ORIGINAL ARTICLE

The Effects of 2100 MHz Radio Frequency Radiation on Thyroid Tissues

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ABSTRACT

Objective: *To reveal the effect of 2100 MHz radio frequency (RF) radiation on thyroid tissues of rats in the 10 days (group E1) and 40 days (group G1) exposure groups.*

Methods: *In this study, 30 healthy female Wistar albino rats, weighing 200−256 g each, were used. The animals were randomly divided into four groups (E1, E2, G1 and G2). Groups E2 and G2 served as the control groups. The exposure groups were exposed to 2100 MHz RF radiation emitted by a generator, simulating a 3G-mobile phone for 6 hours/day, 5 consecutive days/week, at the same time of the day (between 9 am and 3 pm), for 10 days (group E1) and 40 days (group G1).*

Results: *Catalase and xanthine oxidase enzyme activities were compared between the groups E1 and E2; it was found that the difference was statistically significant (*$p < 0.05$*). Between the groups G1 and G2, the difference was found to be significant with respect to catalase activities. Tissue samples of the early and late groups showed no serious pathological findings in the histopathological examination.*

Conclusion: *We believe that comprehensive, clinical and experimental studies are needed to assess the effect of the RF exposure duration and dosage of exposure on thyroid tissues.*

Keywords: Radiation, radio frequency, smart phone, thyroid

INTRODUCTION

The number of smart phone users has been on the rise all over the world. While the old-generation mobile phones propagated radio frequency (RF) radiation in the range of 900–1900 MHz, today's smart phones propagate approximately 2100 MHz RF radiation. Exposure to electromagnetic radiation has an effect, especially on the user. Although many studies have revealed that microwave radiation exposure has no detrimental effect on human health whatsoever (1–6), some studies have shown the contrary (7–9). This study aimed to reveal the effect of 2100 MHz RF radiation on thyroid tissues of rats in the early and late stages.

The thyroid tissue samples taken from the early and late groups were analysed, biochemically and pathologically. Pathologic evaluations were made subjectively. On the other hand, biochemical evaluations were made statistically.

MATERIALS AND METHODS

In this study, 30 healthy female Wistar albino rats, weighing 200–256 g each, were used. The animals were kept on a 14/10-hour light/dark schedule, respectively. During the study, the ambient temperature (22–23°C) and relative humidity (45%) were maintained in the normal range for these animals. The Scientific and Ethics Committee of the Medical Faculty of Gazi University approved the study. The animals were randomly divided into four groups (E1, E2, G1 and G2). Groups E2 and G2 served as the control groups (for 10 and 40 days,

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respectively), and each group included six rats. Groups E1 and G1 were the exposure groups, and each group composed of nine rats.

Exposure conditions

Digitally modulated 2100 MHz 3G signals were produced by a vector signal generator (Rohde & Schwarz SMBV 100A, 9 kHz–3.2 GHz, München, Germany) and a horn antenna (Schwarzbeck, Doppelsteg Breitband Horn antenna BBHA 9120 L3F, 0.5–2.8 GHz, Schönau, Germany) in a temperature-controlled shielded room. The output power and the frequency were controlled by a spectrum analyser (Rohde & Schwarz, FSH 18, 10 MHz–18 GHz, München, Germany) integrated to the signal generator. Measurements were taken during the entire experiment, and data were saved in a computer which was connected to the device *via* a fibre-optic cable. The distance between the horn antenna and the heads of the rats was maintained at 12 cm to provide the far-field condition. The direction of the RF propagation was perpendicular to the Plexiglas cage ($40 \times 25 \times 20$ cm^3). The output level of RF radiation was measured as 16 V/m during the exposure period. Whole-body RF radiation exposure levels of the rats were measured as 0.4 W/kg using the finite-domain time-difference method.

The exposure groups were exposed to 2100 MHz RF radiation emitted by a generator, simulating a 3G-mobile phone for 6 hours/day, 5 consecutive days/week, at the same time of the day (between 9 am and 3 pm), for 10 days (group E1) and 40 days (group G1).

Two days after the last exposure, rats were anaesthetized by ketamine hydrochloride (50 mg/kg, intramuscular). Groups E1 and E2 were sacrificed following an exposure duration of 10 days, and groups G1 and G2 were sacrificed following an exposure duration of 40 days.

Evaluation of oxidative stress

After the sacrifice of the animals, thyroid samples were removed and kept on an ice bath until homogenization. The sample of thyroid was first washed with distilled water, the tissues were homogenized in a physiological saline solution (20% w/v, approximately 1 g in 5 mL for each). Then they were centrifuged 4000*g* for 15 minutes, and upper clear supernatants were used in the assays. Protein levels of the supernatants as mg/mL were measured by using Lowry's method (10), and they were adjusted to equal concentrations before the analyses. Malondialdehyde (MDA) levels were measured by the thiobarbituric acid reactive substance method (11). Xanthine oxidase (XO) activity was determined by measuring uric acid formation from the xanthine substrate at 293 nm (12). Glutathione peroxidase (GSH-Px) activity was measured by following changes in nicotinamide adenine dinucleotide phosphate (NADPH) absorbance at 340 nm (13). Catalase (CAT) activity was determined by measuring the decrease of H_2O_2 absorbance at 240 nm (14). In the activity calculations (international unit), extinction coefficients of uric acid, H_2O_2 and NADPH were used for XO, CAT and GSH-Px, respectively. Superoxide dismutase (SOD) activity was measured by the method based on the nitroblue tetrazolium (NBT) reduction rate (15). One unit for SOD activity was expressed as the enzyme protein amount causing 50% inhibition in the NBT reduction rate.

Histopathological examination

Tissue samples of 1×1 cm^2, which were taken from the peritoneum and from the cecal region with subserosal haemorrhage, were fixed in the 10% formaldehyde solution, and then 5-µm sections were obtained from paraffin-embedded tissues. Tissue sections were examined after being stained with haematoxylen/eosin (H/E) and trichrome stains. The presence of inflammation in H/E-stained sections and the presence of fibrosis in H/E and trichrome-stained sections were evaluated by a semi-quantitative scoring system.

Statistical analyses

Statistical analyses were done using SPSS 15.0 for Windows programme. Numeric values were represented as *n* (number of rats), mean ± standard deviation, mean, and percentage (%). Overall comparison of the groups was made using the Kruskal–Wallis test. Paired comparisons of the groups were made by the Mann–Whitney *U*-test. Based on the results of analyses, a *p*-value of < 0.05 was considered statistically significant. However, the Bonferroni correction was used for the comparison of the probable two groups, and the *p*-value was divided by the number of comparisons.

RESULTS

The biochemical examination results of the tissues are given in Tables 1 and 2. The Mann–Whitney *U*-test was used in the statistical analyses of the results; $p < 0.05$ was considered statistically significant. When CAT and XO values were compared between the groups E1 and E2, it was found that the difference was statistically significant ($p < 0.05$). Between the groups G1 and G2, the difference was found to be significant with respect

Table 1: Biochemical results (10 days/early group)

Enzyme	Groups	n (number of subjects)	Values measured	*p*-value (< 0.05) (Mann–Whitney *U*-test)
SOD (superoxide dismutase) (U/mg)	Exposure Control	9 6	7.56 8.67	0.637
CAT (catalase) (IU/mg)	Exposure Control	9 6	9.89 5.17	*0.040*
GSHPx (glutathione peroxidase) (IU/g)	Exposure Control	9 6	7.50 8.75	0.593
XO (xantine oxidase) (nIU/mg)	Exposure Control	9 6	10.72 3.92	*0.004*
MDA (malondialdehide) (nmol/g)	Exposure Control	9 6	Add texture	Could not be calculated

Statistical analyses of thyroid tissues of the early control and exposure groups' biochemical lab results.

Table 2: The biochemical results (40 days/late group)

Enzyme	Groups	n (number of subjects)	Values measured	*p*-value (< 0.05) (Mann–Whitney *U*-test)
SOD (superoxide-dismutase) (U/mg)	Exposure Control	9 6	7.89 8.17	0.906
CAT (catalase) (IU/mg)	Exposure Control	9 6	11.00 3.50	*0.001*
GSH-Px (glutathione peroxidase) (IU/g)	Exposure Control	9 6	9.44 5.83	0.119
XO (xanthine oxidase) (nIU/mg)	Exposure Control	9 6	9.67 5.50	0.077
MDA (malondialdehyde) (nmol/g)	Exposure Control	9 6	6.64 2.83	0.06

Statistical analyses of thyroids tissues of the late control and exposure groups' biochemical lab results.

to CAT values. In early groups, MDA values were not measured because there were not enough tissues. Blood thyroid-stimulating hormone (TSH) values are shown in Table 3, which suggest that no statistically significant difference was observed between the groups.

The early and late groups' tissue samples showed no serious pathological findings in the histopathological examination. In Fig. 1, in the tissues of the early group vacuolation, the cytoplasm and perivascular cell infiltration were observed.

In Fig. 2, a severe perivascular cell infiltration and a severe vacuolation and desquamation in the cytoplasm were observed. Five samples of G1 tissue showed a severe vacuolation in the cytoplasm.

Table 3: Results of thyroid hormone analyses (early and late groups)

Hormone	Early exposure groups (n = 9)	Late exposure groups (n = 9)	Early control group (n = 6)	Late control group (n = 6)	Measurement unit
TSH	0.007	0.017	0.009	0.017	µUI/mL
	0.010	0.018	0.009	0.020	µUI/mL
	0.008	0.019	0.009	0.020	µUI/mL
	0.008	0.016	0.007	0.018	µUI/mL
	0.007	0.017	0.008	0.016	µUI/mL
	0.007	0.017	0.008	0.018	µUI/mL
	0.010	0.017			µUI/mL
	0.009	0.017			µUI/mL
	0.008	0.017			µUI/mL

TSH measurements in the blood in the groups ($p > 0.05$)

DISCUSSION

So far, many clinical and experimental studies have been carried out on the detrimental effects of RF radiation. In many studies, the devices emitting RF in the range of 900–1800 MHz were used, and it was shown that the EMRs that they emitted had a detrimental effect on the organs and tissues of humans. Histopathological examination of the thyroid gland of the cage control, sham- and RF-exposed rats showed significant pathological change in the thyroid glands of the RF-exposed rat. Exposure to pulse-modulated RF radiation caused striking changes in the thyroid structure. The diameter and area of the

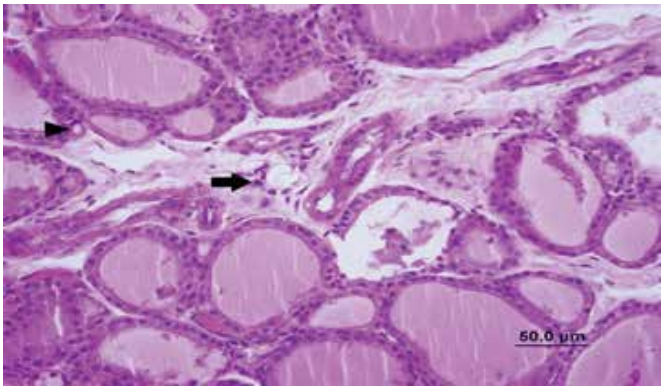

Fig. 1: Microscopic analysis vacuolation in the cytoplasm and perivascular cell infiltration in the tissues of early exposure groups.

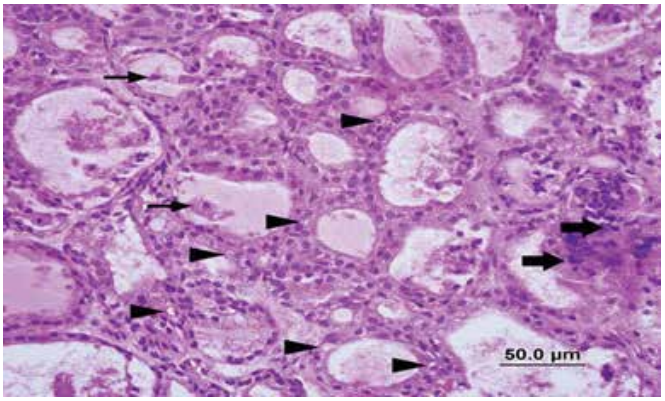

Fig. 2: Microscopic analysis in the tissues of late exposure groups, severe perivascular cell infiltration, a severe vacuolation, and desquamation in the cytoplasm.

colloid in the lumen of the follicles of the RF-exposed group increased significantly. Apoptosis may play an important role in the thyroid homeostasis and autoimmune disease (including the central pathogenic events in the destruction of thyroid follicular cells).

Eşmekaya *et al* (16) divided the rats into three groups. Then, they exposed them to 900 MHz EMR. Caspase-3 and Caspase-9, levels, signs of apoptosis in the tissue were observed to be higher in the exposure groups than those of the control and sham groups, and when the results were compared, it was found that the result was statistically significant.

Koyu *et al* (17) exposed the rats to 900 MHz EMR, and they compared the TSH level with the T3 and T4 levels. The values derived from the rats exposed to RF were relatively low, and when the results were compared, it was found that they were statistically significant (17).

Devrim *et al* (18) exposed the rats to 900 MHz EMR with the supplement of vitamin C for four weeks. Then, the tissues of the rats were exposed to biochemical analyses. With respect to the tissue breakdown and the oxidant and anti-oxidant balance, the results were significant in the exposure groups when compared with other groups. The values of MDA levels in the kidney tissues and those of CAT enzyme activities were statistically significant. It was deduced that EMR exposure had a direct oxidative effect on the kidney tissue. A decrease in the MDA level in the heart tissue and a decrease in the activities of XO, adenosine deaminase, and DNA turnover enzyme were observed. It was found that EMR had a negative effect on the heart tissue. In the liver and ovarium tissues, no important changes were observed. The fact that the same changes were not observed in the tissues of these organs may be attributed to the fact that the tissues in question vary in biochemical, anatomic and functional properties.

Öktem *et al* (19) found that the rats exposed to 900 MHz EMR had an increased MDA level in the kidney tissue and lower activities in the SOD, CAT and GSH-Px levels. Irmak *et al* (20) found that the rabbits after the exposure of seven days had high serum SOD activities, and they associated it with the EMR's oxidative stress promoting property. İlhan *et al* (21) studied the effect of EMS on the brain tissue and found that MDA and XO were on the rise, while there was a decrease in the levels of SOD and GSH-Px.

Moustafa *et al* (22) tried to show that mobile phones have short-term effect on oxidative stress markers. They found that there was a significant increase in plasma levels of MDA from 12 healthy people and a decrease in the SOD and GSH-Px levels in erythrocytes.

Dasdag *et al* (23) examined the effect of 900 MHz EMR on MDA levels in the tissue of the testis, P-53 immune reactivation sperm numbers, and its morphology of the 16 rats. The results were evaluated through the Mann–Whitney *U*-test, and no statistical difference was found between the groups. They concluded that EMR exposure did not cause any structural and morphologic damage to the tissues in the testis. The present study was done by exposing 2100 MHz and high EMR values. After a period of 10 and 40 days' exposures, the thyroid tissue samples were examined biochemically and histopathologically. In biochemical examination, when CAT and XO values were compared between E1 and E2 groups, statistical significance was found ($p < 0.05$. In G1 and G2 groups, statistical difference was found in terms of CAT values ($p < 0.05$). In histopathologic and blood TSH measurement, no statistical significance was found.

CONCLUSION

Given that the thyroid tissue is an indispensable part of the endocrine system, a comprehensive hormonal

function evaluation should be needed along with hormonal measurements of other endocrine organs. We are of the view that smart phones do not bring about significant histochemical and histopathological changes in thyroid tissues. Also, we believe that comprehensive, multi-centred clinical and experimental studies, involving a lot of subjects, are needed to assess the effect of the RF exposure duration and dosage of exposure on thyroid tissues.

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