermatozoa sensitive or vulnerable to exposure. This is due to the fact that spermatozoa are rich in unsaturated fatty acid such as docosahexaenoic acid and has small cytoplasmic volume, resulting in limited intracellular antioxidant enzymatic activities. This increase the likelihood of sperm to produce free oxidants, which in turn resulted in an extensive damage, making the patient infertile.[17] Finally, sperm provides an opportunity for free radical substrates to attack all forms of DNA by damaging sperm membrane, disrupting its motility and ability to fuse with the ovum.[18] Free radicals are produced by the semen, which consists of white blood cells and sperm. Both may trigger extrinsic oxidative reaction and disrupts the integrity of sperm DNA, affecting its motility and DNA integrity.[19] This statement was supported by the findings in de luliis et al. study, where it is evident that exposure to RF-EMW in the same frequency as the one used in mobile phones increased the production of sperm mitochondrial ROS and DNA fragmentation.[20] These ROS were produced in larger number with exposure mobile phonein active mode compared to mobile phones in standby mode.[21]

However, other studies also found contrasting results; studies by Hanom, et al. and Falzone, et al. found no significant defects in sperm DNA integrity after exposure to RF-EMW.[9,22]In contrast, Agarwal et al. studyobserved oxidative stress on sperm due to exposure to this particular electromagnetic wave, but found no significant difference based on the effect of EMW on *in vitro* sperm.[7] Al-Bayyari also found that the habit of keeping mobile phones inside pants pockets significantly and negatively impacts the quality of sperm.[23]

There are limitations to the current study. The use of only one model of mobile phone in this study may yield inaccurate and different result. Due to the large variations of mobile phone models used in the community, the SAR and EMW effects may differ for each mobile phone model. Additionally, thermal and non-thermal effect of RF-EMW mobile phone radiation must also be known beforehand, since only a small number of literature explained which effect might be more dominant.[24,25] False positive results may also be obtained if the sperm is analyzed more than an hour after sample collection. The quality of sperm analyzed after an hour may be reduced due to changes in pH, temperature, and sperm dehydration.[26]

The fact that men tends to make active phone calls with their phones in their pants pocket may cause exposure to mobile phone radiation, which may be detrimental to their reproductive system. It must be noted that natural fertility potential is highly dependent on ejaculates containing high count of spermatozoa, despite the status of DNA integrity.[27,28] DNA fragmentation may play a role in the post-implantation development of an embryo and may cause spontaneous abortion after natural or assisted conception.[29]

Due to these consequences, DNA fragmentation needs to be considered in reproductive problems. However, the resulting damage may also be due to the cumulative effect of repeated exposure and indirect effect of short-term exposure. Further studies need to be conducted, especially to obtain baseline data on radiation exposure to each individual who provided sample for the study.

5. Conclusion

Mobile phone radiation increased sperm DNA fragmentation and may cause reduced sperm quality.

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