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The Effect of Electromagnetic Radiation on the Rat Brain: An Experimental Study

Elektromanyetik Dalgaların Sıçan Beyin Dokusu Üzerine Etkileri: Deneysel Çalışma

Olçay ESER¹, Ahmet SONGUR², Cevat AKTAS³, Ergun KARAVELIOGLU⁴, Veli CAGLAR², Firdevs AYLAK⁵, Fehmi OZGUNER⁶, Mehmet KANTER⁷

¹Afyon Kocatepe University, Faculty of Medicine, Department of Neurosurgery, Afyonkarahisar, Turkey

²Afyon Kocatepe University, Faculty of Medicine, Department of Anatomy, Afyonkarahisar, Turkey

³Namik Kemal University, Faculty of Medicine, Department of Histology and Embryology, Tekirdag, Turkey

⁴Bolvadin Dr. H.I.Ozsoy State Hospital, Department of Neurosurgery, Afyonkarahisar, Turkey

⁵Suleyman Demirel University, Faculty of Medicine, Department of Biochemistry, Isparta, Turkey

⁶Suleyman Demirel University, Faculty of Medicine, Department of Physiology, Isparta, Turkey

⁷Istanbul Medeniyet University, Faculty of Medicine, Department of Histology and Embryology, Istanbul, Turkey

Corresponding Author: Olçay ESER / E-mail: drolcayeser@hotmail.com

ABSTRACT

AIM: The aim of this study is to determine the structural changes of electromagnetic waves in the frontal cortex, brain stem and cerebellum.

MATERIAL and METHODS: 24 Wistar Albino adult male rats were randomly divided into four groups: group I consisted of control rats, and groups II-IV comprised electromagnetically irradiated (EMR) with 900, 1800 and 2450 MHz. The heads of the rats were exposed to 900, 1800 and 2450 MHz microwaves irradiation for 1h per day for 2 months.

RESULTS: While the histopathological changes in the frontal cortex and brain stem were normal in the control group, there were severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei in the EMR groups. Biochemical analysis demonstrated that the Total Antioxidative Capacity level was significantly decreased in the EMR groups and also Total Oxidative Capacity and Oxidative Stress Index levels were significantly increased in the frontal cortex, brain stem and cerebellum. IL-1 β level was significantly increased in the EMR groups in the brain stem.

CONCLUSION: EMR causes to structural changes in the frontal cortex, brain stem and cerebellum and impair the oxidative stress and inflammatory cytokine system. This deterioration can cause to disease including loss of these areas function and cancer development.

KEYWORDS: Electromagnetic waves, Brain tissue, Oxidative stress, Active caspase-3

ÖZ

AMAÇ: Bu çalışmanın amacı, frontal korteks, beyin sapı ve serebellumda elektromanyetik dalgaların yapısal değişikliklerini araştırmaktır.

YÖNTEM ve GEREÇLER: 24 Wistar Albino sıçan rastgele 4 gruba bölündü: grup 1 kontrol grubu ve grup 2-4 900, 1800 ve 2450 MHz elektromanyetik radyasyona maruz kaldı. Sıçanların kafaları 900, 1800 ve 2450 MHz mikrodalgalara 2 ay boyunca her gün 1 saat maruz bırakıldı.

BULGULAR: Kontrol grubunda histopatolojik değişiklikler, beyin sapında ve frontal kortekste normal bulunurken elektromanyetik radyasyon uygulanan gruplarda ciddi dejeneratif değişiklikler, küçülmüş sitoplazma ve geniş koyu piknotik nükleus mevcuttu. Biyokimyasal analizde total antioksidatif kapasite seviyesi elektromanyetik grupta anlamlı olarak azalmış bulunurken, total oksidatif kapasite ve oksidatif stres indeksi seviyeleri frontal korteks, beyin sapı ve serebellumda anlamlı olarak artmış bulundu. Elektromanyetik grupta beyin sapında IL-1 β seviyesi anlamlı olarak arttı.

SONUÇ: Elektromanyetik radyasyon beyin sapında, serebellumda ve frontal kortekste yapısal değişikliklere neden olmakta ve oksidatif stres ve inflamatuvar sitokin sistemini bozmaktadır. Bu bozulma kanser oluşumuna ve bu bölgelerin fonksiyon kaybını içeren hastalıklara neden olabilir.

ANAHTAR SÖZCÜKLER: Elektromanyetik dalga, Beyin dokusu, Oksidatif stres, Aktif kaspaz-3

INTRODUCTION

The frequent using of mobile and cordless phones in our daily life has led to concern regarding possible health effects, cancer risk in particular, from frequent exposure to radiofrequency radiation. However, scientific evidence on

a possible mobile phone-cancer relation has still not clear enough. Therefore, investigators have still been showing high performance to clarify this topic (3). Electromagnetic radiation (EMR) is emitted by both cellular mobile phones and their base stations. Unfortunately, exposure to EMR may have

detrimental effects on the body, depending on the frequency and power of the EMR (27). Analogue phones use frequencies of 400–450 MHz, and digital mobile phones use frequencies of 850–900 MHz (similarly used in our study), and 1850–1990 MHz, whereas microwave ovens use a frequency of 2450 MHz (24).

During embryogenesis, and throughout the lifespan of a multi-cellular organism, apoptosis plays a key role in normal tissue homeostasis (1,8). Perturbations in apoptotic pathways can cause human disease (30). There are several markers currently used to detect apoptosis. In particular, the active (cleaved) caspase-3 is a well-characterized component typically observed during apoptosis, making it an ideal marker for this cellular process (9). Defects in apoptosis signaling pathways are common in cancer cells. Since apoptosis eliminates cells with damaged DNA or dysregulated cell cycle (as is the case for cells with increased malignant potential), deficits in apoptotic signaling may play an important role in tumor initiation (3). Deficits in apoptosis may also promote tumor progression and metastasis by enhancing the survival of tumor cells during transit through the bloodstream and growth in ectopic tissues (17).

Dasdag et al. (4) intended to examine the effects of 900 MHz mobile phone exposure on p53 in the rat brain. They found that 900 MHz microwave radiation emitted from mobile phones do not affect these apoptotic parameters (4). Leszczynski et al. (18) reported that mobile phone exposure can alter heat shock protein-27 (hsp27) by causing a transient increase in its phosphorylation. Hsp27 has many known functions that suggest that mobile phone radiation-induced activation of hsp27 may (i) promote brain cancer by inhibiting the cytochrome c/caspase-3 apoptotic pathway and (ii) enhance the permeability of the blood-brain barrier by stabilizing stress fibers of endothelial cells.

EMR or radiofrequency fields of cellular mobile phones may also affect individuals by increasing free radicals, which enhance lipid peroxidation, or by promoting oxidative stress by changing the antioxidant defense systems of human tissues (24). Oxidative stress is caused by relatively high levels of toxic reactive species, consisting mostly of reactive oxygen species (ROS), reactive nitrogen species (RNS), and the antioxidative defense mechanisms (23). Electromagnetic radiation causes oxidative stress, which in turn induces apoptosis. The signal for apoptosis is possibly generated via lipid peroxidation when radiation acts on cell membranes (23).

IL-1 β , an important component of the cytokine network, and is one of the inflammatory cytokines that introduces prostaglandin E1, matrix metalloproteinase-3, nitrous oxide (NO), and other substances into the intervertebral discs (22). IL-1 β is a pro-inflammatory cytokine elicited following traumatic brain injury; behavioral outcome is improved when this cytokine is decreased (27).

We investigated the effects of cell phone exposure on the antioxidants, oxidants, oxidative stress index, interleukin-1 β

levels and histological structure of rat brain tissue, caspase-3 immunoreactivity in this study.

MATERIAL and METHODS

Animal Model

Twenty-four male Sprague-Dawley rats (8-week-old, 150–200 g body weight) obtained from the Laboratory Animal Production Unit of Suleyman Demirel University were used in the study. The animals were procured, maintained, and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Suleyman Demirel University, Animal Ethical Committee. They were kept in a temperature- and humidity-controlled environment (temperature, 24–26°C; humidity, 55–60%), on a 12:12-h light/darkness cycle for 1 week before the start of experiments. A commercially balanced diet (Hasyem Ltd., Isparta, Turkey) and tap water were provided ad libitum.

Experimental Design

The animals were randomly divided into four equal groups (consisting of 6 rats each): Group I rats were used as the control group (without exposure to EMR); these rats were held in the EMR tube for 60 minutes a day for 60 days (two months) under the same environmental conditions. EMR groups: group II rats were exposed to 900 MHz, group III rats were exposed to 1800 MHz, and group IV rats were exposed to 2450 MHz. All of the rats in the EMR groups were exposed to EMR from the generator for 60 minutes a day for 60 days. The EMR exposure time was from 11:00–12:00 a.m. on each day. At the end of the study, the rats in all of the groups were anesthetized with intraperitoneal administration of ketamine (50 mg/kg)-xylazine (6 mg/kg) after the last dose exposure to EMR. The rats were sacrificed by collecting blood samples from the posterior vena cava. After removing the cranial bones, cerebral cortex and brain stem removed and the brain stem, right frontal cortex and cerebellum tissue were kept in -86°C for biochemical study. Left frontal cortex, cerebellum and brain stem were taken in neutral buffered formalin solution for histopathological study. Histopathological evaluation of left cerebellum was unsuccessful due to staining failure.

Exposure Device

Radiation for the study (900, 1800, 2450 MHz) was provided by an electromagnetic generator continuously for 60 min each day for 2 months (peak power, 2 W; average power density, 1.04 mW/cm²). The predicted average specific absorption rate (SAR) value was measured at 1.04 W/kg. The power density measurements were made with an electromagnetic field meter (Holaday Industries Inc.) produced at the electromagnetic compatibility (EMC) laboratory of the School of Electronic Engineering (Suleyman Demirel University, Isparta, Turkey). The exposure system consisted of a plastic tube cage (length: 12 cm, diameter: 5.5 cm) and a dipole antenna. The entire body of each rat was positioned in close contact above the dipole antenna, and the tube was ventilated from head-to-tail to decrease the stress of the rat while in the tube.

Biochemical Analysis Tissue Sampling and Homogenization

On the last day of the study, brains were removed immediately after euthanization to determine total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI). Prior to biochemical assays, all tissues were weighed and placed in empty glass tubes. Ten milliliters of 140 mM KCl solution per 1 g of tissue were added to each tube containing tissue samples, and the tissue was homogenized in a motor-driven homogenizer. The homogenate was centrifuged at 2800 *g* for 10 min at 4°C, placed in labeled vials and stored at -80°C. Microprotein levels were measured using the Lowry method (20).

Measurement of TAC

TAC levels of all tissues were measured using a novel automated colorimetric measurement method developed by Erel (6,7). This method is based on the Fenton reaction, which involves the colorless substrate *O*-dianisidine reacting with the hydroxyl radical to produce the dianisyl radical, which is bright yellowish-brown. Upon the addition of a plasma sample, hydroxyl radicals present in the reaction mix initiate oxidative reactions that are suppressed by the antioxidant components within the homogenates. This prevents the color change, providing an effective measure of the total antioxidant capacity of the plasma. The assay has excellent precision values (<3% error). Assay results are expressed as $\mu\text{mol Trolox equiv/g protein}$.

Measurement of TOS

TOS levels of all tissues were determined using a novel automated measurement method developed by Erel (7). Oxidants present in the sample oxidize the ferrous ion-dianisidine complex to ferric ion. Glycerol molecules present in the reaction medium enhance the oxidation reaction. In an acidic medium with xylenol orange, the ferric ion forms a colored complex. Spectrophotometric measurement of the color intensity represents the total amount of oxidant molecules present in the sample. Hydrogen peroxide (H_2O_2) is used to calibrate the assay, and the results are expressed as micromolar H_2O_2 equivalents per g protein ($\mu\text{mol H}_2\text{O}_2$ equiv/g protein).

OSI Calculation

OSI is the percent ratio of TOS to TAC and an indicator of the degree of oxidative stress (7). The OSI value was calculated according to the formula: $\text{OSI} = ((\text{TOS}, \mu\text{mol H}_2\text{O}_2 \text{ Equiv/g protein})/(\text{TAC}, \mu\text{mol Trolox equiv/g protein}))$.

Interleukin-1 β Determination

Brain stem tissue weighed in phosphate buffer (pH 7.4) was diluted in 10 volumes. Tissue samples were separated by centrifugation (Eppendorf 5415-R (Germany) brand refrigerated centrifuge) and 4000 rev/min for 15 min, and the supernatant from the IL-1 β was studied. The supernatants were stored at -20°C until the cytokine assays were performed.

Cytokines in the supernatant of tissue homogenates were measured using commercially available enzyme-linked immunosorbent assay according to the manufacturer's instructions. The assay kits for IL-1 β were purchased from RayBio mark (USA).

Histopathologic Evaluation

Frontal cortex and brainstem tissues were harvested and fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at a thickness of 5 μm , and then stained with hematoxylin and eosin (H&E). Sections were photographed and were evaluated using a bright-field microscope (Optiphot 2; Nikon, Tokyo, Japan).

Microscopic Examination

Tissue sections were examined using a bright-field microscope, and the number of neurons was counted within random high-power fields using a Nikon Optiphot-2 light microscope fitted with a square graticule in the eyepiece (eyepiece $\times 10$, objective $\times 40$, a total side length of 0.25 μm^2). The number of neurons was assessed by counting the number of cells in the frontal cortex and brainstem in 400 high-power fields. The number of neurons/ μm^2 in each region was determined. The tissue compartments were used to record neuron distribution in the frontal cortex and brain stem tissues.

Immunohistochemistry

Frontal cortices and brain stems were fixed in 10% neutral buffered formalin solution, embedded in paraffin, and sectioned at a thickness of 5 μm . Immunocytochemistry was performed according to the ABC technique described previously (13). The procedure involved the following steps: (1) incubation in 3% H_2O_2 in distilled water for 30 min to inhibit endogenous peroxidase activity; (2) washing in distilled water for 10 min; (3) incubation in normal goat serum (DAKO X 0907, Carpinteria, CA) with phosphate-buffered saline (PBS) (diluted 1:4) to block non-specific binding of antibodies; (4) incubation in rabbit polyclonal anti-Caspase-3 antibody (1:50; ab13847, Abcam, USA) for 1 h at room temperature; (5) washing in PBS 3 \times 3 min; (6) incubation in biotinylated anti-mouse IgG (DAKO LSAB 2 Kit); (7) washing in PBS 3 \times 3 min; (8) incubation in ABC complex (DAKO LSAB 2 Kit); (9) washing in PBS 3 \times 3 min; (10) detection of peroxidase using an aminoethylcarbazole substrate kit (AEC kit; Zymed Laboratories); (11) washing in tap water for 10 min, followed dehydration; (12) hematoxylin staining to visualize the nuclei; and (13) mounted in DAKO Paramount. All dilutions and washes between steps were performed using PBS and were carried out at room temperature unless otherwise specified. As a negative control, the primary antibody was replaced with PBS.

Immunohistochemical staining was scored in a semiquantitative manner to determine differences between the control group and the experimental groups. Weak (\pm), mild (+), moderate (++) , strong (+++), and very strong (++++) signals was observed and recorded. This analysis was performed in

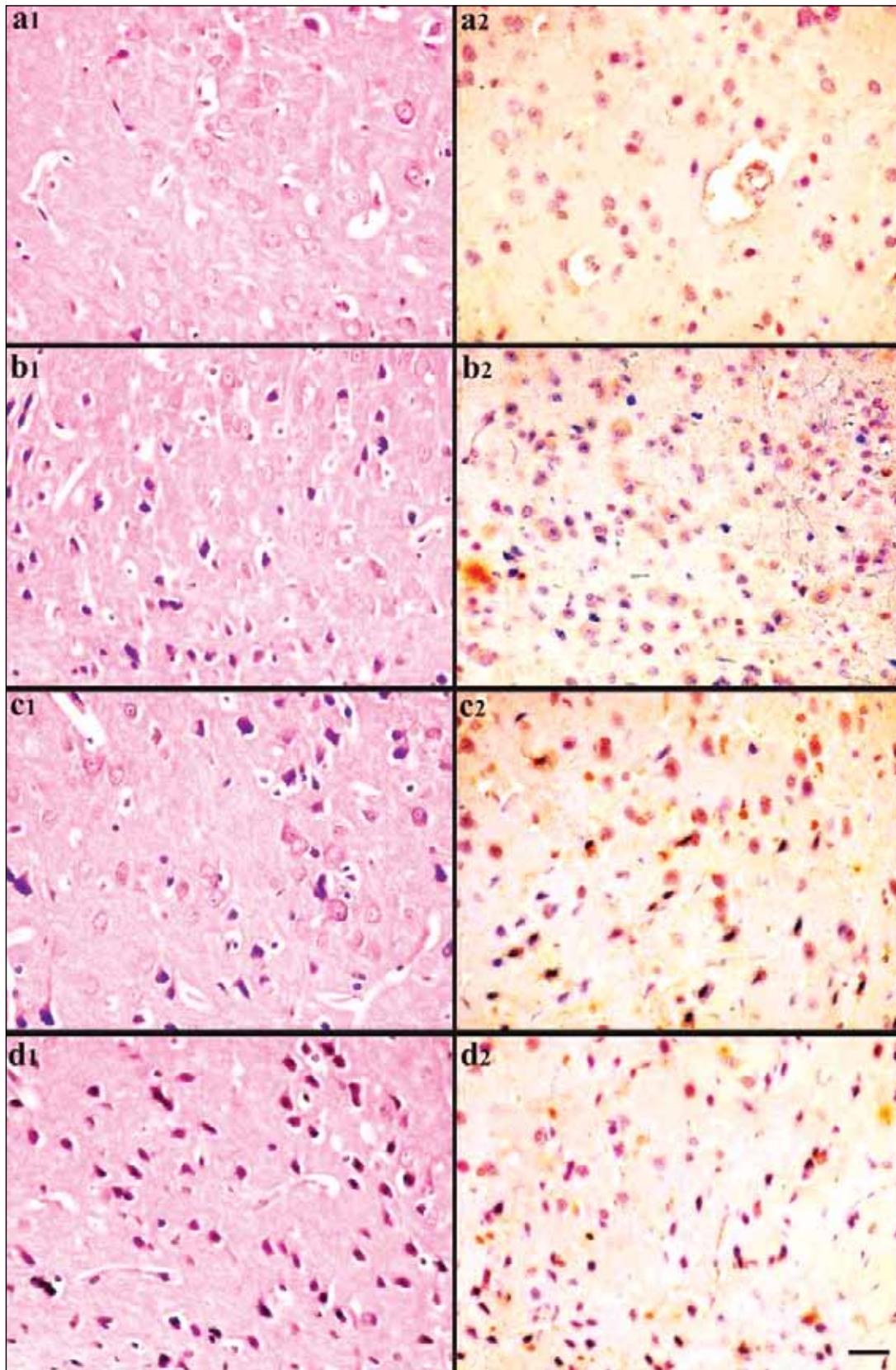


Figure 1: Representative light microphotographs showing the morphology (a1–d1) and apoptosis (a2–d2) of the frontal cortex tissue in control and EMR groups (900, 1800, 2450 MHz) by hematoxylin-eosin and caspase-3 immunohistochemistry. (Immunoperoxidase, haematoxylin counterstain, scale bar, 50 μ m) .

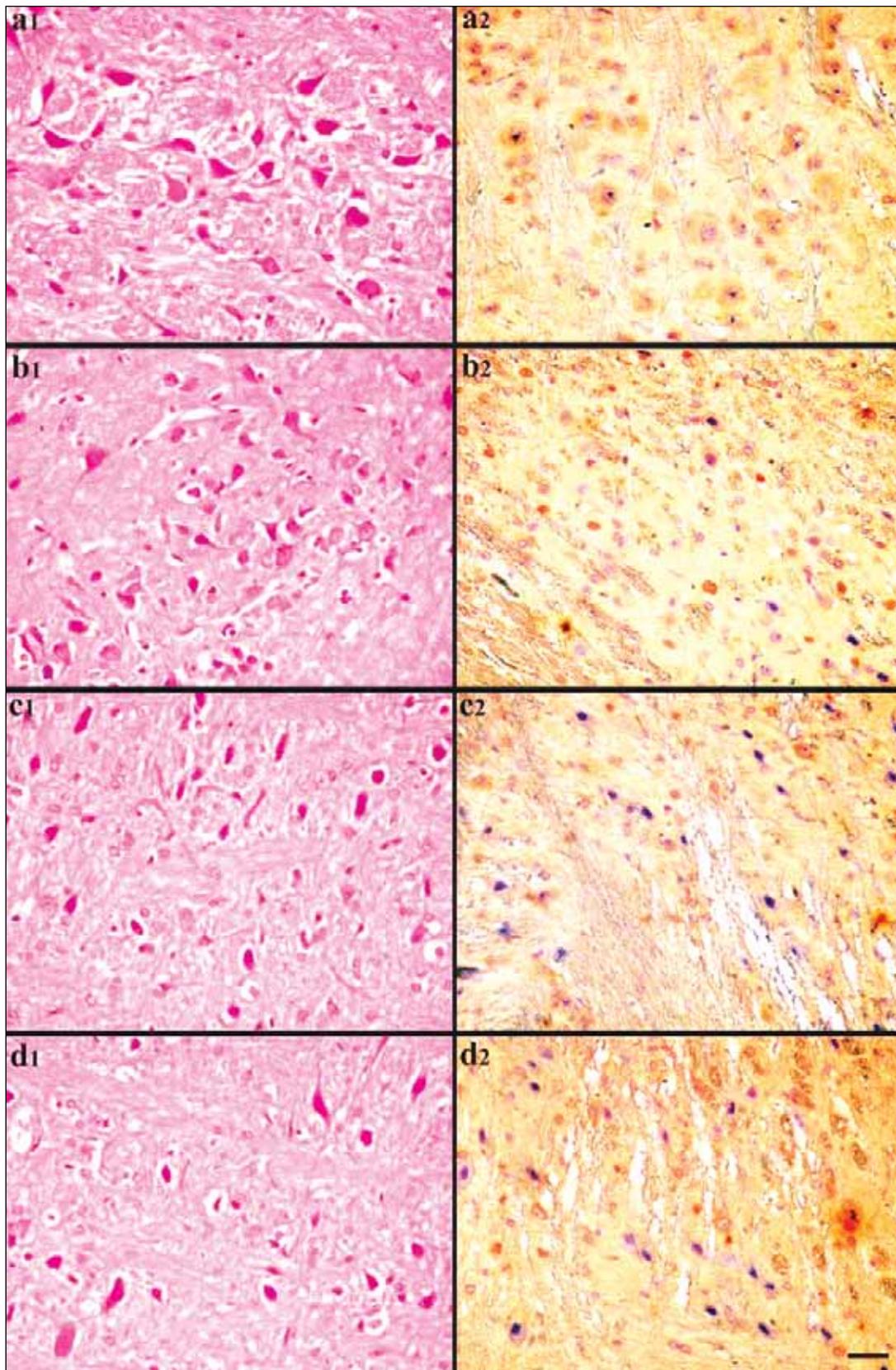


Figure 2: Representative light microphotographs showing the morphology (a1–d1) and apoptosis (a2–d2) of the brain stem tissue in control and EMR groups (900, 1800, 2450 MHz) by hematoxylin-eosin and caspase-3 immunohistochemistry. (Immunoperoxidase, haematoxylin counterstain, scale bar, 50 µm) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16.

at least 8 areas from the frontal cortex and brain stem, in 2 sections from each animal at x400 magnification.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Data from controls and each of the groups were compared using the Mann–Whitney non-parametric test for independent samples. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 11.5 (SPSS Inc. Chicago, IL, USA) and a p < 0.05 was considered significant.

RESULTS

Histopathological Findings

The morphology of neurons in the frontal cortex and brain stem were normal in H&E-stained sections taken from the control group (Figure 1A1; 2A1). In the EMR groups (900, 1800, and 2450 MHz), the most consistent observations were severe degenerative changes, shrunken cytoplasm, and extensively dark pyknotic nuclei in neurons of the frontal cortex and brain stem tissues (Figure 1B1, C1, D1; 2B1, C1, D1). In the EMR groups (900 and 1800 MHz) frontal cortex and brain stem tissues, the intensity of neuronal changes was less than in the EMR group (2450 MHz). The number of neurons in the frontal cortex and brain stem tissues of the EMR groups (900, 1800, 2450 MHz) were also significantly less than in the control group (P < 0.05, P < 0.01 and P < 0.001) (Table I).

Immunohistochemical Findings

Weak caspase-3 immunoreactivity was observed in the cytoplasm of neurons in control rats (Figure 1A2; 2A2). Light micrographs of caspase-3 immunohistochemistry revealed apoptotic neurons after EMR exposure. The caspase-3 signal was more intense in degenerating neurons of the frontal cortex and brain stem tissues following EMR exposure (Figure 1B2–D2; 2B2–D2). In the cytoplasm of neurons in EMR (2450 MHz) group, the strong caspase-3 immunoreactivity was observed (Figure 1D2; 2D2) (Table II).

Biochemical Analysis

Determination of total antioxidative capacity (TAC), total oxidative status (TOS) and Oxidative Stress Index (OSI):

The determination of TAC, TOS and OSI values in the frontal cortex are shown in Table III. The frontal cortex in the EMR groups TAC were lower than in the control group (p<0.05), TOS and OSI were higher than in the control group (p<0.05). In the EMR groups, the group II frontal cortex TAC was significantly different from that of groups III and IV (P<0.05), whereas the group III TAC was not significantly different from that of group IV (P>0.05). The group IV frontal cortex TOS was significantly different from that of groups II and III (P<0.05), whereas the group II TOS was not significantly different from that of group III (P>0.05). For the OSI comparisons, the OSI in frontal cortex group II was significantly different from that of group III and IV (P<0.05), and the group III OSI was not significantly different from that in group IV (P>0.05).

The determination of TAC, TOS and OSI values in the brain stem are shown in Table IV. The brainstem in the EMR groups TAC were lower than in the control group (p<0.05), TOS and OSI were higher than in the control group (p<0.05). In the EMR groups, the group II TAC was not significantly different from that of groups III and IV (P>0.05). The group II TOS was significantly different from that of groups II and III (P<0.05), whereas the group III TOS was not significantly different from that of group IV (P>0.05). For the OSI comparisons, the OSI group II was not significantly different from that of group III and IV (P>0.05).

The determination of TAC, TOS and OSI values in the cerebellum are shown in Table V. The cerebellum in the EMR groups TAC were lower than in the control group (p<0.05), TOS and OSI were higher than in the control group (p<0.05). In the EMR groups, the group II TAC was not significantly different from that of groups III (P>0.05), whereas the group IV TAC was significantly different from that of group II (P<0.05). The group II TOS was significantly different from that of groups III (P<0.05), whereas the group II TOS was not significantly different from that of group IV (P>0.05). For the OSI comparisons, the OSI group II was not significantly different from that of group III and IV (P>0.05), and the group III OSI was not significantly different from that in group IV (P>0.05).

Measurement of Brain Stem IL-1β levels

The brain stem IL-1β levels are showed in table 4. IL-1β levels in the EMR groups were significantly different from those in

Table I: The Numbers (Number/mm²) of Neurons in the Frontal Cortex and Brain Stem Tissue of Control and EMR Groups (900, 1800, 2450 MHz)

Groups	Frontal cortex	Brain stem
Control	77.38±5.13	35.31±1.54
900 MHz	63.82±4.64 ^a	28.12±1.32 ^a
1800 MHz	51.44±3.86 ^b	23.14±1.11 ^b
2450 MHz	42.16±2.98 ^c	18.12±0.94 ^c

Kruskal-Wallis test was used for statistical analysis. Values are expressed as means ± SD, n = 6 for each group; ^aP<0.05 compared to A group, ^bP<0.01 compared to A group, ^cP<0.001 compared to A group.

Table II: Semiquantitative Comparison of the Intensity in Neurons of Caspase-3 Immunoreactivity in the Frontal Cortex and Brain Stem Tissue of Control and EMR Groups (900, 1800, 2450 MHz), (n: 6 for Each Group)

	Frontal cortex	Brain stem
Control	±	±
900 MHz	+	+
1800 MHz	++	++
2450 MHz	+++	+++

The intensity of the staining was recorded as weak (±), mild (+), moderate (++), strong (+++) and very strong (++++).

the control group ($P < 0.05$). In the EMR groups, group II IL-1 β levels were significantly different from those in group III and IV ($P < 0.05$), whereas group III IL-1 β levels were not significantly different from those in group IV ($P > 0.05$).

DISCUSSION

The frequent using of mobile phones and base stations in our daily life has led to concern regarding possible health effects, cancer risk in particular, from frequent exposure to radiofrequency radiation. However, scientific evidence on a possible mobile phone–cancer relation has still not clear enough (3). There fore we aimed to investigate experimentally the biological effect of EMR on the cranial structure. In this study, we demonstrated that EMR affect the oxidative stress, levels of cytokine and induction of the apoptosis on the brain tissue.

Cancer cells often exhibit deficits in apoptotic signaling pathways. These deficits may play a significant role in tumor

initiation because cells with damaged DNA or dysregulated cell cycle (i.e., cells with increased malignant potential) are normally eliminated (17). Moreover, impaired apoptotic function may enhance tumor progression and promote metastasis by enhancing the ability of tumor cells to survive in the circulatory system and to grow in ectopic tissue sites (17). Therefore, the result of this study is of importance in terms of its implication on the dysregulation of apoptotic machinery. Because we found that 900/1800/2400 MHz radiation used in this study altered the number of apoptotic cells, this may potentially be dangerous in the networking of the brain cells.

Radiation is known to induce oxidative stress, which in turn activates the apoptotic pathway (23). Oxidative stress is a cellular or physiological condition of elevated concentrations of reactive oxygen species that cause molecular damage to vital structures and functions (32). It has been reported that the effects of radiation on cell membranes induces apoptotic signal via lipid peroxidation (23). Radiation may also mediate

Table III: The Levels of Total Antioxidatif Capacity (TAC), Total Oxidant Status (TOS) and Oxidant Stres Index (OSI) in Frontal Cortex Groups

Parameters	Control	900MHz	1800MHz	2450MHz
TAC	0.90 \pm 0.05	0.59 \pm 0.01	0.74 \pm 0.02	0.78 \pm 0.03
TOS	3.29 \pm 0.35	4.77 \pm 0.40	4.71 \pm 0.24	6.15 \pm 0.99
OSI	418.48 \pm 41.38	808.52 \pm 69.15	629.38 \pm 28.78	678.01 \pm 83.53

The value represent the mean \pm SD.

TAC compares; Control- 900/ 1800/ 2450 MHz ($p < 0.05$), 900- 1800/2450 MHz ($p < 0.05$), 1800- 2450 MHz ($p > 0.05$).

TOS compares; Control- 900/ 1800/ 2450 MHz ($p < 0.05$), 900- 1800 MHz ($p > 0.05$), 900- 2450 MHz ($p < 0.05$), 1800-2450 MHz ($p < 0.05$).

OSI compares; Control- 900/1800/2450 MHz ($p < 0.05$), 900-1800/ 2450 MHz ($p < 0.05$), 1800-2450 MHz ($p > 0.05$) Arbitrary Units (AU).

Table IV: The Levels of Interleukin-1B (II-1B), Total Antioxidatif Capacity (TAC), Total Oxidant Status (TOS) and Oxidant Stres Index (OSI) in Brain Stem Groups

Parameters	Control	900MHz	1800MHz	2450MHz
IL-1 β	5061.57 \pm 418.75	8189.23 \pm 1151.21	6055.58 \pm 648.34	6039.02 \pm 710.64
TAC	0.37 \pm 0.01	0.26 \pm 0.02	0.27 \pm 0.01	0.28 \pm 0.00
TOS	2.22 \pm 0.13	3.71 \pm 0.30	3.03 \pm 0.14	2.68 \pm 0.11
OSI	661.82 \pm 36.16	1094.94 \pm 108.29	1080.30 \pm 78.29	1074.16 \pm 63.23

The value represent the mean \pm SD.

IL-1 β compares; Control- 900/ 1800/ 2450 MHz ($p < 0.05$), 900-1800/2450 MHz ($p < 0.05$), 1800-2450 MHz ($p > 0.05$).

TAC compares; Control- 900/ 1800/ 2450 MHz ($p < 0.05$), 900- 1800/2450 MHz ($p > 0.05$), 1800- 2450 MHz ($p > 0.05$).

TOS compares; Control- 900/ 1800/ 2450 MHz ($p < 0.05$), 900- 1800/2450 MHz ($p < 0.05$), 1800-2450 MHz ($p < 0.05$).

OSI compares; Control- 900/ 1800/ 2450 MHz ($p < 0.05$), 900-1800/ 2450 MHz ($p > 0.05$), 1800-2450 MHz ($p > 0.05$) Arbitrary Units (AU).

Table V: The Levels of Total Antioxidatif Capacity (TAC), Total Oxidant Status (TOS) and Oxidant Stres Index (OSI) in Cerebellum Groups

Parameters	Control	900MHz	1800MHz	2450MHz
TAC	0.58 \pm 0.08	0.27 \pm 0.01	0.27 \pm 0.02	0.24 \pm 0.01
TOS	2.30 \pm 0.21	3.01 \pm 0.35	2.59 \pm 0.18	2.59 \pm 0.20
OSI	400.85 \pm 80.75	1110.03 \pm 146.43	950.38 \pm 94.75	1077.33 \pm 154.62

The value represent the mean \pm SD.

TAC compares; Control- 900/ 1800/ 2450 MHz ($p < 0.05$), 900- 1800 MHz ($p > 0.05$), 900-2450 MHz ($p < 0.05$), 1800-2450 MHz ($p > 0.05$).

TOS compares; Control- 900/ 1800/ 2450 MHz ($p < 0.05$), 900- 1800 MHz ($p < 0.05$), 900- 2450 MHz ($p > 0.05$), 1800-2450 MHz ($p > 0.05$).

OSI compares; Control- 900/1800/2450 MHz ($p < 0.05$), 900-1800/ 2450 MHz ($p > 0.05$), 1800-2450 MHz ($p > 0.05$) Arbitrary Units (AU).

its apoptotic effects by increasing oxidative stress (24). An increase in free radical formation caused by radiations from mobile phones has been reported in other tissues (14–16). İlhan et al. demonstrated that mobile phones biochemically cause oxidative damage by increasing the levels of NO, MDA, XO, and ADA activities in rat brain used as a model for EMR exposure. These continuously produced ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (CAT) (24). Under some circumstances, these endogenous antioxidant defenses are likely to be perturbed due to overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue (24).

The high number of antioxidant components in tissues hinders individual measurement of each antioxidant component (5). In addition, because of the interaction between various antioxidants, examining one of them separately from the others may not accurately reflect their combined effect (5). Therefore, TAC and TOS, developed by Erel (6,7), are 2 biochemical parameters that seem to appropriately represent the overall oxidant and antioxidant status resulting from antioxidant intake, or production and consumption by increasing oxidative stress level (5).

Hardel et al. (10,11) and Lonn et al. (19) reported that exposure to mobile phone radiation increased the incidence of brain tumors and acoustic neurinoma, depending on the duration of mobile phone use. Belyaev et al. (2) investigated whether rat brain exposure to microwaves of the global system for mobile communication (GSM) induces 1) DNA breaks and 2) changes in chromatin conformation and gene expression. Leszczynski et al. (18) reported that mobile phone exposure results in a transient increase in phosphorylation of hsp27. The function of hsp27 suggests that mobile phone radiation-induced activation of this protein may (i) increase the likelihood of brain cancer by inhibiting the cytochrome c/caspase-3 apoptotic pathway and (ii) increase blood-brain-barrier permeability by stabilizing endothelial cell stress fibers (18). Finally, Dasdag et al. (3) showed that the apoptotic process can be affected by radiofrequency radiation. In this study we demonstrated that in the cytoplasm of neurons in EMR groups, the caspase-3 immunoreactivity which is a well-known feature of typical apoptosis was observed strongly especially in the 2450 MHz group.

Inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, are released by microglial cells during neuropathological states and in response to various neuropathological stimuli (21,25). IL-1 β is a pro-inflammatory cytokine and an important upstream regulator of the inflammatory response that occurs following trauma (27). IL-1 β levels in the cerebrospinal fluid (CSF) positively correlates with high intracranial pressure and poor outcomes in human patients (29). IL-1 β possibly does not directly cause neuronal injury, but may instead act through astrocytes (26) by responding to harmful stimuli (31).

We investigated the effect of EMR on IL-1 β because, as a mediator of the pro-inflammatory response and predictor of

clinical outcome, it plays a key role in injury response. Hirose et al. (12) demonstrated that there was no differences of IL-1 β level between control and 1950 MHz groups in rat brain. Also Rasouli et al (27) demonstrated no differences in IL-1 β level between pulsed electromagnetic fields treated and control groups in brain homogenates after traumatic brain injury in rats. In this study, IL-1 β level was significantly increased in brain stem in EMR groups compared to the control group.

CONCLUSION

Biochemical analysis of this study demonstrated that TAC level was significantly decreased in EMR groups compared to control group while TOS and OSI level were significantly increased. This result revealed that EMR is affective on the oxidant-antioxidant system. The frontal cortex was significantly more affected in 900 MHz EMR. There was not significant difference between other groups. Also in the brain stem and frontal cortex there was not significant differences between EMR groups. IL-1 β level was significantly increased at brain stem in EMR group compared to control group. EMR in terms of IL-1 β levels most affected group was 900 MHz. There were severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei and less neuron in the EMR groups compared to control groups. The most affected EMR group was the 2450 MHz group. In the frontal cortex and brain stem of the EMR groups, caspase-3 immunoreactivity was increased compared to the control group. The most affected group was again the 2450 MHz group.

DECLARATION of INTEREST

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