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ORIGINAL PAPER

The Influence of 1800 MHz GSM-like Signals on Hepatic Oxidative DNA and Lipid Damage in Nonpregnant, Pregnant, and Newly born Rabbits

Arin Tomruk · Goknur Guler · Aylin Sepici Dincel

Abstract The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group III). 1800 MHz GSM-like RF radiation whole-body exposure (15 min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10^6 dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased compared to Group I ($P < 0.05$, Mann–Whitney). No significant differences were found in liver tissue of 8 OHdG/10^6 dG and MDA levels between Group VI and Group V ($P > 0.05$, Mann–Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V ($P < 0.05$, Mann–Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.

Keywords Radiofrequency · Pregnant · 8 OHdG · MDA · Liver · Rabbit

A. Tomruk (✉) · G. Guler
Department of Biophysics, Gazi University, Faculty of Medicine, Gazi Üniversitesi Tıp Fakültesi Biyofizik Abd, Dekanlık Binası 5. Kat, 06500 Besevler, Ankara, Turkey
e-mail: arintomruk@gmail.com

A. S. Dincel
Department of Biochemistry, Gazi University, Faculty of Medicine, Ankara 06500, Turkey

Introduction

Radiofrequency (RF) energy has been widespreadly utilized in almost all of the areas namely, communication (cell phones, personal digital assistant or PDA devices, radars, pagers, TV broadcasting), industry (RF heaters and sealing systems), and health (diathermy unit). The possible effects of RF fields on biological systems are still unclear. However, according to the relevant studies, the biological responses induced by RF exposure may be explained by means of two distinct interaction mechanisms (thermal and nonthermal mechanisms) [1]. RF fields can transfer its energy to biological matter leading to the increment in the medium temperature with the vibration of atoms and molecules [2]. Most of the bioeffects of RF fields associated with the increased temperature of tissue can be clarified by thermal mechanism, but the cellular effects induced by low-level RF exposure are still debated [3–5]. However, the results of recent studies showed that some alterations may be observed at the cellular level through the exposure to low frequency RF radiation. With regard to these alterations, the DNA breakages, chromosomal abnormalities, various cell deaths, activation of endogenous chemical products, cellular stresses, neurological degenerations, aging, and the formation of free radicals can be observed [6–19]. The most important oxygen-free

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radical causing damage to basic biomolecules (proteins, membrane lipids, and DNA) is the hydroxyl radical (HO•). The interaction of HO• with the nucleobases of the DNA strand, such as guanine, leads to the formation of C8-hydroxyguanine (8-OHGua) or its nucleoside form deoxyguanosine (8-hydroxy-2-deoxyguanosine). In nuclear and mitochondrial DNA, 8-hydroxy-2-deoxyguanosine (8-OHdG) or 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) is one of the predominant forms of free radical-induced oxidative lesions, and has therefore been widely used as a biomarker for oxidative stress [20]. Interaction between the unsaturated phospholipids of the biological membranes with reactive oxygen species (ROS) and nitrogen species (RNS) has led to the degradation which is known as lipid peroxidation (LPO).The lipid peroxidation end products, malondialdehyde (MDA) with their relatively long-lived and high activities can also cause the oxygen toxicity at cellular level by interacting easily with basic biomolecules. As the popular TBARS assay has been criticized for its lack of specificity and accuracy, recommendations for the FOX assay as a sensitive and cost-effective alternative for the determination of lipid peroxidation were published [21, 22]. Organoperoxides are early products and MDA is a later product of lipid oxidation of unsaturated lipids such as arachidonic acid [23]. Organoperoxides may increase independently from MDA [24], and the results are raising the question of whether measurement of organoperoxides by procedures such as the FOX assay rather than MDA might provide a more consistent index of oxidative stress.

The most of the studies (laboratory, epidemiological, and modeling studies) conducted on EM radiation have aimed to constitute international standards in order to take precautions for people exposed chronically. With the standards that constituted, it can be enabled to protect people against the possible adverse effects of EM fields and to limit the field exposure of people worked intensively in the industrial and health area. It is important that vulnerable people (pregnants, children, elders) are also exposed to the same degree as the general population. During the pregnancy, RF fields may interact with embryo/fetus resulting in developmental abnormality [25, 26].

In the present study, it is aimed to determine the possible biological effects of RF radiation on nonpregnants, pregnants, and their newly borns. The levels of lipid peroxides and DNA damage based on free radical attacks of all examined subjects' liver tissues were analyzed.

Materials and Methods

Exposure Level and Quality Control

GSM-like signals in 1800 MHz frequency were formed by using a signal generator (Agilent Technologies 8648C, 9 kHz–3.2 GHz) with the integrated pulse modulation unit and horn antenna (Schwarzbeck, Doppelsteg Breitband Horn antenna BBHA 9120 L3F, 0.5–2.8 GHz) in a shielded room. The generated power was controlled by a spectrum analyzer (Agilent Technologies N9320A, 9 kHz–3 GHz) integrated to the signal generator. The signals were amplitude-modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1:8 (pulse width 0.576 ms), corresponding to the dominant modulation component of the GSM.

RFR Generator provided 20 dBm (0.1 W) power during the exposure period. The signal was controlled by means of the spectrum analyzer connected to the signal generator, and NARDA EMR 300 and type 26.1 probe were used for measurement of the output radiation. Measurements were taken during the entire experiment and the data was saved in the computer which was connected to the device via fiber optic cable. The evaluated data was 14 ± 0.5 V/m (Fig. 1).

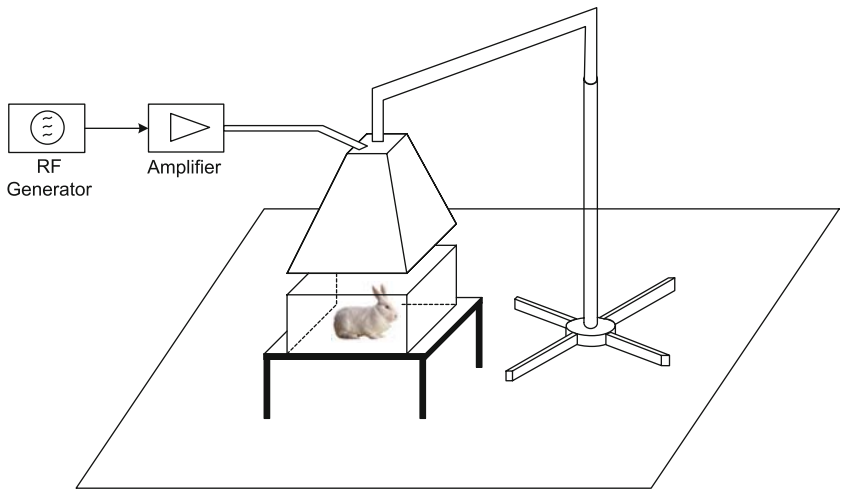

Fig. 1 GSM-like RF exposure system

Animals

New Zealand White rabbits were obtained from the Laboratory Animals Breeding and Experimental Research Center of Gazi University (13-month-old non-pregnant and pregnant rabbits; $n = 36$). The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University (G.U.ET-06.027). All the animal procedures were performed in accordance with the approved protocol. Rabbits were housed under the same conditions in a temperature and humidity-controlled room (20 ± 1°C, 50 ± 10% relative humidity) and 14–16 h light/dark cycle conditions. Except during exposure periods, tap water and standard pelletized food were provided ad libitum. Coeval pregnant and non-pregnant rabbits were adapted to the laboratory conditions for 5 days before the experiment. Quality controls were conducted by the veterinarians to verify that rabbits used in this experiment were healthy at all stages of the experiment. The aim of these controls was to check the health condition of the adult rabbits, condition of the gestation and any malformation or prenatal death of the offspring.

In the breeding process, we selected female rabbits that would get pregnant for the fourth time. Because previous experience of being a mother three times leads to obtain high performance in the number of litter. Besides, mothers are then experienced enough to feed and look after their babies. During the mating process, male ratio was kept constant being one male to 10 female rabbits. Pregnancies were verified by abdominal palpation 10 days after the process of mating.

Pregnant and non-pregnant rabbits are exposed to RFR in the same conditions for 7 days after the adaptation period. Exposure period was between 15th and 22nd days of the gestation for the pregnant rabbits. After the exposure, rabbits were left on their own without any intervention until gestation period of pregnant rabbits, approximately 30 days is over.

After birth, only one newborn, not exposed to RFR (max. 2 days) was randomly selected from each litter and killed immediately after the anesthesia. The main reason for investigating the newborns was to observe the possible effects of intrauterine RFR exposure (between 15th and 22nd days of the gestational period when the transition from embryogenesis to organogenesis takes place).

Only one animal was placed in each cage during each RFR exposure period because placing more than one animal in a cage would create a stress factor. The rectal temperatures were measured before and after the exposure by Digital Thermometer (Elite, Istanbul, Turkey) in both non-pregnant-control and non-pregnant-RFR-exposed rabbits.

Experimental Design

Nonpregnant and pregnant New Zealand White rabbits were randomly divided into four groups:

Group I (Nonpregnant Control): Each nonpregnant rabbit in sham-exposed group ($n = 9$) was kept in plexiglas cage under experimental setup for 15 min/day during 7 days (in the "turned off" arrays).
Group II (Nonpregnant-RFR Exposed): Nonpregnant rabbits in RFR-exposed group ($n = 9$) were exposed individually to 1800 MHz GSM-like RFR for 15 min/day during 7 days.
Group III (Pregnant Control): Pregnant rabbits ($n = 9$) were kept in plexiglas cage under experimental setup for 15 min/day during 7 days (in the "turned off" arrays).
Group IV (Pregnant-RFR Exposed): Pregnant rabbits in RFR-exposed group ($n = 9$) were exposed individually to 1800 MHz GSM-like RFR for 15 min/day during 7 days.

Newborns of the pregnant rabbits were also divided into two groups:

Group V (newborns of Group III): Newly born rabbits ($n = 9$) of pregnant-control group.
Group VI (newborns of Group IV): Newly born rabbits ($n = 9$) of pregnant-RFR-exposed group. They were exposed to 1800 MHz GSM-like RFR for 15 min/day during 7 days in the intrauterine period (between 15th and 22nd days of gestational period).

The day after the last exposure, rabbits were anesthetized and killed with ketamine (35 mg/kg, intramuscular) and xylazine (5–10 mg/kg, intramuscular).

Biochemical Analysis

Liver tissues washed out from contaminating blood with ice-cold buffered saline and stored at −30°C (maximum 10 h) for the various double-blind biochemical analysis. After weighing, the liver was cut into small pieces and then homogenized in four volumes of ice-cold Tris–HCl buffer (50 mM, pH7.4) by using homegenizer (Disperser T10 basic D-79219, IKA-WERKE, GmbH, Staufer).

Malondialdehyde (MDA) Assay

Tissue malondialdehyde (MDA) levels were determined by using TBARS assay which is a major aldehyde species for lipid peroxidation. Difference in absorbance of the two measurements from the butanol phase was used as the MDA value (nmol/g tissue) [27].

Lipid Peroxidation Assay (FOX Assay)

Assay is intended for the quantitative determination of the low levels of lipid hydroperoxide in the samples. It is based on the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) by hydrogen peroxide under acidic conditions. The ferric ion binds with the indicator dye xylenol orange to form a stable colored complex which can be measured at 560 nm as an indirect measure of hydroperoxide concentration. HP Equivalents HPE (nmol/g wet weight) were calculated according to Hermes-Lima et al. [28].

DNA Damage Assay

For the measurement of oxidative DNA damage (lesions/10^6 DNA nucleosides), after the genomic DNA of liver tissues were extracted by Roche DNA extraction kit, it was denatured by heating at 95°C for 3 min and then cooled on ice. 100 µl 2 mM DFAM and 20 mM acetate buffer (pH = 5) were added to the denaturated DNA. DNA content was analyzed spectrophotometrically at 260 nm and then hydrolyzed to nucleotides by incubation with 4 µl of 3.3 mg/ml suspension of nuclease P1. The Tris–HCl buffer (pH = 8.5) was added to the mixture and hydrolyzed to the corresponding nucleosides by incubation with calf intestine alkaline phosphatase for 1 h at 37°C. After adding acetate buffer and 50 mM EDTA/10 mM DFAM solution, the mixture was filtered through a 0.22-µm Millipore filter unit (UltraFree, Bedford, MA) and then centrifuged at 10.000×*g* for 20 min at 4°C. Reverse-phase HPLC-EC was performed as described by [29]. The DNA hydrolysate was injected onto a Waters C18 reverse-phase column (5 µm, 0.46 × 25 cm^2; Waters Assoc., Milford, MA, USA) at a flow rate of 1 ml/min. The mobile phase was 50 mmol/l phosphate buffer (pH = 5.5) with 5% methanol [30, 31].

The eluant was monitored at 290 nm for the ultraviolet detection of dG and at 0.6 V for the electrochemical detection of 8-OHdG. System was calibrated with authentic dG and 8-OHdG standards (Sigma Chemical, St Louis, MO, USA). dG had a retention time of 10–12 min and 8-OHdG had a retention time of 8.7–13.8 min. Standards were run after every fifth sample for verification, and the data were expressed as the ratio of 8-OHdG–10^6 dG.

Statistical Analysis

Data analysis was carried out using the SPSS 11.5 statistical package (SPSS, Chicago, IL, USA). The Kruskal–Wallis (non-parametric) test was applied to evaluate differences among all groups while differences between pairs of groups were evaluated by means of the Mann–Whitney test. The results were expressed as median (interquartile range, IQR) values.

Results

No significant differences were found in the liver tissue of 8 OHdG/10^6 dG amount of exposure groups [Group II—median 1.5043 8 OHdG/10^6 dG (IQR 0.0716-0.11) and Group IV—median 1.5258 8 OHdG/10^6 dG (IQR 0.0501-0.07)] compared to controls [Group I—median 1.5310 8 OHdG/10^6 dG (IQR 0.1025-0.22) and Group III—median 1.5120 8 OHdG/10^6 dG (IQR 0.0811-0.14)] ($P > 0.05$, Mann–Whitney) (Fig. 2).

MDA levels in the same groups [Group II—median 207.0513 nmol/g tissue (IQR 81.73-174.36) and Group IV—median 239.7436 nmol/g tissue (IQR 125.3205-207.05)] were found to increase significantly with respect to non-pregnant controls [Group I—median 65.3846 nmol/g tissue (IQR 40.8654-65.38)] ($P < 0.001$, Mann–Whitney). However, no significant difference was found in Group IV [median 239.7436 nmol/g tissue (IQR 125.3205-207.05)] with respect to Group III [median 196.1538 nmol/g tissue (IQR 114.4231-196.15)] ($P > 0.05$, Mann–Whitney) (Fig. 3).

Increased FOX levels were found in Group II [median 1255.3846 HPE (nmol/g wet weight) (IQR 476.4550-816.31)] and Group IV [median 1035.7202 HPE (nmol/g wet weight) (IQR 952.8810-1407.21)] with respect to non-

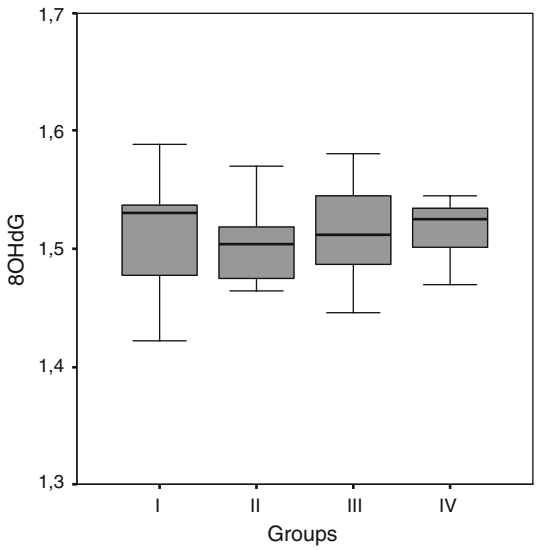

Fig. 2 Changes in the liver 8-hydroxy-2′-deoxyguanosine (8 OHdG, 8 OHdG/10^6 dG) content of both nonpregnant and pregnant New Zealand White rabbits under 1800 MHz GSM-like RF radiation exposure (15 min/day, for 7 days). All values are expressed as median (IQR) values. I: Nonpregnant control, II: Nonpregnant-RFR exposed, III: Pregnant control, IV: Pregnant-RFR exposed

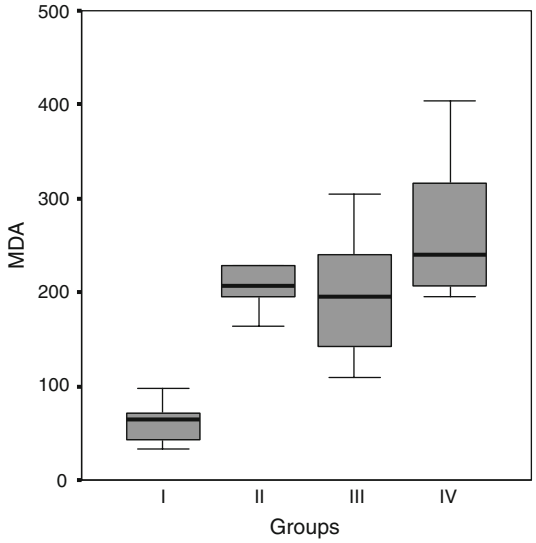

Fig. 3 Changes in the liver malondialdehyde (MDA, nmol/g tissue) content of both nonpregnant and pregnant New Zealand White rabbits under 1800 MHz GSM-like RF radiation exposure (15 min/day, for 7 days). All values are expressed as median (IQR) values. I: Nonpregnant control, II: Nonpregnant-RFR exposed, III: Pregnant control, IV: Pregnant-RFR exposed

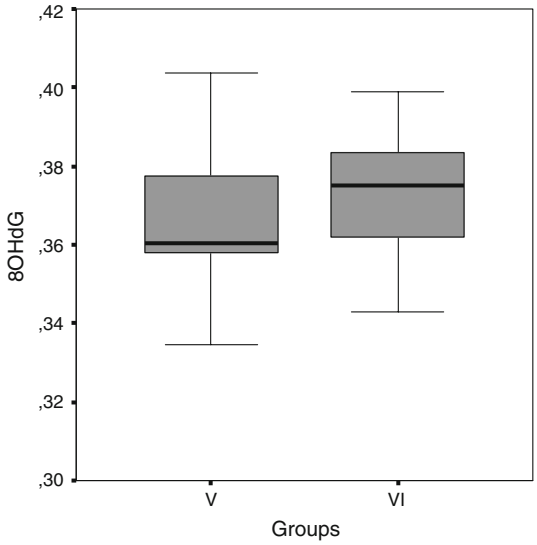

Fig. 5 Changes in the liver 8-hydroxy-2′-deoxyguanosine (8 OHdG, 8 OHdG/10^6 dG) content of newborn New Zealand White rabbits under intrauterine exposure of 1800 MHz GSM-like RF radiation (15 min/day, for 7 days). All values are expressed as median (IQR) values. V: newborns of Group III, VI: newborns of Group IV

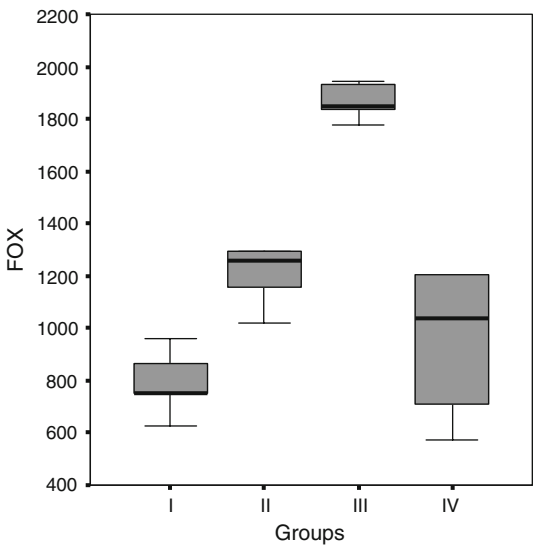

Fig. 4 Changes in the liver ferrous oxidation in xylenol orange [FOX, HPE (nmol/g wet weight)] content of both nonpregnant and pregnant New Zealand White rabbits under 1800 MHz GSM-like RF radiation exposure (15 min/day, for 7 days). All values are expressed as median (IQR) values. I: Nonpregnant control, II: Nonpregnant-RFR exposed, III: Pregnant control, IV: Pregnant-RFR exposed

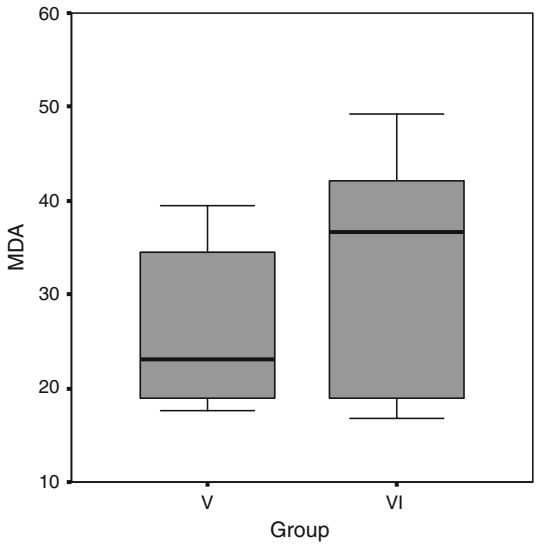

Fig. 6 Changes in the liver malondialdehyde (MDA, nmol/g tissue) content of newborn New Zealand White rabbits under intrauterine exposure of 1800 MHz GSM-like RF radiation (15 min/day, for 7 days). All values are expressed as median (IQR) values. V: newborns of Group III, VI: newborns of Group IV

pregnant controls [Group I—median 753.3659 HPE (nmol/g wet weight) (IQR 224.4351-334.57)] ($P < 0.05$, Mann–Whitney). No statistical difference was found in Group IV [median 1035.7202 HPE (nmol/g wet weight) (IQR 952.8810-1407.21)] with respect to Group III [median 1848.7088 HPE (nmol/g wet weight) (IQR 114.6404-170.10)] ($P > 0.05$, Mann–Whitney) (Fig. 4).

There was no difference in the liver tissue of 8 OHdG/10^6 dG levels between newborns of pregnant-RF exposed [Group VI—median 0.3752 8 OHdG/10^6 dG (IQR 0.0281-0.06)] and newborns of pregnant controls [Group V—median 0.3603 8 OHdG/10^6 dG (IQR 0.0404-0.08)]

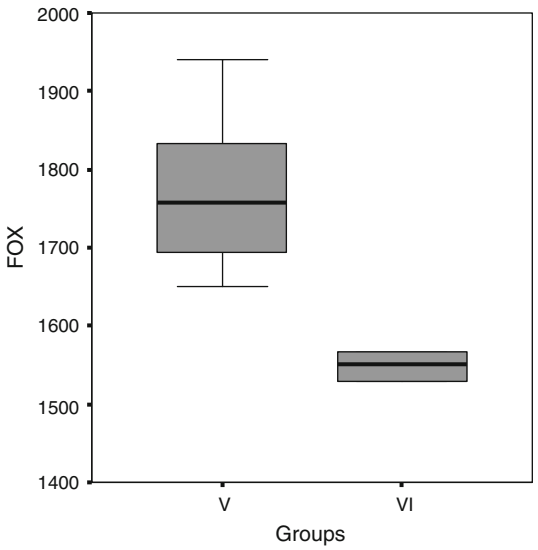

Fig. 7 Changes in the liver ferrous oxidation in xylenol orange [FOX, HPE (nmol/g wet weight)] content of newborn New Zealand White rabbits under intrauterine exposure of 1800 MHz GSM-like RF radiation (15 min/day, for 7 days). All values are expressed as median (IQR) values. V: newborns of Group III, VI: newborns of Group IV

($P > 0.05$, Mann–Whitney) (Fig. 5). Similarly, no significant difference was found in the MDA levels of Group VI [median 36.6154 nmol/g tissue (IQR 23.8109-32.47)] and Group V [median 23.1026 nmol/g tissue (IQR 16.2099-21.79)] ($P > 0.05$, Mann–Whitney) (Fig. 6). However, FOX levels were found significantly decreased in Group VI [median 1551.4904 HPE (nmol/g wet weight) (IQR 108.7421-179.93)] with respect to Group V [median 1758.2277 HPE (nmol/g wet weight) (IQR 177.4887-290.86)] ($P < 0.05$, Mann–Whitney) (Fig. 7).

Discussion

The main finding of our study was the destructive effects of free radicals on membrane phospholipids which can be induced by the exposure of 1800 MHz GSM-like RFR for 15 min/day. However, the formation of radical molecules did not appear to give rise to the oxidative DNA base modification in liver tissue. Besides, intrauterine exposure to RF radiation did not have an effect on the free radical formation and DNA base modification.

The high sensitive population including pregnants, children and elders are exposed to RF fields in the same degree because of the fact that there is no national and international safety limits. It is clear that studies of whether RF radiation has the adverse effects on pregnancy, have been limited due to the ethical difficulties encountered in the use of humans. Therefore, the pregnancy studies are performed by both modeling studies and laboratory experiments by using different animal models.

The International Commission for Non-Ionizing Radiation Protection (ICNIRP) and Institute of Electrical and Electronics Engineers (IEEE) set guidelines to limit the EM exposure of general public and workers. There is no particular restriction for pregnant and their offsprings.

In the intrauterine exposure, the fetus is thought to compensate some detrimental bioeffects of RFR exposure that are related to the rise in maternal body temperature by dissipating heat to the mother or exchanging heat within the umbilical blood vessels [32]. In view of the uncertainty regarding possible effects of raising fetal temperatures directly through RF absorption, a rise in fetal and embryo temperature to less than 38°C should not result in adverse developmental effects [32]. Lary et al. [33] were determined an increment in the colonic temperatures for different pregnancy periods of rats exposed to 27.12 MHz RF radiation. Significant increases in the incidence of fetal malformations and prenatal deaths can occur in parallel to increase in maternal temperature. Temperature-dependent biological responses of fetus have been observed at 41.5°C as the threshold level whereas lethality has dominated above threshold level of maternal colonic temperature. Wangemann and Cleary [34] revealed that rectal temperatures of rabbits which are exposed to 2450 MHz (continuous wave and pulsed mode) were between 38 and 41°C. The threshold for producing developmental toxicity appears if the resting temperature is elevated to 2.5°C more. In this study, pregnant rabbits were exposed to 1800 MHz GSM-like RF on days 15th–22nd of gestation. During the embryogenesis and organogenesis period of fetus, on days 6th–18th of gestation, mothers were exposed to this field for 15 min/day along a week. We aimed to observe the rectal temperature changes in pregnant rabbits in order to determine the possible intrauterine effects of RFR at the very beginning of the experiment. However, rabbits are very sensitive animals especially when they are pregnant. They have a tendency to miscarriage when they are stressed out. Since measuring rectal temperature may stress the pregnant animals out, we measured the temperature change in only non-pregnant group. Moreover, the teratogenic effects of acute RF exposure were investigated by veterinarians, but no malformations on the skeletons of newborns or prenatal deaths were observed. Besides, thermoregulatory mechanisms of rabbits are sufficient to compensate slight changes in body temperatures by increasing respiration rate and ear lobe temperature [35].

Interaction of RF fields with living systems has been characterized by its frequency, polarization, and power density. However, interaction mechanisms are mainly based on the absorption of RF energy by biological matter. In the thermal mechanism, the RF electric field generates an oscillating current and the rapid transfer of the energy of this current into the molecular motion responsible for most

of the heat capacity results in an increase in the local temperature. In contrast to thermal mechanism, nonthermal interaction of RF fields with living systems is associated with two mechanisms, biochemical and electromagnetic. Biochemical mechanism based on the release of secondary chemical messengers can induce biological responses in target cells. Electromagnetic mechanism is based on reemission of secondary photons. Reemitted photons can induce response in other cells if the intercellular distance is shorter than the length of photon absorption [36, 37].

The conformational changes in the structural molecules have been mainly important for the nonthermal interaction mechanisms of RF fields. Conformational changes occurred in structural proteins and enzymes that played key roles in the catalysis of metabolic reactions can cause the important biological responses within the cell. Laurence et al. [38] suggested that RF radiation can change protein denaturation through the biological response occurred against cellular stress. Astumian et al. [39] proposed RF radiation would be responsive to ion movement across the membrane resulting in the conformational change in ATPase. In this study, we argue that 1800 MHz GSM-like signals may induce cellular stress leading to conformational changes that are associated with the excitation of molecular vibration. In their study Erkoc et al. [40] investigated the structural and electronic properties of guanine and guanosine theoretically by performing semi-empirical and ab initio molecular orbital theory calculations and concluded that guanine is a highly polar molecule, therefore it may interact with its surrounding, especially with other polar molecules in the cell more strongly; this makes the guanine molecule as a potential source of damage in the cells. At a frequency of 1000 MHz, the photon energy is 4 meV, and therefore smaller than the energy, 1 eV, required to ionize a typical molecule by a factor of approximately 2×10^5. Challis (2005) suggested that if exposure to RF fields was to damage DNA, it could not be a result of ionization or excitation due to the absorption of single photons to DNA, it would have to be through some other process [1].

Reactive oxygen species generated by external field application may interact with cellular biomolecules, such as DNA, leading to modification and damage [41]. Any modification in DNA molecules generated by radicals has also been important because of its strong responsibility to the repair mechanism of almost all the structural biomolecules [42]. In the present study, we analyzed the ratio of 8 OHdG/10^6 dG in liver tissues as an indicator of DNA damage mediated by 1800 MHz GSM-like signals RFR as a ROS and observed no statistically significant differences between the all groups. Besides recent studies have shown that RFR emitted from mobile phones could increase the release of free radicals. Meral et al. [43] revealed that RFR generated from cellular phone (12 h/day, 30 days) may produce oxidative stress by increasing MDA levels of brain tissues in guinea pigs. Likewise, in this study, we found that whole-body 1800 MHz GSM-like RF exposure for 15 min/day for a week could affect lipid peroxidation by increasing MDA and FOX levels in nonpregnants and pregnant. However, RF exposure has no effect on hepatic lipid peroxidation end product levels of the new borns. Our results are also in consensus with the knowledge that oxidative stress is normally higher during pregnancy than in the nonpregnant state due to increased oxygen intake and energy utilization [44]. Maternal oxidative stress was recently shown to exert adverse effects on birth outcomes [45].

Consequently, to our knowledge, any research on mobile phone-like radiation on oxidative DNA and lipid damage in liver tissue of pregnant animals and their offsprings have not published, yet. With this perspective, our results may constitute a reference for the future pregnancy studies. Moreover, it would be beneficiary to increase number of these studies for establishing international standards for the protection of pregnant women under RF exposure.

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