Evaluation of the Thyroids of Offsprings Exposed to 2450 MHz Radiofrequency Radiation During Pregnancy: A Sixth Month Data

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Abstract

This study aimed to determine whether the exposure to radiofrequency emitted by wireless internet providers (2450 MHz) throughout the day during rats' pregnancy causes a problem in the thyroid tissues of their offspring.

The pregnant rats in the experimental group were exposed to radiofrequency radiation (RFR) (24 hours/day) at a 2450 MHz frequency in pulse wave mode with 1 W output strength by a generator simulating Wi-Fi waves. The offspring in the control and experimental groups were randomized selected (n:8). At the end of the sixth month, the thyroid tissues were removed and evaluated histopathologically and biochemically. Mann–Whitney U-tests and T-tests were used for statistical analysis. The threshold for statistical significance was $p < 0.05$.

There was a significant difference in mononuclear cell infiltration ($p=0.03$) and vascular increase in congestion ($p<0.001$). There was no difference in the TUNEL-positive cell percentage ($p=0.62$) and H2A.X antibody levels ($p=0.68$) between the rats in the control and experimental groups.

In this study, 2450 MHz RFR exposure during the prenatal period did not cause a statistically significant difference in terms of H2A.X levels and TUNEL-positive cell percentages in the thyroid tissue of rats.

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Introduction

The use of wireless communication devices such as mobile phones, computers, tablets, Wi-Fi, Bluetooth, etc., has increased at an incredible rate. As a result, the level of radiofrequency radiation (RFR) exposure along with concerns about its effects on human health has also aggregated. Research on these effects pointed out that there might be a relationship between mobile phone use and various diseases, which in turn, led the International Agency for Research on Cancer (IARC), a branch of the World Health Organization, to consider RFR "possibly carcinogenic to humans" [1].

One of the organs sensitive to radiation is the thyroid gland. However, there are limited number of studies on whether this organ is affected by RFR. Some studies claim that the thyroid gland is sensitive to RFR due to its location [2,3]. The estimated average specific absorption rate (SAR) of the thyroid gland for near-field exposure from the mobile phone is higher than those of many organs and tissues in the body, except for those associated with the brain [4]. Therefore, it is crucial to reveal the effects of RFR on thyroid gland. Prior work on this subject have reported that RFR causes changes in thyroid hormone levels and thyroid follicle cell morphology in rats [5,6]. In a study at the molecular level, the expression and activity of apoptosis regulator proteins were found to be increased in thyroid cells [7]. Moreover, in a study conducted on radio transmitters, television

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transmitters, and radio link technicians exposed to RFRs occupationally, it was concluded that RFRs increased T_3, T_4, and TSH hormones [8].

Ionizing radiation is the only proven environmental risk factor for thyroid cancer [9], and it is not clear whether non-ionizing radiation has an effect on thyroid cancer. Studies addressing the relationship between RFR and carcinogenesis, suggested potential mechanisms of action: DNA repair, oxidative stress, downregulation of mRNA, DNA single-strand break, and DNA double-strand break [10,11,12].

Various physical, chemical, and biological factors can lead to the induction of DNA double-strand breaks. Disruptions in the repair of DNA damage are the most harmful DNA lesions that can lead to critical consequences, such as chromosome abnormalities, genomic instability, and cell death[13]. Inducing DNA double-strand breaks, the H2AX protein is rapidly phosphorylated to form gamma-H2AX. This modified form, called γ-H2AX, is considered a sensitive indicator of DNA double-strand break. Therefore, γ-H2AX is a powerful indicator for monitoring genotoxic events such as cancer development and tumor progression and monitoring the effectiveness of anticancer treatment. Furthermore, the H2AX protein is used in research on other types of radiation[13,14].

Although there are studies investigating the effects of RFR, which is called "possible carcinogenic" according to IARC, on the brain, reproduction and other systems, studies on whether RFR affects the thyroid gland are scarce. In a few studies conducted on this particular area, only those under direct exposure to electromagnetic (EM) rays were discussed. It is not yet clear whether there is a risk for thyroid in the offspring of those exposed to those EM rays during pregnancy. Therefore, it is vital to determine whether this EM exposure will lead to a risky condition in the offspring of those who are exposed to RFRs uncontrollably during pregnancy. For these reasons, this study aims to reveal whether 2450 MHz frequency RFRs created by internet routers and hubs provide a basis for a health risk in thyroid glands of the offspring whose mothers are exposed to RFR during pregnancy. For this purpose, the thyroid tissues of the offspring of mothers exposed to 2450 MHz RFRs during pregnancy were evaluated histopathologically, H2A.FX ELISA method and TUNEL Essay method.

Materials and methods

This study 12 female Sprague Dowley rats (300-350 g), and 2 male Sprague Dowley rats (400 g) were used. Six female rats and one male rat in both control and exposure groups were kept together for five days for mating. Following the detection of pregnancy, male rats were taken from cages. Pregnant rats in experimental group were exposed to 2450 MHz RFR until giving birth. Pregnant rats in the irradiation group were under exposure to 2450 MHz RFR with 1 W output power (24 hours/day) during pregnancy. The control group rats were under the same experimental conditions same as the experimental group, but the RFR generator was turned off. At the end of the 21st day, female rats were placed in separate cages for delivering off-springs. Animals in the experimental group were under exposure to radiation only during pregnancy. The off-spring were kept in a 59 x 38 x 20 cm transparent plexiglass cage for six months without any RFR exposure. To prevent external RFR, both the control and irradiation group were in two different Faraday cages, 75x75x70 cm, for six months. In summary, rats in the experimental group were exposed to 2450 MHz RFR 50 cm high antenna of the RF generator (SET Elektronik LTD, model: FR24X-2 W). To ensure ideal RFR exposure , a 10x10 cm metal reflector was placed on the antenna at a distance of 2.5cm upward (Figure 1). The generator's antenna was placed parallel to the short edge of the cage. The same experimental setup applied in the control group without any RFR exposure.

The off-spring rats were separated from their mothers by sex determination at the end of the lactation process. Offsprings exposed to RFR during the prenatal period were randomly selected to the experimental group (n:8) and offsprings not exposed were randomly selected to the control group(n:8). This study was carried out on male offspring born to rats exposed to Wi-Fi RFRs during pregnancy. They were kept at room temperature (22°C) for six months in standard cages for 12 hours in a dark/light cycle (07:00-19:00 light/ 19:00-07:00 dark). All rats were fed freely with their standard feed, and all groups were given fresh drinking water every day.

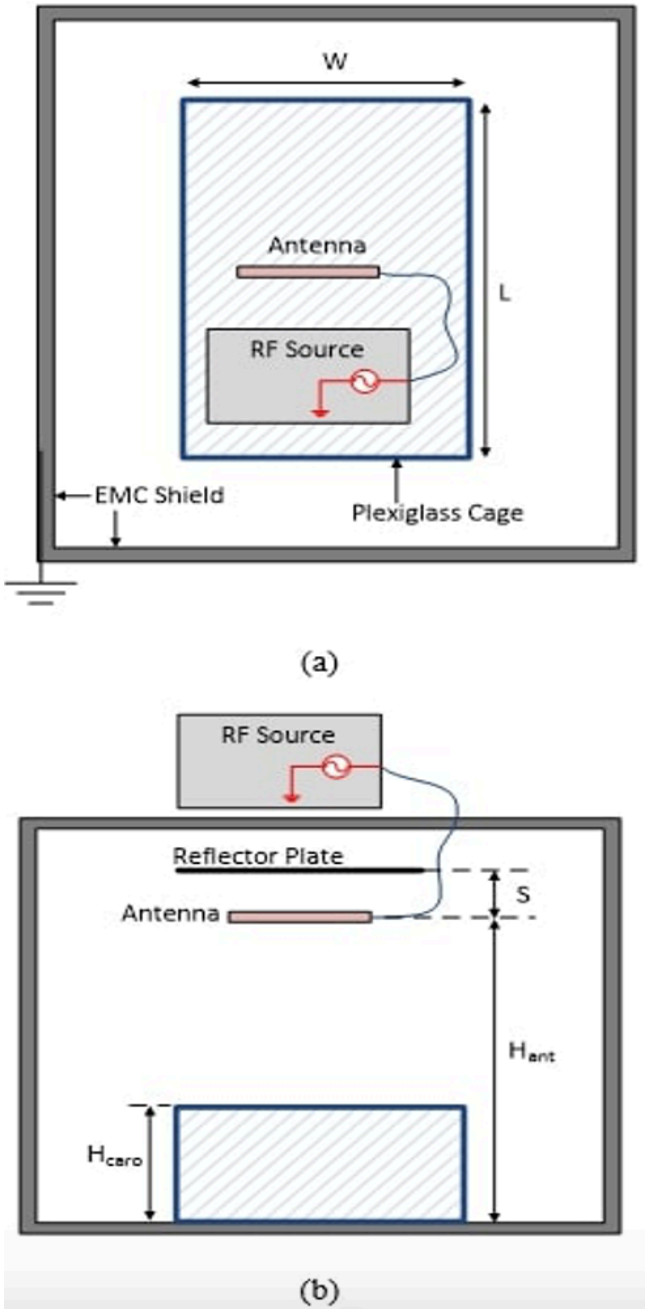


Figure 1. Experimental set-up. (a) Top view, (b) side view (L=38 cm W=59 cm, Hant=50 cm, Hcaro= 20 cm, S=2.5 cm.)

At the end of the sixth month, rats received deep anesthesia with 75 mg/kg ketamine and 10 mg/kg xylazine hydrochloride. Then, they were killed by an intracardiac puncture. The thyroid glands of all rats were carefully dissected.

Ethical Statement

This study was evaluated and approved by the Animal Experiments Local Ethics Committee of the University of Health Sciences Bağcılar Training and Research Hospital (NO: 2018/40)

Histopathology Method

The tissues were in 10% formaldehyde for pathological examination. After routine tissue procedures, they were embedded in paraffin blocks, and 4-micrometer-thick sections were taken. Hematoxylin and eosin stained the preparations and histopathological examination took place. A blinded pathologist using a four-point scale ("0" no change, "1" minimal, "2" moderate, and "3" severe)[15] analyzed these six features; mononuclear cell infiltration, vascular congestion, fibrosis, atypical thyrocytes, thyroid follicle degeneration, and colloid reduction.

Determination of DNA Damage by H2A.FX ELISA Method

For this purpose, the thyroid tissues of the rats were first treated with a protein extraction solution called T-per (Tissue-Protein Extraction Reagent) to 20 ml per 1 g, and protein was obtained from the samples. After centrifugation at 15.000×g for 20 minutes, the supernatants (proteins) were transferred to separate Eppendorf tubes and studied according to the content of H2A.FX ELISA kit. Supernatants were pipetted into 50µl of rat monoclonal H2A.FX antibody-coated strips that recognized H2A.FX in the kit. It was then incubated at 37°C for 30 minutes. At the end of the incubation, after the necessary washing, 75µl of horseradish peroxidase conjugate was added to all samples. Then, room-temperature incubation took place for 30 minutes. At the end of this incubation period, the samples were washed, and immediately afterward, 50µl of "Chromogen Solution A" solution and 50µl of Chromogen Solution B solution were added and incubated at room temperature for 15 minutes.At the end of this period, 50µl of stop solution terminated the reaction, and the resulting color intensity was read spectrophotometrically at 450 nm (FLASHScan S12, Jena, Germany).

Determination of In Situ Apoptosis by TUNEL Assay Method

We used a commercially available terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay kit (ApopTag® Fluorescein In Situ Apoptosis Detection Kit S7110) to detect apoptosis in tiroid tissue. Paraffin sections were incubated overnight at 37°C in an incubator. Sections were deparaffinized in xylene ,

rehydrated through a graded alcohol series and washed with PBS. Subsequently, tissues were digested with 20 mg/mL proteinase K (Sigma-Aldrich, St. Louis, MO) at room temperature for 15 min. Sections were incubated at room temperature for 5 min in 3% H 2O2 in PBS to quench endogenous peroxidase. Sections were incubated with equilibration buffer at room temperature for 30 min, followed by the application of TdT enzyme for 1 h at 37°C. Sections were washed in three changes of PBS for 1 min and anti-digoxigenin peroxidase was applied for 30 min at room temperature. Sections were covered with diaminobenzidine (DAB) for 3–6 min for colour development. DAPI was used to see nucleus morphology.

Specific absorption rate (SAR) value

SAR distribution of rats is simulated using CST, a 3D electromagnetic field solver CST (CST AG, Germany) utilizing finite integration. The volumetric pixel (voxel) model of the rat, which was acquired from CST, was formed using CT scans of a representative rat with subsequent designation of all body details. Thus, it is possible to view SAR distribution over specific organs and glands. The voxel model, 10g and 1g SAR distribution of the rat are displayed in Figure 2. Total body SAR and maximum point SAR are found to be 12 mW/kg and 25 mW/kg, respectively.

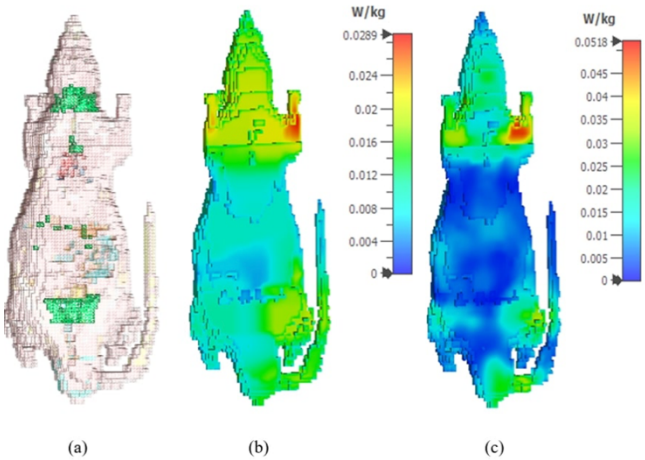


Figure 2. Rat model and SAR distribution, a) rat voxel model with thyroid glands, b) 10 g average, c) 1g average.

Statistical Analysis

The data were evaluated by SPSS (Statistical Package for Social Sciences) 15.0 program. The compliance of the data to a normal distribution was examined using the Shapiro–Wilk test. Mann Whitney U test was used for nonparametric data and t test was used for parametric data. The threshold for statistical significance was $p < 0.05$.

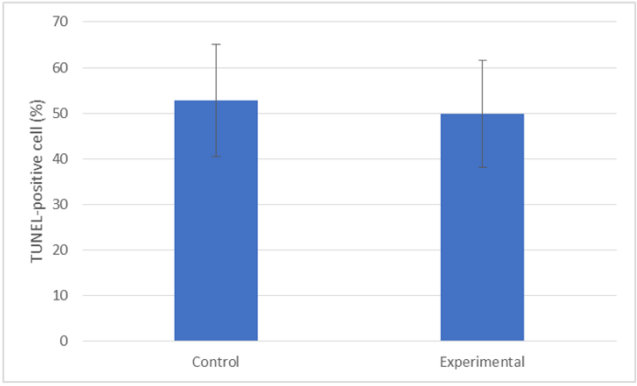
Results

Histopathological Examination

Mononuclear cell infiltration score ($p=0.03$) and vascular congestion score ($p<0.001$) was higher in the experimental group rats. There was no statistically significant difference between the control and experimental groups in terms of fibrosis, atypical thyrocytes, thyroid follicle degeneration, or colloid reduction ($p>0.05$). Histopathological scoring and Mann Whitney U test result are in Table 1.

	Control group (n:8)				Experimental group (n:8)				p
	0	1	2	3	0	1	2	3	
Mononuclear cell infiltration	7	1	0	0	2	6	0	0	0.03
Vascular congestion	8	0	0	0	0	8	0	0	<0.001
Fibrosis	8	0	0	0	5	3	0	0	0.23
Atypical thyrocytes	8	0	0	0	5	3	0	0	0.23
Thyroid follicle degeneration	8	0	0	0	5	3	0	0	0.23
Colloid reduction	8	0	0	0	8	0	0	0	1.00

Table 1. Histopathological scoring and Mann Whitney U test result.
0: no change, 1: minimal, 2: moderate, 3: severe



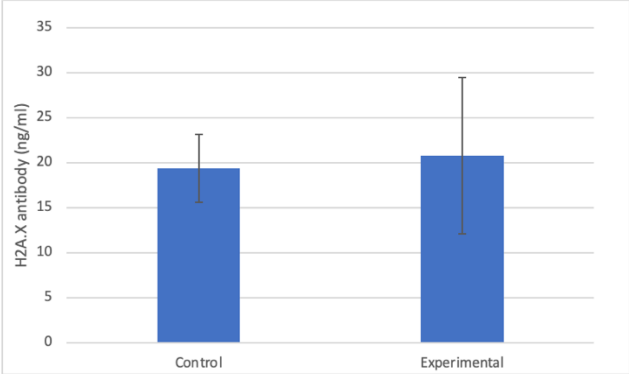
Graphic 1. TUNEL-positive cell percentage of the rats in the experimental and control groups.

TUNEL Assay and ELISA Examination

The TUNEL-positive cell percentage was (mean±SD) 52.8±12.3 in the control group and 49.8±11.7 in the experimental group ($t[df]=0.502[14]$; $p=0.62$). The TUNEL-positive cell percentage of the rats in the experimental and control groups are given in graphic 1.

According to the ELISA results, the mean (±SD) H2A.X antibody level was 19.39±3.8 ng/ml in the control group, while this value was 20.81±8.7 ng/ml in the experimental group. There

was no statistically significant difference in terms of H2A.X antibody levels in rats in the control and experimental groups (t[df]=-0.425[14] ; p=0.68). The mean H2A.X antibody levels of the control and experimental groups are displayed in Graphic 2.



Graphic 2. The mean H2A.X antibody levels of the control and experimental groups.

Discussion

Considering widespread use of wireless devices today, it is important to examine the possible effects of RFR. Although there are many studies on the biological effects caused by RFR exposure, the effects of intrauterine exposure to RFR are not clear. It is known that the fetus is sensitive to external factors during the prenatal period. We aimed to investigate whether RFR, as an external factor, has an effect on the thyroid gland.

Several studies have claimed that RFR can cause DNA fractures at the cellular level, an increase in gene expression, an indicator of DNA strand breaks, and an increase in apoptosis percentages[12,16,17]. In this study, as a result of the examination of the thyroid tissues of the rats by the TUNEL assay method to determine intracellular apoptosis, there was no difference found between the control and experimental groups. Eşmekaya et al. observed that the expression and activity of caspase-3 and caspase-9, which are apoptosis-regulating proteins, increased in the thyroid of rats exposed to RFR at 900 MHz for 20 minutes a day for three weeks[7]. Although the radiation level and duration in the study of Eşmekaya were lower than those in the RFR exposure in our study, the detection of apoptotic effects may be associated with direct exposure. The fact that RFR exposure in the intrauterine period did not affect apoptosis in the study can be due to the tissue layers outside the fetus. As RFR progresses through the tissue environment, the tissue absorbs the energy, and RFR exposure gradually decreases[18]. Therefore, the tissue layers may absorb RFR before reaching the fetus, and consequently, its effect on the fetus may be lower.

In the study of Agustino et al., similar to the results of this study, there was no evidence of apoptosis in the thyroid tissue of rats exposed to 2450 MHz radiation. RFR can change the levels of cellular stress in the thyroid gland of rats without changing their anti-apoptotic capacities[19]. In the study of Güler et al., in parallel with this study's findings, there was no apoptotic change detected by TUNEL staining in the brain tissues of animals after intrauterine and extrauterine exposure to RFR[20]. Daşdağ et al. reported that 900 MHz RF wave exposure did not cause apoptosis in rat testicular tissue[21].

H2AX protein, a significant indicator for DNA double-strand break detection resulting from ionizing radiation, was not associated with non-ionizing radiation exposure according to our study. In the literature, the effect of RFR exposure in different tissues on DNA double-strand break was investigated by evaluating the H2AX protein. A 50 Hz magnetic field exposure in lens epithelial cells was examined in vitro, and there was no significant difference in H2AX proteins[22]. Ji et al. reported no significant difference in γ-H2AX in bone marrow stromal cells of mice exposed to 900 MHz RFR[23]. Xu et al. examined the effects on DNA in four cell types exposed to 1800 MHz mobile phone radiation. They found a statistically significant increase in the level of γ-H2AX in hamster lung cells and human skin fibroblast cells after 24 hours of exposure to radiofrequency-electromagnetic field (RF-EMF). Yet, there was no significant change after 1 hour of exposure[24]. The impact of non-ionizing radiation on DNA strand break may depend on the cell type, radiation type, and duration of exposure.

In this study, according to the histopathological examination of thyroid tissues, there was a significant difference in the rats in the experimental group in terms of mononuclear cell infiltration and vascular congestion compared to those in the control group. There was no significant difference between the control and experimental groups in fibrosis, atypical thyrocytes, thyroid follicle degeneration, or colloid

reduction. In the study of Koyu et al., similar to our findings in this study; mononuclear cell infiltration increased significantly in the thyroid tissue of rats that underwent radiation for 1 hour a day for 28 days at a frequency of 2450 MHz, there was no significant difference in terms of colloid reduction, fibrosis or atypical cells in thyroid follicles. Unlike our findings, degeneration in thyroid follicles increased in the RFR group, and there was no difference in vascular congestion[25]. In the study of Rajkovic et al., the colloid content of rats exposed to a 50 Hz EM field decreased, there was vascular congestion, and thyroid stromal connective tissues thickened[26]. Although the dose and duration of non-ionizing radiation applied in the studies are different, the most significant difference in this study is the application of exposure in the intrauterine period. In a systematic review investigating the harmful effects of mobile phone use on the thyroid, the volume of thyroid gland cells decreases[27].

The most important feature that distinguishes this study from other studies in the literature is the examination of effects of RFR on the thyroid gland of exposure in intrauterine life. Histopathological examination revealed a minimal increase in mononuclear cell infiltration and vascular congestion. RFR exposure did not lead to a statistically significant difference in terms of H2A.X levels and TUNEL-positive cell percentages.

Declaration of Interest

The authors report no conflict of interest.

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