Original Article

The Effect of Mobile Radiation on the Oxidative Stress Biomarkers in Pregnant Mice

Nargess Moghadasi; M.Sc.[1], Iraj Alimohammadi; Ph.D.[1], Ali Safari Variani; Ph.D.[2], Azadeh Ashtarinezhad; Ph.D.[1]

1 Department of Occupational Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
2 Department of Occupational Health Engineering, School of Public Health, Qazvin University of Medical Sciences, Qazvin, Iran

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Abstract

Objective: Due to the growing use of communication instruments such as cell phones and wireless devices, there is growing public concern about possible harmful effects, especially in sensitive groups such as pregnant women. This study aimed to investigate the oxidative stress induced by exposure to 900 MHz mobile phone radiation and the effect of vitamin C intake on reducing possible changes in pregnant mice.

Materials and methods: Twenty-one pregnant mice were divided into three groups (control, mobile radiation-exposed, and mobile radiation plus with vitamin C intake co-exposed (200 mg /kg)). The mice in exposure groups were exposed to 900 MHz, 2 watts, and a power density of 0.045 µw /cm^2 mobile radiation for eight hours/day for ten consecutive days. After five days of rest, MDA (Malondialdehyde), 8-OHdG (8-hydroxy-2' -deoxyguanosine), and TAC (Total Antioxidant Capacity) levels were measured in the blood of animals. The results were analyzed by SPSS.22.0 software.

Results: The results showed that exposure to mobile radiation increased MDA (P=0.002), and 8-OHdG (P=0.001) significantly and decreased Total Antioxidant Capacity in the exposed groups (P=0.001). Taking vitamin C inhibited the significant increase in MDA and 8-OHdG levels in exposed groups.

Conclusion: Although exposure to mobile radiation can cause oxidative stress in the blood of pregnant mice, vitamin C as an antioxidant can prevent it.

Keywords: Radio Waves; Pregnancy Outcome; Oxidative Stress; Ascorbic Acid

Introduction

Electromagnetic pollution is one of the major health and environmental problems in recent decades caused by human-made resources, especially in terms of communications, diathermy and medical imaging, and high-voltage power lines. Electromagnetic waves with a frequency of 100 kHz-300GHz are commonly called radiofrequency radiation. These waves are emitted at many communication stations and wireless devices such as cell phones (1). One of the most common standards of radio communication for telecommunications is GSM (Global System for Mobile Communication), which is widely used in Europe and Asia, utilizing frequencies of 850, 900, 1800 and 1900 MHz and 217 Hz modulation (2). The increasing use of communication devices, especially

Correspondence:
Dr. Azadeh Ashtarinezhad
Email: ashtarinezhad.a@iums.ac.ir

cell phones, and the lack of clarity on the health effects of their radiation have raised public concern, especially in sensitive groups such as pregnant women and children. Moreover, the exposure limits for pregnant women, in particular, are not set by relevant organizations such as the International Commission on Non-Ionizing Radiation Protection (ICNIRP). Meanwhile, the International Agency for Research on Cancer (IARC) has identified electromagnetic waves as a possible carcinogen for humans, based on research into the effects of electromagnetic fields on brain cancer (3), and some animal studies have shown the negative impact of mobile radiation such as malformation and developmental abnormality on the fetus (4, 5).

Many studies have shown that as a non-thermal effect, exposure to mobile radiation produces free radicals in various body tissues such as the brain (6, 7) and endometrium (8) that can cause diseases such as cancer. Following an increase in the number of free radicals and a lack of antioxidant levels, the balance between the number of oxidants and antioxidants in the body is disturbed, called oxidative stress, which can harm macromolecules such as lipids, proteins, and DNA and finally causes Cell death, DNA damage and lipid peroxidation. Malondialdehyde (MDA) is one of the toxic by-products of lipid peroxidation (9), and 8-hydroxy-2' -deoxyguanosine (8-OHdG) is a common biomarker of DNA damage (10), both of which are used as biomarkers to identify oxidative stress (11, 12). Total Antioxidant Capacity (TAC) shows the cumulative amount of antioxidants present in plasma and body fluids that protect the body cells against diseases (13). Many studies have investigated the effect of various antioxidants on reducing the harmful effects of exposure to cellular radiation and have recommended their use to aid the antioxidant system (8, 14, 15).

Inflammation could be found under normal physiological conditions, such as pregnancy. There is a strong association between oxidative stress and preeclampsia as a manifested complication during pregnancy (16). Additionally, ROS's redundant production correlated with the fetus's low antioxidant capacity appears to play a fundamental role in the risk of preterm birth and low birth weight due to suboptimal conditions in intrauterine life (17). Oxidative stress may predispose the fetus to a higher risk of large for gestational age, increased adiposity in childhood (18), and cardiovascular disease later in life (17).

Consumption of vitamins is necessary for supporting physiological circumstances, especially during pregnancy for normal infant growth and development. Maternal death, poor birth outcomes such as intrauterine growth restriction and low birth weight, and increased risk of infections and child stunting are the expected adverse consequences of vitamin deficiencies during pregnancy (19). During pregnancy, intaking food with high antioxidant activity might decrease the possibility of birth defects (20). According to some research, positive birth outcomes would be the result of intaking nutrients like vitamin C (21), vitamin A, folic acid, and selenium (17).

Vitamin C, as one of the most potent water-soluble antioxidants, has been shown to play an important role in improving the antioxidant system in tissues such as the liver, testis, kidney, brain, and eye (22, 23). This vitamin, which has been proven to have an antioxidant role, can reduce oxidative stress in placental tissue, inhibiting preeclampsia and gestational diabetes (24, 25). Due to more water content and tissue conductivity to radiation in the pregnancy period, and few studies have been performed on the effects of 900 MHz mobile radiation during pregnancy, especially in crucial organogenesis period, this study aimed to evaluate the oxidative stress induced by GSM in the low power and density GSM range during the first ten days of pregnancy and the role of vitamin C as a water-soluble antioxidant in reducing the potential effects of mobile radiation and its effectiveness during pregnancy of the rats.

Materials and methods

Animals: In this study, 15 adult male and 30 adult female mice, about 4-6 weeks of age, weighing 25-30 gram were obtained from the Center for Experimental and Comparative Studies of Iran University of Medical Sciences. Animals were kept in Plexiglas cages in the standard situation (temperature: 21± 1 °C, light: 12 hours darkness: 12 h, humidity: 40-45%) and fed with special food and water. The animals were kept in a special radiation chamber for a 7-day adaptation period. Male and female rats were mated overnight. The next morning, day zero of pregnancy was determined for female mice with vaginal plaque. Pregnant mice were then randomly divided into three groups (7 mice in each group); control group (I), mobile radiation-exposed group (II) and mobile radiation plus with vitamin C intake co-exposed group (III). The control group had no exposure to mobile radiation. Groups II and III were exposed to 900 MHz mobile radiation, 2 watts, 8 hours/day for 10 consecutive days. Moreover, group III received vitamin C (200mg/kg) by gavage (23) for 10 exposure days.

Groups I and II received distilled water. This study was approved by the Ethics Committee of Iran University of Medical Sciences (Code of Ethics: 9311139007.REC1394.IUMS.IR) and under the guidelines of the Committee for the Care and Use of Laboratory Animals (ACUC) of the Center for Experimental and Comparative Studies of Iran University of Medical Sciences.

Exposure Set up: A radiofrequency radiation generator (built at the Electrical Engineering Department of K.N. Toosi University of Technology, Tehran, Iran) was used for producing waves in the frequency of 900 MHz with an output power of 2 watts and power density of 0.045 $\mu w/cm^2$ in 20 cm from pregnant mice. It has a central antenna, capable of producing waves in the frequency of 860 to 960 MHz, 1-6 watts of power, and modulation of 100-217 Hz. Before the start of the experiment, the power, frequency and possible reflectance were evaluated with the Wave Control device (made in Spain). To prevent possible interference of the ambient waves and control the reflection of other waves, animals were housed in a radiation chamber with absorbent walls during the experiment (Figure 1).

Biochemical tests: On the 15th day of gestation, after 5 days rest, mice were anesthetized using ketamine (80 mg/kg) and xylazine (10 mg/kg) and then blood was taken from their hearts. Blood was collected in EDTA-containing tubes and centrifuged for 10 minutes at 3000 rpm (Hettich, DRE Universal, Germany) and their plasma was separated and stored in a microtube at -80 ° C until the test day. Plasma malondialdehyde concentration was measured according to TBRAS method using a commercial kit (ZellBio GmbH, Germany) at 535 nm with ELISA Reader (Awareness Technologies Stat Fax 2100 Microplate ELISA Reader, USA) and the concentration was expressed in µM. Total antioxidant capacity was measured using a commercial kit (ZellBio GmbH, Germany) at 490 nm with ELISA reader and its concentration in plasma was expressed in mM. The concentration of 8-OHdG in plasma was measured using a commercial enzyme-linked immune sorbent assay kit (ZellBio GmbH, Germany) using an ELISA reader at 450 nm and expressed in ng/ml.

Statistical analysis: All data were analyzed with SPSS 22.0 software. Data were expressed as mean±SD. The normal distribution of data was evaluated using the Kolmogorov-Smirnov test. ANOVA test was used to compare differences between study groups. A P-value less than 0.05 was considered as a significant level.

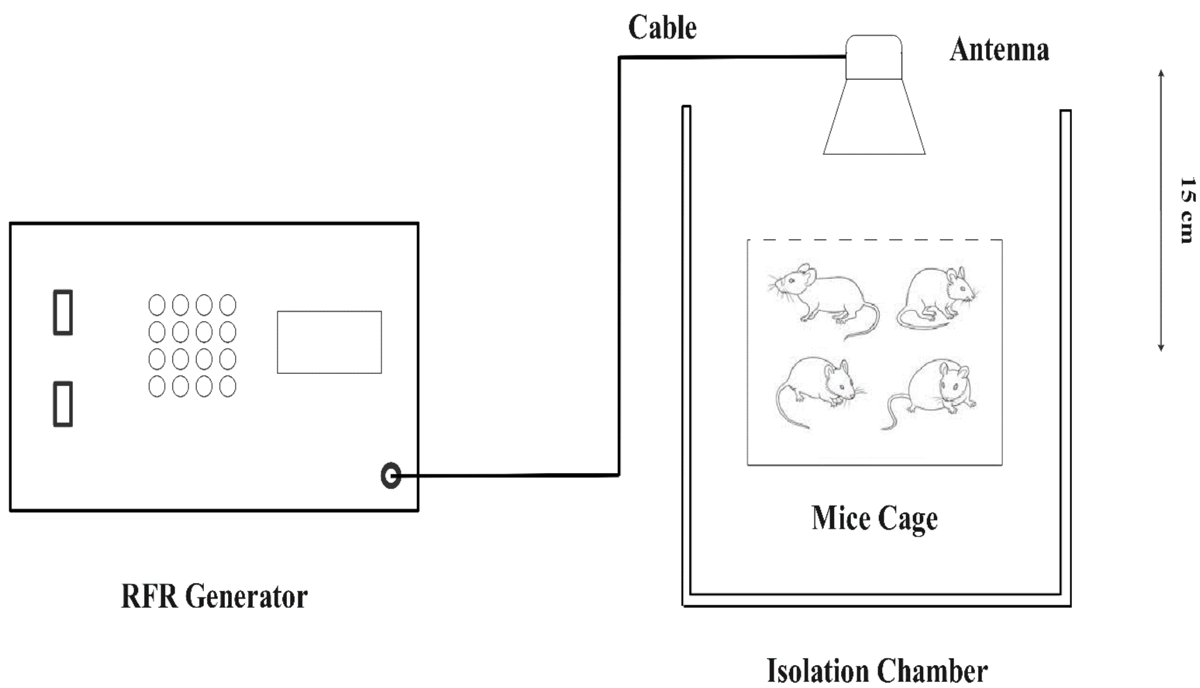

Figure 1: Exposure setup

Table 1: Comparison of biomarkers with a normal distribution (Mean± SD)

Biomarker	Group I (n=7)	Group II (n=7)	Group III (n=7)	P-value
MDA(μM)	6.711±0.936	8.28±0.590[a]	7.134±0.273	0.002
8-OHdG(ng/ml)	33.301±2.061	45.325±4.851[a]	34.708±3.708	0.001
TAC(mM)	0.827±0.07	0.479±0.048[a]	0.575±0.03[a]	0.001

Results

Table 1 shows the concentration of three biomarkers including MDA, TAC and 8-OHdG in the three study groups. The results showed that the level of MDA in the exposed to mobile radiation group (group II) was significantly higher than the other two groups (P=0.002). MDA levels were higher in the exposed to mobile radiation and taking vitamin C group than in the control group but this difference was not significant (P=0.56). Also, the level of 8-OHdG in the exposed to mobile radiation group (group II) was significantly higher than the control group and co exposure to mobile radiation and vitamin C (P=0.001). However, the concentration of this biomarker in group III was higher than the control group, but this difference was not significant (P=0.09). On the other hand, TAC concentration in groups 2 and 3 were significantly lower than the control group (P=0.001).

Discussion

Today, the potential adverse effects of exposure to radiofrequency waves and other environmental pollutants on the health of a pregnant mother and her fetus are of paramount importance. With the increasing use of wireless communication devices and the conflicting information on the health effects of these waves, there is a growing need for further studies in this area. Moreover, some research has shown the association between oxidative stress and abnormal pregnancy outcomes or childhood development. For instance, Olufunmilayo Arogbokun et al. illustrated a link between third-trimester oxidative stress and lower birth weight and higher early childhood weight and BMI (26).

Some studies have suggested that exposure to electromagnetic waves can contribute to cell and DNA damage, ovarian dysfunction during pregnancy and developmental abnormality (27-29). However, some other in-vivo and in-vitro studies have not shown the negative effects (30-32). For example, the results of a study by Kim et.al on the local exposure of C57BL / 6N mice to radiofrequency radiation in 849 and 1763 MHz for 6 and 12 months showed no cellular damage or cell death in the brain tissue (33).

This study investigated the oxidative stress induced by exposure to 900 MHz mobile radiation at 2 watts power and power density of 0.045 $\mu w/cm^2$ for 8 h/day, 10 consecutive days, in pregnant mice and the effect of vitamin C intake on reducing possible changes. Exposure to these waves was found to significantly increase the levels of MDA and 8-OHdG in the blood plasma of pregnant mice, but vitamin C consumption did not significantly increase these biomarkers in the recipient group. Exposure to mobile radiation also significantly decreased the total antioxidant capacity in both mobile radiation-exposed group (II) and mobile radiation-exposed with vitamin C intake group. Several studies have confirmed the relationship between exposure to electromagnetic waves at frequencies of 2045 GHz, 900 and 1800 MHz and the production of free radicals that result in oxidative stress in cells such as the brain and sperm production (34-36).

Zosangzuali et al. has reported the possible effects of exposure to mobile phone base station (MPBS) emitting 1800-MHz radiofrequency electromagnetic radiation on oxidative stress biomarkers in the brain, heart, kidney, and liver of Swiss albino mice underexposures below thermal levels. The glutathione (GSH) levels and activities of glutathione-s-transferase (GST) and superoxide dismutase (SOD) significantly plummeted in mice brain after exposure for 12 hours and 24 hours per day. Exposure of mice to radiofrequency radiation for 12 hours and 24 hours per day also resulted in a considerable increase in MDA as a lipid peroxidation index in mice brains. While, any significant change in various oxidative stress-related parameters in the heart, kidney, and liver of mice was found. Exposure to radiation from MPBS might cause harmful effects in mice brains by inducing oxidative stress arising from the generation of reactive oxygen species (ROS) as indicated by enhanced lipid peroxidation and reduced levels and activities of antioxidants (37). These findings are in line with the results of our study; however, their exposure period was longer. This decrease of antioxidant enzymes and parameters can be related to significant ROS increase and the more need for antioxidant supplements.

Studies have shown that exposure to low-intensity electromagnetic waves can cause oxidative stress and structural alterations of the DNA molecule. Due to the production and binding of some free radicals to the DNA molecule, the 8-OHdg biomarker, which indicates DNA damage by attack free radicals, was increased. Mutation in this molecule can also occur following the pairing of organic DNA bases with this biomarker and can be transferred from generation to generation (38, 39). This can be considered as one of the most important health damages during pregnancy because the mother and the fetus are more vulnerable to the waves during this period and DNA damage and possible mutations may cause fetal abnormality or birth defect. It can also cause some diseases, such as gestational diabetes or preeclampsia in the pregnant mother.

Exposure to electromagnetic waves directly relates to the creation of free radicals, the bonding of these compounds to macromolecules such as lipids, and increased lipid peroxidation biomarkers such as MDA (32). However, some studies have shown that antioxidant supplements increase antioxidant enzymes such as GSH-PX and CAT in liver and heart cells, which increases the potency of the antioxidant system to prevent the increase of MDA (40). Also, in a study in 2007, vitamin C intake reduced the oxidative damage of endometrial tissue in female rats exposed to 900 MHz (8). Vitamin C plays a significant role in regulating antioxidant enzyme activity and maintaining balance in the body by eliminating hydrogen peroxidase and free radicals. This vitamin can improve the antioxidant system and reduce lipid peroxidation by reproducing alpha-tocopherol and glutathione from free radicals (41). A study by Jelodar and colleagues showed that prolonged exposure (45 days, four hours/day) to 900 MHz mobile phone radiation could increase MDA and decrease levels of antioxidant enzymes such as GPX, CAT, and SOD, but consumption of 200 mg/kg of vitamin C during this period increased the levels of antioxidant enzymes and decreased MDA. However, in the present study, the total antioxidant capacity was decreased, which may be justified by more excellent resistance of rats to radiation or lower exposure hours per day and more significant opportunity for recovery of the animal's body (23). In the study of Alchalabi et al., it was shown that exposure to 1800 MHz radiofrequency waves increased MDA levels and decreased GSH-PX as one of the antioxidant enzymes in the ovary and uterine tissues and caused some pathological changes in both tissues. It has been suggested that oxidative stress is caused by 30 and 60 days of exposure to these waves. The biochemical results of present study are in line with the results of this study and can be attributed to the decrease in total antioxidant capacity in the present study due to increased levels of MDA and 8-OHdG oxidative stress biomarkers and increased activity of antioxidant system to eliminate free radicals and oxidant products (42).

In the present study, although consumption of vitamin C in group III prevented a significant increase in MDA and 8-OHdG biomarkers compared to the control group, the total antioxidant capacity in both II and III groups is significantly lower than the control group. The amount and duration of vitamin C intake to increase the activity of antioxidant enzymes in the production of antioxidants may have been low, and it would have been preferable to continue with vitamin C up to 15 days of gestation (blood sampling day) after ten days of exposure. There was more time for mice to get back to normal condition. In addition, due to changes in the body during pregnancy and placental activity, ROS levels increase and thereby cause oxidative stress (43), which may decrease antioxidant capacity in both exposure groups.

The present study had several limitations. It was done only during ten gestation days. Antioxidant factors such as GPx, SOD, and CAT were not measured. However, it was done in the organogenesis period. It is suggested that a study with different frequencies be performed on the whole period of pregnancy, and the gene expression be evaluated in some cases.

Conclusion

It can be stated that exposure to radiofrequency radiation at 900 MHz results in DNA damage and increased lipid peroxidation during pregnancy in mice. Nevertheless, vitamin C intake as a potent and water-soluble antioxidant can significantly reduce these damages along with enzymes in the antioxidant system. Due to the sensitivity of pregnant women and their fetuses and babies to radiofrequency radiation, it is recommended to study the effects of different frequencies of various intensities on different fetal body tissues and gene expression of specific diseases such as ADHD (Attention Deficit Hyperactivity Disorder) and autism. It can help related organizations to determine exposure threshold limit value for pregnant women.

Conflict of Interests

Authors have no conflict of interests.

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