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Original Article

Effects of 900 MHz electromagnetic field on prenatal locomotor development in rats

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Abstract: Introduction: The present study investigated the effects of 900 MHz electromagnetic field (EMF) on locomotor system development in pregnant rats. Materials and methods: Following impregnation, the rats were divided into a study group (exposure to 900 MHz EMF) and control group (no exposure). A total of 28 rat pups were used in the study. On the 60th postnatal day, bone, nerve, and muscle tissues were evaluated by radiological, biochemical, and histological methods. Results: The study group showed significantly decreased femoral medullary canal diameter to cortical thickness ratio (15.3% in females, 8.5% in male pups, $p<0.05$), lower alkaline phosphatase levels (18.24% in females, $p>0.05$. 22.25% in male pups, $p<0.05$), and higher calcium (2.37% in females, 0.09% in male pups, $p>0.05$) and creatinine kinase levels (16.86% in females, 3.01% in male pups, $p>0.05$). Neuronal nitric oxide synthase (nNOS) immunoreactivity of sciatic nerve tissues and calcineurine staining of bone tissues indicated significantly higher anti-nNos and anti-calcineurin a antibody levels in the study group ($p<0.05$). Conclusion: Prenatal exposure of rat pups to 900 MHz EMF may negatively affect the development of sciatic nerves, muscles, and bones.

Keywords: Electromagnetic field, prenatal period, mobile phone, locomotor development, bone

Introduction

Parallel with advancements in telecommunication technology in the world over the last 2 decades, the long-time use of mobile phones (MP) has become widespread. For this reason, MP is among the leading electromagnetic sources of exposure [1]. Children meet with MP at earlier ages and, since their life duration is longer than that of adults, the duration of their exposure to MP is longer [2]. MP radiates electromagnetic radiation (RF-EMR) as a radio frequency. Radio signals emitted by MP are between 800 and 1900 Mhz. In tissues of the body exposed to RF, some physiopathologic changes can result in harmful changes [3]. Therefore, the effects of electromagnetic radiation on human health has drawn increasing attention from researchers [3-5].

In studies carried out, spermatogenesis in the reproductive system [5] and subjective symptoms, such as headache, lack of appetite, itching [6], and increased risk of leukemia [7], have been reported. Moreover, an increase in cancer risks along with an increase in duration of speaking has been observed [8, 9].

When exposed to RF, that has the potential of creating such a wide range of pathologic consequences, they are more sensitive than adults [1, 3]. Compared to adults, it was observed that the number of studies regarding the effects of RF sensitivity of children caused from MP on the formation and development of the locomotor system is limited.

The present study histologically, radiologically, and biochemically analyzed the positive and negative effects of 900 MHz EMF on pregnant rat cubs locomotor systems, as well as bone, peripheral nerve, and muscle development.

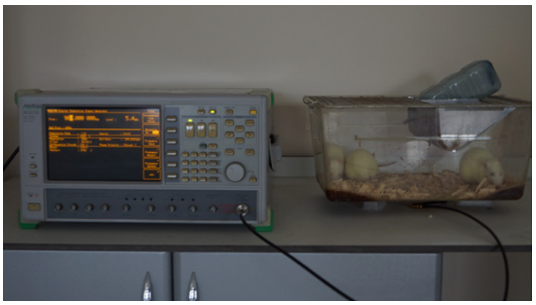

Figure 1. 3 pregnant sprague-dawley rats were exposed to radiation emitted.

Materials and methods

This study was approved by the laboratory animal local ethics committee of medical faculty of Recep Tayyip Erdoğan University, rize, on 01.08.2014 with decision number 2014/3. Upon approval, the experiment was started. Procedures carried out were designed according to national institute of health guidelines for the care and use of laboratory animals.

Animals and experimental procedures

A total of 6 pregnant Sprague-Dawley rats, weighing 290-320 grams, were included in the present study. Rats were placed in cages sized 36 cm x 23 cm x 21 cm as triples to conceive. One male rat was placed in each cage for mating with the three female rats. Two days after, male rats were removed from the cages. Following impregnation, they were segregated into two groups. One group was exposed to EMF for 24 hours while the other remained as unexposed control. Newborns were further segregated in accordance with gender, with each group consisting of 7 anmals. A total of 28 rats were used in the study. The rats were maintained on a 12-hour light-dark cycle regimen at a constant temperature of 21-23°C with 50-55% relative humidity. All animals were fed ad libitum with free access to food and tap water.

Exposure to electromagnetic radiation

After the mating day, 3 pregnant Sprague-Dawley rats were exposed to radiation emitted from the digital signal generator (**Figure 1**). The generator (Antritsu MG3670 B type, Japan), which produces 900 MHz Radiofrequency radiation, was used in the present study to represent exposure of global systems for mobile communications. Regarding the digital signal generator used in this study, the carrier frequency was 900 MHz and maximal peak power was 2 W. The study was performed on two groups, the control group and the exposed group. A generator with an external antenna was closely placed under the cage, centrally. Study group rats were exposed to radiation on talking mode for 24 hours a day. For the cage control, no radiation was applied.

At the end of 60 days, rats (110-140 g) were intraperitoneally administered a combination of 6 mg/kg of 2% xylazine hydrochloride (Rompun) and 75 mg/kg ketamine hydrochloride (Ketalar). Following anesthesia, the animals were sacrificed by intracardiac perfusion with 4% formaldehyde. Blood samples (3.0 mL) were removed from the left ventricle of each animal on decapitation. Bones, nerves, and muscles were excised and the specimens were prepared for radiological and histopathological studies.

Histopathological evaluation

Bone tissues and muscle tissues were removed for the preparation of specimens. These tissues were fixed in 10% formalin solution for 48 hours. After standing in a fixative material, the bones were put in a Müller solution for 24-48 hours to be decalcified. Decalcification was completed by the addition of a 3% Müller solution. The muscle tissues were combined with bone tissue and washed for 1-2 hours with running distilled water.

Tissue blocks were dehydrated in a series of alcohol, cleared in xylene, and embedded in paraffin. 4-5 μm sections were taken using a rotary microtome (Leica RM2255 Rotary Microtome, Leica Biosystems, Nussloch, Germany). The sections were stained with H&E and then examined under a light microscope (BX51; Olympus, Tokyo, Japan) with a digital camera (DP72; Olympus, Tokyo, Japan). They were photographed at relevant magnifications.

Immunohistochemical study

For immunohistochemical staining 3 μm thick sections of bone and nerve tissues were cut and allowed to stand in xylene for 20 minutes before the application of an alcohol series (50-100%). They were then allowed to stand for 10 minutes in an 3% H_2O_2 solution. After being washed with PBS, these sections were heated in a citrate buffer solution at 800 W for

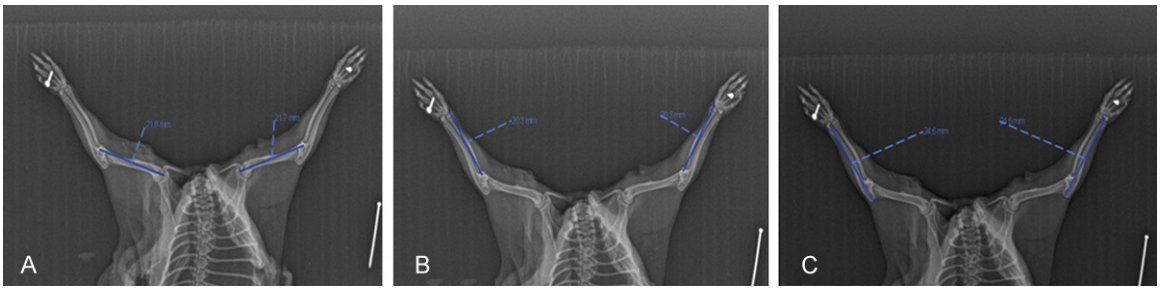

Figure 2. Anteroposterior radiograph showing the lines needed for measurement and calculation of the humerus (upper row, A), radius (upper row, B), ulna (upper row, C).

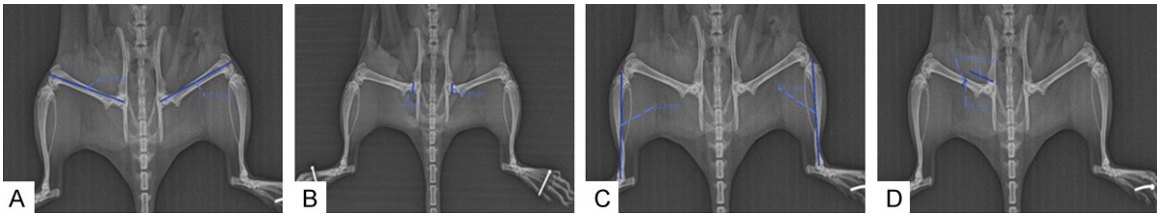

Figure 3. Anteroposterior radiograph showing the lines needed for measurement and calculation of the femoral shaft (lower row, A), femoral head (lower row, B), tibia (lower row, C), ratio of femur medulla to the femur cortex (lower row, D).

4-5 minutes and allowed to stand in secondary blocker substance for 20 minutes. After being washed with phosphate-buffered saline (PBS), the samples were heated with 800 W in acitrate buffer solution (heated four minutes (repeated five times) and allowed to stand in a secondary blocker substance for 20 minutes. For every preparation to be stained with anti-calcineurine (CN) a (Code: ab-93875, Abcam plc, Cambridge CB4 0FL UK) (Anti-CN A 1/100) and anti-nNOS (ab1376, Abcam Plc, Cambridge, UK) (anti-nNOS 1/300), they were put into various dilutions for 75 minutes. Diaminobenzidine solution was used as a chromogen and Mayer's hematoxylin was used as a counterstain for 5 minutes. PBS was used as a negative controller. The preparations were photographed after being covered with a suitable substance. Results of the immunohistochemical stained tissues were subdivided into four categories according to the percentage of immunopositivity reactions, as follows: mild (+), moderate (++), severe (+++), and very severe (++++). Two histologists blindly scored and evaluated the preparations with regards to statistical analysis.

Biochemical processes

Blood samples (3.0 mL) were removed from the left ventricle of the animals to measure the amount of electrolytes, such as calcium (Ca), alkaline phosphatase (ALP), and creatine kinase (CK), using a standard autoanalyzer technique (Architect c16000 Autoanalyzer, Abbott Diagnostics, Waltham, Massachusetts, USA).

Bone measurement method

After being sacrificed, the internal organs were extracted and fixed on an x-ray board to clearly to display the skeletal system. Their anteroposterior x-ray graphics were checked using the G.E. X-R 6000 device. They were photographed before being sent to a digital photography program (Akgun PACS Viewer 4.0) to perform measurements.

The means (with fallibility of ±0.01 mm) of the following distances were measured bilaterally using Akgun PACS Viewer Program. Diameters of femur medulla were measured from physeal cartilage level. Femur lengths were measured between femur medulla and femur condyle. Tibia lengths were measured between anterior eminence and ankle. Ulna lengths were measured between olecranon and wrist. Femur bicortical distance and medullary distances were measured from the distal of minor trochanter (1/3 proximal of femur length) (**Figures 2** and **3**). All measurements are given in separate **Table 1**.

Table 1. Morphometric analysis of femur, tibia, humerus, radius and uina bone tissues

Measurement Site	Side	Male		Female	
		Study Group (med ± SD)	Control Group (med ± SD)	Study Grou (med ± SD)	Control Group (med ± SD)
Weight (gr)		132±13.21	139±8.43	121±11.32	127±7.22
Femur length (mm)	R	28.70±0.80	29.10±0.40	27.30±0.86	27.70±0.63
	L	28.60±0.83	29.10±0.45	27.70±0.83	27.60±0.60
Tibia length (mm)	R	31.9±0.09	32.1±0.06	31.00±0.07	30.80±0.07
	L	31.7±0.09	31.9±0.06	31.10±0.07	30.70±0.07
Femur head diameter (mm)	R	3.60±0.04[a]	3.80±0.05	3.30±0.03[e]	3.50±0.07
	L	3.60±0.04[b]	3.80±0.05	3.30±0.03[f]	3.50±0.07
Ulna length (mm)	R	25.30±0.06	25.80±0.03	24.70±0.07	24.60±0.05
	L	25.30±0.07	25.70±0.03	24.20±0.09	24.60±0.05
Radius length (mm)	R	20.50±0.05	21.00±0.04	20.80±0.06	20.30±0.04
	L	20.50±0.06	21.00±0.03	20.60±0.06	20.30±0.03
Humeral length (mm)	R	22.80±0.05	23.10±0.03	22.90±0.08	21.80±0.05
	L	22.70±0.05	23.20±0.04	22.80±0.07	21.70±0.05
Femur medulla/cortex ratio	R	0.58±0.01[c]	0.54±0.03	0.58±0.01[g]	0.50±0.01
	L	0.60±0.01[d]	0.54±0.04	0.60±0.01[h]	0.50±0.01

[a,b,e,f]p=0.001 (p<0.05); [c,d,g,h]p=0.001 (p<0.05).

Statistical analysis

Statistical analyses were performed using SPSS software 17.0 (IBM, Chicago, IL, USA) version. Data were analyzed. Median ± Standard Deviation (SD) was used for non-parametric data, while mean ± SD was used for parametric data. To determine significant differences between the groups, One-Way Analysis of Variance (ANOVA) was used. As a post-hoc test, the tukey's HSD and LSD were utilized for multiple comparisons. Regarding histopathological examination, non-parametric data analysis was performed with blind grading and examined via Mann-Whitney U-Test. For all comparisons, the statistical significance was set at $p<0.05$.

Results

Femur distal tissues of female and male study and control groups were examined from a histopathological aspect (**Figure 4A**).

Distal femoral epiphyseal plaque was observed, showing no significant variation among the study and control groups. It was determined that vacuolization (v) increased in regions of transition to trabecula and they were stained mildly eosinophilic. Hematopoietic system cell density was observed to decrease in bone marrow. Slight decreases were observed in volumes of both medial femoral condyle and lateral femoral condyle regions. Chondrocyte necrosis and mildly apoptotic cells related with proteoglycan losses were also determined (**Figure 4A3**).

The distal femoral epiphyseal plate and femur medulla region did not show significant changes in male control and study groups. They had more normal morphology than female groups. It was observed that, in regions of trespassing to trabecula, vacuolization (v) increased, the apoptotic chondrocyte cells became more significant, and they were stained significantly eosinophilic. In bone marrow, it was observed that the density of hematopoietic system cells decreased but the intensity of fatty tissue increased.

While slight decreases were observed in volumes of medial femoral condyle and lateral femoral condyle regions, chondrocyte necrosis and mildly apoptotic cells related with proteoglycan losses were determined (**Figure 4A4**).

Gastrocnemius muscles of male and female study and control groups were examined from a histopathological aspect (**Figure 4B**).

It was observed that dilatations (d) between the muscle fibers increased and edema-like (e) liquids accumulated in dilatation regions. Despite local swelling in myocytes, no mono-

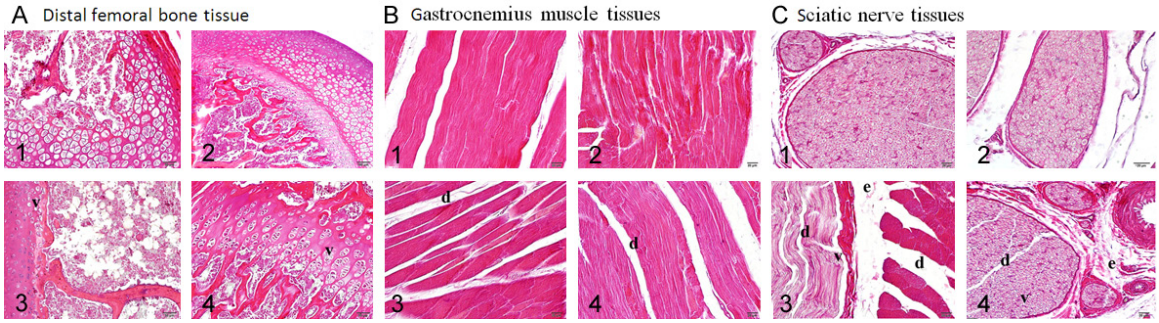

Figure 4. Histopathological staining of bone, muscle, and nerve tissue following irradiation with 900 MHz radiofrequency. female (A1, ×200) control, (A3, ×400) study group and male (A2, ×200) control, and (A4, ×100) study group; gastrocnemius muscle tissues of 900 MHz RF-applied female (B1, ×400) control, (B3, ×400) study group and male (B2, ×400) control, and (B4, ×400) study group; sciatic nerve tissues of 900 MHz applied female (C1, ×200) control, (C3, ×200) study group and male (C2, ×200) control, and (C4, ×200) study group; d: dilatation, v: vacuolization, e: edema. Hematoxyloneosin.

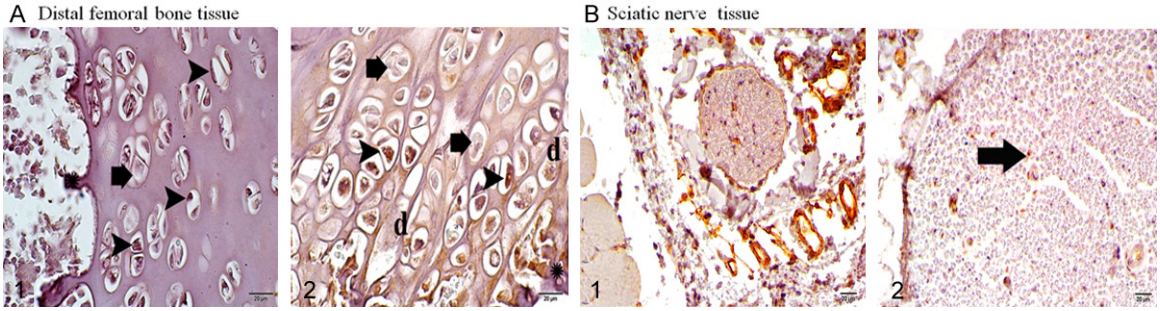

Figure 5. Immunohistochemical staining of bone and nerve tissues. Left: Anti-CN A staining of distal femoral tissue of the study group (A2, ×600) shows more degenerative cells compared to the control group (A1, ×600). Right: anti-nNOS staining of sciatic nerve of the study group (B2, ×600) and control group (B1, ×600) arrow head: intense immune reactivity, arrow: mild immune reactivity, d: degenerative cell.

Table 2. Analysis of histopathological results of muscle tissue

Groups N:7	Myelin degeneration (med ± SD)	Hypertrophic and necrosis cartilage (med ± SD)	Epiphyseal plate degeneration (med ± SD)	Apoptotic myocyte (med ± SD)	Muscle fiber dilatation (med ± SD)	Myocyte swelling and degeneration (med ± SD)
Male Control Group	0.00±0.42	0.00±0.32	0.00±0.48	0.00±0.42	0.00±0.48	0.00±0.42
Female Control Group	0.00±0.32	0.00±0.32	0.00±0.00	0.50±0.53	0.00±0.42	0.00±0.42
Male Study Group	2.00±0.67[a]	2.00±0.52[a]	1.00±0.32[a]	2.00±0.67[a]	2.00±0.42[a]	2.00±0.79[a]
Female Study Group	2.00±0.70[b]	2.00±0.95[b]	1.00±0.52[b]	2.00±0.32[b]	2.00±0.63[b]	2.00±0.48[b]

[a]In statistical investigation between male control and study group in terms of myelin degeneration, hypertrophic and necrotic cartilage, epiphyseal plate degeneration, apoptotic myocyte, muscle fiber dilatation and myocyte swelling by using Mann-Whitney U Test, statistically significant difference was found between the groups (P<0.05). [b]In statistical investigation between female control and study group in terms of myelin degeneration, hypertrophic and necrotic cartilage, epiphyseal plate degeneration, apoptotic myocyte, muscle fiber dilatation and myocyte swelling by using Mann-Whitney U Test, statistically significant difference was found between the groups (P<0.05).

nuclear cell infiltration was observed in ligament tissues. Moreover, edemas developing in dilatation regions between the muscles and epimysium and slight thickening was observed in epimysium (**Figure 4B3**).

It was observed that dilatations (d) between the muscle fibers increased and slight edemas accumulated in dilatation regions. Although congestion was observed in veins, it was also determined that swelling developed in myocytes (**Figure 4B4**).

Sciatic nerves of female and male study and control group rats were examined from a histopathological aspect (**Figure 4C**).

Table 3. Analysis of histopathological nerve and bone tissue results

Group N:7	Anti-CN A (med ± SD)	Anti-nNOS (med ± SD)
Male Control Group	3.00±0.70	3.00±1.05
Female Control Group	3.00±0.94	4.00±0.67
Male Study Group	1.00±1.35[a]	2.00±0.67[c]
Female Study Group	2.00±0.79[b]	2.00±0.84[d]

[a]In statistical investigation between male control and study group in terms of Anti-CN A immune reactivity by using Mann-Whitney U Test, statistically significant difference was found between the groups, p=0.027 (p<0.05). [b]In statistical investigation between female control and study group in terms of Anti-CN A immune reactivity by using Mann-Whitney U Test, statistically significant difference was found between the groups, p=0.039 (p<0.05) [c]In statistical investigation between male control and study group in terms of Anti-nNOS immune reactivity by using Mann-Whitney U Test, statistically significant difference was found between the groups, p=0.003 (p<0.05). [d]In statistical investigation between female control and study group in terms of Anti-nNOS immune reactivity by using Mann-Whitney U Test, statistically significant difference was found between the groups, p=0,009 (p<0.05).

Even though mild vacuolization (v) developed in sciatic nerves, it was determined that the level of degeneration in axons was low. It was found that the dilatation (d) in intermediary tissue of nerves was mild and showed homogenous distribution. Dilatations were observed tobe higher in female groups.

It was determined that swelling-related degeneration occurred in osteoprogenitor cells and the cells were significantly basophilic stained (**Figure 4C3**).

While no mononuclear cell infiltration was observed in interstitial ligament tissue, low levels of axonal degeneration were observed besides the mild vacuolization (v) in sciatic nerves near the muscle. While intense edemas (e) developed in dilatation regions between the muscles and epimysium, slight thickening and disconnection from the nerve were observed in epimysium. It was determined that the dilatation of intermediary tissue of nerve was low and showed homogenous distribution. It was also observed that less dilatation developed in the proportion of female groups (**Figure 4C4**).

In addition, the tissues were examined by immunohistological staining (**Figure 5A** and **5B**). Statistical analyses were used to compare the different groups.

In proportion to the female and male baby rats in control groups, it was determined that their myelin degeneration, hypertrophic and necrotic cartilage, epiphyseal plate degeneration, apoptotic myocyte, muscle fiber dilatation, myocyte swelling, and degeneration increased. These increases in both study groups were statistically significant. Results are presented in **Table 2**.

CN levels in bone tissues were lower in male and female study groups, compared to control groups. nNOS levels in nerve tissues were lower in male and female study groups than those in control groups. Decreases in CN and nNOS levels were statistically significant. Results are presented in **Table 3**.

Serum ALP, Ca, and CK values are presented in **Table 4**. ALP in the male study group was lower than that of the control group. This decrease was statistically significant. There was a decrease in the female study group, but it wasn't statistically significant.

The weights of female and male baby rats were lower than that of cubs in the control group. In both genders, femur, humerus, and ulna lengths and femur medulla diameters were lower in study groups when compared to control groups. In the male study group, lengths of the tibia and radius were shorter than the control group. It was higher in the female study group. Femur medulla and cortex diameter ratios were higher in study groups than in control groups for both genders. Results are presented in **Table 1**.

Discussion

The present study examined how the development of bone, muscle, and peripheral nerve systems in locomotor system of female and male prenatal rats exposed to 900 MHz EMF are affected. During analysis, histopathologic changes in long bones, gastrocnemius muscles, and sciatic nerves of rats, as well as morphometric bone tissue changes and blood biochemistry parameters were examined. According to the literature review, it was found that most of the animal experiences have examined the postnatal-period effects of EMF on the locomotor system [10-13]. The present study determined that the number of studies examining the effects of EMF on locomotor development of rats exposed in prenatal period was limited [14].

Table 4. Serum biochemistry value changes by control and study groups

	Male		Female	
	Study Group (med ± SD)	Control Group (med ± SD)	Study Group (med ± SD)	Control Group (med ± SD)
Alkaline phosphatase (U/L)	234±33.25*	301±49.68	121±30.91	148±31.31
Creatine kinase (U/L)	862±212.12	836±227.85	557±234.33	670±156.05
Calcium (mMol/L)	10.75±0.22	10.74±0.34	10.30±0.25	10.51±0.13

*($p<0.05$).

Many recent studies have been carried out to determine the effects of EMF on bone [10-12], muscle [15, 16], and peripheral nerve tissue [16, 17]. However, there is not a consensus concerning the effects of EMF on bone, muscle, and peripheral nerve tissue development [10-12, 22].

In this study, from the bones of female and male baby rats exposed to 900 MHz EMF in prenatal period, the negative effects were determined using radiological measurements of femur, tibia, humerus, radius and ulna lengths, femur medulla diameter, femur medulla/cortex diameter ratio. The effects were significant in male baby rats. In addition to those results, it was determined that, among female baby rats in the study group, when compared to those in the control group, the femur medulla diameters and femur medulla/cortex diameter ratios were negatively affected by 5.8% and 15.3%, respectively. Compared to the control group, femur medulla diameters and femur medulla/cortex diameter ratios of male baby rats were negatively affected by 5.3% and 8.5%, respectively. Even if it is not statistically significant in the female study group, in radiological measurements of lengths of femur, tibia, humerus, radius, and ulna bones, no negative changes occurred in proportion to the male study group. Results indicate that the gender factor may be effective while evaluating the effects of exposure to EMF in prenatal period on lengths of long bones of baby rats.

Moreover, according to the literature review, besides studies [11, 12, 18] asserting that low-frequency EMF has positive effects on bone tissue, there also studies asserting that they have negative effects [10, 14].

In the present study, negative changes in femur medulla diameter and femur medulla to cortex diameter ratios in female and male study groups were statistically significant. Since the femur medulla diameter and ratio of femur medulla to cortex diameter is an indicator of bone quality [19], results showed that EMF has negative effects on the development process of bone quality in both genders. Van der Jagt OP et al. [10] carried out a study on the effects of EMF on bone microarchitecture and bone fracture healing in osteoporotic and healthy rats. They determined no effects on cancellous bone and fracture healing. However, Yıldız M et al. [20] determined that bone density decreased in irradiated group and bone density increased in the treatment group. Regardless of the gender factor, it was determined that the bone quality parameter was negatively affected in study groups.

When compared to female and male baby rats in the control group, those in study groups were determined to have increased myelin degeneration, hypertrophic and necrotic cartilage, epiphyseal plate degeneration, apoptotic myocyte, muscle fiber dilatation, and myocyte swelling and degeneration. At the same time, these histopathological changes were statistically significant.

Cellular changes corroborating the histopathological findings of the present study have been observed in a study of Türedi S et al. under light microscope. They determined irregularity and apoptotic changes in myocardium fibrils of baby rats exposed to 900 MHz radiation [21]. One of the possible reasons for these histopathological changes in study groups has been reported by Hancı H et al. [21] and Türedi S et al. [22] to be the significantly higher oxidative stress markers in the blood of baby rats. These findings indicate that baby rats might be exposed to harmful effects of EMF through oxidative stress in the wombs of their mothers. CN is the protein phosphatase. For enzyme activa-

tion, Ca/calmodulin is needed. It is an effective protein for cell proliferation. It has been found to exist in structure of many organs. It plays important role in cell development and proliferation within cell tissues [23]. For this reason, the present study examined the bone calcineurin relationship.

The effects of CN activity on the bone have been shown by Li et al. [24], reporting that CN inhibitors led to osteoporosis in bone. Thus, CN immunohistochemical staining was performed on femur medulla sections. Statistically significant differences were determined between male control and study groups (p=0.027) and female control and study groups (p=0.039) in terms of anti-CN a immune-reactivity. Results showed that EMF negatively affected the proliferation in bone structure and cell production. Results corroborating present findings have been reported by Erkut et al. [14]. Neural nitric oxide structure of nNOS is utilized as the indicator of active neuronal regeneration and myelination [25, 26]. By determining the nNOS level according to immune reactivity in cell degenerations, axon losses, and nerve defects, the presence of cell regeneration can be revealed. For this reason, nerve-nNOS relationship was examined in the present study.

Nitric oxide (NO) is the signal molecule in the form of free gas. It plays a role in heart, vein, nerve, and immune system regulation. NO, in vertebrates, are synthesized by the L arginine from nitric oxide synthase (NOS). NOS has three isoforms, namely neuronal, endothelial, and immunologic. nNOS (neuronal nitric oxide synthase) is one of them. It mainly exists in nerve tissue. Its neuronal and endothelial forms are named NOS. For neuronal transmission, it synthesizes low levels of NO interruptedly. When intracellular Ca increases, Ca integrates with calmodulin and activates the nNOS [26, 27]. nNOS is predominantly present in sarcolemma of skeletal muscle fibrils. Activation of nNOS in skeletal muscles is organized by developmental, myogenic, and neurogenic factors [26]. Functional studies have shown NO, as a modulator in skeletal muscle contraction, mitochondrial respiration, carbohydrate metabolism, and neuromuscular transmission [25].

For this reason, this study performed neuronal nitric oxide synthase (nNOS) immunohistochemical staining in sciatic nerve sections. In staining, it was that found statistically significant differences between male control and study groups (p=0.003) and female control and study groups (p=0.009) existed in terms of Anti-nNOS immune reactivity. This result indicates that EMF affected the neural structure in terms of axon degeneration, myelin degeneration, and cell proliferation.

Moreover, Kim et al. [28] reported the positive effects of EMF on nerve tissues. They investigated the effects of EMF on rat laryngeal nerve model after anastomosis. They determined the positive effects of nNOS on nerve regeneration. Moreover, they found that axonal regeneration was enhanced and enzyme activation was also increased. Despite the positive effects on peripheral nerve tissue in the postnatal period, it was determined that it negatively affected development in prenatal period through decreases in nNOS levels.

On the other hand, Aydın MA et al. [16] investigated the effects of PFEF (power frequency electric field) on nerve regeneration in an experimental model, where they created crush injuries in peroneal nerve. They did not determine any effects in morphometric analyses. Similarly, in their study, Güneş et al. [17] investigated the electrophysiological effects of EMF on sciatic nerves. They did not determine any significant differences. In the present serum biochemistry analyses, it was determined that, in proportion to the control group, ALP in the study group was 22.25% lower in males and 18.24% lower in females. ALP is the enzyme secreted from osteoblast cells in bones. Its activity causes increases in bone development and repair [29]. In both histopathological examinations and radiological analyses, it was determined that bone tissue was qualitatively and quantitatively affected in study groups, when compared to control groups. This result was in accord with the ALP values (**Table 4**). The level of Ca in the study group, when compared to control group, was 0.09% higher in males and 2.37% lower in females. The differences were statistically non-significant. CK in the study group, compared to the control group, was 3.01% higher in males and 16.86% lower in females. In cases of any muscle damage, concentrations of CK in the blood increases [30]. However, in the present study, despite histopathological muscle changes in study gro-

ups, there were very low levels of CK concentrations. A decrease was observed in female groups, but it was statistically insignificant.

Conclusion

Peripheral nerve, muscle, and bone tissues of female and male baby rats, exposed to 900 MHz EMF in the prenatal period and then grown for 60 days, were affected negatively from a histopathological aspect. Furthermore, development of long bones was negatively affected in quality, in both genders, and in quantity in male baby rats.

The present study is a pilot study explaining this phenomenon. Further molecular and electron-microscopic studies on this topic are needed.

Disclosure of conflict of interest

None.

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