

The Effects of 2100 MHZ Radio Frequency Radiation on Thyroid Tissues

S Tuncal¹, AK Önalın¹, F Aydođan², V Unsal³, N Yumuřak⁴, EÖ Büyükatalay⁵, G Öztürk⁵, N Seyhan⁵, S Celepli¹, ED³, B Kılıçođlu,¹

ABSTRACT

Background: The study aims to reveal the effect of 2100 MHz radio frequency radiation on thyroid tissues of the rats in early and late groups.

Material and Method: In this study, 30 healthy female Wistar albino rats, weighting 200 to 256 g each were used. The animals were randomly divided into four groups (groups E1, E2, G1, G2). Groups E2 and G2 served as the control groups. The exposure groups were exposed to 2100 MHz radiofrequency 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-3pm), 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 G-1 and G-2 the difference was found to be significant with respect to catalase activities. The early and late groups tissue samples showed no serious pathological findings in the histopathological examination.

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

Keywords: Radiation, radiofrequency, smart phone, thyroid

From: ¹Ankara Training and Research Hospital General Surgery Department, ²Ankara Training and Research Hospital Head and Neck Surgery Department, Ankara University Faculty of Medicine Medical Biochemistry Department, ⁴Harran University Faculty of Veterinary Medicine, Pathology Department ⁵Gazi University Faculty of Medicine Biophysics Department.

Correspondence: Dr B Kilicoglu, Ankara Training and Research Hospital, General Surgery Department, Ulucanlar, Altindag Altindađ, Ankara, Turkey 06370. E-mail: kilicoglubulent@yahoo.com

INTRODUCTION

The number of smart phone users has been on the rise all over the world. While the old generation mobile phones propagated radiofrequency radiation in the range of 900-1900 MHz, today's smart phones propagate approximately 2100 MHz radiofrequency radiation. The electromagnetic radiation emitted 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). The study aims to reveal the effect of 2100 MHz radio frequency radiation on thyroid tissues of the rats in early and late stages.

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

MATERIAL AND METHOD

In this study, 30 healthy female Wistar albino rats, weighting 200 to 256 g each were used. The animals were kept on a 14/10-h light/dark schedule. 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 (groups E1, E2, G1, G2). Groups E2 and G2 served as the control groups (for 10 days and 40 days, 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 analyzer (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 fiber optic cable. The distance between the horn antenna and the heads of the rats were 12 cm to provide far-field condition. The direction of the radiofrequency propagation was perpendicular to the Plexiglas cage (40 x 25 x 20 cm). Output level of radiofrequency radiation was measured as 16 V/m during the exposure period. Whole body radiofrequency radiation exposure levels of the rats were measured as 0.4 W/kg using the Finite Domain Time Difference (FDTD) method.

The exposure groups were exposed to 2100 MHz radiofrequency 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-3pm), for 10 days (group E1) and 40 days (group G1).

Two days after the last exposure, rats were anesthetized 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 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 (20% w/v, approximately 1 g in 5 ml for each) physiological saline

then they were centrifuged 4000xg for 15 min and upper clear supernatants were used in the assays. Protein levels of the supernatants as mg/mL were measured by using the Lowry's method (10) and they were adjusted to equal concentrations before the analyses. Malondialdehyde (MDA) levels were measured by the thiobarbituric acid reactive substances (TBARS) method (11). Xanthine oxidase activity was determined by measuring uric acid formation from xanthine substrate at 293 nm (12). Glutathione peroxidase (GSH-Px) activity was measured by following changes in NADPH absorbance at 340 nm (13). Catalase (CAT) activity was determined by measuring decrease of H₂O₂ absorbance at 240 nm (14). In the activity calculations (IU-international unit), extinction coefficients of uric acid, H₂O₂ and NADPH were used for XO, CAT and GSH-Px, respectively. Superoxide dismutase (SOD) activity was measured by the method based on nitro blue 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 1x1 cm, which were taken from the peritoneum and from cecal region with subserosal hemorrhage, were fixed in 10% formaldehyde solution and then 5µm sections were obtained from paraffin-embedded tissues. Tissue sections were examined after being stained with Hematoxylen/Eosin (H/E) and Trichrom stains. Presence of inflammation in H/E stained sections and presence of fibrosis in H/E and Trichrom stained sections were evaluated by semi-quantitative scoring system.

Statistical analyses

Statistical analyses were done using SPSS 15.0 for Windows program. Numeric values were represented as n (number of rats), mean ± standard deviation, mean, and percentage (%). Overall comparison of the groups was done using "Kruskal Wallis Test". Paired comparisons of the

groups were done by "Mann Whitney U Test". Based on the results of analyses, a p value <0.05 was considered statistically significant. However, Bonferroni correction was used for the comparison of probable two groups and p value was divided by the number of comparisons.

RESULTS

The biochemical examination results of the tissues are given in Table 1 and Table 2. Mann-Whitney U test was employed in the statistical analyses of the results. $p<0.05$ was defined as significant level. 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 G-1 and G-2 the difference was found to be significant with respect to CAT values. In early groups, MDA values were not measured due to not having enough tissues. Blood TSH values were shown in Table 3 and no statistical difference was observed between the groups.

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

In figure II, a severe perivascular cell infiltration and a severe vacuolation and desquamation in the cytoplasm were observed. 5 samples of G-1 tissue samples showed a severe vacuolation in the cytoplasm.

DISCUSSION

So far many clinical and experimental studies have been carried out on the detrimental effects of radio frequency radiation. In many studies, the devices emitting radio frequency in the range of 900-1800 MHz were used and it was shown that the EMR that they emitted had a detrimental effect on the organs and tissues. Histo-pathological examination of the thyroid gland of the cage control, sham and RF exposed rats showed significant pathological changes 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 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 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 high in the exposure groups compared with those of the control and sham groups and when the results were compared, it was found that the result was statistically significant (16).

Koyu et al exposed the rats to 900 MHz EMR and they compared TSH with 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. exposed the rats to 900 MHz EMR with supplement of Vitamin C for four weeks. Then, the tissues of the rats were exposed to biochemical analyses. With the respect of tissue breakdown and 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 of MDA level in the heart tissue and a decrease in the activities of XO and ADA, DNA turn-over 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 the organs may be attributed to the fact that the tissues in question vary in biochemical, anatomic and functional properties. (18)

Öktem et al. found that the rats exposed to 900 MHz EMR had a increased MDA level in the kidney tissue and a lower activities in SOD ,CAT and GSH-Px levels.(19) Irmak et al. found that the rabbits after the exposure of 7 days had a high serum SOD activities and they associated it with EMR's oxidative stress promoting property.(20) İlhan et al. 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. (21).

Moustafa et al. 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 (22).

Dasdag et al. examined the effect of 900 MHz EMR on MDA levels in the testis tissue, P-53 immune reactivation sperm numbers and its morphology of the 16 rats. The results were evaluated through Mann- Whitney U-Test and no statistical difference was found between the groups. They concluded that EMR exposure didn't cause any structural and morphologic damage to the testis tissues. (23) This 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 histo-pathologically. 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 idea that smart phones do not bring about significant histochemical and histo-pathological changes in thyroid tissues. Also, we believe that comprehensive, multi-centered clinical and experimental studies, involving a lot of subjects, are needed to assess how effective the RF exposure duration and dosage of exposure on thyroid tissues.

REFERENCES

1. Fritze K, Sommer C, Schimtz B, Mies G, Hossmann KA, Kiessling M et al. Effect of global system for mobile communication (GSM) microwave exposure on blood-brain barrier permeability in rat. *Acta Neuropathol* 1997; **94**: 465–70.
2. Stagg RB, Thomas WJ, Jones RA, Adey WR. DNA synthesis and cell proliferation in C6 glioma and primary glial cells exposed to a 836.55 MHz modulated radiofrequency field. *Bioelectromagnetics* 1997; **18**: 230–36.
3. Li JR, Chou CK, McDougall JA, Dasgupta G, Wu HH, Ren RL et al. TP53 Tumor suppressor protein in normal human fibroblasts does not respond to 837 MHz microwave exposure. *Radiat Res* 1999; **151**: 710–16.
4. Gos P, Eicher B, Kohli J, Heyer W-D. No mutagenic or recombinogenic effects of mobile phone fields at 900 MHz detected in the yeast *Saccharomyces cerevisiae*. *Bioelectromagnetics* 2000; **21**: 515–23.
5. Grant FH, Schlegel RE. Effects of an increased air gap on the in vitro interaction of wireless phones with cardiac pacemakers. *Bioelectromagnetics* 2000; **21**: 485–90.
6. Maes A, Collier M, Verschaeve L. Cytogenetic effects of 900 MHz (GSM) microwaves on human lymphocytes. *Bioelectromagnetics* 2001; **22**: 91–6.
7. Freude G, Ullsperger P, Eggert S, Ruppe I. Effects of microwave emitted by cellular phones on human slow brain potentials. *Bioelectromagnetics* 1998; **19**: 384–7.
8. Dasdag S, Akdag MZ, Ayyıldız O, Demirtas O C, Yayla M, Sert C. Do cellular phones alter blood parameters and birth weight of rats? *Electro- and Magnetobiology* 2000; **19**: 107–13.

9. Schirmacher A, Winters S, Fischer S, Goeke J, Galla HJ, Kullnick U, Ringelstein EB, Stoßgbauer F. Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the bloodbrain barrier in vitro. *Bioelectromagnetics* 2000; **21**: 336–45.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265.
11. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. *Arch Biochem Biophys* 1962; **98**: 253–61.
12. Hashimoto S. A new spectrophotometric assay method of xanthine oxidase in crude tissue homogenate. *Anal Biochem* 1974; **62**: 426–35.
13. Paglia DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; **70**: 158–69.
14. Aebi H. Catalase. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. New York and London: Verlag Chemie Weinheim Academic Press, Inc 1974, p. 673–7.
15. Durak İ, et al. Activities of total cytoplasmic and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. *J Clin Lab Anal* 1996; **10**: 17–20.
16. Eşmekaya MA, Seyhan N and Ömeroğlu S. Pulse modulated 900 MHz radiation induces hypothyroidism and apoptosis in thyroid cells: a light, electron microscopy and immunohistochemical study. *Int.F.Radiat.Biol.*, 2010, Vol 86, No 12, pp 1106–1116
17. Koyu A, Cesur G, Özgürer F, Akdoğan M, Mollaoğlu H, Özen S. Effects of 900 MHz electromagnetic field on TSH and thyroid hormones in rats. *Toxicoll Letters* 2005; **157**: 257–62.

18. Devrim E and Ergüder İB, Kılıçoğlu B, Yaykaşlı E, Çetin R, Durak İ. Effects of electromagnetic radiation use on oxidant/antioxidant status and DNA turn-over enzyme activities in erythrocytes and heart, kidney, liver and ovary tissues from rats: possible protective role of vitamin C. *Tox Mech and Met* 2008; **18**: 679–83.
19. Öktem F, Özgüner F, Mollaoğlu H, Koyu A, Uz E. Oksidative damage in the kidney induced by 900-MHz-emitted mobile phone: protection by melatonin. *Arch Med Res* 2005; **36**: 350–55.
20. Irmak MK, Fadıllıoğlu E, Güleç M, Erdoğan H, Yağmurca M, Akyol O. Effects of electromagnetic radiation from a cellular telephone on oxidant and antioxidant levels in rabbits. *Cell Biochem Funct* 2002; **20**: 279–83.
21. İlhan A, Gürel A, Armutçu F, Kamışlı S, Iraz M, Akyol O, Özen S. Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. *Clin Chim Acta* 2004; **340**: 153–62.
22. Moustafa YM, Moustafa RM, Belacy A, Abou-El-Ela SH and Ali FM. Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. *J Pharm Biomed Anal* 2001; **26**: 605–8.
23. Dasdag S, Akdağ MZ, Aksen F, Yılmaz F, Bashan M, Dasdag MM et al. Whole body exposure of rats to microwaves emitted from a cell phone does not effect patients with lung cancer. *J Clin Lab Anal* 2003; **10**: 17–20.

Table 1: Biochemical results (10.Day / Early Group)

Enzyme	Groups	N(The Number of Subjects)	Values Measured	p Value (<0.05) (Mann-Whitney U)
SOD (Superoxide Dismutase) (U/mg)	Exposure	9	7.56	0.637
	Control	6	8.67	
CAT (Catalase) (IU/mg)	Exposure	9	9.89	<u>0.040</u>
	Control	6	5.17	
GSHPx (Glutathione Peroxidase) (IU/g)	Exposure	9	7.50	0.593
	Control	6	8.75	
XO (Xantine Oxidase) (nIU/mg)	Exposure	9	10.72	<u>0.004</u>
	Control	6	3.92	
MDA (Malondialdehyde) (nmol/g)	Exposure	9	Add Texture	could not be calculated
	Control	6		

Statistical Analyses of thyroids tissues of the early control and exposure groups' biochemical lab results.

Table 2: The Biochemical results (40.Day / Late Group)

Enzyme	Groups	N (The Number of Subjects)	Values Measured	p value (<0.05) (Mann-Whitney U)
SOD (Superoxide-Dismutase) (U/mg)	Exposure	9	7.89	0.906
	Control	6	8.17	
CAT (Catalase) (IU/mg)	Exposure	9	11.00	<u>0.001</u>
	Control	6	3.50	
GSH-Px (Glutathione peroxidase) (IU/g)	Exposure	9	9.44	0.119
	Control	6	5.83	
XO (Xanthine Oxidase) (nIU/mg)	Exposure	9	9.67	0.077
	Control	6	5.50	
MDA (Malondialdehyde) (nmol/g)	Exposure	9	6.64	0.06
	Control	6	2.83	

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

Table 3: Thyroid Hormon Analyses Results (Early and Late Groups)

Hormon	Early Exposure Groups N(9)	Late Exposure Groups N(9)	Early Control Group N(6)	Late Control Group N(6)	Measurement Unit
TSH (Thyroid Stimulan Hormon)	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)

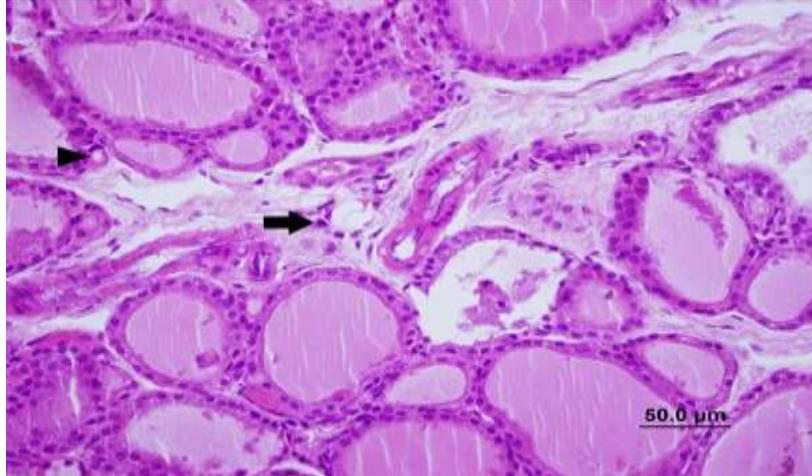


Fig. 1: Microscopic analysis vacuolation in the cytoplasm and perivascular cell infiltration in the tissues of early exposure groups.

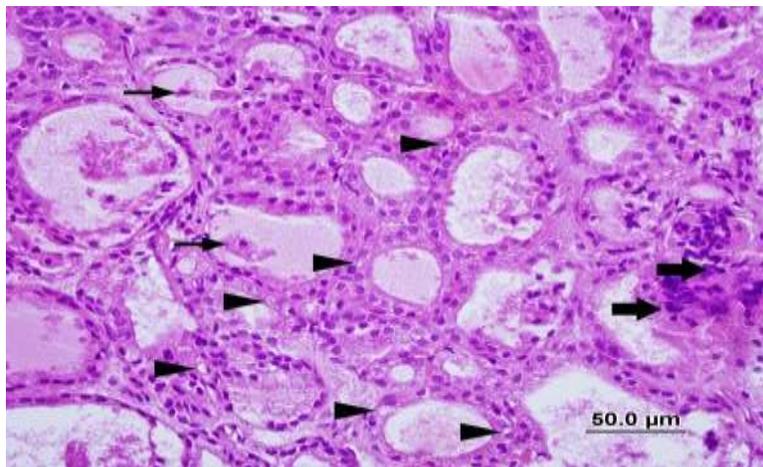


Fig. 2: II- Microscopic analysis in the tissues of late exposure groups, severe perivascular cell infiltration, a severe vacuolation and desquamation in the cytoplasm.