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Original Research

Effects of Exposure to Radiofrequency at 2.45 GHz on Structural Changes Associated with Lipid Peroxidation in Prepubertal Rat Testicular Tissue

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ABSTRACT

Objective: The increasing use of electronic devices, accompanied by advancing technologies, has led to heightened exposure to non-ionizing electromagnetic radiation (EMR). This exposure instigates the accumulation of free radicals and oxidative damage in tissues, consequently impacting biological systems. Notably, the testis is among the tissues adversely affected by EMR. Numerous studies have highlighted the pivotal role of the testis in sperm production, emphasizing the potential implications of any damage on the reproductive system. This study aims to assess the levels of lipid peroxidation through histological evaluation in the testicular tissue of prepubertal male rats exposed to electromagnetic radiation at varying electric field intensities within the 2.45 GHz radiofrequency (RF) range.

Methods: The experimental group comprises six subdivisions, including a sham control group, as well as groups exposed to varying electric field strengths (EFS) of 0.6 V/m, 1.9 V/m, 5 V/m, 10 V/m, and 15 V/m, respectively. Excluding the sham control group, the remaining subgroups were subjected to a daily 2.45 GHz RF exposure for 1 hour starting immediately after fertilization. This exposure to different electric field intensities continued for 45 days post-birth.

Results: The samples obtained from the RF radiation-exposed rats exhibited elevated malondialdehyde (MDA) values and decreased glutathione (GSH) values in the testicular tissue. Furthermore, a comparative analysis between the microwave radiation-exposed group and the control group revealed distinct histological alterations in the testicular tissue.

Conclusion: In conclusion, our findings indicate that exposure to microwave radiation at an electric field intensity of 15 V/m can lead to significant histopathological and oxidative parameter changes in Wistar rats. These results underscore the potential effects of such exposure on human health.

Keywords: Testicular Injury, Prepubertal Rats, Malondialdehyde, Glutathione, Radiofrequency

INTRODUCTION

The widespread use of wireless communication devices, radar systems, communication gadgets, cell phone base stations, high-voltage power lines, radio and television transmitters, transformer substations, electrical appliances in homes and workplaces, and numerous electrical systems worldwide emit a broad spectrum

of electromagnetic radiation (EMR) [1, 2]. Particularly, today's mobile phone models (1800MHz - 2200MHz), laptop computers (1000 MHz - 3600MHz), and wireless networks operate at high frequencies, emitting RF (2.45 GHz) [1, 2]. Numerous studies encompassing both animal and human research have investigated the potential health effects of EMR, shedding light on the need to create and maintain a protected environment during the prenatal, neonatal, and postnatal periods for a healthy childhood. Given the ongoing uncertainties surrounding the impact of EMR, the Council of Europe recommends the implementation of restrictions on internet access and mobile phone usage in educational institutions, as a precautionary measure to safeguard the younger generation from the potential risks of radiation exposure [3]. Due to the increasing sensitivity among children, the World Health Organization (WHO) led a comprehensive study on electromagnetic fields (EMF) between 2006 and 2010, pioneering research on the effects of early exposure to microwave radiation. [4]. The WHO, declaring that the potential adverse effects of EMR on health are more significant in children compared to adults, organized a crucial workshop in 2004 focusing on examining children's sensitivity to electromagnetic fields and addressing related research topics. Scientists from 43 different countries discussed research on this topic and decided to increase the number of studies on the effects of EMF on children. [5]. Thus, international authorities have focused their attention on radiation exposure during childhood.

In assessing the potential environmental impacts on the development of childhood diseases, it is crucial to recognize not only that childhood exposures differ from those of adults, but also that age plays a significant role in sensitivity to these exposures [6]. Our literature reviews revealed that studies investigating the effects of prenatal EMR exposure are widespread, whereas studies examining the effects on infants and young children post-birth are scarce [7]. It has been reported that exposure to 2.45 GHz RF radiation, even at low doses, in the fetal period will lead to profound and definite consequences on health [8-11]. Zhang et al. have identified neurobehavioral disorders associated with exposure and gender-specific learning and memory deficiencies in their study on microwave radiation exposure during the fetal period in mice [12]. On the other hand, Othman et al. have determined that prenatal exposure to the radiofrequency of conventional WiFi devices leads to postnatal neurodevelopmental disorders and oxidative stress [13]. Additionally, various studies indicate that radiation exposure has adverse effects on brain or liver development, causing mutations in DNA and disturbances in the structure of membrane lipids [14-16]. Furthermore, our study's focus on testicular tissue is crucial due to its high sensitivity, as spermatogenesis is regulated by a complex and delicate regulatory mechanism [17]. Several studies have indicated that EMR leads to reductions in spermatogenesis and sperm motility by affecting Leydig cells that produce the testosterone hormone [18, 19]. There are limited studies evaluating the effects of 2.45 GHz RF on prepubertal rat testicular tissue. Hence, this study aims to determine the levels of lipid peroxidation and histologically evaluate the testicular tissue of male rats exposed to electromagnetic radiation at 2.45 GHz RF, starting from prenatal stages up to the prepubertal period, under different electric field intensities.

Main Points;

- Consistent with studies on lipid peroxidation, we observed an increase in MDA level and a decrease in GSH level, especially at 15 V/m electric field intensity. This suggests that EMR exposure causes oxidative stress and biological changes may occur.
- As a result of histological examinations, disruptions in the organization of spermatogenic cells and lack of spermatogonia were detected in seminiferous tubules at 10 and especially 15 V/m electromagnetic field values. These findings are similar to the pathological data reported in testicular tissue after radiation exposure. This disruption in spermatic cell organization will also affect spermatogenesis and sperm quality.

MATERIALS AND METHODS

Experimental Design, Animals and Groups

Male Wistar rats, 45 days old, were obtained from the Ondokuz Mayıs University Animal Experiments Theoretical Ethics Committee (OMÜ HAYDEK) (Samsun, Türkiye). The experimental phases of the study were conducted in accordance with the guidelines of the National Institutes of Health (NIH) for the care and use of laboratory animals. The study protocol was approved by HAYDEK under decision number 2019-23. All animals were kept under a constant 12:12-hour light-dark cycle and were provided ad libitum access to food.

The experimental groups and the procedures applied to these groups are explained below.

Group I (G1) (n=9): The sham group, which did not expose rats to any RF radiation.

Group II (G2) (n=8): The group was subjected to 1 hour/day of 0.6 V/m at 2.45 GHz RF radiation.

Group III (G3) (n=8): The group was subjected to 1 hour/day of 1.9 V/m at 2.45 GHz RF radiation.

Group IV (G4) (n=8): The group was subjected to 1 hour/day of 5 V/m at 2.45 GHz RF radiation.

Group V (G5) (n=8): The group was subjected to 1 hour/day of 10 V/m at 2.45 GHz RF radiation.

Group VI (G6) (n=8): The group was subjected to 1 hour/day of 15 V/m at 2.45 GHz RF radiation.

The 2.45 GHz RF has been utilized in this study due to its widespread use in industrial and medical applications, scientific research, military fields, and household devices in today's. In our study, although radiation exposure can occur for different durations in real-life situations, we adopted the exposure duration from Shokri et al.'s studies as a reference (one hour) [20]. Mantiply et al. measured the minimum EFS value at ground level from microwave towers as 0.6 V/m, and this value was determined as the minimum EFS applied in our study (G2) [21]. Joseph et al. determined that the most dominant Electric Field Strength (EFS) value detected in outdoor settings originated from GSM900, with an EFS magnitude of 1.9 V/m. This value was established as the applied EFS for our G3 group [22]. Additionally Nassiri et al. determined the electric field intensities of many schools and hospitals to be 5-15 V/m [23]. In our study, when determining the EFS values applied to groups G4, G5, and G6 we were inspired by the minimum and maximum values identified by Nassiri et al. Accordingly, we applied them systematically, increasing in order, as 5, 10, and 15 V/m.

The RF exposure was initiated on the first day of pregnancy for the rats in the exposure group (G2, G3, G4, G5, G6), and it continued for 45 days in male offspring rats after birth. The male rats in the sham control group were allowed to grow under normal conditions until reaching prepubescence. The day after the final exposure, prepubescent male rats from all groups were sacrificed under anesthesia, and testicular tissues were collected for lipid peroxidation analyses and histological examinations, which were stored under appropriate conditions. The schematic representation of the conducted applications is summarized in Figure1.

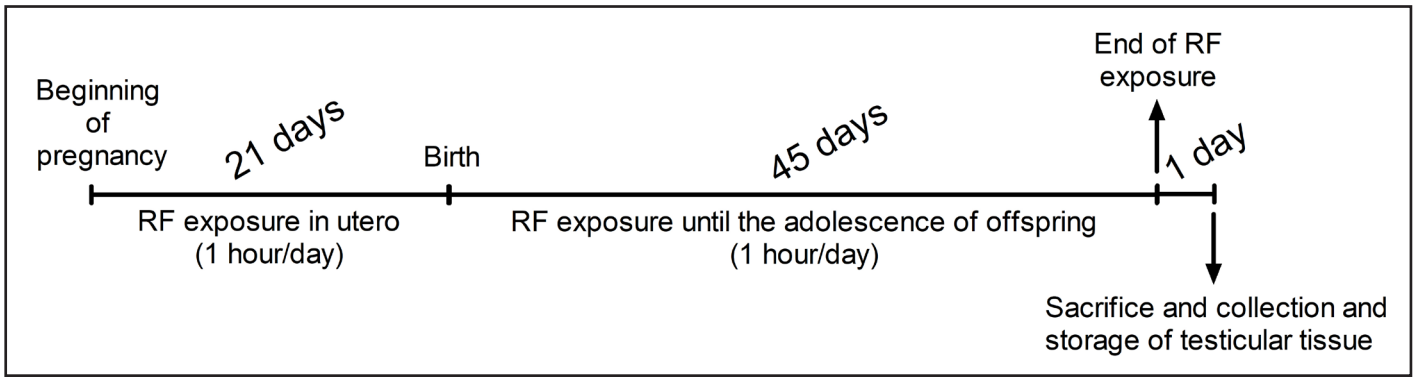

Figure 1. Schematic representation of radiofrequency (RF) exposure application processes.

SAR Evaluation

In this study, a 2004X-RF Wi-Fi system generator (2004X-RF Wi-Fi system generator, Everest Co., Adapazarı, Türkiye) with a Monopole antenna and a maximum output power of 0-1 Watt was utilized to produce 2.45 GHz RF at various electric field intensities. Before the study, Spectran NF-5035 spectrum analyzer (Spectran NF-5035, AARONIA AG, Germany) was used to identify frequencies in the range of 1 Hz to 1 MHz, and RF-Explorer 6G Combo (EMRSS,Germany) was employed for the range of 15 MHz to 6.1 GHz to determine the frequencies present in the environment. According to the Spectran NF-5035 measurements, the highest magnetic field strength level in the environment was found to be 286 μA/m, while the measurement using the RF-Explorer 6G Combo indicated the

highest measurement value as -70 dBm. The isotropic electric field probe for the Narda EMR-300 in the 100 kHz-3 GHz frequency band (EMR300 Probe model, Type 8C, 2244/90.21, Germany) and the Narda SRM-3006 isotropic Model 3501/03 probe in the 27 MHz-3 GHz frequency band range were utilized to measure the ambient electric field and magnetic field strengths before exposure. The lowest and highest ambient electric field measurements were determined to be 92 mV/m and 103 mV/m, respectively.

Different RF levels were applied daily to the five experimental exposure groups, with a plexiglass holder positioned in the center of a monopole antenna to ensure uniform distribution of electric field values. 66-day electric field strength (EFS) measurements were conducted on the head and back regions of each rat for 6 minutes in accordance with the regulations of the Information Technologies Authority. The EFS values measured using the Narda EMR-300 were used to calculate specific absorption rate (SAR) values in 10 g tissue using Computer Simulation Technology (CST) program (CST Studio Suite, version of 2018, USA), which helps the discretization of Maxwell equations by Finite Integration Technique (FIT). Subsequently, the SAR distributions for the whole body and testis were calculated with the EFS values and utilized for dosimetric evaluation.

Biochemical Analysis

The isolated testicular tissues for biochemical analyses were stored -80°C. On the day of the study, all samples were thawed at room temperature and homogenized to obtain the homogenate. Testicular tissue malondialdehyde (MDA) level measurements were conducted using the Mihara and Uchiyama method [24] on the SPECTROstar device (BMG Labtech, Germany), with the results reported in nmol/g protein. Testicular tissue glutathione (GSH) levels were determined using the Ellman method [25] on the SPECTROstar device (BMG Labtech, Germany), with the data presented as mg/g tissue.

Histological Analysis and Johnsen Criteria

For histological analysis, the tissues were fixed in 10% formaldehyde at +4ºC for 24 hours and embedded in paraffin blocks. Sections of 4 µm thickness were taken and stained with Hematoxylin & Eosin. These sections were examined using a BX51 microscope (Olympus) in a blind manner, with all images captured using the DP72 (Olympus) camera. The dimensions of seminiferous tubules, spermatogenic cells, their organization, as well as Sertoli and Leydig cells were evaluated. Furthermore, the testicular biopsy preparations were examined under a microscope, and the Johnsen scoring system was applied for each preparation based on the degree of germ cell maturation, with at least 50 tubules assessed per preparation [26]. The criteria formulated by Johnsen were used to evaluate testicular biopsies. According to these criteria, a ten-point scoring system was described as follows: 10- fully organized germinal epithelium with numerous sperm cells, 9- presence of numerous sperm cells with irregular germinal epithelium, 8- presence of a few sperm cells, 7- no sperm cells but numerous spermatids, 6- absence of sperm cells, but a few spermatids present, 5- no sperm cells or spermatids, but numerous spermatocytes present, 4- a few spermatocytes, 3- only spermatogonia, 2- only Sertoli cells, no germ cells, and 1- acellular seminiferous tubules [27].

Statistical Analysis

The data were analyzed using Prism software (GraphPad, version 6.07, USA) and Microsoft Excel (Microsoft 365 Apps, USA). The number of experimental animals for each group was determined using the Power Analysis method. The normality of the data distribution was evaluated with the Shapiro-Wilk normality test, and the homogeneity of variances was assessed with the Levene test. All data showing a normal distribution were presented as mean ± standard deviation (M ± SD). One-way analysis of variance and the Tukey post-hoc test were used for the statistical analysis data. p-values <0.05 were considered as statistically significant.

RESULTS

SAR Results

The whole-body SAR values of the exposure groups are 0.48 µW/kg, 0.53 mW/kg, 3.44 mW/kg, 15.1 mW/kg, and 34.9 mW/kg. The testicular tissue-specific SAR values for 10g of tissue are 0.0054 mW/kg, 0.0605 mW/kg, 0.4070 mW/kg, 1.7345 mW/kg, and 4.1091 mW/kg, respectively (Figure 2).

Biochemical Results

The MDA levels in Group 6 were significantly higher than the other groups (Group 1, Group 2, Group 3, Group 4, and Group 5) ($p < 0.05$). In comparison to the sham control group (Group 1), there was an increase in all exposure groups (Groups 2, 3, 4, and 5), although it was not statistically significant ($p > 0.05$) (Figure 3).

GSH levels in Group 6 were significantly lower than the other groups (Group 1, Group 2, Group 3, Group 4 and Group 5)

($p<0.05$). Compared to the sham control group (Group I), there was a decrease in all exposure groups (Groups 2, 3, 4 and 5), although not statistically significant ($p>0.05$)(Figure 4).

Histological Results and Johnsen Criteria

No significant morphological deterioration was observed in groups 1, 2, 3 and 4 in the H&E images examined. It was observed that all cell development stages were present, from spermatogonia, primary spermatocytes, spermatids and spermia. The organization of spermatogenic cells was smooth. In Groups 5 and 6, slight disruptions in the organization of spermatogenic cells and lack of spermatogonia were observed in some seminiferous tubules. No significant difference was observed in seminiferous tubule diameters in any of the groups. Leydig cells were present in the intertubular areas in all groups (Figure 5).

According to the Johnsen scoring results of seminiferous tubular sections, a statistically significant difference was detected

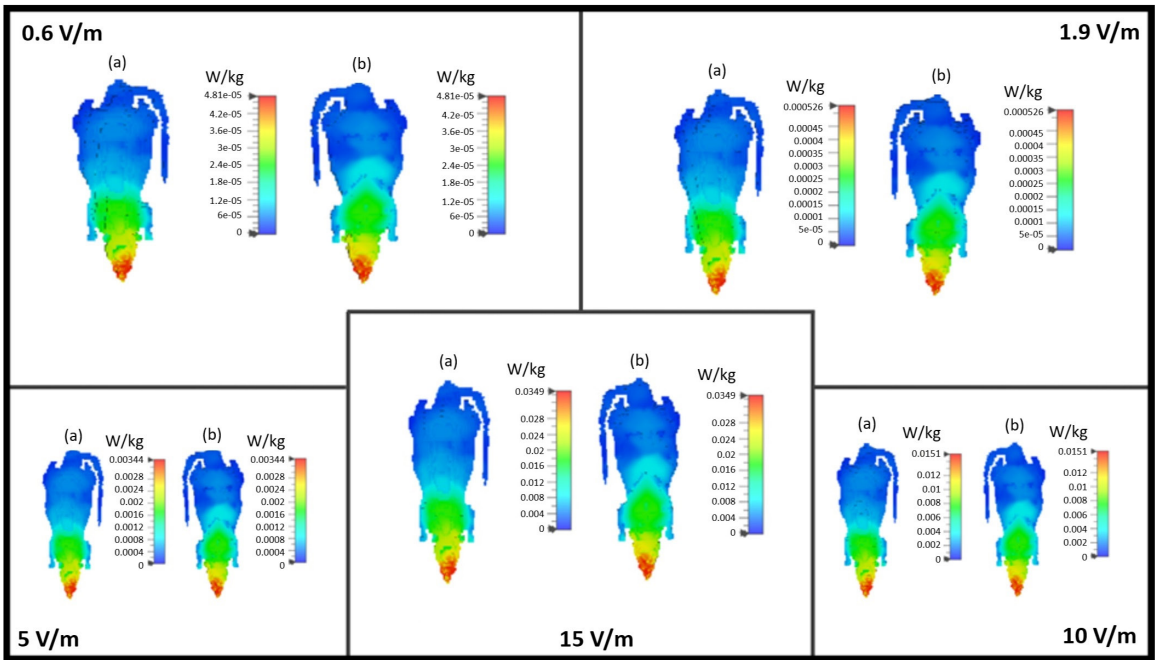

Figure 2. SAR 10g distribution in the rat model. a) Top view b) bottom view

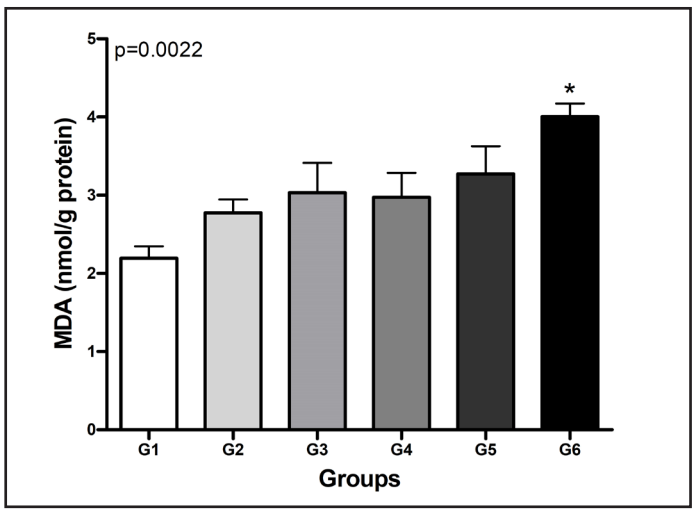

Figure 3. MDA (nmol/g protein) values of testicular tissues of all experimental groups. MDA (nmol/g protein) values of testicular tissues are shown as mean ± standard error. * indicates the degree of significance between the groups at the $p<0.05$ level.

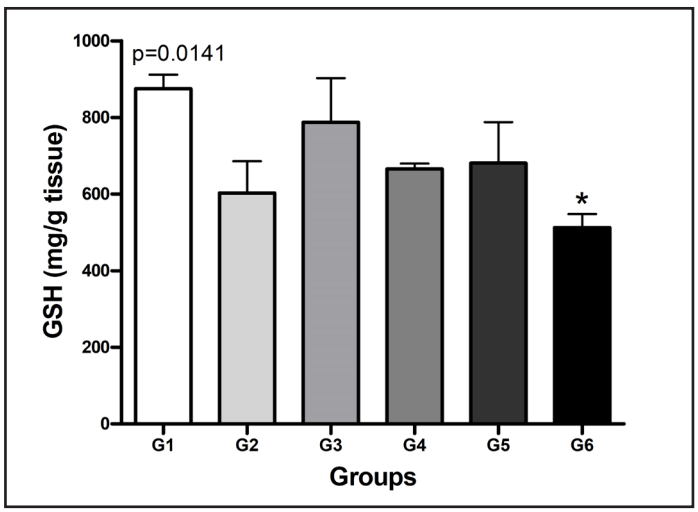

Figure 4. GSH (mg/gr tissue) values of testicular tissues of all experimental groups. GSH (mg/gr tissue) values of testicular tissues are shown as mean ± standard error. * indicates the degree of significance between the groups at the $p<0.05$ level.

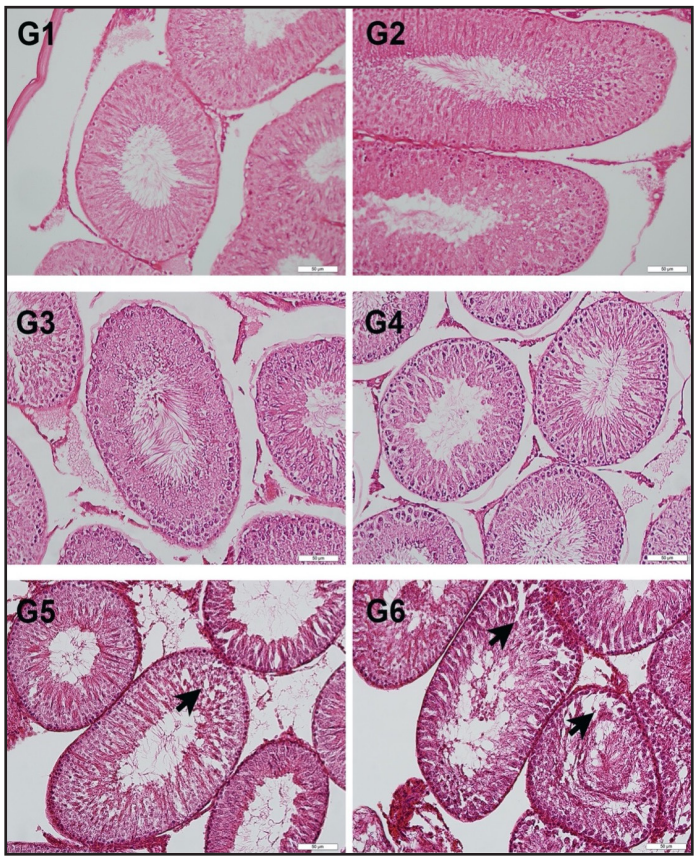

Figure 5. Testicular tissues of groups 1, 2, 3, 4, 5 and 6 are shown at 40x magnification. G1, G2, G3 and G4: Normal seminiferous tubule morphology is observed in Group1, 2, 3 and 4. G5, G6: Disorganization appears in some seminiferous tubules in Groups 5 and 6 (black arrow).

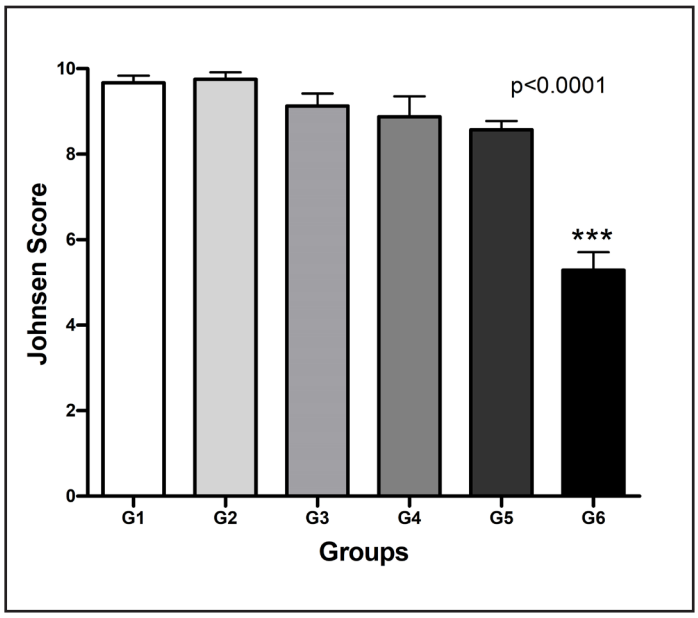

Figure 6. Johnsen score of seminiferous tubular sections. *** indicates significance at p<0.0001 level for G6 vs other groups.

between the G6 group and all other groups (Figure 6).

DISCUSSION

With the advancement of technology, electronic devices that hold a significant place in our daily lives and the associated technical infrastructures have made radiation exposure inevitable. Our study focused on the effects of 2.45 GHz RF exposure, particularly at an intensity of 15 V/m EFS during the prepubertal phase in 45-day-old male rats. Histological examination confirmed that this exposure led to lipid peroxidation. The findings indicate that EMR can significantly impact testicular tissue, particularly at an intensity of 15 V/m.

In recent years, the discussion surrounding the potential harmful effects of EMR exposure on children has gained significant attention. These concerns are particularly relevant during fetal development, as adverse pregnancy outcomes may result from such exposure. The absorption of EMR is influenced by various factors, including the individual's size, the dielectric properties of organs, as well as the frequency and polarization of the radiation [28, 29]. Moreover, the level of EMR exposure is known to impact the cell membrane's penetrative ability, leading to alterations in cellular and metabolic activities. Studies have indicated that EMR exposure induces oxidative stress by producing an excess of reactive oxygen species (ROS), thereby contributing to tissue damage [30-33]. Several researchers have reported a correlation between EMR exposure and the generation of free radicals, which exhibit strong oxidizing capabilities, ultimately interfering with normal cellular functions. These findings align with previous studies that have highlighted the oxidative damage caused by EMR, leading to reduced sperm count, motility, and daily sperm production, alongside significant testicular histopathological changes [14, 34, 35].

Some studies on microwave radiation have indicated that exposure can lead to a reduction in the size of testicular organs and the diameter of seminiferous tubules, potentially resulting in significant impacts on testosterone hormone levels [36-40]. The effects of exposure to 900, 1800, and 2100 MHz RF radiation on DNA breaks and oxidative changes in rat testicular tissue have been investigated. As a result, it has been demonstrated that particularly at high frequencies (1800 and 2100 MHz), there is an increase in the MDA (malondialdehyde) levels, indicating a potential induction of oxidative stress in rat testicular tissue [40]. EMR has the potential to alter biological lipid membranes and these changes are seen in the structural and functional properties of the cell [41]. In another study using 1 gigahertz

electromagnetic field on rats, oxidative stress parameters were investigated and as a result, it was found that MDA level increased and GSH concentration decreased significantly [42]. In a study investigating the effect of 2.45 GHz electromagnetic radiation (EMR) on oxidant and antioxidant status in rats, glutathione peroxidase (GSH-Px) decreased and the concentration of MDA levels increased. It has shown that the role of oxidative mechanisms in EMR-induced tissue damage can improve oxidative damage through antioxidant substances [43]. Studies have shown that EMR can increase lipid peroxidation and thus increase MDA level [44, 45]. In this study, we observed an increase in MDA level (Figure 3) and a decrease in GSH level (Figure 4), especially at 15 V/m electric field intensity, in line with the studies conducted with lipid peroxidation.

Our study evaluated the histopathological effects of exposure to various levels of electromagnetic fields on prepubertal rats by examining the presence of pathological lesions in the testis. Upon examination of the histological structure of the testicular tissue, no significant histological deterioration was observed at electromagnetic field values of 0.6 V/m, 1.9 V/m, and 5 V/m. However, at electromagnetic field values of 10 and 15 V/m, disruptions in the organization of spermatogenic cells in certain tubular structures, along with a lack of spermatogonia, were detected (Figure 5). These findings are consistent with the pathological data reported by Hasan et al., which indicated similar changes in testicular tissue following exposure to 4G mobile phone radiation in mice [39]. The observed disruptions in the organization of spermatic cells are likely to affect spermatogenesis and sperm quality. As a matter of fact, previous studies have demonstrated impaired sperm motility and quality [13, 14], suggesting that exposure to electromagnetic radiation triggers oxidative stress and significant biological changes.

Limitations

In this study, only male rats at 45 days of age (prepubertal) were utilized. Although different electric field intensities were applied, the RF effects were examined only at a single frequency (2.45 GHz), and frequency-dependent effects were not evaluated. While the testicular damage in our data was associated with oxidative stress, ultrastructural evaluations regarding mitochondria, which play a key role in oxidative damage, were not conducted due to our study constraints. It is believed that these limitations, which will be elucidated in future studies, will lead to a better understanding of the damage induced in the testicular tissue by RF exposure.

CONCLUSION

In this study, the evaluation of lipid peroxidation levels and histology in prepubertal male rat testicular tissue exposed to electromagnetic radiation at different electric field intensities at 2.45 GHz RF revealed a dose-dependent negative effect. These findings have the potential to lead to significant changes in the quality of life for individuals exposed to EMR over extended periods. Notably, this exposure may result in infertility due to disruptions in the reproductive systems of individuals or potentially transmit adverse effects to the next generations.

Conflict of interest: The authors declare that they have no potential conficts of interest to disclose.

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Ethical Approval: This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animals Ethics Board of Ondokuz Mayıs University's Experimental Medicine Research and Application Center (Approval number: 2019-23). This research was performed on the animals (rat).

Author Contributions: Conception: AK, NAU – Design: NAU – Supervision: NAU, AK, EGM, BKE – Funding: None – Materials: AK, NAU, EGM, BKE, AA – Data Collection and/or Processing: NAU, AA – Analysis and/or Interpretation: NAU, SV, AA – Literature: AK, NAU – Review: AK, NAU, EGM, AA – Writing: NAU, AK, AA – Critical Review: NAU.

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